History of Prostatic Diseases

Chairman

D. SCHULTHEISS (GERMANY)
R. S. WALDBAUM (USA)
Leonardo da Vinci (1452-1519) was the first master of anatomical and medical illustration, although his outstanding work on the anatomy and physiology of the human body was almost unknown to his contemporaries. The incident that he never gave a description of the prostate gland can be explained by the fact, that most of his knowledge was drawn from anatomical dissections of castrated oxen only having a small and atrophic prostate.

The "Tabulae Anatomicae" of the anatomist Andreas Vesalius (1514-1564) from 1538 gave the first illustration of the prostate gland but omitted the seminal vesicles. A more detailed illustration can be found in “De Humani corporis fabrica Libri septem” from 1543.
In his “Dix Livres de la Chirurgie” from 1564 the master of French Renaissance surgery Ambroise Paré (1510-1590) dedicated one chapter to “des chaudes pisses, des pierres et des rétentions d’urine”. He described obstructive urinary symptoms and related them to “carnosities” or “caruncles” deriving from the prostate.

Ambroise Paré even suggested catheters or sounds with sharp ridges on their surfaces designed to remove these “carnosities” or “caruncles” by repeatedly passing these instruments through the urethra.

In his “Tractus de virorum organis generationi inservientibus, de clysteribus et de usu siphonis in anatomia” (1668) the Dutch physician Regnier de Graaf (1641-1673) gave a very detailed and exact anatomical description and illustration of the prostate, the seminal vesicles and the ejaculatory ducts.
The famous surgeon and anatomist John Hunter (1728-1793) from London is well-known for his contributions on venereal diseases, the prostate and urethral strictures. In his book “A Treatise on the Venereal Disease” from 1786 he illustrated an enlarged middle lobe of the prostate. In his later works he precisely described obstructive symptoms caused by enlargement of the prostate and the resulting effects on the bladder muscularis and of dilation of the upper urinary tract. Hunter also realized that these changes did not occur in castrates.

Jean Civiale (1796-1867) from Paris devised the “kiotome” around 1830 for incision of the obstructing bladder neck. In 1836, his contemporary Louis-Auguste Mercier (1811-1882) introduced several instruments to incise the median bar or even to remove small tissue particles similar to a punch.

In 1874 Enrico Bottini (1835-1903) from Pavia in Italy was the first to apply electric surgery to the prostate. His “Cauterio termogalvanico” facilitated destruction and incision of a prostate lobe and median bar without producing hemorrhages.
Based on the “Cauterio termo-galvanico” of Bottini several modified galvano-cautery and –incision instruments were suggested by Freudenberg (A), Chetwood (B) and Wishard (C).

Sir Henry Thompson (1820-1904) from London won the prestigious Jacksonian Prize in 1860 with his monography “The enlarged Prostate, its Pathology and Treatment”. He gained reputation as the world’s greatest specialist surgeon after he successfully removed the bladder stone of King Leopold I, King of Belgium, in 1863.

Theodor Billroth (1829-1894) was one of the greatest surgeons of his time and before coming to Vienna he most likely performed the first planned removal of carcinomatous prostatic tissue as partial perineal excision of the prostate in 1867 while still working in Zurich.
George Goodfellow (1855-1910), from Tombstone and Tucson performed the first perineal enucleation of the enlarged prostate in 1891. In 1904 (July and November issue of JAMA) he first reported on 72 cases which he had operated with only 2 fatalities. Many surgeons followed him and contributed various technical aspects to perineal prostatectomy. His definition of a good surgeon was: “A surgeon should have the eye of an eagle, the heart of a lion, and the touch of a woman.”

Eugene Fuller (1858-1930) from New York published suprapubic transvesical enucleation of the prostate in 1895 as performed by him the previous year. He accomplished for the first time not only removal of the intravesical but also of the intraurethral enlargement of the prostate by digital enucleation.

Peter Freyer (1852-1921) from St. Peter’s Hospital in London must have learned about Fuller’s technique of transvesical prostatectomy through a visit of Fuller’s assistant Ramon Guitéras in 1900. One year later Freyer then claimed priority for the technique and by this gave ground to some dispute. Freyer however became the major prostatectomist of the time and deserves credit for popularizing the operation and making it a standard procedure.
During the 1890s men with severe symptoms of prostatic hypertrophy were occasionally castrated. Mansell Moullin from London described the effect of castration in 1894: “Removal of the testes is followed in a large proportion of cases by complete and rapid absorption of the enlarged prostate. The gland entirely disappears; nothing is left but a little fibrous mass.” The symptomatic improvement in over a half of patients with an enlarged prostate treated with castration was reported by William White from Philadelphia in 1895 and 1904.

Searching for alternatives to castration for the treatment of enlarged prostate, vasectomy was firstly suggested by James Ewing Mears in 1890, although Felix Guyon of Paris was also cited as the first to achieve this doubtful distinction. This treatment was en vogue for a short period of time as it was claimed to be a method with minimal morbidity and great effectiveness. Further applications of this technique soon resulted in less enthusiastic reports (e.g. by Harrison and Wood both in 1900), showing an only 15% improvement in micturition. Shortly later the method fell into disrepute.

Hugh H. Young (1870-1945) from Baltimore performed the first radical perineal prostatectomy for prostate cancer on the 7th April 1904. The respective illustrations are taken from a later publication of Young from 1919. In the early 1940’s he reported on 184 patients he had operated with this technique of whom 34 had lived cancer-free and died from unrelated disease 5 to 27 years later.
Robert Proust (1873-1935), brother of the famous French author Marcel Proust, was the most important early promoter of perineal prostatectomy in France or even Europe at the beginning of the 20th century. At that time the operation was called “Proustatectomie” in France. The starting point was his doctoral thesis in 1900, which was written under the guidance of Felix Guyon and Joaquin Albarran at the Hôpital Necker in Paris. Later Proust designed a special operating table to put the patient in perfect position for perineal surgery.

As in the US by Young perineal prostatectomy was not only performed in benign disease but even for localized prostatic cancer in France by Joaquin Albarran (1860-1912). Notice wide excision at the bladder neck and anastomosis. These illustrations are taken from the textbook “Médecine opératoire des voies urinaires” published by Albarran in 1909.

Edmond Papin, like Proust working at the Hôpital Necker in Paris, wrote an extensive book of over 200 pages on “Sexual Function after Prostatectomy” in 1908. On the basis of anatomical examinations he analyzed sexual dysfunction after prostatectomy, including 55 documented case reports.
“Prostatectomia suprapubica extravescialis” had already been performed by W. J. van Stockum (1860-1913) from Rotterdam, The Netherlands, in 1908 and published one year later in the German journal “Zentralblatt der Chirurgie”. However, the wide clinical establishment of retropubic approach to the prostate after 1945 must be credited to the Irish surgeon Terence J. Millin (1903-1980), who was working in London.

In 1909 Hugh H. Young (1870-1945) performed the first “cold punch” resection of the median bar of the prostate under direct vision control. In 1913 he already reported on 100 patients treated with this new technique.

James Buchanan „Diamond Jim“ Brady (1856-1917) made money with selling railroad equipment and loved diamonds. Besides from being obese, hypertensive and diabetic he suffered from prostatic obstruction. Hugh H. Young (1870-1945) performed the cold punch on him in April 1912 and his patient later donated the Brady Urological Institute at The Johns Hopkins Hospital.
In 1914 Georges Luys from Paris – depicted here as the great explorer in a sketch of the magazine Chanteclair - reported endoscopic electrocoagulation of the prostate, which he called “forage de la prostate (drilling of the prostate)“.

In January 1926 Maximillian Stern (1873-1946) from New York presented his “resectoscope” before the Genito-Urinary Section of the New York Academy of Medicine on the basis of 46 treated patients. The instrument had two lens systems, one indirect vision for examination and a direct vision lens for bipolar resection.

Joseph McCarthy (1874-1965) from New York incorporated a bakelite non-conducting sheath and his foroblique lens system to improve the Stern resectoscope in 1931. It was the first instrument to move the loop from the bladder towards the instrument as in today’s instruments.
Some alternatives were looked for during that era, e.g. in 1928 the endothermal prostatic excisor of Frederic Foley (1891-1966) from Minneapolis. A conical section of the prostate was burned out with a thin steel music wire under electric current by rotating the instrument.

Finally, the improvements of Stern and McCarthy were united in the classical Stern-McCarthy resectoscope which was then in use for many decades.

Other countries had different and individual developments of TURP. Maximillian Stern came to Berlin in 1927 to present his new resectoscope before the Berlin Urological Society and operated on two patients. As both of them died after surgery the new technique of TUR was not well adopted in Germany initially. Alexander von Lichtenberg (1880-1949, Berlin) realized the importance of TURP and developed his own resectoscope, the “Prostata-Cutor” in 1932, which was later improved as the “Lichtenberg-Heywalt-Elektrotom”.
After World War II several European urologists were trained in the US and transferred the technique of TURP to Europe. One of them was Wolfgang Mauermayer (1919-1994) from Munich, Germany, who designed a famous resectoscope with two light sources and for one-handed use in 1952.

Edward L. Keyes and Russell S. Ferguson from New York City had performed radioorchietomy in several patients since 1932. In the 6th edition of their textbook “Urology” from 1936 they pronounced the “Extension of the life of the patient in comfort, even in the face of widespread metastatic disease”. In some of their patients roentgen castration combined with local irradiation was successful in arresting the primary tumor and the metastatic lesions.

The experimental studies which finally established the knowledge about androgen control of malignant prostatic growth were initiated in 1939 by Charles Brenton Huggins (1901-1997) at that time working in Chicago. He demonstrated that castration in man decreases the height of prostatic epithelial cells in normal prostatic tissue, that testosterone stimulates secretory activity of dogs’ prostatic cells and diethylstilbestrol inhibits this activity. He further proved that acid phosphatase was elevated in metastatic prostate cancer and that castration produced a relief of pain and a stabilization or regression of local and metastatic osseous lesions.
In the beginning Huggins won a gold medal of the American Medical Association for his work in benign prostatic hyperplasia in 1940 and only a merit for his study on prostate cancer the year after. Finally, these fundamental investigations on the influence of the endocrine system onto the development of a human malignancy were honored with the Noble Prize for Medicine and Physiology in 1966.

R. Paschkis from Vienna devised the first cystoscopic radium applicator in 1911. The radium capsule was situated at the very tip of the instrument and no external fixation of the cystoscope holding it in the correct position was used. In 1913 O. Pasteau and Degrais from Paris reported several cases of prostate cancer which had been successfully treated by the use of radium introduced through a simple coudé gum catheter.

Benjamin Barringer (1877-1953) Chief at Memorial Hospital New York was the first to perform transperineal implantation of radium into the prostate in hundreds of cases between 1915 and 1930; first published in JAMA in 1917. Initially, Barringer used radon-tipped needles introduced through the perineum and left for several hours. Later gold-encapsulated Radon seeds were applied by the same route as permanent implants.
In the 1930’s Barringer also experimented with the open approach for radon seed application already using a template for controlled placement of the seeds as in today’s technique. He even combined the perineal and suprabupic approach for brachytherapy of the prostate.

Immediately after Barringer’s reports Hugh H. Young adopted and investigated various techniques of prostate brachytherapy at his institute in Baltimore.

Young placed needles with Radium tips alternatively intraurethrally and transrectally for short periods of time over several weeks. For this he designed his own radium applicator.
Rubin Flocks (1906-1975) from Iowa State University treated more than 400 prostate carcinoma patients in the 1950’s by injection of a colloidal suspension of radioactive gold into the prostate. Injection was either performed by the closed perineal or the open transvesical route.

Since 1970 Willet F. Whitmore from the Memorial Sloan-Kettering Cancer Center in New York combined open retropubic implantation of permanent Iodine 125 seeds with bilateral pelvic lymph node dissection allowing a more refined treatment.

Hiroki Watanabe from Sendai, Japan, obtained the first clinically useful tomogram of intrapelvic organs via the rectum in 1967. This equipment was used in 400 patients with one examination being accomplished in 15 minutes.
Holm and Gammelgaard from the University of Copenhagen performed the first ultrasound-guided perineal prostatic biopsies in 1982 and first Iodine 125 seed implantation was reported from the same institution in 1983.

REFERENCES

Ballenger EG, Frontz WA, Hamer HG, Lewis B (1933) History of Urology. Williams and Wilkins, Baltimore


CORRESPONDENCE

Dirk Schultheiss, M.D.
Chairman, History Office of the European Association of Urology
Department of Urology, Protestant Hospital Giessen
Paul-Zipp-Str. 171
35398 Giessen, Germany

Robert S. Waldbaum, M.D., F.A.C.S.
Historian of the American Urological Association
Clinical Professor of Urology, Cornell University Medical School
Chairman, Department of Urology, North Shore University Hospital
535 Plandome Road
Manhasset, New York 11030, USA
Chapter 10

Committee 13

New Therapeutic Targets and Treatments for Metastatic Prostate Cancer

Chairman

W.G NELSON (USA)

Vice-Chair

F. SAAD (CANADA),

Members

F.M.J DEBRUYNE (THE NETHERLANDS),

M. A EISENBERGER (USA),

R. FOURCADE (FRANCE),

E.P FRENKEL (USA),

P.W KANTOFF (USA),

H. KUMON (JAPAN),

Y. NASU (JAPAN),

S. OUDARD (FRANCE),

M. WIRTH (GERMANY)
### CONTENTS

| I. NATURAL HISTORY OF PROSTATE CANCER PROGRESSION TO METASTASIS |
| II. THERAPY TARGETING ANDROGEN SIGNALING |

1. **Androgen Deprivation Therapy**
2. **Anti-Androgens and “Complete” Androgen Blockade**
3. **When Should Men with Prostate Cancer Start Androgen Deprivation Therapy?**
4. **Intermittent Androgen Deprivation Therapy**
5. **Salvage Endocrine Therapy**

| III. BONE-TARGETED TREATMENTS |
| IV. CYTOTOXIC CHEMOTHERAPY |

1. **Bisphosphonates**
2. **Endothelin Receptor Antagonists**
3. **Bone-Targeted Radionuclides**

| V. IMMUNOTHERAPY |

1. **PSA Vaccines**
2. **Antigen-Loaded Dendritic Cell Vaccines**
3. **Prostate Cancer Cell Vaccines**

| VI. “TARGETED” THERAPIES |

1. **Retargeted Cytotoxic Chemotherapy Drugs**
2. **Signal Transduction Pathway Inhibitors**
3. **Epigenetics as a Target for Prostate Cancer Treatment**

| VII. CONCLUSIONS AND RECOMMENDATIONS |

---

**ALGORITHMS**

- Post-Prostatectomy recurrence
  - No clinical metastasis on Work-up
- Post-Radiotherapy Rise in PSA
  - No Clinical Metastasis on Work-up
- Progression after First-line Hormone therapy
  - At least 2 consecutive serum PSA increases
  - Newly Diagnosed Metastatic Prostate Cancer (N+ and/or M+)

**REFERENCES**
With an increased prevalence of prostate cancer screening, made possible via the ready availability of serum prostate-specific antigen (PSA) tests and digital rectal examinations, the mode of presentation of prostate cancer has progressively changed. For example, before extensive prostate cancer screening, men tended to present with prostate cancer complicated by systemic metastases. Currently, the majority of men are first diagnosed with clinically-localized prostate cancer. Most of these men are then treated with radical prostatectomy or radiation therapy. Even men who initially pursue “watchful waiting” often eventually receive local prostate cancer treatments. For men who undergo local therapy for prostate cancer, the first manifestation of extraprostatic spread of prostate cancer is usually a rise in the serum PSA (often termed “biochemical” relapse). Occasionally, a rising serum PSA reflects local treatment failure; more often, the PSA increases serve as a remarkably sensitive indicator of systemic metastases that are not yet evident by imaging studies, and not yet causing symptoms. Androgen deprivation therapy, accomplished via several different strategies, has been the mainstay systemic treatment for prostate cancer for half a century. Unfortunately, men with metastatic prostate cancer treated with androgen deprivation therapy frequently ultimately develop androgen-independent prostate cancer. Thus, physicians now confront the systemic spread of prostate cancer in several “disease states” with different treatment considerations: (i) androgen-dependent prostate cancer without detectable systemic metastases (a rising serum PSA after adequate local therapy), (ii) androgen-dependent prostate cancer with obvious metastases, (iii) androgen-independent prostate cancer without detectable metastases (a rising serum PSA despite adequate androgen deprivation therapy), (iv) androgen-independent prostate cancer with obvious metastases, and (v) symptomatic androgen-independent prostate cancer.

Although prostate cancer mortality rates have been declining in the developed world, a phenomenon that has been attributed to prostate cancer screening, to more widespread use of prostate cancer treatments, or to some other poorly understood reason(s), many men continue to die from the disease. Recent autopsy studies have attempted to characterize the “lethal phenotype” of prostate cancer in the modern era [1,2]. In one of the series, residual tumors were present in the prostate, though often small, and there were frequent metastases to the skeleton (including the skull and dura), liver, lung, lymph nodes, adrenal, and pelvic soft tissues, but not the brain [1]. Remarkably, although for each prostate cancer case, different metastatic cancer deposits at different sites contained similar somatic genome abnormalities, a marked heterogeneity in phenotype, assessed via morphologic appearance, immunohistochemical staining for androgen receptor and PSA, and transcriptome profiling, was characteristic [2-5]. These findings might be consistent with a mechanism by which prostate cancer cells capable of metastasis form early during the prostatic carcinogenesis, but continue to evolve and adapt at metastatic sites, perhaps in response to interactions with stromal elements, leading to malignant disease progression [6]. One prediction of such
a mechanism is that “poor prognosis” features should be able to be discerned in primary cancers at the time of presentation; an alternative explanation is that prostate cancers at specific metastatic sites, such as the bone, might respond differently to certain treatments that prostate cancers at other metastatic sites, such as in soft tissues. Also, if primary cancers prone to metastasis are continuously or intermittently contributing carcinoma cells to the circulation and to lymphatics, adequate control of the primary cancer by surgery or radiation therapy should result in a reduced risk for clinical metastases and for life-threatening prostate cancer progression [7]. In the well-publicized Scandinavian randomized clinical study of surgery versus watchful waiting (n = 695), local therapy for prostate cancer was associated with a reduced risk of prostate cancer metastasis (RR = 0.60 with a 95 percent confidence interval of 0.42 to 0.86) and a reduced risk of prostate cancer mortality (RR = 0.56 with a 95 percent confidence interval of 0.36 to 0.88) as well as overall mortality [8,9].

Several clinical features appear to discriminate poor prognosis prostate cancer at the time of presentation and diagnosis, including pathologic grade (Gleason score), tumor stage, tumor volume, serum PSA level, and rate of serum PSA rise. These clinical features have been incorporated into a variety of clinical tools that can predict pathologic stage at radical prostatectomy and/or risk of prostate cancer recurrence or progression after local prostate cancer treatments. For clinically localized prostate cancer diagnosed by prostate biopsy, these tools are widely used to select candidates for surgery or for radiation therapy. However, in the modern era of serum PSA testing for prostate cancer screening, aggressive treatment of all men discovered to have prostate cancer has been questioned. Men with prostate cancer often face the threat of other life-threatening illnesses, and thus age, co-morbidities, and quality-of-life, as well as prostate cancer prognosis, must all contribute to decision-making regarding prostate cancer treatment. For some men, when the available prognostic tools predict a low risk for malignant prostate cancer progression, a watchful waiting approach is considered in place of immediate surgery or radiation therapy. In the future, men with clinically localized prostate cancer at a high risk for prostate cancer progression or mortality may also be stratified to receive adjuvant chemotherapy and/or androgen deprivation therapy along with local treatment. At present, men with high risk prostate cancer (clinical stage T2c-T4, Gleason score of 8-10, or serum PSA > 20 ng/mL) routinely receive adjuvant androgen deprivation therapy along with external beam irradiation. One of the best risk stratification tools appears to be the temporal pattern of serum PSA changes. In a study of men undergoing radical prostatectomy for prostate cancer (n = 1095), the rate of serum PSA rise (PSA “velocity”) in the year before diagnosis was correlated with the risk of death from prostate cancer [10]. When compared with a PSA velocity of 2.0 ng/mL per year, a yearly PSA rise of > 2.0 ng/mL portended a shorter time to death from prostate cancer (p < 0.001) and from any cause (p = 0.01). Similarly, for men treated with radiation therapy, a yearly PSA rise of > 2.0 ng/mL before diagnosis was also accompanied by a shorter time to prostate cancer-specific mortality (hazard ratio = 12.0 with a 95% confidence interval of 3.0 to 54.0) and all-cause mortality (hazard ratio = 2.1 with a 95% confidence interval of 1.3 to 3.6) [11].

Prostate cancer that has recurred after adequate local treatment, or has progressed after adequate androgen deprivation therapy, exhibits a remarkably variable, and often indolent, natural history. In a study of men (n = 379) who suffered a rising serum PSA after undergoing radical prostatectomy for clinically localized prostate cancer, the median survival had not been reached after 16 years of follow-up. However, the PSA doubling time (< 3.0 months versus 3.0-8.9 months versus 9.0-14.9 months versus > or = 15.0 months), the pathological Gleason score (< or = 7 versus. 8-10), and the time from surgery to biochemical recurrence (< or = 3 years versus. >3 years) were all significant predictors for time to prostate-specific mortality [12]. The relative risk for death from prostate cancer was 28-fold higher for men with a rising serum PSA at a doubling time < 3 months, who have a median survival of 6 years, versus at a doubling time > 15 months (RR = 27.38 with a 95% confidence interval of 10.66 to 70.85), who face very little threat of prostate cancer mortality at all after radical prostatectomy [12]. Similar data have been reported in other case series [13-16]. These data argue that for men with prostate cancer recurrence manifest as a rising serum PSA after definitive local therapy, men with a rapidly rising PSA may be in more urgent need of systemic treatment than men with a slowly rising PSA. Also, PSA doubling time, Gleason score, and time to recurrence can likely be used as a stratification tool for systemic treatment [12]. For men suffering with a rising serum PSA despite adequate androgen deprivation therapy, a recent clinical study (n = 201) revealed that after 2
years, only 33% of the men had developed bone metastases, and that the median bone metastasis-free survival was 30 months, while the median time to first bone metastases and overall survival were not reached [17]. Again, the rate of PSA rise, along with the baseline serum PSA, was a significant predictor of the time to clinically-significant prostate cancer progression [17]. Thus, for men with systemic spread of prostate cancer manifest only as a rising serum PSA, before or after androgen deprivation therapy, the rate of serum PSA change appears to stratify men at greater risk for worrisome prostate cancer progression, identifying men who might most benefit from aggressive treatment intervention.

II. THERAPY TARGETING ANDROGEN SIGNALING

The first systemic treatment offered most men with prostate cancer targets the androgen signaling axis, accomplished using androgen deprivation, anti-androgens, or a combination of androgen deprivation and anti-androgens [18-20]. In the prostate, testosterone, produced by Leydig cells in the testes upon stimulation by leutinizing hormone (LH), is converted to dihydrotestosterone (DHT) by the action of 5α-reductase [21]. DHT, a more potent androgen than testosterone, binds to intracellular androgen receptors to activate the expression of target genes, such as PSA [22,23]. In the normal prostate epithelium, androgenic hormones principally drive differentiation to a columnar secretory phenotype. However, in prostate cancer cells, androgen signaling axis contributes to cell growth and survival as well as to differentiation. As a consequence, most men enjoy an initial benefit to treatment targeting androgen signaling, with a fall in serum PSA and relief of symptoms attributable to prostate cancer. Unfortunately, the ultimate emergence of androgen-independent prostate cancer is common. In most cases, androgen-independent prostate cancers maintain the expression and function of androgen receptors despite therapeutic reduction of serum androgen levels [24-27]. For human prostate cancer cells studied in xenograft models, progression to androgen-independence appears to be associated with increased expression of androgen receptor transcripts and increased abundance of androgen receptors, presumably contributing to an increased sensitivity of the receptors to low levels of androgenic hormones [28]. Whether this phenomenon occurs commonly in men suffering androgen-independent prostate cancer has not been established. Nonetheless, AR, encoding the androgen receptor, is a known target for somatic genome alterations in prostate cancer, especially upon progression of the disease to androgen-independence [24,28-41]. AR mutations, encoding androgen receptors with altered ligand specificity, can result in agonist activity for anti-androgens, providing one molecular explanation for the “anti-androgen withdrawal” syndrome, in which men with prostate cancer progression despite treatment with a combination of androgen deprivation and anti-androgens benefit from discontinuation of the anti-androgen [42-44]. Finally, androgen-independent prostate cancer cells containing wild-type androgen receptors appear to be capable of androgen receptor signaling, even in the context of reduced androgen levels, as a result of post-translational modifications of the androgen receptor and/or androgen receptor co-activators in response to other growth factor signaling pathways [24,45-48].

1 ANDROGEN DEPRIVATION THERAPY

Androgen deprivation therapy for prostate cancer involves reduction of circulating testosterone levels to < 50 ng/mL, accomplished via surgical removal of the testis (bilateral orchiectomy), by inhibition of the synthesis and release of pituitary gonadotropins by leutinizing-hormone-releasing hormone analogues (LHRH analogues) or antagonists (LHRH antagonists), or by the administration of pharmacological doses of estrogens. Bilateral orchiectomy results in a rapid decline of testosterone to 5-10% of normal values; LHRH analogue suppression of testosterone production, though comparable to that achieved by castration, does not reach its nadir until after 3-4 weeks of treatment. By acting as LHRH agonists, the LHRH analogues first trigger LH release by the pituitary, rarely associated with a symptomatic “flare” of prostate cancer, then after chronic administration suppress both LH and testosterone production [49-51]. Currently, long-acting depot preparations of LHRH analogues (administered monthly, every 3 or 4 months, or yearly) are most commonly used. LHRH antagonists appear to achieve suppression of testosterone production without the brief flare associated accompanying initiation of treatment with LHRH analogues [52]. Although there are no long-term studies testing the efficacy of LHRH antagonists for prostate cancer, in comparison to bilateral orchiectomy or LHRH analogues, because LHRH antagonists can fairly rapidly lower testosterone levels without a risk for a symptomatic disease flare, the
agents may offer an advantage over LHRH analogues in a clinical setting where such a flare might carry a threat of significant morbidity. Finally, the administration of pharmacological doses of synthetic estrogens, such as diethylstilbestrol (DES), represented the earliest strategy for drug treatment of prostate cancer [53]. Accumulated data from several prospective randomized clinical trials for men with metastatic prostate cancer have revealed comparable efficacy of bilateral orchietomy, DES, and LHRH analogues, regardless of the outcome measure used [54-61]. However, when LHRH analogues were found to have fewer serious treatment complications, such as congestive heart failure and thromboembolic events, than DES, estrogens were virtually abandoned in favor of LHRH analogues for the initial treatment of metastatic prostate cancer. Also, many men find LHRH therapy more acceptable than bilateral orchietomy.

2. Anti-Androgens and “Complete” Androgen Blockade

Anti-androgens directly interact with the androgen receptor, interfering with its trans-activation of target gene transcription [24]. These agents have been used as monotherapy, in an attempt to spare side effects of androgen deprivation, and along with androgen deprivation therapy as “complete” androgen blockade. The anti-androgen bicalutamide, given as monotherapy, has been reported to provide a similar survival benefit as bilateral orchietomy for men with locally-advanced, but non-metastatic, prostate cancer (stage T3 and T4), but to be inferior to androgen deprivation for men with metastatic disease [62]. Side effects of bicalutamide monotherapy at a 150 mg daily dose include significant gynecomastia, and although libido can be preserved, few men remain fully potent [62]. The efficacy of anti-androgens as adjuvant therapy for men with high-risk prostate cancer treated with radical prostatectomy, or as treatment for men with a rising serum PSA after adequate local therapy remains to be established.

Weak androgenic hormones such as androstenedione and dehydroepiandrosterone are produced in the adrenal glands. In an attempt to neutralize the effects of adrenal androgens, a combination of bilateral orchietomy (or LHRH analogues) and a non-steroidal anti-androgen was promoted as “complete” androgen blockade [63,64]. Initial reports of the efficacy of this treatment combination prompted the conduct of a large number of clinical trials testing whether “complete” androgen blockade offered an advantage over androgen deprivation alone for men with metastatic prostate cancer. Some 7,987 men with metastatic prostate cancer have been enrolled in 27 prospective randomized clinical trials comparing the efficacy bilateral orchietomy (or LHRH analogues) alone to combinations of bilateral orchietomy (or LHRH analogues) and anti-androgens [65,66]. A review of these trials reveals that 24 of the 27 studies reported no significant differences in survival, and 3 studies showed only modest improvements, which were statistically significant, in favor of “complete” androgen blockade [66] (Figure 1). In 1995, the Prostate Cancer Trialists’ Collaborative Group (PCTCG) reported the results of a meta-analysis from 22 of the randomized trials comparing “complete” androgen blockade to androgen deprivation alone for 5710 men with prostate cancer, finding a 2.1% difference in survival in favor of “complete” androgen blockade (with a 6.4% reduction in annual risk of death) that was not statistically significant. The Agency for Health Care Policy and Research (AHCPR; results published at http://www.ahcpr.gov/clinic/index.html#evidence as AHCPR report No.99-E012 ) also conducted a meta-analysis of all published “complete” androgen blockade clinical trials, finding no difference in 2-year survival rates.
(hazard ratio = 0.970 with a 95% confidence interval of 0.866 to 1.087). For the 10 trials that reported 5-year survival data, the meta-analysis revealed a minimal 5-year survival difference in favor of “complete” androgen blockade (hazard ratio = 0.871 with a 95% confidence interval of 0.805 to 0.9887).

3. WHEN SHOULD MEN WITH PROSTATE CANCER START ANDROGEN DEPRIVATION THERAPY?

Androgen deprivation therapy is the standard treatment for men with metastatic androgen-dependent prostate cancer. However, for men with androgen-dependent prostate cancer manifest only as a rising serum PSA, the optimal timing for the initiation of androgen deprivation has not been fully established. There are 3 randomized trials of “early” versus “late” androgen deprivation therapy with apparently conflicting results. In the first study, VACURG Study 1, men with advanced prostate cancer were randomized to immediate treatment with bilateral orchiectomy plus a 5 mg daily dose of DES, bilateral orchiectomy plus a placebo, 5 mg DES per day alone, or placebo alone, with the possibility of a cross-over from the placebo arm at the time of cancer progression [53,57]. There was no survival benefit to any treatment arm assignment, suggesting that “early” androgen deprivation therapy was not superior to “late” treatment. In a second study, the Medical Research Council randomized men with prostate cancer (n = 934 men; 434 men with and 500 men without prostate cancer metastasis) to either “early” androgen deprivation or to androgen deprivation offered for symptomatic prostate cancer progression [67]. Using death from prostate cancer as a study endpoint for the men who had overt prostate cancer metastases, no significant difference was detected between the early (65% prostate cancer deaths) versus late treatment groups (69% prostate cancer deaths). In contrast, men with non-metastatic prostate cancer appeared to have fewer prostate cancer deaths (32%) when treated with “early” androgen deprivation therapy than when not treated “early” (49%), though some 54% of the men given immediate androgen deprivation therapy never received any hormonal therapy. In a more recent report, reflecting greater follow-up time, no statistically significant differences were evident for men with prostate cancer treated with early versus delayed androgen deprivation therapy. The Eastern Cooperative Oncology Group (ECOG) carried out a randomized prospective trial of immediate androgen deprivation therapy versus observation in men (n = 98) who underwent radical prostatectomy and were found to have lymph node metastases [68]. After a median of 7.1 years of follow-up, a significant difference in survival, favoring immediate androgen deprivation therapy, was detected [68].

Unfortunately, there is not any clear mechanistic explanation for the different apparent benefits of “early” versus “late” androgen deprivation among the 3 trials. Currently, many men consider initiating androgen deprivation therapy at the time of prostate cancer recurrence after adequate local therapy, most often manifest as a rising serum PSA. Beginning treatment at that time might exploit any added benefit attributable to “early” initiation of androgen deprivation, but will likely increase the chance for the adverse consequences of androgen deprivation, such as bone loss, loss of libido, cognitive decline, and worsening quality-of-life. With the median survival of such men likely greater than 16 years, such adverse treatment-associated consequences are of great concern [12]. In contrast, waiting for the appearance of overt prostate cancer metastases before beginning androgen deprivation might miss an “early” androgen deprivation advantage, but permit a longer period of time without treatment-associated symptoms. The PSA doubling time may provide a tool for stratifying men with a rising serum PSA for androgen deprivation therapy: men with a PSA shorter doubling times require treatment earlier than men with longer PSA doubling times[12].

4. INTERMITTENT ANDROGEN DEPRIVATION THERAPY

Provocative findings from animal model studies have hinted that intermittent reductions in serum testosterone levels might offer an advantage over continuously maintained androgen deprivation in delaying prostate cancer progression to androgen-independence, stimulating a significant body of clinical research on intermittent androgen deprivation therapy [69]. In the animal studies, mice carrying 3 gram androgen-dependent cancers were either treated with bilateral orchiectomy (continuous androgen deprivation), or were treated with bilateral orchietomy and then subjected to tumor harvest after the tumors had regressed at least 30%. The regressed tumors were then transplanted into intact mice and then treated again with bilateral orchietomy after the tumors had again grown to 3 grams.

This treatment cycle (intermittent androgen depriva-
tion) was continued until the cancer became androgen-independent. Intriguingly, androgen-independent cancer emerged 51 days after initiation of continuous androgen deprivation versus 147 days after initiation of intermittent androgen deprivation. The mechanism for this difference, attributed to a superiority of intermittent androgen deprivation as cancer treatment, has not been fully elucidated. However, other pre-clinical animal model studies have yielded conflicting results. When rats carrying a transplantable androgen-dependent prostate cancer were treated with immediate bilateral orchiectomy, with continuous high- or low-dose DES, or with intermittent high- or low-dose DES, rats treated androgen deprivation continuously survived 38-50% longer than rats treated with intermittent androgen deprivation [70].

The clinical translation of the intermittent androgen deprivation therapy concept, most often accomplished via careful monitoring of the serum PSA, may also provide a significant reduction in androgen deprivation side effects. The results of large randomized clinical trials of intermittent versus continuous androgen deprivation therapy will test whether either approach is associated with a benefit in prostate cancer survival and/or in overall survival.

5. SALVAGE ENDOCRINE THERAPY

The increasing understanding of the biology of prostate cancer progression to androgen independence has triggered renewed interest in various endocrine manipulations as salvage therapy (Figure 2). As an example, some 20-25% of men treated with anti-androgens (such as with “complete” androgen blockade) exhibit an “anti-androgen-withdrawal” syndrome, characterized by an improvement after stopping the anti-androgen but maintaining androgen deprivation [71–73]. For some of these men, AR mutations, encoding androgen receptors with altered ligand binding properties, may permit anti-androgens to function as receptor agonists [42,43,74]. As such, several agents which potentially interfere with ligand activation have been reported to provide beneficial responses, such as a drop in the serum PSA or an improvement in cancer symptoms, in a fraction of men progressing despite androgen deprivation therapy, including bicalutamide (20-24%), megestrol acetate (8-13%), DES (26-66%), ketoconazole with hydrocortisone (27-63%), and glucocorticoids alone (18-22%) [75,76]. In general, responses to second-line endocrine therapies are brief, with median durations ranging between 3-4 months.

---

**III. BONE-TARGETED TREATMENTS**

Metastasis to bone, with destruction of normal bone architecture, is a major feature of malignant prostate cancer progression [1]. In addition, androgen deprivation therapy commonly results in osteopenia [77,78]. In a retrospective analysis of men (n = 50,613) with prostate cancer who appeared in the Surveillance, Epidemiology, and End Results and/or Medicare databases in 1992 through 1997 and survived at least 5 years after diagnosis, 19.4% of men treated with androgen deprivation therapy suffered with a bone fracture, versus 12.6% of men not treated with hormonal manipulation (p < 0.001) [78]. Thus, both progressive prostate cancer and its treatment increase the risk of skeletal-related complications, such as pain, fracture, and spinal cord compression, which pose a significant threat to quality-of-life. The skeletal complications of bone metastases can be particularly painful and debilitating, and can have a profound effect on quality-of-life. Indeed, Weinfurt et al. assessed the effect of skeletal-related
Early-generation bisphosphonates (comitantly with cytotoxic chemotherapy agents. patients with cancer, even when administered con-

nally well-tolerated as drugs for long-term use in

nate compounds target bone surfaces and are gener-

for osteoporosis, for hypercalcemia of malignancy,

lasms, have not been well-established [83]. Animal

iments in serum lactate dehydrogenase levels were sig-

though not associated with significant toxicity, nev-

lantases from prostate cancer may cause such severe pain and functional limitation as to

require hospitalization for treatment and palliation, placing a greater burden on patients and caregivers alike. Clinical studies have also suggested that skele-

tal destruction may hasten life-threatening prostate cancer progression: in one series, men (n = 195) treated with androgen deprivation therapy for prostate cancer who suffered a skeletal fracture (n = 24) exhibited worse survival than men with no frac-
tures (median survivals of 121 months versus 160 months) [80]. Prostate cancer cells are known to appear in bone marrow in many prostate cancer cases, including clinically localized disease [81,82]. Unfortunately, the molecular mechanisms of prostate cancer progression in the bone, accompanied by dys-

regulated activation of both osteoblasts and osteo-

clasts, have not been well-established [83]. Animal

studies, limited by the lack of a model that fully reca-
pitulates all features of human prostate cancer bone metastasis, have hinted that PTHrP, RANKL, osteo-

protegerin, TGFβ, bone morphogenetic proteins (BMPs), matrix metalloproteinases (MMPs), and endothelin-1 might all be expressed at bony metastatic sites and contribute to skeletal destruction [84-86].

1. BISPHONATES

Bisphosphonates have emerged as effective modula-
tors of ossification in bones, providing benefit both for osteoporosis, for hypercalcemia of malignancy, and for cancer bone metastasis [87-90]. Bisphospho-
nate compounds target bone surfaces and are generally well-tolerated as drugs for long-term use in patients with cancer, even when administered con-
comitantly with cytotoxic chemotherapy agents. Early-generation bisphosphonates (eg. etidronate and clodronate) were demonstrated to have limited efficacy for men with advanced prostate cancer. As compared with men treated with a placebo, men receiving daily oral clodronate (2080 mg) for bone

pain from prostate cancer in a randomized clinical trial (n = 311) showed a trend toward increased bone progression–free survival (p = 0.066) and a signifi-
cantly lower rate of performance status decline. Unfortunately, gastrointestinal toxicity and fluctua-
tions in serum lactate dehydrogenase levels were sig-
nificantly worse for the oral clodronate group (p = 0.002). Intravenous clodronate (1500 mg monthly), though not associated with significant toxicity, nev-
ertheless failed to demonstrate any significant pallia-
tive benefit, when compared to placebo, in phase 3 clinical testing for men with painful bone metastases from prostate cancer. Later-generation bisphospho-
nate drugs have greater potency and may exhibit increased efficacy in such a setting. Ibandronate demonstrated significant pain palliation in a small uncontrolled trial targeting men (n = 25) with painful bone metastases from prostate cancer, and pamidronate has also showed some benefit in that setting, although the benefit of pamidronate treat-

ment in randomized trials failed to reach statistical significance [91,92]. More recently, zoledronic acid (4 mg in a 15-minute infusion every 3 weeks; Zometa®) demonstrated significant objective bene-

fits and received widespread regulatory approval in the setting of painful bone metastases from prostate cancer. In a 24-month placebo-controlled trial for men with bone lesions from prostate cancer that had progressed during androgen deprivation therapy (n = 643), 4 mg zoledronic acid reduced the proportion of patients who experienced skeletal complications by 22% (38% with zoledronic acid vs. 49% with place-
bo, a difference of -11.0% with a 95% confidence interval of -20.2% to -1.3% and p = 0.028) [93]. These results are similar are similar to those obtained in placebo-controlled trials using intravenous bis-
phosphonates for women with bone metastases from breast cancer, which led to a recommendation for the use of bisphosphonates in that setting. Compared with placebo, 4 mg zoledronic acid decreased the mean annual incidence of skeletal complications in men with androgen-independent prostate cancer by 48% (0.77 events/year vs. 1.47 events/year for place-
bo, p = 0.005), and significantly prolonged the medi-
an time to first skeletal-related adverse events (SRE) by more than 5 months (488 days vs. 321 days, p = 0.009) [93]. Zoledronic acid also significantly

reduced the ongoing risk of skeletal complications by 36% (risk ratio = 0.64 with a 95% confidence interval of 0.485 to 0.845 and p =.002) [93]. Throughout the study, as compared with placebo, zoledronic acid consistently reduced bone pain, with differences reaching statistical significance at the 3-,
9-, 21-, and 24-month time points (p < 0.05 for each time point) [93]. Finally, although the study was not powered for survival, a provocative survival advantage of 2.6 months was observed (p = 0.1) [93].

In addition to preserving bone mineral density and preventing skeletal morbidity from bone metastases in patients with prostate cancer, preclinical evidence has hinted that bisphosphonate treatment of early-stage prostate cancer might reduce the incidence of bone metastases. The potential of bisphosphonates to prevent bone metastasis is currently being investigated in clinical trials in patients with breast cancer, prostate cancer, renal cell cancer, and other solid tumors. Pre-clinical evidence of synergy between zoledronic acid and docetaxel has also been reported and needs to be confirmed in the clinic. Among all of the bisphosphonates studied thus far, only zoledronic acid has been shown to reduce skeletal-related adverse events (SREs) in men with androgen-independent prostate cancer and bone metastases. The superiority of zoledronic acid over other bisphosphonates at reducing SREs in prostate cancer has been attributed to increased potency and to therapeutic effects on osteoblasts as well as osteoclasts [94,95]. Nonetheless, the other bisphosphonates can likely act to prevent osteoporosis associated with androgen deprivation. In one randomized trial, men (n = 47) with advanced or recurrent prostate cancer and no bone metastases were treated either with leuprolide alone or with leuprolide and pamidronate (60 mg intravenously every 12 weeks) for as long as 4 years, and monitored for bone mineral density via dual-energy x-ray absorptiometry (DEXA) of the lumbar spine and proximal femur, and trabecular bone density via quantitative computed tomography (CPA) of the lumbar spine [96]. Findings were a decrease in bone mineral density (3.3 +/- 0.7% in the lumbar spine, 2.1 +/- 0.6% in the greater trochanter, and 1.8 +/- 0.4% in the total hip) and in trabecular bone density (8.5 +/- 1.8% in the lumbar spine) attributable to leuprolide therapy, but no decline in any of the bone density measurements when pamidronate was administered along with androgen deprivation [96]. Bisphosphonate therapy can be complicated by significant side effects, including renal dysfunction, that mandate careful monitoring during treatment. In addition, osteonecrosis of the jaw has been reported in the majority of cases of osteonecrosis associated with bisphosphate treatment [97]. Currently, men with low bone mineral density, assessed via DEXA scans, should be considered for bisphosphonate therapy when treated with androgen deprivation therapy. Men with androgen-independent prostate cancer and bone metastases should be considered for treatment with zoledronic acid. While receiving bisphosphonates, men should also be evaluated and counseled regarding risks associated with dental problems and procedures.

2. ENDOTHELIN RECEPTOR ANTAGONISTS

The recognition that endothelin-1, acting via the endothelin-A receptor, is a major mediator of osteoblastic bone metastasis has focused attention on the possible role of selective endothelin-A antagonists in treating men with prostate cancer and bone metastasis [85,86] (Figure 3). In a seminal study, men with metastatic prostate cancer were found to have significantly elevated plasma endothelin-1 levels [98]. Most prostate cancer cells secrete endothelin-1, which may act in an autocrine fashion by binding to endothelin A receptors to increase response to growth factors and to attenuate apoptosis [99,100]. Such cells are often devoid of endothelin-B receptors, thought to act as clearance receptors that remove endothelin from the local milieu [99]. The absence of endothelin-B receptors in prostate cancer cells appears attributable to abnormal DNA hypermethylation changes affecting the EDNRB transcriptional promoter; in localized prostate cancer cases, EDNRB promoter methylation has been correlated with increased tumor stage and grade [5,101,102]. A selective endothelin-A receptor antagonist, atrasentan (Xinlay®), has been introduced into clinical tri-
als for men with metastatic prostate cancer [103]. In one double-blinded, randomized, placebo-controlled trial, men (n = 288) with asymptomatic, metastatic, androgen-independent prostate cancer were randomized to treatment with 2.5 mg atrasentan daily, 10 mg atrasentan daily, or placebo until disease progression [104]. Trial results suggested that endothelin-A receptor blockade resulted in an attenuation of prostate cancer-associated increases in markers of bone deposition (total alkaline phosphatase and bone alkaline phosphatase) and of bone resorption (N-telopeptides, C-telopeptides, and deoxypyridinoline) [104]. For the 10 mg daily atrasentan group, a trend toward an increase in time-to-cancer-progression was also seen [105]. In a second randomized trial, men (n = 809) with androgen-independent prostate cancer also showed a trend toward a delay in time-to-progression when treated with 10 mg atrasentan versus placebo [106]. Atrasentan will be reviewed by the United States Food and Drug Administration in the fall of 2005 for use in androgen-independent prostate cancer treatment.

3. BONE-TARGETED RADIONUCLIDES

The disproportionate action of osteoblasts at the site of prostate cancer bone metastases has stimulated interest in radioemitting calcium mimetics as treatments for progressive metastatic prostate cancer [107,108] (Table 1). 89Sr, given intravenously as 89SrCl, has been found to provide pain palliation in the setting of bone metastases, though a prolongation of survival from such treatment has not been demonstrated [109]. The agent, a β-emitter with a t1/2 of 50.5 days, accumulates at the site(s) of bone metastases, and is ultimately cleared in the urine. The main toxicity of treatment is myelosuppression, with nadir blood counts some 4-6 weeks after administration, limiting the use of the radioemitter to men with adequate blood counts and retreatment to an interval of at least 6 months. As a palliative treatment, 89Sr may not be better than targeted external beam radiation therapy: in a recent randomized trial in which men (n = 203) with painful bone metastases were treated with 89Sr or with external beam radiation therapy, subjective responses were similar (34.7% for [89Sr and 33.3% for external beam radiation) and there was a borderline significant survival advantage for external beam radiation therapy [110]. However, when used in combination with chemotherapy, 89Sr may be more effective. In a provocative clinical trial, men (n = 72) with androgen-independent prostate cancer and bone metastases who had responded to induction chemotherapy were randomized to receive 89Sr and doxorubicin versus doxorubicin alone as consolidation treatment [111]. The findings were an improvement in survival from a median of 16.8 months (with a range of 4.4 to 34.2 months) for chemotherapy alone to a median of 27.7 months (4.9 to 37.7 months) for combined treatment (p = 0.0014) [111]. Newer bone targeted radiopharmaceuticals, including 188Re-hydroxyethylidene diphosphonate (188Re-HEDP), which emits both β and γ particles with a t1/2 of 16.9 hours, and 153Sm-ethylene diamine tetramethylene phosphonate (153Sm-EDTMP), which emits both β and γ particles with a t1/2 of 46.3 hours, appear to provide palliation of painful bone metastases without as much myelosuppression as 89SrCl, which may permit easier use in combination with cytotoxic chemotherapy [112,113].

IV. CYTOTOXIC CHEMOTHERAPY

The progression of metastatic androgen-independent prostate cancer constitutes a significant threat of morbidity and mortality. Systemic chemotherapy with mitoxantrone and corticosteroids had previously been shown to improve bone pain and quality-of-life for men with metastatic androgen-independent prostate, but not to provide a survival advantage, in randomized trials [114-116]. Based on the results of

---

**Table 1. Bone-targeted radionuclides in clinical use.**

<table>
<thead>
<tr>
<th>Isotope</th>
<th>t1/2</th>
<th>Maximum energy (keV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>89Sr</td>
<td>50.5 days</td>
<td>β = 1495</td>
</tr>
<tr>
<td>186Re (HEDP)</td>
<td>3.8 days</td>
<td>β = 1070, γ = 137</td>
</tr>
<tr>
<td>153Sm (EDTMP)</td>
<td>46.3 hours</td>
<td>β = 233; γ = 103</td>
</tr>
</tbody>
</table>
these trials, mitoxantrone and corticosteroids became a standard, though not widely-used, treatment for prostate cancer, and a reference treatment arm for future randomized trials. Last year, two well-publicized clinical trials demonstrated that docetaxel, alone or in combination with estramustine, improved the survival of men with metastatic androgen-independent prostate cancer in comparison to mitoxantrone and corticosteroids [117,118]. With these reports, the role of systemic chemotherapy in prostate cancer treatment became fully established, ushering in a new era of systemic treatment development [119].

1. Taxanes

Paclitaxel (Taxol®), derived from the bark of the Pacific yew tree (Taxus brevifolia), and docetaxel (Taxotere®), derived from the leaves of the European yew tree, are taxanes that interfere with microtubule function. Microtubule function is required for mitotic chromosome segregation, and taxanes, as well as vinca alkaloids and colchicine, are well-known to trigger arrest at the G2/M phase of the replicative cell cycle and to promote apoptosis in rapidly dividing cells [120,121]. Taxanes have exhibited considerable activity against prostate cancer in clinical trials, even though most prostate cancers are not composed of rapidly dividing cells, prompting consideration of other mechanisms of taxane action. Drug disruption of microtubules and microtubule dynamics have been proposed to inhibit nuclear-cytoplasmic shuttling of regulatory proteins, to undermine angiogenesis, and to stimulate signaling pathways leading to phosphorylation and inactivation of the anti-apoptotic regulator Bcl-2 [122-125]. Whether these different consequences of microtubule damage and dysfunction might favor certain microtubule-targeted drugs, different microtubule-targeted drug doses and schedules, or selected microtubule-targeted drug combinations, has not been determined.

Paclitaxel has been evaluated as monotherapy for androgen-independent prostate cancer, showing hints that its activity may be highly schedule-dependent. In one study, men (n = 23) with prostate cancer were treated with paclitaxel, given as a continuous infusion over 24 hours (135-170 mg/m²) every 3 weeks for as many as 6 treatment cycles, with little benefit (a single partial response lasting some 9 months) [126]. In another trial, when men (n = 18) with prostate cancer received paclitaxel as a weekly hour-long infusion (150 mg/m²) for 6 of 8 weeks, more responses were seen (4 major responses and 3 partial responses among 8 men with measurable disease and significant serum PSA declines in 7 of 18 men) [127]. Paclitaxel has also exhibited considerable activity in combination with other inhibitors of microtubule function. A phase 2 trial of the combination of paclitaxel (120 mg/m² by 96-hour infusion every 3 weeks) and estramustine (Estracyt®; n = 34) reported a serum PSA response rate of 53%, a measurable disease response rate of 44% and a median survival of 17.3 months [128]. Paclitaxel given at a different dose and infusion schedule (225 mg/m² by 3-hour infusion every 3 weeks) combined with estramustine provided a similar serum PSA response rate of 62% in another trial (n = 28) [129]. Weekly paclitaxel (90 mg/m² by 1-hour infusion) combined with daily oral estramustine also gave significant rates of serum PSA declines (42%; n = 66) [130].

Like paclitaxel, docetaxel has also been assessed in a series of clinical trials for androgen-independent prostate cancer both as monotherapy and in combination with estramustine. When given every 3 weeks (75 mg/m²) to men with prostate cancer (n = 35), docetaxel therapy resulted in a 46% serum PSA response rate [131]. In several studies of weekly docetaxel (35-40 mg/m²), undertaken to make treatment more tolerable to elderly men with prostate cancer, serum PSA response rates ranged from 41% to 64% [132-135]. In a pooled analysis of men (n = 86) treated with weekly docetaxel in 2 different clinical trials, men > 70 years of age (n = 52) were as likely as men < 70 years of age (n = 34) to enjoy serum PSA responses and no more likely to suffer side effects [136]. Docetaxel and estramustine combinations have shown consistently high response rates in phase 1 and 2 trials for men with androgen-independent prostate cancer, both for serum PSA responses (45-74%) and for measurable disease responses (11-57%) [137-141]. Myelosuppression was the dose-limiting toxicity for the docetaxel-estramustine combination in the phase 1 trial: the recommended docetaxel dose to be given in combination with estramustine was 70 mg/m² for minimally pretreated men and 60 mg/m² for extensively pretreated men 140. In the Cancer and Leukemia Group B (CALGB) phase 2 study, men with androgen-independent prostate cancer (n = 47) treated with docetaxel, estramustine, and low dose hydrocortisone, had serum PSA response rate of 68%, a measurable disease response rate of 50%, and a median survival of 20 months [137]. Because docetaxel as monotherapy and in combination with estramustine produced high
response rates in men with androgen-independent prostate cancer, phase 3 randomized trials against mitoxantrone and corticosteroids were initiated.

The two resultant large, randomized, controlled phase 3 trials both showed improvements in overall survival for men with androgen-independent prostate cancer treated with docetaxel-based chemotherapy over mitoxantrone-based chemotherapy. One of the studies (TAX-327) randomized men (n =1006) to one of 3 treatments arms, directly comparing 2 different schedules of docetaxel and prednisone (with docetaxel at 30 mg/m² given weekly or at 75 mg/m² given every 3 weeks) with the historical standard treatment of mitoxantrone and prednisone [118] (Figure 4). The median survival was 18.9 months (with a 95% confidence interval of 17 to 21.2 months) for men on the docetaxel (75 mg/m² every 3 weeks) and prednisone treatment arm, compared with 16.5 months (with a 95% confidence interval of 14.4 to 18.6 months) for men treated with mitoxantrone and prednisone (p = 0.009). The difference in median survival between men treated with weekly docetaxel and prednisone 17.4 months (with a 95% confidence interval of 15.7 to 19 months) versus with mitoxantrone and prednisone was not statistically significant. In addition, docetaxel (75 mg/m² every 3 weeks) and prednisone provided a better median serum PSA response rate (45% versus 32%) and better pain control (35% versus 22%) than mitoxantrone and prednisone (p < 0.01). The other study (SWOG 99-16) randomized men (n = 770) to treatment with docetaxel and estramustine versus mitoxantrone and prednisone, using the same randomization and eligibility criteria as in the TAX-327 trial [117] (Figure 5). The median overall survival was longer for men given docetaxel and estramustine than for men given mitoxantrone and prednisone (17.5 months versus 15.6 months, p = 0.02). In each study, docetaxel-based chemotherapy caused more side effects, including edema, gastrointestinal symptoms, neuropathy, and nail changes, than mitoxantrone-based treatment, and the combination of docetaxel and estramustine was also associated with a higher incidence of cardiovascular events.

The improvement in median survival attributable to docetaxel in the large phase 3 trials has established docetaxel, given every 3 weeks, and prednisone as the standard-of-care for men with metastatic androgen-independent prostate cancer. Further evidence in support of docetaxel-based chemotherapy for prostate cancer has come from a recently reported

Figure 4. Docetaxel treatment of men with metastatic androgen-independent prostate cancer results in improved overall survival when compared to treatment with mitoxantrone (reference 188).

Figure 5. The combination of estramustine and docetaxel prolongs progression-free and overall survival for men with metastatic androgen-independent prostate cancer versus mitoxantrone and prednisone (reference 117).
randomized phase 2 study for men (n = 130) with androgen-independent prostate cancer [139]. In the trial, men were treated with docetaxel (at 2 different schedules), estramustine, and prednisone, versus mitoxantrone and prednisone. Results demonstrated the superiority of docetaxel-based therapy for the primary endpoints of serum PSA response and time-to-PSA-progression, and hinted at an overall survival advantage for each of the docetaxel treatment arms (18.6 and 18.4 months compared to 13.4 months for mitoxantrone and prednisone). A meta-analysis of men in the 3 randomized trials (a total of 1807 men, with 1092 treated with docetaxel and 715 with mitoxantrone) revealed that docetaxel significantly reduced the risk of death by 8-21% persisting at least 3 years after the start of chemotherapy [142]. With these compelling data, the US FDA granted an indication to docetaxel for the treatment of androgen-independent prostate cancer in May of 2004. However, key questions still remain, such as what is the contribution of estramustine to docetaxel efficacy? Recently, a randomized phase 2 trial comparing docetaxel and estramustine to docetaxel alone (n = 92) found a serum PSA response rate of 68% for the combination versus 29% for docetaxel monotherapy, though no survival data were reported [143]. A large randomized clinical study will likely be required to assess the balance of efficacy and toxicity in combination versus monotherapy.

2. ESTRAMUSTINE

Estramustine phosphate is a nitrogen mustard derivative of estradiol-17-β-phosphate; its mechanism of action in prostate cancer likely combines the hormonal effect of estrogen with a cytotoxic action through disruption of microtubule function and/or nuclear matrix binding [144-147]. Combinations of estramustine with other microtubule-targeted drugs, such as vinblastine, paclitaxel, and docetaxel, have shown synergistic cytotoxicity in preclinical studies, and have been explored in clinical trials [148]. As monotherapy, estramustine has been approved by the FDA for use in men with prostate cancer, though the drug has not been demonstrated to prolong the survival of men with androgen-independent metastatic prostate cancer. Side effects, mostly attributable to the estrogenic properties of the drug, are significant: men treated with estramustine alone or in combination with docetaxel have had venous and arterial thromboembolic events, ranging from deep vein thrombosis to pulmonary embolism to stroke and death. A meta-analysis (n = 896 men from 23 studies) has revealed that 7% of men (with a 95% confidence interval of 5-11%) treated with estramustine-containing chemotherapy regimens are at risk for a thromboembolic complication [148]. As a result, daily aspirin or low-dose daily coumadin has been recommended to reduce the risk accompanying estramustine therapy [141]. However, the phase 2 trials using such prophylaxis have yielded mixed results [129,139,141]. Because a reduction in thromboembolic events using these strategies has not been proven in a randomized trial, the risk of estramustine has to be weighed against potential benefits when considering estramustine treatment. Although randomized trials of estramustine in combination with other microtubule-targeted drugs, such as vinblastine, paclitaxel, and docetaxel, have shown improved serum PSA responses attributable to combination therapy, the impact of estramustine on overall survival has not been clearly demonstrated [143, 149,150]. Estramustine may not be appropriate for men with prostate cancer at increased risk for thromboembolic events.

3. VINCA ALKALOIDS

Vinca alkaloids are microtubule-targeted drugs derived from the periwinkle plant (Cantharanthus roseus) [121]. Two vinca compounds, vinblastine (Velban®) and vinorelbine (Navelbine®), have been evaluated for treatment of prostate cancer in clinical trials. Vinblastine has shown activity against androgen-independent prostate cancer when tested as monotherapy, in combination with estramustine, and in multicomponent chemotherapy regimens [150-153]. For vinorelbine monotherapy, studies have found PSA response rates in 13 to 17%, and durable clinical benefit, as defined by improvement in pain and/or performance status, in 32 to 39%, of men with androgen-independent prostate cancer [154-156]. In a recent randomized trial comparing vinorelbine and hydrocortisone with hydrocortisone alone (+/−aminogluthethimide) in men (n = 414) with progressive androgen-independent prostate cancer, a statistically significant difference of 1 month (3.7 versus 2.8 months; p = 0.005) in median progression free-survival favoring vinorelbine treatment, with no difference in overall survival (median 15 months), was detected [157]. PSA response rates were also significantly higher for vinorelbine treatment (30.1% versus 19.2%; p = 0.01), as was clinical benefit, defined as a decrease in pain intensity or analgesic consumption or an improvement in performance status (30.6% versus 19.2%; p = 0.008). Also, vinorelbine
therapy was generally well tolerated in the trial, with a less than 7% incidence of significant neutropenia and less than 1% incidence of cardiotoxicity. Currently, combinations of vinorelbine with estramustine, docetaxel, and other agents, are under clinical development [158,159].

4. EPOTHILONES

The epothilones are a new class of microtubule-targeted agents obtained from fermentation of the myxobacterium Sorangium cellulosum. Provocatively, while the epothilone mechanism of action is similar to that of the taxanes, anti-cancer activity has been seen against taxane-resistant cancers [121]. In prostate cancer cell lines, epothilone B is the most active epothilone, inhibiting growth some 7 to 10-fold more potently than paclitaxel in vitro and in vivo [160,161]. Two epothilones, BMS-247550 (ixabepilone) and EPO906 (patupilone), are currently being developed for men with androgen-independent prostate cancer. In a phase 2 trial (n = 41), ixabepilone demonstrated a PSA response rate of 34% and an objective clinical response rate of 16%; however, a significant amount of neuropathy was observed, with 29% of men going off study due to toxicity [162]. When ixabepilone (35 mg/m² every three weeks) was given with or without estramustine (280 mg orally 3 times daily on days 1 to 5) to men with prostate cancer (n = 92) as part of a randomized phase 2 trial, partial responses were observed in 32% (with a 95% confidence interval of 14% to 50%) of men treated with ixabepilone monotherapy, and 48% (with a 95% confidence interval of 27% to 68%) of men treated with ixabepilone and estramustine [163]. The time-to-PSA-progression was 4.4 months (with a 95% confidence interval of 3.1 to 6.9 months) on the ixabepilone-alone arm and 5.2 months (with a 95% confidence interval of 4.5 to 6.8 months) on the combination arm. Toxicities experienced by more than 5% of patients included neutropenia, fatigue, and neuropathy; estramustine seemed to increase toxicity, particularly nausea, vomiting and thrombosis. Peripheral neuropathy was very common (67 to 73% in the two treatment arms; mainly grade 1-2), and the median time-to-onset of grade 1 or 2 neuropathy was 52 or 118 days, respectively. In all, the activity of ixabepilone against prostate cancer seen thus far appears comparable to that achieved in phase 2 and 3 trials of docetaxel. A randomized phase 2 trial of ixabepilone versus mitoxantrone in men with taxane-refractory prostate cancer has been completed and awaits analysis, and ECOG is conducting a phase 2 trial of weekly ixabepilone (in order to reduce neurotoxicity) in three cohorts of men: chemotherapy-naïve, post-taxane therapy, or post-mitoxantrone therapy. Patupilone, an epothilone which is not a substrate for the multidrug resistance transporter, has demonstrated activity against androgen-independent prostate cancer in preclinical models [164.] In a phase 2 trial (n = 37), patupilone, which was well-tolerated with grade 1-2 peripheral neuropathy reported in 14% and 5% of men, was associated with serum PSA responses in 22% and measurable disease responses in 20% of men with androgen-independent prostate cancer [165].

5. NEW DOCETAXEL COMBINATIONS

With the established efficacy of docetaxel in the treatment of metastatic androgen-independent prostate cancer, and the uncertain overall benefit of the addition of estramustine to docetaxel, several new docetaxel combinations are under active development and evaluation [119] (Tables 2 and 3). For example, a phase 2 study of high-dose calcitriol (0.5 mcg/kg orally administered weekly) and docetaxel with dexamethasone (given weekly for 6 consecutive weeks of an 8-week cycle) for men (n = 37) with androgen-independent prostate cancer found an 81% serum PSA response rate accompanied by only mild toxicity [166]. Based on this experience, a double-blind, randomized, multicenter trial (named ASCENT: Androgen-independent prostate cancer Study of Calcitriol ENhancing Taxotere), using docetaxel with DN-101 (a high-dose calcitriol formulation) or with placebo, was undertaken. The preliminary results, presented at the American Society of Clinical Oncology (ASCO) 2005 Annual Meeting, hinted at a trend toward more PSA responses with

Table 2. Phase 2 combination chemotherapy trials for prostate cancer featuring docetaxel.

<table>
<thead>
<tr>
<th>Phase 2 Taxane Combination Trials (completed, in progress, or proposed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>docetaxel (Taxotere®) + bevacizumab (Avastin®)</td>
</tr>
<tr>
<td>docetaxel (Taxotere®) + thalidomide (Thalomid®)</td>
</tr>
<tr>
<td>docetaxel (Taxotere®) + bortezomib (Velcade®)</td>
</tr>
<tr>
<td>docetaxel (Taxotere®) + antisense Bcl-2</td>
</tr>
<tr>
<td>docetaxel (Taxotere®) + mTOR inhibitors</td>
</tr>
<tr>
<td>docetaxel (Taxotere®) + EGFR inhibitors</td>
</tr>
<tr>
<td>docetaxel (Taxotere®) + KDR inhibitors</td>
</tr>
<tr>
<td>docetaxel (Taxotere®) + calcitriol</td>
</tr>
</tbody>
</table>
DN-101 and docetaxel treatment versus placebo and docetaxel treatment (63% versus 52%, p = 0.07), with no increase in toxicity [167]. In a multivariate analysis of the trial data, the overall survival was improved for DN-101-treated men (HR = 0.67, p = 0.035), with a 16.4 month median survival for men who received placebo and docetaxel and an estimated > 23.5 month median survival for DN-101 and docetaxel (the median survival was not reached in the trial).

Docetaxel has also been combined with anti-angiogenesis agents such as thalidomide and bevacizumab. Thalidomide, a potent teratogen which inhibits blood vessel growth in the developing fetal limb bud, has been used to treat highly vascular Kaposi’s sarcoma [168,169]. In phase 2 studies of androgen-independent prostate cancer, serum PSA response rates were below 20%, and side effects, including sedation, constipation, and sensory peripheral neuropathy, were reported [170,171]. When thalidomide (200 mg daily dose) was combined with weekly docetaxel versus docetaxel alone in a randomized phase 2 trial, men (n = 75) with androgen-independent prostate cancer had a higher PSA response rate (53% versus 37%) and longer progression-free survival (5.9 months versus 3.7 months) with the docetaxel and thalidomide than with docetaxel alone [172]. Furthermore, after a median follow-up of 46.7 months, the overall survival of the combination treatment arm was 25.9 months versus 14.7 months for docetaxel alone (p = 0.04). Venous thrombotic events were reported in the combination arm which disappeared with the introduction of anticoagulation. In a follow-up trial, combination treatment with docetaxel, estramustine, and thalidomide has resulted in serum PSA responses in 12 of 12 men treated thus far [173]. Bevacizumab, a neutralizing human monoclonal antibody against vascular endothelial growth factor (VEGF), has been combined with docetaxel and estramustine for men androgen-independent prostate cancer (n = 79) in a phase 2 trial by the CALGB, given on day 2 (15 mg/kg) of treatment cycles repeated every 3 weeks. Findings were a PSA response rate of 77% and a radiological response rate of 44%. Based on these results, the CALGB has initiated a phase 3 randomized trial (CALGB 90401) to compare overall survival with docetaxel and prednisone to that with the combination of docetaxel, prednisone, and bevacizumab in patients with metastatic androgen-independent prostate cancer; approximately 1020 men will be accrued for to study over 3 years.

6. SECOND-LINE CHEMOTHERAPY FOR ANDROGEN-INDEPENDENT PROSTATE CANCER

With the use of docetaxel increasing for men with androgen-independent prostate cancer, a need has arisen for agents that can be used for men who progress despite docetaxel treatment. Unfortunately, there is no therapy with proven clinical efficacy for such men. Questions also remain regarding the efficacy of docetaxel-based chemotherapy for men with androgen-independent prostate cancer after treatment with mitoxantrone. Several reports addressed this question at the 2005 ASCO Annual Meeting. In one retrospective analysis (n = 83), a 44% PSA response rate was reported when docetaxel was used after mitoxantrone, while when mitoxantrone was given after docetaxel, the PSA response rate was only 15% [174]. A second retrospective analysis (n = 68) found similar results, with more second-line responses when docetaxel was used after mitoxantrone (60.6%) than vice versa (5.9%) [175]. In an ongoing prospective study (n = 30), some 85% of men have thus far responded to docetaxel (75mg/m² every 21 days) after having progressed on mitoxantrone and prednisone [176]. Thus, the available

Table 3. Randomized trials of docetaxel combinations for prostate cancer.

<table>
<thead>
<tr>
<th>Combination</th>
<th>Size</th>
<th>Locations</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taxotere® +/- Gleevec®</td>
<td>n = 140</td>
<td>MDACC, DFHCC, MSKCC, UCSF, U. Michigan</td>
<td>nearly completed</td>
</tr>
<tr>
<td>Taxotere®/prednisone +/- Avastin®</td>
<td>n = 800</td>
<td>opened Taxotere® +/- calcitriol n = 240</td>
<td>completed with survival prolonged</td>
</tr>
</tbody>
</table>
data so far suggest that docetaxel might provide a reasonable therapeutic alternative to men previously treated with mitoxantrone. However, men progressing despite docetaxel treatment should consider investigational therapy.

7. Expanded Use of Chemotherapy for Early Prostate Cancer

The activity of docetaxel-based regimens in metastatic androgen-independent prostate cancer has prompted testing of the drug earlier during the natural history of the disease. For example, several tools have been developed with the potential to stratify men diagnosed with localized prostate who are at high risk for cancer progression despite adequate local therapy and who might benefit from active systemic treatment [177-180]. Similar risk stratification tools have become available for men with recurrent prostate cancer after adequate local therapy, or after androgen-deprivation therapy [12,17]. These tools have facilitated a new wave of clinical research targeting men at high risk for prostate cancer morbidity and mortality.

Several groups have investigated the role of neoadjuvant chemotherapy in men with high-risk localized prostate cancer. In general, these phase 2 trials have been small and have not yet demonstrated that chemotherapy, either alone or in combination with hormonal therapy, can induce markedly significant pathologic responses in primary tumors. However, there is evidence that chemotherapy, particularly regimens containing docetaxel, can reduce tumor volumes assessed via radiographic imaging, significantly decrease serum PSA levels, and induce histopathologic changes. When men (n = 21) with locally advanced prostate cancer were treated with docetaxel and estramustine for up to 6 treatment cycles, 3 of 10 men who underwent surgery had organ-confined prostate cancer at radical prostatectomy [181]. In a trial of weekly docetaxel for 6 weeks for men (n = 29) with locally advanced prostate cancer, serum PSA responses were reported in 24%, thought desmoplastic reactions slightly increased technical difficulties with radical prostatectomy [182]. Weekly docetaxel was given for 6 months to men (n = 15) with locally advanced prostate cancer, with PSA responses in two thirds of the men in another trial [183]. Based on these results, a phase 3 trial (CALGB 90203) is soon to be initiated comparing neoadjuvant docetaxel (75 mg/m² every three weeks for 6 cycles) plus an LHRH analogue followed by radical prostatectomy to radical prostatectomy alone. Eligibility for this trial is based on a nomogram prediction targeting men that have a 60% chance or less of being disease-free 5 years after surgery; a total of 750 men will be accrued to the study. In addition, 2 other phase 3 randomized trials are ongoing. The first one, conducted by Anthony D’Amico and colleagues, is comparing neoadjuvant docetaxel followed by androgen deprivation and radiotherapy versus androgen deprivation and radiotherapy alone. The second study, conducted by the Groupe d’Études des Tumeurs Uro-Génitales (GETUG), is comparing docetaxel, estramustine and androgen deprivation therapy versus androgen deprivation therapy, before pre-defined local treatment (either prostatectomy or radiotherapy).

Docetaxel has also been explored as post-surgical adjuvant therapy. In a phase 2 study, men (n = 77) at high risk for serum PSA recurrence following radical prostatectomy were treated with 6 cycles of docetaxel (35 mg/m², days 1, 8, 15 every 28 days) [180, 184]. With a median follow-up of 17 months (10 months to 31 months), the median time-to-PSA recurrence has not yet been reached. Also, the chemotherapy was well tolerated. Based on this study, a large international multicenter randomized phase 3 trial (Tax 3501/ATLAS Study) comparing immediate adjuvant hormonal therapy in combination with docetaxel versus hormonal therapy alone versus deferred therapy followed in men with prostate cancer at high risk of relapse after radical prostatectomy was started and is ongoing.

A rising serum PSA level after surgery and radiation likely reflects the presence of residual or recurrent prostate cancer. Men with a rising PSA defined to be at high risk of distant metastasis have been treated with docetaxel in two phase 2 studies, with serum PSA response rates of 43-9% [185,186]. Phase 3 trials of docetaxel and androgen deprivation versus androgen deprivation alone have been initiated.

V. IMMUNOTHERAPY

The transcendant rationale of cancer immunotherapy is that cancer cells contain antigens recognizable by the immune system is such a way as to permit their selective destruction [187]. For T-cells, such antigens are peptide fragments of cellular proteins presented on specialized molecules at the surface of cancer cells, usually class I major histocompatibility complexes (MHCs). Cancer-associated antigens may be neo-antigens, formed as a result of somatic
genome alterations, such as mutations and translocations, or may be differentiation antigens, unique to the lineage of origin of the cancer cells. An emerging body of evidence suggests that while immune surveillance against cancer, involving innate and adaptive immune responses, may occur early during carcinogenesis, most established cancers elude immune responses principally by mediating immune tolerance to the cancer-associated antigens [188]. For prostate cancer, lineage antigens, such as PSA, prostate-specific acid phosphatase (PAP), prostate-specific membrane antigen (PSMA), NKX3.1, and others, shared between normal and neoplastic prostate cells, have become attractive targets for vaccine immunotherapy [189]. The major vaccine strategies taken to the clinic thus far have featured the delivery of these antigens to antigen presenting cells (APCs), like dendritic cells (DCs) in the skin, which can stimulate specific T-cells to mediate rejection of cancer cells expressing the antigens [187] (Figure 6). In general, the clinical experience to date with prostate cancer vaccine immunotherapy has been that measurable immune responses can be elicited with vaccination using a number of different strategies, and that these immune responses are occasionally accompanied by disease responses that may be associated with clinical benefit [190].

Why has vaccine immunotherapy not been more effective? Animal model studies have suggested that T-cells capable of recognizing prostate lineage antigens are present after puberty, and in animals without prostate cancer, circulate in an inactive state that can be stimulated by vaccination [191]. However, as prostatic carcinomas develop, the prostate-antigen-specific T-cells become tolerant, a state in which they are inactive and refractory to stimulation by vaccination [191]. Intriguingly, androgen deprivation therapy for prostate cancer appears to create a transient increase in T-cell recognition of the prostate antigens, sufficient to abrogate CD4 T-cell tolerance and allow better responses to vaccination [191]. These data, and the accumulating clinical experience with vaccine immunotherapy, indicate that immune tolerance is a significant barrier to the full clinical activity of prostate cancer vaccine therapy. New approaches, targeting the regulation of T-cell activation, have the potential to overcome immune tolerance and increase the utility of vaccine approaches for prostate cancer [192]. Because the prostate is not a vital organ, the risks of new approaches for abrogating immune tolerance are thought to likely be acceptable.

1. PSA VACCINES

With its restricted expression among normal tissues, and its consistent expression in prostate cancers, PSA has been an attractive antigen for prostate cancer immunotherapy. Furthermore, measurable T-cell responses against PSA peptides have been detected in 6% of men, and antibody responses to PSA proteins in 11% of men, with prostate cancer [193,194]. Peptide fragments from PSA have been shown to elicit CD4 and/or CD8 T-cell reactivity on various class I and class II MHC backgrounds [195-201]. Anti-PSA vaccination strategies employed thus far have included inoculation with “naked” DNA encoding PSA, with PSA-loaded DCs, with PSA-derived peptides, and with poxviruses genetically-modified to permit PSA expression; all have induced measurable immune responses against PSA [202-208]. Poxvirus-PSA vaccines have been taken the farthest thus far in clinical development. In phase 1 trials for men with prostate cancer, vaccinia-PSA vaccines have appeared safe, with skin reactions and low grade fevers found to be the most common side effects [205,207,208]. In one of the trials (n = 33), serum PSA rises appeared to stabilize for at least 6 months in 42% of men with a rising serum PSA after radical prostatectomy [208]. A randomized phase 2
trial of 3 different prime-boost schedules of vaccinia-PSA and fowlpox-PSA vaccines for men (n = 64) with a rising PSA after local therapy was conducted by ECOG [209]. Results of the trial indicated that some 45.3% of men remained free of PSA progression at 19.1 months and 78.1% remained free of clinical or symptomatic progression, with a statistical trend favoring one of the treatment schedules [209]. Also, although no significant increases in antibody titers against PSA were detected, 46% of men in the trial exhibited an increase in PSA-reactive T-cells [209]. To augment the immunogenicity of poxvirus-PSA, the vaccine preparations have been further genetically-modified to allow expression of molecules, such as B7.1, that tend to activate T-cells [210,211]. Such a strategy was tested in a phase 2 randomized trial of adjuvant vaccine immunotherapy given along with prostate radiation treatment [211]. In this trial, men (n = 30) with prostate cancer were randomized in a 2:1 ratio to receive vaccine plus radiotherapy or radiotherapy alone. The vaccination strategy featured a priming inoculation of vaccinia-PSA plus vaccinia-B7.1 followed by monthly booster vaccines with fowlpox-PSA, administered with local granulocyte-macrophage colony-stimulating factor (GM-CSF) and low-dose systemic interleukin-2 (IL-2); external beam radiation therapy in the combination treatment arm was given between the fourth and the sixth vaccinations. Findings from the study were that 13 of 17 men who completed all of their vaccinations showed increases in PSA-specific T-cells of at least 3-fold versus no detectable increases for men treated with radiation therapy alone (p < 0.0005) [211]. Another trial randomized men (n = 42) with a rising serum PSA after local therapy to treatment with a similar poxvirus-PSA and B7.1 vaccination regimen versus treatment with nilutamide monotherapy, with few differences seen in time-to-progression between the two treatment arms [210].

Definitive phase 3 trials for fowlpox-PSA vaccines are under consideration.

2. ANTIGEN-LOADED DENDRITIC CELL VACCINES

DCs are the APCs capable of activating T-cells to mediate anti-cancer immune responses. DC precursors are found in most tissues; mature DCs can present antigens on both class I and class II MHC molecules along with a variety of co-stimulatory molecules that can stimulate cancer-specific T-cells to proliferate, secrete cytokines, and kill tumor cells. To create DC vaccines, immature DC precursors are typically recovered from blood mononuclear cells via leukapheresis, incubated with cytokines, and then loaded with antigens. As such, DC vaccine construction can be considered more of a patient-specific procedure than a product. A variety of antigen-loading strategies for the construction of anti-prostate cancer DC vaccines have been pursued, ranging from transfection of DNA, or of RNA, encoding antigens of interest, into the DCs to direct addition to DCs of proteins, protein fragments, or cancer cell lysates [203,212-219]. DCs loaded with PSMA peptides have been introduced into phase 1 and 2 clinical trials for prostate cancer [218-222]. In one of the phase 2 trials, men (n = 33) with androgen-independent prostate cancer were treated with PSMA peptide-loaded DCs, with 6 men showing partial responses and 2 men showing complete responses as evidenced by a 50% reduction of PSA or a resolution of measurable lesions imaging studies [218]. Phase 3 trials of this immunotherapy strategy are planned. Loading of DCs with PA-2024, a recombinant protein consisting of PAP fused to GM-CSF, to create APC-8015 (Provenge®), has undergone the most extensive clinical development for prostate cancer treatment. In the first phase 1 and 2 trials reported for this immunotherapy approach, men (n = 31) with androgen-independent prostate cancer tolerated DC treatment quite well, with fever as the most common adverse event [223]. Immune responses to PA-2024 were detected in all of the men in the trials, with 38% of the men exhibiting immune responses to PAP 223. PSA responses were seen in 3 of the men and the median survival was 29 weeks for men on the phase 2 trial [223]. With these data, a phase 3 trial of APC-8015 randomized 2:1 versus placebo for men (n = 127) with asymptomatic metastatic androgen-independent prostate cancer was undertaken, using a primary endpoint of objective time-to-progression on imaging studies [224]. The trial results hinted at a trend toward improved time-to-progression for the APC-8015 arm (HR = 1.39 with a 95% confidence interval of 0.95 to 2.04 and p = 0.085). For men with a Gleason score at the time of diagnosis of 7 or less, the median time-to-progression was 16 weeks for men treated with APC-8015 versus 9 weeks for men treated with placebo (HR = 2.2 with a 95% confidence interval of 1.3 to 3.7 and p = 0.002) [224]. A phase 3 trial selectively targeting men with androgen-independent prostate cancer and a Gleason score at the time of diagnosis of 7 or less has been initiated. Also, combinations of APC-8015 and bevacizumab have been explored: a trial in men (n = 22) with prostate cancer manifest as a rising serum PSA after local treatment revealed an increase in PSA.
doubling time from 6.7 months before combination APC-8015/bevacizumab treatment to 12.7 months while on treatment (p = 0.004) [225].

3. Prostate Cancer Cell Vaccines

Because the optimal antigens for prostate cancer immunotherapy have not yet been defined, whole prostate cancer cells, which presumably contain all, or very nearly all, of the critical antigen targets, have been used as vaccine cells. The key to the use of cancer cells generally as vaccine cells has been to enhance the immunogenicity of the cells via genetic modification to permit secretion of immunostimulatory cytokines [226]. For prostate cancer, the first clinical application of this strategy involved genetic modification of autologous prostate cancer cells recovered at radical prostatectomy with GM-CSF cDNA [227-229]. In a phase 1 trial (n = 8 men) of irradiated GM-CSF-secreting autologous prostate cancer cell vaccines, 7 of the vaccinated men exhibited delayed-type hypersensitivity (DTH) reactions against unmodified autologous tumor cells [228]. Biopsies of the reactive DTH sites showed infiltrates of effector cells consisting of CD45RO+ T-cells and of degranulating eosinophils, consistent with activation of both Th1 and Th2 T-cell responses [228]. In addition, sera from 3 of the vaccinated men contained new antibodies recognizing polypeptides of 26, 31, and 150 kDa in protein extracts from prostate cells [228]. Despite these encouraging findings, autologous prostate cancer cell vaccines appeared to have limited clinical utility for prostate cancer treatment because an adequate number of autologous prostate cancer cells could not be reliably recovered and expanded from all men with the disease. To generate a more widely useful prostate cancer cell vaccine, vaccine cells were prepared from the prostate cancer cell lines LNCaP and PC-3 via genetic modification to permit high level secretion of GM-CSF (GVAX®) [230]. In a recent phase 2 study of this vaccine approach, men (n = 80) with androgen-independent metastatic prostate cancer were treated with various doses and dose-schedules of GM-CSF-secreting LNCaP/PC-3 vaccines [230]. After a median of 15 months of follow-up, the median survival had not been reached [230]. Also, some 43% of the men had stable or improved bone scans [230]. Antibody responses to vaccination were common, with the most frequently occurring immunoreactive species (in 29% of men) similar in size on immunoblots (~280 kD) to filamin, an antigen previously identified in a man in an earlier clinical trial who obtained a complete response after vaccine therapy; a trend towards improved survival was seen for men who generated this response versus men who did not (p = 0.09) [230]. A phase 3 trial of this vaccine approach, randomized against treatment with docetaxel, for men with androgen-independent metastatic prostate cancer, has been started.

VI. “Targeted” Therapies

Great excitement has been generated for the future of cancer treatment by the discovery and development of “targeted” cancer treatments. The hope and promise of such treatments is that “targeted” drugs might have a very high therapeutic index, causing “specific” killing of cancer cells by interacting with targets not present in normal cells and tissues, rather than exerting “selective” cancer cytotoxicity by interfering with processes, such as cell proliferation, shared by normal cells and cancer cells. “Selectively” toxic drugs, which have a fairly low therapeutic index, include most cytotoxic chemotherapy, which must be administered at or near the maximally-tolerated dose to have maximal efficacy. In contrast, the new “targeted” drugs can be administered at doses far less than the maximally-tolerated dose and still possess significant anti-cancer activity. This provides a great opportunity for cancer treatment with less debilitating side effects, but also presents a great challenge to clinical drug development: rather than defining the maximally-tolerated dose and dose-limiting toxicity in phase 1 trials, the “optimal biologic dose,” where the agent specifically interacts with the desired target, will need to be discerned in early clinical studies. Two major classes of agents, antibodies recognizing key cell surface species on cancer cells, and small molecules, inhibiting signaling networks in cancer cells, have been recently introduced to clinical oncology as “targeted” drugs. Of these, the most widely publicized is imatinib (Gleevec®), which can inhibit the tyrosine kinase activity of the Bcr-Abl fusion gene responsible for chronic myelogenous leukemia (CML). When used to treat CML in chronic phase, the majority of patients respond spectacularly to imatinib treatment [231-233]. However, after long-term therapy, relapses can occur, attributable to mutations at kinase target or to the contribution of alternative growth signaling pathways, such as those active in CML stem cells [234-237]. Thus, it is likely that intrinsic and acquired drug resistance will undermine the efficacy of “targeted” cancer treatments in a manner similar to conventional cytotoxic chemotherapy.
1. RETARGETED CYTOTOXIC CHEMOTHERAPY DRUGS

Cytotoxic chemotherapy drugs for cancer are typically administered at doses near the maximally-tolerated dose and at schedules that permit recovery from dose-limiting toxicities. A number of attempts at improving the therapeutic index of such drugs, by altering drug distribution and activation, have been undertaken. An example is the delivery of the taxane paclitaxel in a preparation where the drug is bound, in nanoparticle form, to albumin (ABI-007, Abraxane®) [238,239]. In women (n = 66 evaluable so far) with breast cancer previously treated with taxanes, ABI-007 has shown a 20% response rate with acceptable side effects [240]. The drug, now approved by the FDA for women with breast cancer, will be tested also in men with prostate cancer. The albumin-containing nanoparticle appears to be taken up by tumor blood vessel cells in a pathway featuring the 60-kD albumin-binding glycoprotein (gp60) and caveolin-1 [241]. Another retargeting strategy unique to prostate cancer has been to link cytotoxic drugs to peptides that are substrates for enzymes selectively expressed on or near prostate cancer cells (Figure 7). PSA is a proteolytic enzyme that can cleave at HSSKLQ amino acid sequences [242]. When secreted into the extracellular milieu by prostate cancer cells, PSA remains enzymatically active until binding to α-1-anti-chymotrypsin or to α-2-macroglobulin [243]. When cytotoxic drugs are conjugated to HSSKLQ, the resultant “pro-drugs” are essentially non-cytotoxic until cleaved by PSA specifically in the vicinity of prostate cancer cells [244-247]. PSA-specific pro-drugs have been synthesized from flurouracil, doxorubicin, and thapsigargin [244-247]. Each of these has exhibited striking preclinical activity in animal modes, often eradicating PSA-secreting tumors while not affecting non-PSA-producing tumors [244-247]. In a phase I trial of L-377202, a peptide conjugate of doxorubicin that releases the active metabolites leucine-doxorubicin and doxorubicin upon cleavage by PSA, for men (n = 19) with androgen-independent prostate cancer, 2 of 5 men treated at the highest dose level exhibited PSA responses [248]. PSMA, a folate hydrolase, has also been targeted for prodrug activation [249].

2. SIGNAL TRANSDUCTION PATHWAY INHIBITORS

One of the most inviting signal transduction pathways for “targeted” prostate cancer drugs is the phosphatidylinositol 3'-kinase/protein kinase B (PI3K/Akt) signaling pathway, which can connect cell surface receptor tyrosine kinases to oncogenic cell growth and survival responses [250] (Figure 8). PTEN, a tumor suppressor gene that is commonly altered in prostate cancer cells, encodes a phos-
phatase active against both proteins and lipid substrates that inhibits PI3K/Akt signaling [251-263]. PTEN is expressed by normal prostate epithelial cells and is diminished or absent in many prostate cancer cells [264]. Somatic PTEN defects may be more common in metastatic prostate cancers than in non-metastatic cancers [258,259,265-267]. In mouse models, haploinsufficiency for Pten is associated with marked growth dysregulation in normal and neoplastic prostate cells: (i) Pten+/- mice display prostatic hyperplasia and dysplasia, (ii) crosses of Pten+/- mice with Nkx3.1+/- mice lead to intraepithelial neoplasia in progeny male Pten+/- Nkx3.1+/- and Pten+/- Nkx3.1+/- mice, (iii) crosses of Pten+/- mice with Cdkn1b+/- mice, devoid of p27, lead to rapidly progressive prostate cancer in progeny male Pten+/- Cdkn1b+/- mice, and (iv) when TRAMP mice, which carry SV40 T-antigen under the control of a prostate-specific promoter and develop prostate cancer, were crossed with Pten+/- mice, the Pten+/- TRAMP progeny had a poorer survival from prostate cancer than Pten+/+TRAMP littermates [268-273]. The growth promoting effects of Pten insufficiency in the mouse prostate cells is likely attributable to Akt activation, as prostate-specific expression of Akt in transgenic mice also causes intraepithelial neoplasia [274]. As for the discovery of small molecule drugs which “target” the PI3K/Akt pathway in prostate cancer cells, a variety of drugs are under development for many cancers, including prostate cancer, which inhibit cell surface receptor tyrosine kinases [275]. Akt inhibitors have yet to be introduced into clinical trials [250]. Rapamycin, and its analogs CCI-779 (temsirolimus) and RAD001 (everolimus), inhibit signal transduction through mTOR (originally described at the target of rapamycin), and appear to antagonize many of the phenotypic consequences of PTEN loss and Akt activation [250]. In transgenic mice over-expressing Akt in prostate cells, mTOR inhibitor treatment triggered apoptosis in the prostate epithelium and a complete reversal of the neoplastic phenotype [276]. Preclinical studies of CCI-779, given to immunodeficient mice carrying human prostate cancer xenograft tumors, revealed that the drug, alone or with chemotherapeutic drugs, caused significant tumor growth delay [277]. Early clinical trials, with pharmacodynamic endpoints assayable in prostate cancer tissues, are underway with mTOR inhibitors in men with prostate cancer who are candidates for radical prostatectomy.  

3. Epigenetics as a Target for Prostate Cancer Treatment

Of all the somatic genome changes that accumulate during the pathogenesis of human prostate cancer, only changes in DNA methylation appear to occur consistently (>90% of cases), to arise early (first appearing in prostate cancer precursor lesions), and to be potentially reversible (the DNA sequence remains intact) [278-281]. One such change in DNA methylation, increased CpG dinucleotide methylation at “CpG islands” encompassing the transcr-
tional regulatory regions of critical genes, leads to the transcriptional “silencing” of critical cancer genes [282]. In prostate cancer cells, hypermethylation of “CpG island” sequences has been reported for GSTP1, RASSF1a, MDR1, PTGS2, and EDNRB; the sensitive detection of these somatic genome alterations is under active exploration for the molecular diagnosis, detection, and staging of prostate cancer [283]. “CpG island” hypermethylation has been reported to inhibit gene transcription by interfering with the binding and/or function of transcriptional trans-activators, or by recruiting methyl-CpG-binding domain (MBD) family proteins capable of mediating transcriptional repression via effects on chromatin structure [284]. How can somatic “CpG island” hypermethylation and associated gene “silencing” be targeted for prostate cancer treatment? (Figure 9) One rational strategy, features the use of inhibitors of DNA methyltransferases (DNMTs), such as 5-aza-cytidine, 5-aza-deoxycytidine, zebularine, procainamide, or hydralazine, to reduce 5-mCpG density at the “CpG island” sequences in dividing cells [285-288]. Another approach has been the use of inhibitors of histone deacetylases (HDACs), such as sodium phenylbutyrate, valproic acid, or suberoylanilide hydroxamic acid (SAHA), pyroxamide, N-acetyl dinaline (CI-994), and depsipeptide, to limit the formation of repressive chromatin conformation near the genes carrying abnormally methylated “CpG islands” [289-292]. In addition, combinations of DNMT inhibitors and HDAC inhibitors also appear to have intriguing activity in pre-clinical models [293,294]. Thus far, only phenylbutyrate and SAHA have been used to treat men with prostate cancer, along with patients with other solid tumors, in phase 1 trials [290,292].

**VII. CONCLUSIONS AND RECOMMENDATIONS**

Systemic treatments for prostate cancer are now considered for (i) men who have localized prostate cancer at high risk for prostate cancer recurrence after adequate local treatment, (ii) men who have androgen-dependent prostate cancer with or without detectable systemic metastases, and (iii) men who have androgen-independent prostate cancer with or without obvious metastases and troublesome symptoms. Treatment considerations vary for these different disease “states.” Adjuvant androgen-deprivation therapy has an established role given along with radiation therapy for men with high risk localized prostate cancer; androgen-deprivation therapy and chemotherapy are currently being tested as adjuvant therapy for men at high risk for relapse after radical prostatectomy. Androgen-deprivation therapy remains an effective treatment for men with metastatic prostate cancer, though the optimal timing for initiation of treatment has not been established. Chemotherapy, using docetaxel, can prolong survival of men with androgen-independent metastatic prostate cancer.

![Figure 9. Epigenetically “silenced” chromatin as a target for drug treatment of prostate cancer. Inactive chromatin conformations at sites of critical genes are maintained by DNA methyltransferases (DNMTs), histone deacetylases (HDACs), histone methyltransferases (HMTs), and 5-meC-binding domain proteins (MBDs).](image-url)
1. Failure of PSA to fall to undetectable levels or PSA > 0.3 ng/ml and rising on 2 or more determinations

2. Lower risk of metastasis:
   - PSA < 2 ng/ml or
   - Positive margin or
   - PSA doubling time > 10 months

3. Higher risk of metastasis
   - Positive seminal vesicles or lymph nodes or
   - PSA doubling time < 3 months
   - High-risk patients have better survival with active immediate treatment

4. Watchful waiting until symptomatic or signs of rapid progression (such as PSA doubling time < 3 months) or change of risk category
   - Patients with life expectancy < 10 years and with low or intermediate risk disease have a similar survival rate with watchful waiting as with active treatment
   - Patients with life expectancy > 10 years have a better survival with active treatment

5. Orchiectomy or LHRH agonist (Antiandrogen to Prevent initiation Symptomatic Flare)
   - Orchiectomy or LHRH agonist + antiandrogen
   - Clinical trials

---

See algorithm “Progression after first-line hormone therapy”
1. 3 consecutive PSA increases at least 3 months apart
2. Candidate for local therapy:
   - original clinical stage T1-T2, NX or N0
   - Life expectancy > 10 y
   - PSA now < 10 ng/ml
3. Not a candidate for local therapy
   - original clinical stage > T1-T2, NX or N0
   - Life expectancy <10 y
   - PSA now > 10 ng/ml
4. Watchful waiting until symptomatic:
   - Patients with life expectancy <10 years and with low or intermediate risk disease have a similar survival rate with watchful waiting as with immediate active treatment
   - High risk patients do better with active treatment
5. Orchiectomy or LHRH agonist (Antiandrogen to prevent initiation symptomatic flare)
   - Orchiectomy or LHRH agonist + antiandrogen
   - Clinical trials
Progression after First-line Hormone therapy
At least 2 consecutive serum PSA increases

Maintain androgen deprivation

Assessment
- Serum PSA
- Bone scan
- Abdominopelvic CT scan or MRI
- Chest radiograph
- Bone mineral density determination

Bone mineral density

Zoledronic acid for bone metastasis

Watchful waiting is an acceptable option for asymptomatic patients

Mitoxantrone provides symptom palliation for some men

Clinical trials are to be encouraged at any stage after failure of first-line hormonal therapy

Consider second-line hormone manipulation
- Add ketoconazole, estrogens, alternative anti-androgen

chemotherapy
- Docetaxel-based regimens are standard
- Zoledronic acid

Localized or Systemic radiotherapy (samarium/strontium) and/or
- Clinical trials
  - Docetaxel combinations
  - New agents
- Supportive measures

Watchful waiting

Progression

No clinical metastasis

Clinical metastasis
Newly Diagnosed Metastatic Prostate Cancer (N+ and/or M+)

Androgen Deprivation Therapy¹

- Bone Mineral Density Determination at Baseline then Each Year

Biphosphonate + Ca and vit D for osteopenia

Monitoring: Serum PSA every 3 months

Progression
(At least 2 consecutive serum PSA increases)

- Check serum testosterone if not at castrate levels, consider orchiectomy
- Add antiandrogen if not in use
- Androgen withdrawal if already in use

Assessment²

Failure of First-line Hormone therapy

Maintain androgen deprivation add Zoledronic acid for bone metastasis³

Asymptomatic or unfit for chemotherapy

- Watchful waiting⁴
  - Consider second-line hormone manipulation
  - Add ketoconazole, estrogens alternative anti-androgen

Progression

Symptomatic and fit for chemotherapy

- Chemotherapy ⁵
  - Docetaxel-based regimens are standard

Progression

- Other chemotherapy regimens⁶
  - Clinical trials⁶
    - Docetaxel combination
    - New agents
  - Localized or Systemic radiotherapy (samarium or strontium) for pain relief
  - Supportive measures

¹ Orchietomy or LHRH agonist (Antiandrogen to Prevent initiation Symptomatic Flare)
  - Orchietomy or LHRH agonist + anti-androgen
  - Clinical trials

² Assessment: Bone scan, Abdominopelvic CT scan or MRI, Chest radiograph, Bone mineral density determination

³ To prevent bone complications or treat osteopenia

⁴ Watchful waiting until symptomatic is an option for patients with long PSA doubling time

⁵ Mitoxantrone provides symptom palliation for some men.
  If neuroendocrine : cisplatin or carboplatin/etoposide

⁶ Clinical trials are an option to be encouraged at any stage of metastatic disease
REFERENCES


33. Taplin ME, Bubley GJ, Ko YJ, Small EJ, Upton M, Rajeshkumar B and Bark SP: Selection for androgen receptor mutations in


127. Ernst DS, Tannock IF, Winquist EW, Venner PM, Reyno L, Moore MJ, Chi K, Ding K, Elliott C and Parulekar W: Ran-


251. Mammalian target of rapamycin (mTOR) inhibitor reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-
254. Kim MJ, Cardiff RD, Desai N, Banach-Petrysko WA, Parsons R, Shen MM and Abate-Shen C: Cooperation of Nks3.1 and Pten loss of function in a mouse model of prostate carcinogene-
264. Brooks JD, Weinstein M, Lin X, Sun Y, Pin SS, Bova GS, Epstein JI, Isaacs WB and Nelson WG: CG island methylation changes near the GSTP1 gene in prostatic intraepithelial neo-
265. Nakayama M, Bennett CJ, Hicks JL, Epstein JI, Platz EA, Nelson WG and De Marzo AM: Hypermethylation of the Human Glutathione S-Transferase-pi Gene (GSTP1) CpG Island Is Pre-
266. Beckley KE and Bird A: Number of CpG islands and genes in

251. Wu X, Senechal K, Neshat MS, Whang YE and Sawyers CL: The PTEN/MMAC1 tumor suppressor phosphatase functions as a negative regulator of the phosphoinositide 3-kinase/Akt path-
255. Myers MP, Pass I, Barry AH, Van der Kaay J, Stolarow JP, Hem-
257. Cairns P, Okami K, Halachmi S, Halachmi N, Esteller M, Her-
258. Suzuki H, Freije D, Nusskern DR, Okami K, Cairns P, Sidran-
259. Roberts TM and Sellers WR: Regulation of G1 progression by the PTEN tumor suppressor protein is linked to inhibition of the PTEN phosphatase activity of PTEN is critical for its tumor suppressor function. Proc Natl Acad Sci U S A. 95: 1375-8., 1998.


Committee 14

Androgen Therapy in Men at Risk for Prostate Disease

Chairman

*C. Schulman (Belgium)*

Members

*S. Horie (Japan),
J.M Kaufman (Belgium),
C. Mahler (Belgium)
A. Morales (Canada),
A. Morgentaler (USA),
J. Tostain (France)*
CONTENTS

I. POTENTIAL MOLECULAR EFFECT OF TESTOSTERONE ADMINISTRATION ON THE PRECANCEROUS LESIONS

1. Proliferative inflammatory atrophy and testosterone
2. Testosterone and p27

II. ANDROGEN THERAPY AND PROSTATE DISEASE

1. Introduction
2. Testosterone replacement therapy and benign prostatic hyperplasia
3. Testosterone therapy and prostate cancer
4. Prostate cancer prevalence among men with low serum testosterone
5. Conclusions

III. DEHYDROEPIANDROSTERONE (DHEA) and DEHYDROEPIANDROSTERONE SULFATE (DHEA-S).

IV. GROWTH HORMONE (GH)

V. SELECTIVE ANDROGEN RECEPTOR MODULATORS (SARMs)

1. Steroidal androgen receptor modulator: MENT
2. Nonsteroidal selective androgen receptors modulators
3. Conclusion on SARMs

VI. ANTIESTROGENS AND SELECTIVE ESTROGEN RECEPTOR MODULATORS (SERMs)

1. Antiestrogens: Clomiphene citrate and tamoxifen
2. Selective estrogen receptor modulators (SERMs)

VII. INDICATIONS AND CONTRAINDICATIONS FOR TESTOSTERONE TREATMENT

1. Management of men with BPH and SLOH.

RECOMMENDATIONS (insofar as indications for androgen substitution)

1. Monitoring – prostate
2. Prostate and breast safety – I
3. Prostate safety – II
4. Prostate safety – III

V. SELECTIVE ANDROGEN RECEPTOR MODULATORS (SARMs)

1. Steroidal androgen receptor modulator: MENT
2. Nonsteroidal selective androgen receptors modulators
3. Conclusion on SARMs

III. DEHYDROEPIANDROSTERONE (DHEA) and DEHYDROEPIANDROSTERONE SULFATE (DHEA-S).

IV. GROWTH HORMONE (GH)

V. SELECTIVE ANDROGEN RECEPTOR MODULATORS (SARMs)

1. Steroidal androgen receptor modulator: MENT
2. Nonsteroidal selective androgen receptors modulators
3. Conclusion on SARMs
Currently the major concern for the therapy for male late-onset hypogonadism is whether androgen replacement affects the safety of prostate. This section discusses the molecular aspects of the potential effect of androgen replacement on the prostate of aging male.

Prostate cancer is the absolute contraindication of androgen replacement. However recent studies show that low androgen milieu itself contributes to more aggressive prostate cancer [1, 2, 3]. These findings may invite speculation that androgen level in the prostate might modulate the oncogenic process of prostate cancer. To this date, there is no proof of evidence that androgen replacement therapy does increase the chance of prostate cancer. Thus the practical approach would be to discuss the potential molecular effect of testosterone administration on the precancerous lesions.

I. POTENTIAL MOLECULAR EFFECT OF TESTOSTERONE ADMINISTRATION ON THE PRECANCEROUS LESIONS

Currently the major concern for the therapy for male late-onset hypogonadism is whether androgen replacement affects the safety of prostate. This section discusses the molecular aspects of the potential effect of androgen replacement on the prostate of aging male.

Prostate cancer is the absolute contraindication of androgen replacement. However recent studies show that low androgen milieu itself contributes to more aggressive prostate cancer [1, 2, 3]. These findings may invite speculation that androgen level in the prostate might modulate the oncogenic process of prostate cancer. To this date, there is no proof of evidence that androgen replacement therapy does increase the chance of prostate cancer. Thus the practical approach would be to discuss the potential molecular effect of testosterone administration on the precancerous lesions.

I. PROLIFERATIVE INFLAMMATORY ATROPHY AND TESTOSTERONE

Several lines of evidence suggest that chronic inflammation establishes a microenvironment for a precancerous lesion of many types of malignancies in humans, including prostate cancer. Inflammatory cells produce an array of oxygen-containing mutagenic chemicals, including superoxide, nitric oxide (formation of peroxynitrite), hydrogen peroxide, and hypochlorous acid [4, 5]. Oxygen radicals can cause tissue damage, and moreover they can directly attack DNA, which results in the accumulation of potentially pro-mutagenic oxidized DNA bases, such as 8-hydroxydeoxyguanosine [6]. Oxygen radicals are now considered to have a place in the prostate carcinogenesis since several studies show that antioxidants, such as carotenoid lycopene, vitamin E and selenium, decrease oxidative genomic damage in prostate cells, and may also reduce the risk of prostate cancer [7,8,9]. When a tissue becomes damaged as a result of inflammation, the consequent tissue regeneration processes usually involve increased cellular proliferation. De Marzo et al. proposed that a prostatic lesion called proliferative inflammatory atrophy is a precursor to prostatic intraepithelial neoplasia and prostate cancer) [10]. The frequent association of lesions of proliferative inflammatory atrophy with chronic inflammation suggests that these lesions arise as a consequence of the regenerative proliferation of prostate epithelial cells in response to injury caused by inflammatory oxidants [10]. Indeed PIA lesions that are exposed to inflammatory oxidants induce GSTP1 expression as a defense against oxidative genome damage [11]. In aging males, the tissue level of androgens decreases as the blood level of testosterone does [12]. A thorough deprivation of circulating androgens results in diffuse prostatic atrophy. However whether partial decrease in androgen signal triggers focal atrophy which may be related to PIA remains unknown. Testosterone probably has two opposite effects on the prostate with inflammation. First of all, in relatively low androgen level, testosterone has anti-inflammatory effects. For example, in patients with hypogonadism, supplementation of androgens reduces serum inflammatory cytokines [13]. In an animal model, the inflammation
2. Testosterone and p27

Testosterone exerts strong proliferative effect on developing prostate epithelial cells. Prostate epithelium chronically requires physiological levels of androgens for their maintenance to avoid apoptosis [22]. After castration, a majority of secretory cells of the rat prostate are rapidly lost (60%–70% within 7 days of androgen deprivation). Androgen administration to mature castrated rats can trigger the regrowth of the prostate gland, which will eventually return to its original size [23]. However, this regenerative process is timely regulated since proliferation rates decline to the baseline levels once the organ has attained adult size despite further androgen treatment [24]. At the same time that androgens exerts mitogenic activity, androgens induce glandular differentiation [25,26]. Recent study utilizing cDNA microarray identified that androgen responsive genes are involved in metabolism, chaperoning, trafficking, cell cycle, apoptosis, protein synthesis, extracellular matrix and scaffold, which are presumably involved in prostate differentiation [27]. In this switch from proliferation to differentiation, a cyclin kinase inhibitor, p27, plays a significant role in controlling the regrowth by limiting the proliferation of epithelial cells and inducing their differentiation [28,29,30]. p27 belongs to the Kip (Kip1) family of CKIs and is involved in multiple fundamental cellular processes, including cell proliferation, cell differentiation, and apoptosis. p27 was initially identified as an inhibitor of cdk2/cyclin E complex activity, and it was found to induce cell cycle arrest when overexpressed in cultured cells [31]. Moreover, p27Kip1 is a putative tumor suppressor gene that appears to play a critical role in the pathogenesis of several human malignancies, and its reduced expression has been shown to correlate with poor prognosis in cancer patients [32]. Androgens regulate the expression of p27 in both normal and neoplastic prostate epithelial cells. This regulation is dependent on the androgen receptor and achieved through modulation of its specific degradation by the ubiquitin-proteasome proteolytic system. p27 is widely expressed in differentiated prostate secretory cells. The expression of p27 is lost in cancer, PIN, and PIA [33]. Especially, it is noteworthy that PIN features hyperproliferative or intermediate state. The specific mechanism by which androgens regulate p27 degradation is unknown. However in the loss of p27, the transitional process from proliferation and differentiation would be disrupted, and androgen might direct towards proliferation in precancerous and malignant lesions. Back to 1964, Franks, LM et al. reported that prostates in old mice showed the atrophy and hyperplasia of the spontaneous nonbacterial chronic prostatitis of rats is worsened by castration, and it is blocked by replacement with testosterone [14,15,16]. Estrogen induces exaggerated inflammation and activates proinflammatory genes in the rat prostate [17], while testosterone inhibits the inflammation [18]. These experimental data indicate that testosterone may have anti-inflammatory effect on the prostate with low androgen milieu.

However, testosterone also has prooxidative effects which lead to activate AP-1 and NF-kB [19]. AP-1 is a transcriptional factor stimulating cell proliferation. NF-kB is a pro-inflammatory molecule and plays significant role in carcinogenesis. Furthermore, testosterone also activates MAPK and PI3K-Akt cascades, which contribute to cell proliferation [20]. These facts propose a serious question whether addition of testosterone to PIA, a potential precancerous lesion of prostate cancer, further enhances inflammation, aberrant cell proliferation, and genetic instability. In experimental carcinogenesis, short-term treatment of rats with chemical carcinogens or chronic treatment with testosterone produces a low incidence of prostate cancer. Thus testosterone itself may not be genotoxic enough to initiate carcinogenesis. However, combination of chronic treatment with testosterone following administration of carcinogens such as N-methyl-N-nitrosourea (MNU) and 3,2’-dimethyl-4-aminobiphenyl (DMAB) results in a high carcinoma incidence. Testosterone markedly enhances prostate carcinogenesis even at doses that do not measurably increase circulating testosterone. These data indicate that testosterone is a strong tumor promoter for the rat prostate [21].
REFERENCES


351
1. INTRODUCTION

One of the main concerns regarding testosterone therapy is the possibility of causing or promoting prostate disease, especially prostate cancer. This concern originates from two historical observations:

Metastatic prostate cancer regresses with castration or with pharmacologic lowering of serum testosterone to castrate levels, [1] and under experimental conditions, normal and malignant prostate cells die or regress in the absence of testosterone, and resume growth with restoration of physiologic levels of testosterone.

These observations have led to the concern that higher testosterone levels, via the use of testosterone replacement therapy (TRT) may cause growth of occult prostate cancer, converting these into clinical cancers. Since benign prostatic hyperplasia is also androgen-dependent, [1] there is an additional concern that testosterone supplementation may cause progression of benign prostatic hyperplasia and associated lower urinary tract symptoms (LUTS). Although these beliefs are widely held, clinical results largely fail to support these concepts. Key studies are briefly reviewed below.

2. TESTOSTERONE REPLACEMENT THERAPY AND BENIGN PROSTATIC HYPERPLASIA

Eugonadal men who received supra-physiologic doses of testosterone failed to demonstrate any increase in PSA, [2] suggesting that there exists a threshold, or saturation level for testosterone-dependent stimulation of prostatic tissue. In contrast, several studies have shown that TRT in hypogonadal men does result in a rise in PSA and prostate volume [3-6]. This increase is modest, at approximately 15%. Of interest is that the PSA and prostate volumes rise to levels similar to those of eugonadal men, [3] again suggesting a saturation point for stimulation of prostate growth. Clinically, however, these studies have shown no evidence of worsening of voiding symptoms, with unchanged prostate symptom scores. Urine flow rates and post void bladder residual urine measurements were also unchanged, and there has been no evidence that complications such as urinary retention occurred more frequently among TRT patients than for placebo control patients.

Although this experience with TRT and BPH is reassuring, individuals with severe voiding symptoms may note exacerbation of such symptoms with TRT. Consideration should therefore be given to treatment of LUTS prior to initiation of TRT in men with severe voiding symptoms.

3. TESTOSTERONE THERAPY AND PROSTATE CANCER

a) Clinical trials

Although there are no long-term, large-scale placebo-controlled clinical trials of TRT, the available data fail to support the contention that TRT results in a substantially increased risk of prostate cancer, or cancer progression [7]. A compilation of TRT trials revealed a cancer detection rate of 1%, which is similar to detection rates in large-scale screening programs for prostate cancer [7].

Moreover, one year of TRT among hypogonadal men at high risk for prostate cancer based on the presence of high grade prostatic intraepithelial neoplasia (PIN) resulted in only one case of cancer among 20 men (5%) [8]. The natural history of men with PIN is the development of cancer in 25% over three years. Although one must be cautious in comparing one-year to three-year data, it would certainly appear that TRT failed to cause a substantially increased rate of prostate cancer in a high-risk population.

b) Population-based studies of endogenous testosterone levels and prostate cancer risk

In 2001 Hsing reviewed twelve prospective population-based studies examining the relationship of endogenous testosterone levels to prostate cancer risk [9]. The hypothesis of such studies is that men who develop prostate cancer would demonstrate higher testosterone levels than men without cancer based on frozen serum samples from years prior to the clinical diagnosis of cancer. None of these twelve studies showed that men who developed prostate cancer had higher testosterone levels than men who did not develop cancer.

Moreover, men with the highest testosterone levels did not exhibit a greater risk of prostate cancer compared to men with the lowest testosterone levels. Although one study did claim an association for higher testosterone and prostate cancer, [10], this result was obtained only after simultaneous adjustment for four other hormones, representing a statistical manipulation that is unlikely to have clinical relevance.
4. PROSTATE CANCER PREVALENCE AMONG MEN WITH LOW SERUM TESTOSTERONE

If high testosterone is believed to represent a risk factor for prostate cancer, then it follows that low testosterone should be protective against prostate cancer. However, this does not appear to be true. Prostate biopsy performed in a group of 77 hypogonadal men with normal digital rectal exam and PSA <4.0 revealed cancer in 11 men, for a detection rate of 14% [11]. This rate is similar to the 15% cancer detection rate among men with PSA <4.0 in the placebo arm of the Prostate Cancer Prevention Trial who underwent biopsy as part of the study protocol. [12]. Although cancer rates in that latter trial were reduced by 25% among men receiving finasteride (which lowers dihydrotestosterone), [13] this drug does not substantially alter serum testosterone levels, and thus one cannot draw any conclusions from that study regarding the potential impact of TRT on prostate cancer risk.

5. CONCLUSIONS

In the absence of large-scale, long-term clinical trials of TRT, one must be cautious in assessing the risk of TRT with regard to prostate cancer. Nevertheless, the effect of testosterone on the prostate has been investigated extensively, and from a variety of research approaches. As noted above, normalization of testosterone among hypogonadal men causes a modest rise in PSA and prostate volume to levels seen with eugonadal men, but no higher, and does not impact voiding except in rare cases. Clinical trials of TRT have shown a low prevalence of cancer detection with treatment, and even high-risk populations fail to demonstrate any striking increase in cancer detection or progression. Moreover, multiple studies have failed to show that higher endogenous testosterone is a risk factor for subsequent cancer development. Furthermore, the presence of low testosterone does not appear to reduce this risk.

How does one resolve the lack of worrisome clinical data with the original observations that prostate cancer regresses with castration? The answer is that TRT is not the opposite of castration, since most hypogonadal men presenting for treatment already have substantially higher circulating testosterone levels than castrates. Saturation of testosterone receptors in cancer cells at hypogonadal levels would explain why no further stimulation of prostate cancer growth has been shown in clinical trials. This hypothesis merits laboratory investigation.

Although it is possible that further research may ultimately provide evidence that higher testosterone levels or TRT increases prostate cancer risk, to date there is no compelling evidence to support this contention. Indeed, this belief flies in the face of the inescapable, yet rarely acknowledged fact that prostate cancer becomes prevalent at a time of life when testosterone levels are in decline, and that clinical prostate cancer is almost never seen during the early decades of life when testosterone levels are highest. As others have previously postulated, [14] this observation leads to the possibility that long-term studies may even show that TRT is protective against the development of prostate cancer.

REFERENCES

Serum levels of the adrenal androgen dehydroepiandrosterone (DHEA) peak in men in the 3rd decade of life and thereafter decrease progressively with age. This decreased secretion of DHEA and DHEA-S by the adrenals is responsible for a parallel decrease in androgen and oestrogen formation in peripheral tissues by the steroidogenic enzymes specifically expressed in each cell type in individual target tissues [3]. The consequences of decreased DHEA production are still matter of debate. Because DHEA can serve as a precursor to more potent androgens and estrogens, like testosterone (T), dihydrotestosterone (DHT), and oestradiol (E2), supplemental DHEA use may pose a cancer risk in patients with nascent or occult prostate cancer.

Adrenal Androgens Function as an Androgen Source Within Prostate and Androgen Target Tissue

Up-to-date only a few studies have investigated the effects of DHEA on the prostatic cell. Experiments in male Wistar-Unilever rats demonstrated that non-toxic doses of DHEA confer significant protection against prostate carcinogenesis in rats. The efficacy of delayed administration of DHEA suggested that the compound confers protection against later stages of prostate cancer induction and could suppress the progression of existing preneoplastic lesions to invasive disease [1].

Koh E. et al. [4] compared the ability of three human prostatic cancer cell lines to metabolise the adrenal androgens, DHEA, and androstenedione under living culture conditions. Androgen-independent cell lines PC-3 and DU145 and androgen-dependent cell line LNCaP were investigated [2]. The findings show that the adrenal precursor pool has the potential to contribute to the regulation of prostatic cells. In another study with steroid responsive human LNCaP prostate cancer cells, containing a functional, but mutated androgen receptor (AR) the effects of DHEA were compared with those of T, DHT, and E2 on cell proliferation, and protein and/or gene expression of AR, PSA, IGF-I, IGF-I receptor (IGF-IR), IGF-II, IGF binding proteins -2, 3, and 5, (IGFBPs-2-5), and oestrogen receptor-beta (ERbeta). Cell proliferation assays revealed significant stimulation by all four steroids. DHEA and E2-induced responses were similar, but delayed and reduced, compared with those of T and DHT.

Since the drug is unregulated and easily available as over the counter “nutritional supplement” in some countries, the use of DHEA should be considered to have the same contraindications that apply to T regarding prostate safety. Further studies of the mechanisms of DHEA effects on prostate cancer epithelial cells of varying AR status, as well as on prostate stromal cells, will be required to discern the implications of DHEA supplementation on prostatic health.

REFERENCES


IV. GROWTH HORMONE (GH)

Relatively few studies have examined the effects of GH on the prostate. Prostate hypertrophy is found in acromegaly and reduced size of the prostate in patients with GH deficiency. Overall and cancer mortality in acromegaly have been shown to correlate with the degree of GH control; if post therapy GH is controlled, both the overall and cancer mortality do not appear to differ from that of the normal population. Neither prostate nor breast cancers have been consistently shown to have an increased prevalence in acromegaly, but larger prospective epidemiological studies are required to study this further [1].

The effects of GH are transmitted through the insulin-like growth factor 1 (IGF-1). Although IGF-1 appears to exert a permissive effect on tumorigenesis, there is no clear evidence that tumour initiation is triggered by IGF-1 in acromegaly. However IGF-1 and its main binding protein, IGFBP-3, modulate cell growth and survival, and are thought to be important in tumour development. An evaluation of 21 studies comparing 3609 cases and 7137 controls showed that circulating concentrations of IGF-I might be associated with an increased risk of cancer, whereas IGFBP-3 concentrations could be associated with a decreased cancer risk, but these associations were modest and varied between sites [2].

Although laboratory methods need to be standardised, these epidemiological observations could have major implications for assessment of risk and prevention of cancer. In men below 59 years of age however P. Stattin [3] found a significant rise in prostate cancer risk with increasing IGF-1 levels. This amounted to odds ratio of 1.67 for those with the highest compared to the lowest levels. Even after adjustment for IGFBP-3, the ratio remained at 1.47. For men who were aged less than 59 years at recruitment, the odds ratio was 4.12. Moreover, the odds ratio for advanced cancer was 2.87 times greater in men with the highest versus the lowest IGF-1 levels. Thus the researchers conclude that the association appears particularly strong “for IGF-1 measurements made at a comparatively young age and for advanced disease”.

GH has been shown to increase the rate of cell proliferation in prostate cancer cell lines and the co-expression of GH and GH receptor (GHR) mRNA isoforms in the ALVA41, PC3, DU145, LNCaP prostate cancer cells by reverse transcription poly-merase chain reaction has been demonstrated. Sequence analysis confirmed that these cell lines express the pituitary form of GH mRNA and also the placental mRNA isoform. The presence of GH and GHR proteins in these cell lines by immunohistochemistry was also shown [5]. Weiss-Messer et al. [6] in a recently published study demonstrated mRNA expression of GHR and of its exon 9-truncated isoform (GHR(tr)) in benign prostate hyperplasia (BPH) and prostate adenocarcinoma patient tissues, as well as in LNCaP, PC3 and DU145 human prostate cancer cell lines. GH-induced activation of signalling pathways, and its effects on AR protein in LNCaP cells and the isoform-specific regulation of GHR in prostate cancer patient tissues, suggest that GH, most likely in concert with other hormones and growth factors, may play an important role in progression of human prostate cancer.

Colao et al [4] investigated whether GH replacement therapy in adult patients with GHD has adverse effects on the prostate. The effects of 12-month GH or GH plus testosterone replacement on prostate pathophysiology in 24 adult patients with GHD (11 euandrogenemic and 13 hypoandrogenemic), compared to 24 age-matched healthy controls were evaluated. GH replacement restored prostate size to normal in both young and elderly patients, with no increase in prostate abnormalities. Prostate-specific antigen (PSA) and free PSA did not change, whereas PSA density was significantly reduced after treatment in hypoandrogenemic patients. Because the simultaneous treatment with GH and testosterone induces an increase of prostate size by 50% of baseline on average, care is suggested in elderly patients with prostate hyperplasia to avoid any risk of prostate symptoms. In these cases, GH replacement might be performed sequentially to reduce the hypertrophic effect of combining GH and testosterone.

Very recent, as yet unpublished data from the KIMS study database following more than 6400 patients receiving GH replacement therapy showed a very slightly increased rate of prostate cancer incidence, but this might be biased by the relatively high age of the treated patients (personal communication).

RECOMMENDATIONS

Surveillance for prostate cancer in elderly males with high IGF-1, especially if also receiving testosterone replacement therapy, is recommendable, by measurement of serum PSA, rectal examination and/or prostatic ultrasound [7].

However, GH administration is contraindicated in
the presence of any cancer to which prostate is not an exception; as it is suspected to play a causal role in CaP. It is prudent, therefore, to consider that the same concerns and contraindications apply to GH as to androgens in relation to prostate health.

**REFERENCES**


**V. SELECTIVE ANDROGEN RECEPTOR MODULATORS (SARMS)**

The term selective androgen receptor modulator (SARM) was introduced by A. Negro-Vilar in 1999 to name synthetic androgen agonists targeting the androgen receptor with a great degree of tissue selectivity, designed to eliminate undesired side effects (i.e. prostate stimulation, aggressive behavior…) and to maintain or enhance the positive, protective effects (i.e. anabolic) normally regulated by endogenous androgens [17]. The complete action of testosterone on male sex accessory organs, especially prostate and seminal vesicles, depends upon its reduction to 5α-dihydrotestosterone (DHT) whereas the action on most other tissues does not. Because DHT is a 6-7 times more potent androgen than testosterone, its action on proliferation is amplified in tissues where such reduction occurs. Since DHT mediates several untoward actions, including prostate diseases, the ideal steroid for therapeutic androgen replacement in the adult male would be, like SARMs, a potent testosterone agonist that does not undergo 5α-reduction to DHT but can be aromatized to estradiol.

**1. STEROIDAL ANDROGEN RECEPTOR MODULATOR : MENT**

The synthetic androgen 7α-methyl-19-nortestosterone (MENT) is believed to be more biopotent overall than testosterone both because 19-nor-testosterone derivatives in general demonstrate this property [12] and also because the 7α-methyl group in MENT greatly enhances its binding affinity for the androgen receptor and increases subsequent nuclear retention [15]. In contrast to testosterone, MENT shows no binding to sex hormone binding globulin [13]. In a study on castrated rats, MENT was 4 times more potent than testosterone in maintaining the weights of ventral prostate and seminal vesicles but was 10 times the effect of testosterone on the weights of bulbocavernous plus levator ani muscles and 12 times that of testosterone in the suppression of gonadotropin levels [11]. The physiologic half-lives of MENT and testosterone are similar, suggesting that the increased biological activity of MENT is not determined by reduced clearance. The use of a 5α-reductase inhibitor had no influence on the activity of MENT, whereas cyproterone acetate, an antian- drogen that competitively binds to the androgen receptors, inhibited the action of MENT and testosterone on the prostate as well as on the muscle. The response of seminal vesicles or kidney weights to either testosterone or MENT replacement may differ according to different animal strains [18]. Similar results were obtained in non human primates in which MENT has 10 times more antigonadotropic and anabolic potency than testosterone, but is only 2-3 times more potent at stimulating prostate growth [5]. In the castrated male monkey, the minimal testosterone dose required to suppress LH stimulated prostate growth to twice normal size, whereas the minimal MENT dose for LH suppression maintained approximately normal prostate size [5]. Moreover, these effects were associated with unaltered HDL and increased HDL2 [5]. In hypogonadal men previously treated with testosterone, prostate volume and serum PSA were found to fall during MENT administration on a 24 weeks trial [2]. In the latter study,
according to MENT dosage, lean mass increased whereas fat mass and serum leptin concentration were shown to fall.

The effects of MENT on behavior seem not to be uniform. The sexual behavior in male castrated rats or mice, as judged on the percent of animals with mounts and intromissions, is fully restored at a dose between 1/2 and 1/20 of the effective dose of testosterone [16, 18]. In the castrated male hamster, MENT is approximately 4 to 5 times more potent than testosterone to sustain mating behavior [25], equivalent to the relative binding affinities of MENT and testosterone for the androgen receptor. In contrast, testosterone was much more effective than MENT to restore aggressive behavior in male castrated mice [18]. However, the relative contributions of androgenic and estrogenic metabolites in male sexual and aggressive behaviors cannot be generalized. In a short term study in hypogonadal men, MENT was as effective as testosterone in improving spontaneous nocturnal and waking erections, and sexual interest [1]. The ability of MENT to provide adequate support to sexual behavior and erectile function was confirmed in a 24 weeks replacement study [2] and in another one for contraceptive purpose [23]. The effects on mood were less consistent and partly conditioned by cultural differences [1].

Thus, in a clinical setting, MENT may maintain important androgenic, anabolic, antigonadotropic and behavioral functions without hyperstimulation of the prostate or promotion of aggressiveness. The dose required for androgen replacement in human is estimated to be 300-700µg/day, according to the severity of hypogonadism and the tissue selectivity [21]. This quantity could be delivered subdermally in currently available sustained release formulations, such as subdermal implants of MENT acetate, that should last for up to 8-12 months [1, 20].

As with others androgen derivatives, it is unclear how to determine with certainty androgen replacement in the absence of an ability to measure serum testosterone. Normal hemoglobin concentration and hematocrit seemed to be sensitive markers of an adequate androgen stimulus in a mid-term study in hypogonadal men [2]. Part of the effect of testosterone on the maintenance of BMD in men is mediated by conversion to estradiol. Although MENT is a substrate for aromatase [14], it was shown that physiological androgen replacement with MENT did not provide adequate support to the skeleton with a fall in BMD at the lumbar spine [2]. The high potency of MENT as an androgen, by reducing the dosage requirement, might become disadvantageous when metabolism by aromatase is required. On the other hand, aromatization end-products of MENT might be less potent activators of estrogen receptors than estradiol itself [2]. Obviously, more data are needed to further evaluate the effects and long-term safety of MENT.

2. NONSTEROIDAL SELECTIVE ANDROGEN RECEPTORS MODULATORS

Although MENT is considered one of the first tissue-selective androgens, some suggest to reserve the acronym SARM for androgens with a partial agonist/antagonist profile like the classical selective estrogen receptor modulator (SERM) raloxifene [6]. The steroidal compound mifepristone (RU34486) has partial agonist and antagonist actions [3]. Nonsteroidal ligands have a better receptor selectivity than steroidal ligands and demonstrate tissue-selective actions with diverse activity profiles that may serve specific therapeutic needs. Nonsteroidal androgens can neither be potentiated upon 5α-reduction nor aromatized to estrogenic compounds. The tissue selectivity of nonsteroidal androgen action may depend upon ligand-induced AR conformation and recruitment of a tissue-specific repertoire of coregulatory factors that function as coactivators or corepressors [4].

The tissue selectivity of nonsteroidal SARMS has been demonstrated in animal models, with a partial agonist action in prostate and seminal vesicles and a full anabolic action in the levator ani muscle [26]. In intact rats, the tissue selectivity of the SARM designated S1 decreased prostate weight with efficacy similar to that of the 5α-reductase inhibitor finasteride without affecting the levator ani muscle or altering the plasma levels of T, LH, or FSH [7]. When administered to orchidectomized rats, LGD2226 [19] and S-40503 [8] showed an osteoanabolic effect with an increase of BMD and biomechanical strength of femoral cortical bone but no elevation of prostate weight over the normal. Both products also exerted anabolic activity on the levator ani muscle [8, 19].

Thus, the strong agonist activity of tissue-selective nonsteroidal androgens in DHT-independent tissues could be used to help to reduce androgen depletion syndromes and to treat muscle wasting, osteoporosis and age-related frailty. However, the development of these compounds is at an early stage, and no information is yet available on the effects of these new androgens in humans.

357
3. CONCLUSION ON SARMs

In theory, SARMs should be safer than testosterone with regard to prostate hypertrophy and with regard to stimulating the growth of incipient prostate cancer. But the results of the Prostate Cancer Prevention Trial [22] raises an important question concerning a possible useful physiological function mediated by 5α-dihydrosteroids that would not be subserved by an androgen that resists 5α-reduction. The incidence of prostate cancer in the finasteride group was 18.4%, as compared with 24.4% in the placebo group. Moreover, the incidence of tumors with a Gleason score 7-10 was 37.0% in the finasteride group vs 22.2% in the placebo group [22]. The second estrogen receptor (ER-β) may have a role in this outcome [9]. ER-β is silenced in human cancers that are not well differentiated [27] and knock-out mice for ER-β show failure of the prostatic epithelium to differentiate fully, whereas the epithelial cells continue to proliferate [10]. The natural ligand for ER-β may be a steroid, 5α-androstan-3β,17β-diol (3 β-androstenediol), a metabolite of DHT [24]. Imamov et al. [9] suggest that finasteride, by blocking the conversion of testosterone to DHT, inhibits the production of 3 β-androstenediol, thus suppressing ER-β and preventing the differentiation of epithelium, accounting for the higher incidence of poorly differentiated tumors in the finasteride group in the Prostate Cancer Prevention trial. While the tissue selectivity and prostate-sparing effect of SARMs have been demonstrated in animal models, clinical trials with SARMs suppressing endogenous testosterone and 5α-reduction to DHT should address this potential major drawback in the future.

REFERENCES


VI. ANTIESTROGENS AND SELECTIVE ESTROGEN RECEPTOR MODULATORS (SERMs)

A diagnosis of primary gonadal failure is made when a decreased serum level of testosterone is associated with an elevated level of gonadotropin and, on the other hand, normal or low level of gonadotropin in the presence of a lowered serum level of testosterone lead to a diagnosis of secondary or hypogonadotrophic hypogonadism. In the latter group of patients, it is necessary to exclude patients with hyperprolactinemaia and patients with a structural lesion of the hypothalamic-pituitary area.

However, a group of men exists who have a low level of serum testosterone and normal or low levels of prolactin and gonadotropin with no demonstrable anatomic abnormality of the hypothalamus or pituitary gland and no conditions known to cause hypogonadotrophic hypogonadism (physical stress, psychotic illness, head injury, acute critical illness, alcohol intake, multiple medications...). This phenomenon of testosterone levels that decline without an increase in the gonadotropins has been reported to be related to age. Secondary hypogonadism refers to a low free testosterone level that is not compensated for by an increase in LH secretion.

The normal LH and FSH responses to GnRH suggest that the cause of reduced basal gonadotropins in the presence of reduced testosterone is a result of inadequate stimulation of pituitary gonadotrophs by properly timed release of GnRH pulses [4].

1. ANTIESTROGENS: CLOMPHIENE CITRATE AND TAMOXIFEN

Clomphiene [8] and tamoxifene [7] are believed to stimulate endogenous production of GnRH by the hypothalamus by their antiestrogen action, probably reflecting the primacy of estrogen over testosterone in the negative feedback regulation of male gonadal function. In men with erectile complaints and secondary hypogonadism, administration of clomphiene resulted in elevation of the levels of LH, FSH, and free and total testosterone similar to that reported in patients with intact pituitary and gonadal function [3, 4]. Clomphiene citrate was effective on both clinical and biological data in hypogonadotrophic hypogonadism induced by exercise in a male endurance athlete [1] and by steroid abuse in a 30-year-old male [9]. Tamoxifen had the same favourable effect in a man with hypogonadism caused by sports activity together with an impaired testicular function (cryptorchidy) [7]. The hypothalamus can be challenged with 50-100mg of clomphiene citrate for 5-7 days [3, 9] and the treatment resumed to 25-50mg 3 times a week [5].

2. SELECTIVE ESTROGEN RECEPTOR MODULATORS (SERMS)

Recent data suggest that estrogen deficiency may play an important role in age-related bone loss in elderly men. Lasofoxifene, a selective estrogen receptor modulator, has been shown to prevent bone loss by inhibiting bone turn-over associated with aging and orchidectomy in adult male rats [6]. Further, lasofoxifene did not affect the prostate weight. Raloxifen, a SERM with an agonist action on bone with an impaired testicular function (cryptorchidy) and the hypothalamus can be challenged with 50-100mg of clomphiene citrate for 5-7 days [3, 9] and the treatment resumed to 25-50mg 3 times a week [5].
3. CONCLUSION ON SERMS

Endogenous testosterone elevation from antiestrogens stimulation in hypogonadotropic hypogonadism raises PSA levels as exogenous testosterone does [5]. Determining PSA levels before and during treatment with an anti-oestrogenic drug is therefore strongly recommended.

REFERENCES


VII. INDICATIONS AND CONTRAINDICATIONS FOR TESTOSTERONE TREATMENT

In clinical practice there are only a few valid and specific reasons not to consider treatment for men with documented symptomatic late onset hypogonadism SLOH. In fact, the case for treatment is compelling, although some simply consider androgen substitution to be a quality of life issue. In a retrospective historical review of castrati who lived between the XVI and XIX Centuries, Nieschlag et al [1] found that life expectancy among these 50 men was not different than that of contemporary controls. However, a recent retrospective study suggests a contrary view: standardized mortality ratio was significantly higher in men with untreated gonadotropin deficiency as compared to those treated (with sex steroids) and healthy controls [2].

This section deals primarily with long-term androgen treatment. As explained later, treatment should not be instituted until a satisfactory work up has been completed. It is also understood that this section focuses on the adult male –the common victim of both prostate diseases and hypogonadism- eligible for androgen supplementation in whom androgen deficiency has been clearly diagnosed. The physician should be satisfied that the patient has signs and symptoms of SLOH and that the clinical picture is supported by biochemical findings. The decision tree summarizes in a practical way the views expressed here. In general terms hormone replacement therapy aims to substitute the deficient hormone with a perfect copy of the natural hormone, with a dose schedule that generates physiological hormone levels over 24 hours of the day. Currently, it is not known whether the treatment schedule should ideally mimic the circadian rhythm of production of the deficient hormones.

1. MANAGEMENT OF MEN WITH BPH AND SLOH.

As with most clinical situations where evidence-based guidelines do not exist, knowledge and good medical judgment play a crucial role. From the considerations on the effect of androgens on BPH, it is clear that some modest increase in the volume of the gland is to be expected after T treatment in a hypogonadal man. Men with modest residual urine volumes and minimal symptoms of bladder outlet obstruction due to BPH (I-PSS < 8) can be safely treated with supplemental androgens. The situation
is less well defined in the moderate category (I-PSS between 8-15). Obviously, a man with severe symptoms and significant residual volumes can be tipped over into urinary retention by the administration of T; therefore, ART is contraindicated. But the benefits of ART should not be denied to a properly selected candidate because of the presence of mild, stable lower urinary obstructive symptoms. Treatment should only be postponed in those with moderate to severe obstructive symptoms until they have been successfully treated according to well established criteria [3].

2. MANAGEMENT OF MEN WITH SLOH AND AT RISK OF PROSTATE CANCER.

Current standard of practice establishes categorically that the administration of androgens is absolutely contraindicated in men suspected of or harboring prostate (and breast) cancer. This includes those with an abnormal digital rectal examination (DRE) and/or abnormal prostate specific antigen (PSA) in whom the diagnosis of carcinoma has not been excluded beyond doubt. However, a sub-clinical cancer can easily escape detection [4]. The presence of prostatic intraepithelial neoplasia (PIN) represents a major dilemma for the urologist. The experience on this is minimal and the information available limited to the extreme. Although Rhoden et al [5] concluded that ART “is not contraindicated in men with a history of PIN” their study included only 75 men followed for 12 months. These results must be viewed with caution until further independent information is available.

The increasing prevalence of localized CaP results in a large number of men undergoing curative procedures. Some of these men will present with SLOH. This presents a truly challenging situation. Should they receive androgen supplementation? If so when? Let us start admitting that today’s physicians have an ingrained desire for deterrence of T use in men with a history of prostate cancer. However, if one such man is considered cured and suffers from SLOH, should he be denied treatment? The facts are

1) most men undergoing curative surgery for CaP do not undergo simultaneous castration,
2) most men undergoing radical surgery have normal serum T levels,
3) although not fully recognized, serum T levels increase after radical prostatectomy and
4) early evidence is being presented indicating that no detrimental effects have occurred in patients receiving T after radical prostatectomy.

With these facts and a commitment for close follow-up, the prudent treatment of SLOH with testosterone supplementation appears warranted. Once again, definitive evidence is simply not available.

The following recommendations are adapted from the Consensus Conference held in Paris in 2003 [8]. They reflect the current state of knowledge but must be seen as “work in progress”. As new and better documented information becomes available the recommendations must be adapted to fresh realities. They are provided only as a general guide with global applicability.
1. **Monitoring – Prostate**

In men over the age of 40 years, digital rectal examination (DRE) and determination of serum prostatic specific antigen (PSA) are mandatory as baseline measurements of prostate health prior to therapy with androgens, every three (3) to six (6) months for the first 12 months, and yearly thereafter. Transrectal ultrasound guided biopsies of the prostate are indicated only if the DRE or the PSA are abnormal.

2. **Prostate and Breast Safety – I**

Androgen administration is absolutely contraindicated in men suspected of harboring carcinoma of the prostate or breast.

3. **Prostate Safety – II**

Men successfully treated for prostate cancer and suffering from symptomatic hypogonadism may become candidates for androgen therapy, after a prudent interval, if there is no evidence of residual cancer. The risk and benefits must be clearly understood by the patient and the follow-up must be particularly careful. No reliable evidence exists in favor or against this recommendation. The clinician must exercise good clinical judgment together with adequate knowledge of the advantages and drawbacks of androgen therapy in this situation.

4. **Prostate Safety – III**

Androgen supplementation is contraindicated in men with severe bladder outlet obstruction due to an enlarged, clinically benign prostate. Moderate obstruction represents a partial contraindication to ART. After successful treatment of the obstruction, the contraindication can be lifted.

---

**REFERENCES**

Committee 16

Patient’s Perspectives in Prostate Diseases

Chairman

T. Hudson (Ireland)

Members

L. Faulds Wood (UK),
R. Muntz (France),
J. Page (USA),
J. Pais (Belgium),
K. Redmond (Italy),
H. Tavio (Finland)
To ensure that we made a comprehensive report on the patient’s perspective in Prostate disease, the committee was structured to include representatives of Europa Uomo the European Prostate Cancer Coalition representing 18 National Prostate Cancer Patient Groups from across Europe, ECPC the European Cancer Patients Coalition representing 160 patient groups covering all cancers, US Too the leading prostate patients group in the United States and finally the Editor of ‘Cancer World’.

The Committee would like to acknowledge the vision of ICUD who have made welcome patients representatives at the consultation for a number of years. It is one of the very few international forums in which patients are given a voice and can receive accurate information on the latest advances in patient care.

It is also reassuring to Europa Uomo and other prostate cancer patient organisations around the world to have the opportunity to collaborate with the International Urological Community in advancing the patient / physician partnership.

EUROPA UMOM

Europa Uomo is the European Coalition of National Prostate Cancer Patient Organisations; it is a legal Non-Government Organisation (NGO) representing 182 member countries with 4 more waiting for their application for membership to be approved.

Membership requires that a group:
Must be legally recognised,
Have patient support activities.
Adhere to the ten objectives of Europa Uomo.

THE TEN OBJECTIVES OF EUROP A 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 emphasise the need for appropriate early detection;

5. To campaign for the provision of and access to optimum treatment;

6. To ensure quality, supportive care throughout and after treatment;

7. To promote multi-professional 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.

Of the above; four points are of particular relevance to our current report, number 1: To find ways and means to promote quality of life for prostate cancer patients and their families. 4: To emphasise the need for appropriate early detection.
much on the agenda for patient and non-patient alike. Number 5: To campaign for the provision of and access to optimum treatment. And Number 6: To ensure quality, supportive care throughout and after treatment, this point and No1 provide a particular focus for PCa patient groups.

THE PATIENTS PERSPECTIVE

A simple quotation describes a patient’s perspective and provides the start point for our report.

‘For the busy physician the details of diagnosis and treatments are all familiar territory.’

‘For the frightened patient, it’s uncharted territory and a potential minefield.’

Illustrating the existing gulf between physician and patient and highlighting where the work of Europa Uomo and other similar regional organisations starts.

PATIENT ORGANISATIONS

To prevent misunderstandings, we believe that it is necessary to clearly outline the role of a patient support organisation, such a body would:

Represent the patient community to health decision and policy makers.

Provide a source of information, guidance, comfort and camaraderie that ultimately make the physicians’ job easier.

Patient groups do NOT condone ‘doctor bashing’ nor do they provide medical ‘advice’.

Patient groups help patients make better informed treatment decisions by relaying personal experience and helping set more realistic expectations (accepted medical/clinical definitions vs. patient definitions can be very different).

It is interesting to note that clinical research has validated the benefit of a patient joining a support group.

For our committee, it has been a pleasure to listen to the many reports and recommendations made at this consultation: specifically with those offered by Committee 10 (Screening & Early detection)

PATIENT INFORMATION

The male public (including those who subsequently become patients) need BALANCED information material.

We look forward to the opportunity to work together with the International Urological Community to develop ‘user friendly’ material, it is only too easy to get caught up in the use of ‘big ’important sounding words’, we really need to remember to work to the level of the patient and the publics comprehension.

Together we can approach national health authorities to change the awareness of the need for better national and global prostate health care.

Europa Uomo has also developed a ‘Prostate Passport’ in co-operation with its medical advisors in a number of countries. The prime aim of this passport is to provide a vehicle for professionals to record information and to communicate their technology to patients and for patients to communicate their needs to the professionals, neither patronising the other. It will provide a means for recording clinical information in an easily accessible form, being pocket size. It will also support patients in asking the right questions of their consultants/medical practitioners.

Many clinicians see an advantage in requesting the appropriate patient-led support group to issue the passport to recently diagnosed patients thus cementing the desired bond between the clinicians and support organisations.

FUNDING MECHANISMS

Money is always a problem; there is a need for funding to:

Encourage better informed early detection education.

Provide for routine payment for PSA testing and further diagnostic testing and follow-up treatment if required.

BETTER EARLY DETECTION

Important to both Physician and Patient but from very different view points.

Patients’ organisations would like to see the systematic use of PSA ‘screening’

The establishment of a baseline PSA with appropriate monitoring of velocity of PSA change.

Increased use of Risk Assessed diagnosis (including PSA velocity) vs. use of absolute threshold (4.0 ng/ml)

Recognition that PSA is more specific to PCa in younger men and the disease is more curable in this age group.
Studies clearly show that screening with early detection reduces M+ at initial diagnosis and with appropriate intervention delays M+ progression.

Patients are becoming better educated, even if we do not guide them, they can pick up information from the internet and other sources which are not always accurate or reliable, this underlines the reasons why it is so important that patient groups and organisations work collectively with the professionals to ensure the accuracy of all information made available to the public and the patient.

**OVER TREATMENT**

Over treatment vs. Over diagnosis

The need to focus research on better detection of aggressive vs. indolent disease

PSA history, velocity of change and doubling time

Family history

Development of better markers and tests

Improved use of diagnostic tools

The need to develop more appropriate treatment protocols, for example: is it appropriate to treat a patient over 65 years of age with low grade disease by radical prostatectomy? What consideration of that patient’s Quality of Life?

*This has become a patient issue, something that is very real to them.* Without the proper information we could be encouraging them to go in the wrong direction.

**PATIENT EDUCATION AND INFORMED CONSENT**

A Patient / Physician Partnership? This is the real world where the patient goes to the physician for help and guidance.

Leading to:

Better patient CHOICE / Patient-directed care.

The better informed patient equals truly INFORMED CONSENT. Patient CONTROL of Treatment Decisions:

Educated about options and after effects.

By evaluating the situation – framed by the patients own value system – the ‘best’ course of treatment can be selected by the patient and his physician.

Decisions should be right for the INDIVIDUAL. A patient might choose to have a different form of treatment to enable him to retain his particular lifestyle; therefore it is important to recognize his interpretation of Quality of Life.

Improved patient satisfaction and patient – identified QoL - patient defined QoL indicators vs. standardized clinically defined QoL scores.

**EXPECTATIONS OF TREATMENT**

‘Outcome regret’ can be better avoided by fully disclosing risks and creating realistic expectations.

Patients need *time* to digest the diagnosis and decide on a course of treatment.

Survival vs. QoL is an individual and personal decision.

Patients wish to maintain an ongoing relationship with their Urologist even if surgery is not the treatment option selected. This is important to them because they both respect and see the Urologist as having a lifesaving opinion and they want to continue this relationship.

Patients need help to understand what will make the physicians’ job easier and their (the patients) experience and treatment outcome more positive.

This is the responsibility of *both* the patient and physician.

**MULTIPROFESSIONAL TEAM**

Urologists need to work collaboratively with other professionals (i.e. Radiation and Medical Oncologists) the inability to work together is harmful to the patient, because the patients very quickly pick up on this, they find that their consultant is not referring them, or that for some reason they are not discussing the case with some other people that they, the patient, consider to be important and this can be very damaging.

Medical professions need to work on Joint Treatment Protocols to better support patients.

Need to include the patients’ spouse-partner or family member.

PCa affects the man but impacts on the entire family.

A spouse/partner included in interviews provides enhanced understanding of true patient status and improves retention of information.

Allied health professionals need to be included to
deal with other patient issues. E.g. Nurse / Nurse educator, Social worker, Pharmacist, Nutritionist, Psychologist / Psychiatrist.

WHY?

Clearly there are many questions yet to be answered. We found when we were researching and preparing our presentation that there were so many questions some of which we have discussed and some that we really need to revisit. Our conclusion was that there are a series of questions that need to be posed to you, as in many cases you have the answer.

So from the patients’ perspective and to summarize what we have been talking about:

If early detection is important for effective treatment: Why not develop better patient-specific early detection guidelines? This is in both your hands and ours as an organization where we need to work with you and if necessary to remind you that this is urgent.

If evidence suggests patients are overtreated: Why not develop better treatment protocols?

If patients are faced with so many different choices: Why not provide a better education for the patient and the public? This is something that we can do, but we can only do it in consultation with you because we need your guidance and to be able to tap into your knowledge to ensure that the information that we are passing on to the patient and the public is the right information and we can only get that in consultation with you.

If prostate cancer represents different diseases: Why not increase use of multiprofessional teams and a more holistic patient outlook?

If treatment changes so rapidly: Why not increase use of centers of excellence in complicated cases?

If evidence shows age-based treatment decisions can be effective: Why do physicians continue to routinely suggest invasive treatments on so many men over the age of 65? This comes down to Quality of Life issues which you are well aware of.

If patients control the choices they must make: Why are they largely uninformed about the wide range of available treatment options?

If prostate cancer is a slow growing tumor: Why are patients not given the time to absorb the diagnosis get other opinions and evaluate the correct course to take?

These questions can be well addressed if Patient and Physician Groups work together! This it the key! We believe that by finding the time to work together we will be able to make positive progress for everybody’s benefit.

THE FUTURE

Europa Uomo is currently working on the formation of a world wide PCA coalition, a ‘Global Prostate Cancer Coalition’, Every-body has a shared interest in working together and by doing so, we believe that we can help bring about change for the better.

Changes in Policy and Protocols alone can positively save lives.
CHAPTER 2

Committee 3A

Epidemiology and Natural History of Prostate Cancer

Chairmen

M. Barry (USA),
M. Murai (Japan)

Members

R. Bosch (The Netherlands),
C. Cheng (Singapore),
O. Cusenot (France),
J. Donovan (UK),
R. Etzioni (USA),
S. Jacobsen (USA),
R. Khouri (Lebanon),
E. Lee (Korea),
R. Sahabudin (Malaysia),
K. Sasidharan (India),
J. Stanford (USA),
T. Tsukamoto (Japan),
L. Zhou (China)
## CONTENTS

<table>
<thead>
<tr>
<th>I. INTRODUCTION</th>
<th>IV. NATURAL HISTORY</th>
</tr>
</thead>
<tbody>
<tr>
<td>II. DESCRIPTIVE EPIDEMIOLOGY</td>
<td>V. SPECIAL SECTION: GENETIC MARKERS OF RISK AND PROGRESSION FOR PROSTATE CANCER</td>
</tr>
<tr>
<td>III. RISK FACTORS</td>
<td></td>
</tr>
<tr>
<td>1. SMOKINGS</td>
<td>1. INTRODUCTION</td>
</tr>
<tr>
<td>2. ALCOHOL</td>
<td>2. PROSTATE CANCER SUSCEPTIBILITY GENES</td>
</tr>
<tr>
<td>3. PHYSICAL ACTIVITY</td>
<td>REFERENCES</td>
</tr>
<tr>
<td>4. DIET AND NUTRITIONAL SUPPLEMENTS</td>
<td></td>
</tr>
<tr>
<td>5. MEDICATIONS</td>
<td></td>
</tr>
<tr>
<td>6. THE IGF SYSTEM</td>
<td></td>
</tr>
<tr>
<td>7. OVERALL SUMMARY</td>
<td></td>
</tr>
</tbody>
</table>
Epidemiology and Natural History of Prostate Cancer

M. Barry, M. Murai
R. BOSCH, C. CHENG, O. CUSSENOT,
J. DONOVAN, R. ETZIONI, S. JACOBSEN, R. KHAULI, E. LEE, R. SAHABUDIN, K. SASIDHARAN,
J. STANFORD, T. TSUKAMOTO, L. ZHOU

I. INTRODUCTION

Prostate cancer is a common cause of morbidity and mortality in developed countries worldwide, and particularly in Europe and North America. Prostate cancer differs from many other solid tumors in that the latent prevalence of the disease – the number of men with prostate cancer – far exceeds the number of men diagnosed with, or dying from, the disease.

Autopsy studies show that cancerous cells can be found in the prostates of 30-40% of men at age 60 [1], rising to 60-70% by age 80[2], yet the eventual risk of death from prostate cancer is only about 3% for a 50-year-old man in the United States [1].

The high prevalence of latent prostate cancer complicates the study of its epidemiology, as incidence rates are driven by surveillance intensity [3, 4]. Surveillance intensity is in turn related to the intensity of direct screening efforts with digital rectal exams and prostate specific antigen (PSA) tests, and indirect screening through the performance of prostatectomies for presumed BPH, which can uncover incidental prostate cancers.

Even prostate cancer mortality data seems to be influenced by the intensity of surveillance efforts. For example, in the United States, both prostate cancer incidence (as expected) and mortality (unexpected) increased with the introduction of widespread PSA testing in the late 1980s and early 1990s [5]. The influence of surveillance intensity on prostate cancer incidence and mortality data needs to be kept constantly in mind in interpreting epidemiologic data.

II. DESCRIPTIVE EPIDEMIOLOGY

Prostate cancer is a worldwide public health problem, with an estimated 679,000 cases diagnosed in 2002 (11.7% of all new cancers) and 221,002 deaths (5.8% of all cancer deaths in men) [6]. The highest incidence in the world is among African Americans, with Chinese men having the lowest incidence (Figure 1). Hsing et al. [7], summarized international trends in incidence between 1973-77 and 1988-92 and noted the greatest increases in the U.S., Canada, Australia, France and Asian countries. Screening and early detection clearly contributed to this pattern in the developed countries. For example, survey results from a representative sample of U.S. men completed in 2000 showed that 57% had ever had a prostate-specific antigen (PSA) test and 30% had three or more PSA tests within the past five years [8]. In Asian populations, prostate cancer incidence also has been increasing over the past two decades [9]. These trends may be due to enhanced diagnostic efforts in developing countries as well as changes in environmental and lifestyle exposures such as increased calorie and fat consumption [9].

Blacks in the U.S. also have the highest mortality from this disease, followed by men in the Caribbean, Southern, Middle, Eastern and Western Africa [6]. Worldwide, prostate cancer claims more men with African ancestry than any other group (Figure 1). In Asian populations with the lowest mortality, the death rate increased between 1973-77 and 1988-92 [7]. In contrast, mortality rates have been declining in many of the developed countries. This latter pattern may be attributed to a combination of PSA
screening and better treatment of clinically localized as well as advanced stage disease [10].

In developed countries such as the U.S., where PSA screening is widely used, the survival of prostate cancer patients diagnosed with localized or regional stage disease has increased over the past few decades, with current relative 5-year survival approaching 100%. For distant stage disease, however, there has been little improvement in survival over the past 30 years, with 5-year relative survival remaining around 30% [11].

**III. RISK FACTORS**

The incidence of prostate cancer is strongly linked to age, race/ethnicity, and family history of the disease [11, 12]. A number of factors such as lifestyle habits, dietary intake, nutritional supplement use, and medication use have been examined in relation to prostate cancer in ongoing efforts to identify modifiable exposures that may reduce the risk of the disease. Recent publications relevant to some of these modifiable factors will be highlighted as an update to the International Consultation on Prostate Cancer report published in 1999 [13].

**I. SMOKING**

A number of studies have examined cigarette smoking as a risk factor for prostate cancer [14]. Although overall results have not been consistent, a consensus conference report in 1996 noted that “the weight of the evidence does suggest an excess risk” [15]. More recent data indicate that heavy smoking may be related to more aggressive prostate cancer and disease progression. In analyses of data from a large cohort of men participating in the Health Professionals Follow-up Study, Giovannucci et al. found that men who had smoked 15 or more pack-years within the past 10 years had a significant 80% (95% CI 110% - 310%) increase in risk for distant stage prostate cancer and a 200% (95% CI 110% - 390%) increase in risk of fatal prostate cancer relative to nonsmokers [16]. Risk abated ten years after smoking cessation. The Physicians’ Health Study, however, found no associations between smoking and prostate cancer incidence or mortality [17]. This latter study was unable to adjust for prostate cancer screening or dietary intake, which may have confounded the results.

Two subsequent studies support an association between smoking and prostate cancer, particularly more aggressive phenotypes. In an analysis of men under age 55 years at the time of radical prostatectomy for prostate cancer, current smokers compared to
non-smokers had an odds ratio (OR) of 3.9 (95% CI 1.4-10.3) for having extraprostatic and Gleason score > 7 disease [18], and risk increased significantly with increasing pack-years of smoking. In another population-based study of men under age 65 years, Plaskon et al [19] found a 40% (95% CI 0%-200%) increase in risk among current vs. non-smokers, and there was a dose-response relationship between number of pack-years smoked and risk (trend p= 0.03), which was stronger for men with more aggressive prostate cancer (OR= 2.0, 95% CI 1.3-3.1 for men with Gleason scores of 8-10 or regional/distant stage disease and > 40 pack-years of smoking). Further analyses from the same study revealed that the excess risk of prostate cancer was most apparent in heavy smokers with the GSTM1-null genotype (Figure 2). Approximately 50% of the general population is GSTM1-null, lacking this enzyme that metabolizes carcinogenic substances in tobacco smoke [20]. Among GSTM1-null Caucasian men, the risk of prostate cancer increased with increasing pack-years of smoking (trend p= 0.007). Consistent with data from the Health Professionals Follow-up Study [16], the risk of prostate cancer declined with increasing years since smoking cessation (trend p=0.02) [19].

Taken together, recent studies suggest that smoking is a risk factor for prostate cancer, particularly more aggressive disease. As summarized by Hickey et al [14], four biological mechanisms may explain how smoking could alter the risk of prostate cancer: cadmium exposure from tobacco, alterations in the hormonal milieu, immune system suppression, and mutations in genes such as p53. In addition, recent data indicate that men lacking the GSTM1 gene may be especially susceptible to the adverse effects of smoking [21].

2. ALCOHOL

Over 60 epidemiologic studies have evaluated the association of alcohol consumption with prostate cancer with mixed results. Several reviews of the literature have been published, and two meta-analyses have been completed in the last five years. Most studies of moderate alcohol consumption (3 drinks/day) have found no increased risk of prostate cancer, as summarized by Dennis and Hayes in their review of studies published between 1971 and 1999 [22]. One U.S. population-based case-control study found heavy users (≥8 drinks/day) had a significantly increased risk of prostate cancer (relative risk, RR= 1.9; 95% CI 1.3-2.7). This risk was elevated for both blacks and whites, for recent and former drinkers, and for beer (RR= 2.1; 95% CI 1.4-3.1) and liquor use (RR= 1.9; 95% CI 1.4-2.7), but not significantly for wine use (RR= 1.4; 95% CI 0.9-2.2) [23].

A meta-analysis of 11 epidemiologic studies published from 1966-2000 found a pooled relative risk of prostate cancer of 1.05 (95% CI 1.00–1.08) for 25g of alcohol per day and 1.19 (95% CI 1.03–1.37) for 100g of alcohol per day [24]. Another meta-analysis of 35 epidemiologic studies of alcohol use and prostate cancer risk published from 1976 to 1997 found a pooled relative risk of 1.05 (95% CI 0.98-1.11) for ever consuming alcohol, and 1.21 (95% CI 1.05-1.39) for men who drank four drinks per day [25]. Among five studies that reported risk by type of alcohol, the pooled risk estimate for beer was 1.18 (95% CI 1.07-1.29), for liquor was 1.08 (95% CI 0.99-1.19), and for wine was 1.06 (95% CI 0.96-1.18) [25].

Few epidemiological studies have examined type of wine (red vs. white) in relation to risk of prostate cancer. The first epidemiological study to examine

![Figure 2. Odds ratios for prostate cancer according to GSTM1 genotype and pack-years of smoking. Models adjusted for age, family history of prostate cancer, PSA test, lifetime alcohol consumption and education. Among GSTM1-null p-trend=0.007; among GSTM1-present p-trend=0.77. Numbers in parentheses are 95% confidence intervals of risk estimates.]
red vs. white wine in relationship to prostate cancer was the Netherlands Cohort Study. Schuurman et al [26] collected a self-administered questionnaire on diet and alcohol use from 58,279 Dutch men in 1986; with over 6.3 years of follow-up, 680 were diagnosed with prostate cancer. Alcohol use was queried as “usual consumption” during the year preceding the study. Compared to ex-drinkers and abstainers, the risk for prostate cancer associated with the highest level of consumption (≥15 g/day or about 5 oz/day) was 3.3 (95% CI 1.2-9.2) for white wine and 0.8 (95% CI 0.4-1.7) for red wine; neither p-value for trend across level of intake was significant. When stratified by stage of disease, the highest level of red wine consumption (≥ 15 g/day) was associated with a 20% reduction in the relative risk of advanced stage prostate cancer whereas a similar intake of white/fortified wine was associated with a relative risk of 2.2 for advanced stage disease [26]. These estimates were not adjusted for total alcohol intake.

Another study that examined red vs. white wine and risk of prostate cancer was recently published [27]. This population-based case-control study was the first to assess lifetime consumption of red wine and white wine separately, and to adjust estimates for total alcohol intake and other confounders such as PSA screening history. These analyses revealed that each 4-ounce glass of red wine was associated with a 6% decrease in prostate cancer risk (OR= 0.94; 95% CI 0.90-0.98; p-trend= 0.02). About a 50% reduction in risk was observed for men who consumed 4 or more glasses of red wine per week (Figure 3), and this decrease in risk with increasing intake was most apparent in the subgroup of men with more aggressive (Gleason score 8-10 or regional/distant stage) disease (OR= 0.40; 95% CI 0.2-0.9). No significant associations were found for intake of white wine, beer or liquor.

Prostate cancer incidence according to white and red wine consumption was also examined in the Health Professionals Follow-up Study [28]. Alcohol intake was assessed as usual intake over the prior two years. No significant association was found for white wine (hazard ratio, HR= 1.14, 95% CI 0.98-1.33) or red wine (HR= 0.93, 95% CI 0.77-1.13) at a level of 2-5.9 g/day. Unfortunately, lifetime wine consumption was not collected so this finding reflects only recent intake.

In summary, these studies suggest that red wine may be associated with a decrease in prostate cancer risk and highlight the need for further study, particularly based on the unique chemical composition of red wine. Red wine is a rich source of polyphenols, one of which is resveratrol, that has been shown to have significant biological effects relevant to prostate cancer chemoprevention [29-31].

3. Physical Activity

Epidemiological studies of physical activity and prostate cancer have not consistently shown a beneficial effect, which may be related to difficulties in measuring lifetime physical activities. A literature review by Lee et al [32] noted that most studies found no association. More recently, Torti and Matheson[33] reviewed studies conducted between 1976 and 2002 and found an average risk reduction ranging from 10%-30% associated with increased physical exercise. Further, several recent studies reviewed below suggest that vigorous physical activity may be associated with a reduced risk of more advanced prostate cancer.

Data from the Health Professionals Follow-up Study [34] showed no overall association for prostate cancer and physical activity, but in men aged 65 and older there was a significant reduction in risk of advanced (RR= 0.33, 95% CI 0.2-0.6) and fatal
Several reviews have summarized the biological effects of physical activity that may be relevant to its potential effect on risk of prostate cancer. Plausible beneficial effects of exercise include reduced androgen and IGF-1 levels, reduced obesity, enhanced immune function, and improved antioxidant defense systems [38-40].

4. DIET AND NUTRITIONAL SUPPLEMENTS

Dietary intake and dietary supplements have been widely studied in efforts to identify modifiable factors that may either enhance or reduce the risk of prostate cancer. A complete review of this voluminous literature is beyond the scope of this chapter, but some recent findings warrant comments. Dietary fat and meat consumption have been associated with modestly elevated relative risks of prostate cancer in some, but not all studies. Several recent reviews summarized these inconsistent findings, noting that a reduction in overall fat and red meat intake may decrease prostate cancer risk [41-43]. Also noted was the fact that diets high in fat and meat tend to be low in plant foods, which may offer some protection against prostate cancer. In particular, recent evidence suggests that vegetables (especially cruciferous vegetables and tomato products) may be associated with a reduced risk of prostate cancer [44-48]. Studies of Japanese men who have migrated to Hawaii and their progeny reveal that they rapidly develop an incidence of prostate cancer intermediate between the low rates in Japan and the high rates among Caucasian Hawaiians, [49] suggesting the traditional diet in Japan may have a protective effect. A case-control study suggests the factors in this traditional Japanese diet that may be protective are the low meat intake and high fish and soy product intake [50]. Other recent dietary associations of interest include a reduction in risk related to intake of fish (as in Japan) [51], and increased risk estimates in relation to higher levels of total energy intake [52, 53] and calcium intake [44, 54, 55]. Kolonel [41] highlights the need for future studies of dietary factors to also consider potential interactive effects of genetic polymorphisms in genes responsible for metabolism of dietary exposures.

Interest in dietary compounds associated with lower cancer incidence has fueled interest in evaluation of nutritional supplements as chemopreventive agents [56]. Results from two randomized, placebo controlled trials demonstrated a 52% (RR= 0.48, 95% CI 0.3-0.8) reduction in prostate cancer risk in men taking 200 µg of selenium per day [57, 58], and a 36% (95% CI -56% to -6%) lower incidence in men taking 50 mg of α-tocopherol per day [59]. These results were from secondary data analyses, but combined with other evidence were sufficiently compelling to lead to the launching of the Selenium and Vitamin E Prostate Cancer Prevention Trial (SELECT). This randomized, double-blind placebo controlled clinical trial was designed to test the efficacy of selenium and vitamin E alone and in combination for prostate cancer prevention. Randomization of over 32,400 men has been completed and results from SELECT are anticipated in 2013 [60].

5. MEDICATIONS

A number of medications may lower prostate cancer incidence. Recent results highlight the potential role of drugs that alter androgenic exposure, inflammation, or cholesterol metabolism. Based on the evidence that androgens are important in prostate cancer pathogenesis, drugs that inhibit 5α-reductase, the enzyme in the prostate that converts testosterone to its potent metabolite dihydrotestosterone, are of special interest [61]. The Prostate Cancer Prevention Trial (PCPT) randomized 18,882 men aged 55 years and older with normal DRE and PSA (≤ 3 ng/ml) to 5 mg/day of finasteride (a 5α-reductase inhibitor) or placebo [62]. After 7 years of follow-up, there was a statistically significant 25% (95% CI 18.6% to 30.6%, p <0.001) lower cumulative incidence of prostate cancer in men on the active drug compared to placebo. This dramatic beneficial effect is mitigated, however, by the observation that more patients in the finasteride group than in the placebo group had Gleason score 7-10 tumors (p <0.001), and the active treatment group experienced more sexual side effects. Based on these concerns, finasteride is unlikely to be widely accepted as a chemopreventive agent in the general population [63]. Notably, this
study does provide direct evidence for the role of androgens in prostate cancer etiology.

Nonsteroidal anti-inflammatory (NSAIDs) drugs and aspirin (ASA) have been available and widely used over the past 20 years to treat chronic or recurrent inflammatory conditions as well as for prevention of cardiovascular disease. Further, chronic inflammation and infectious agents that induce an inflammatory response have been linked to several types of cancer [64]. The first epidemiological data on these medications in relation to prostate cancer were from a population-based case-control study conducted in New Zealand [65]. Norrish et al. [65] found that regular use of NSAIDs (OR= 0.7, 95% CI 0.5-1.07) or ASA (OR= 0.7, 95% CI 0.5-1.08) was associated with a reduced risk of advanced prostate cancer relative to nonusers. Recently, a systematic review and meta-analysis of 12 studies published before 2003 reported that most investigations of ASA showed a negative association with prostate cancer (summary OR= 0.9, 95% CI 0.8-0.99), particularly advanced disease (summary OR= 0.7, 95% CI 0.5-0.9). The risk estimates for nonaspirin NSAIDs (OR= 0.87, 95% CI 0.6-1.2) and total NSAIDs (OR= 0.68, 95% CI 0.4-1.2) were less than one, but were not statistically significant [66]. Most of these studies did not collect data on lifetime use of these medications, some were based on pharmacy records that likely underestimated exposure, few collected data on dose or duration, and some did not account for potential detection bias.

The most recent data on NSAIDs and prostate cancer incidence comes from the Baltimore Longitudinal Study of Aging [67]. Platz et al [67] found that ever compared to never use of aspirin (RR= 0.8, 95% CI 0.5-1.07), NSAIDs (RR= 0.8, 95% CI 0.5-1.2), or both medications (RR= 0.7, 95% CI 0.5-1.02) was associated with nonsignificant reductions in risk. These investigators also showed that “current” use of these drugs in men without a diagnosis of prostate cancer was unrelated to serum PSA level. However, the study did not evaluate duration of use or dosage. Potential mechanisms through which these drugs may act as chemopreventive agents include their ability to enhance apoptosis, reduce cell proliferation, and inhibit prostaglandin synthesis [68].

A third class of medications that has a potential role in prostate cancer prevention is statins [69]. These agents inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and thereby block mevalonate production, the rate-limiting step in cholesterol biosynthesis. In addition to modifying cholesterol metabolism and significantly reducing cardiovascular mortality, statins have been shown to reduce prostatic cell proliferation, enhance apoptosis, and may regulate androgen receptor activity [70-72]; they also have anti-inflammatory and antioxidant activities [73].

An early hospital-based study by Coogan et al [74] found no association between prostate cancer and ≥ 3 years of statin use. Two other studies that ascertained statin use through pharmacy databases reported nonsignificant risk estimates of 0.37 (95% CI 0.1-1.3) [75], and 0.87 (95% CI 0.6-1.2) [76], although the average follow-up period for these studies was limited (3.3 and 7.2 years, respectively). Recent data from the Health Professionals Follow-up Study [77], however, showed a significant reduction in the incidence of advanced prostate cancer (HR= 0.54, 95% CI 0.3-0.95) and incidence of advanced and fatal prostate cancer combined (HR= 0.34, 95% CI 0.2-0.95); and, risk decreased with increasing duration of statin use (p-trend= 0.008). No associations were observed for men with organ-confined prostate cancer. Given that statins were first marketed in 1987 and only became widely used in the mid-1990s, additional studies of these agents are clearly needed to evaluate potential effects of longer-term use on prostate cancer risk. It will be key to see if future studies can confirm the results of Platz et al [77], which demonstrate that statin use can significantly reduce the incidence of clinically important prostate cancer.

6. The IGF System

Insulin-like growth factors (IGF-I and -II) are nutritionally regulated peptides, with a structure similar to proinsulin, that play a key role in somatic growth and organ development in early-life and in tissue repair, cell proliferation, metabolic regulation and apoptosis throughout life in a wide variety of cells and tissues [78], including the prostate [79]. IGF-I levels are regulated by growth hormone (GH) and are influenced by both genetic [80] and environmental factors, including early growth, diet in both child and adulthood, exercise and ethnicity [81-87]. In the circulation, most (>99%) IGF-I and -II form complexes with one of six different binding proteins (IGFBP-1 to -6), the vast majority (>90%) being with IGFBP-3 and an additional acid-labile protein subunit. Several epidemiological studies suggest that elevated circulating IGF-I levels are associated with 2-4 fold higher risks of prostate cancer when the top versus bottom quartiles of IGF concentrations are
compared [88]. Case-control studies generally suggest 50-150% increased risks of prostate cancer associated with the highest levels of IGF-I [89-93], though in one study a halving of the risk of prostate cancer was observed [94]. In case-control studies it is difficult to control for the effects of selection bias where clinically defined cases are used, or reverse causality when blood is drawn around the time of case diagnosis. Early prospective studies suggested odds ratios (ORs) for prostate cancer for the highest versus lowest levels of IGF-I of 4.3 [95], and 3.1[96], but others indicate more modest effects (OR = 1.32 [97]), and several demonstrate protective effects of high IGF-I levels (ORs = 0.81 [98], 0.6 [99], and 0.52 [100]). One explanation for variations in results could be that IGF-I is more important in advanced stage – extraprostatic and distant metastatic – disease than in the development of cancer localised to the prostate gland [91, 101].

The role of IGFBP-3 in modulating the effects of IGF-I on prostate cancer risk remains unclear, with some studies suggesting an inverse relationship of IGFBP-3 with newly diagnosed prostate cancer [89], and advanced prostate cancer [101-105], but others fail to demonstrate any association [89, 91, 94, 96, 100, 106, 107]. One population-based prospective cohort study showed a positive association between IGFBP-3 and prostate cancer [108]. Circulating levels of IGF-II and IGFBP-2 have been proposed as potential tumour markers as prostate cancers overexpress these factors [79, 88]. In two case-control studies, raised serum levels of IGF-II were associated with a doubling of the risk of prostate cancer, and the association was stronger with advanced compared with localised disease [89, 91]. Clinical and epidemiological studies have shown substantially increased serum levels of IGFBP-2 in men with prostate cancer [103, 107, 109], particularly where it is more advanced [109], though this finding has not been replicated in screen detected prostate cancer [91], or in a prospective study [97]. There is little evidence that measurement of the IGF axis enhances the specificity of prostate cancer detection in clinical practice beyond that achievable using the free/total PSA ratio [110].

Further research in prospective cohort studies is needed to clarify the extent to which the IGF system is involved in prostate cancer development, rather than reflecting the presence of pre-clinical tumour (reverse causality) [95, 101], and whether IGFs have value in predicting the risk of developing locally-advanced or metastatic prostate cancer.

7. **OVERALL SUMMARY**

The findings from recent studies of modifiable exposures that may influence the risk of prostate cancer suggest that substantial progress has been made in identifying factors that may generate novel prevention approaches. Especially encouraging are data on specific dietary components and nutritional supplements, including red wine intake, and medications that have low toxicity profiles and biological activities that favor a reduction in risk of prostate cancer. Several of these lifestyle factors and medications may reduce prostate cancer incidence as well as progression, and some may have therapeutic benefits. Future studies should aim to collect data on potential confounding factors related to the above exposures and clinical features of disease so that exposures can be examined in relation to more clinically important prostate cancers. In addition, these factors should be examined in studies that also consider the role of genetic variants that may alter biological effects of these lifestyle and environmental exposures in subsets of the population.

IV. NATURAL HISTORY

The vast discrepancy between the autopsy prevalence and the clinical incidence of prostate cancer is attributable to the generally long latency period or preclinical duration of the disease (Figure 4). Several studies of prostate cancer latency have been conducted, with fairly consistent results. Broadly speaking, these studies can be broken down into three types: (1) Studies based on retrospective analysis of PSA levels in serial serum samples, stored prior to prostate cancer diagnosis; (2) Statistical or model-based analyses of prospective screening cohorts; and (3) Epidemiologic analyses, comparing latent prevalence based on autopsy studies, with disease incidence. Results of these studies point to an average latency period of 10 years or more.

![Figure 4. Schematic of preclinical duration of prostate cancer.](image-url)
Retrospective studies of PSA analyze serial PSA samples stored prior to diagnosis of prostate cancer. Piecewise linear trajectories are fit to the observed PSA measurements; these allow PSA growth to accelerate beginning at some point prior to diagnosis. The latency period is then estimated as the duration of accelerated PSA growth. Estimates of the latency period from these studies range from 7 to 13 years, depending on the study and the stage of disease. The first such analysis, based on data from the Baltimore Longitudinal Study of Aging (BLSA) [111, 112], found the average preclinical duration to be 7 years for localized cases and 9 years for advanced cases; a subsequent analysis estimated the preclinical duration to be approximately 13 years on average for cases subsequently diagnosed with stages B through D disease [113].

A recent meta-analysis [114] of data from three retrospective PSA studies [111, 115, 116], utilized a larger case group and, in contrast to the prior BLSA study, found that both PSA growth and progression from occult to clinically apparent disease were significantly faster among cases eventually diagnosed with clinically advanced disease than among localized cases (Figure 5). This study also found that cases with moderate to high Gleason scores (7-10) tended to progress faster from latent to clinical disease than did cases with lower Gleason scores. These findings have implications for early detection because they suggest that tumors destined to become metastatic may be biologically different from localized tumors at times prior to diagnosis, and perhaps, even from the point of disease onset.

Statistical analyses of prospective screening cohorts estimate latency distributions that best match observed rates of detection by screening and other methods. Draisma et al [117] used a computer simulation model to project diagnosis frequencies in the Rotterdam section of the European Randomized Study of Screening for Prostate Cancer. They varied the latency period duration until the projected frequencies closely matched those observed in the trial. In practice, their model broke down the latency period into nine states defined by combinations of grade (less than 7, 7, greater than 7) and stage (local, regional, distant). The model estimated transition rates between the states and used the estimates to provide estimates of the overall preclinical duration, which was 12.7 years on average.

Recently, Tsodikov et al [118] presented a statistical analysis of prostate cancer incidence in the US following the advent of PSA screening. Their model analytically estimated the latency distribution that best matched observed incidence given annual PSA screening frequencies in the US. These authors noted that the latency period has declined over time, due to practice trends prior to the PSA era that led to progressively earlier diagnosis of disease. For example, the sojourn time for a case with disease onset in 1973 was estimated to be 11.8 years on average, whereas the sojourn time for a case with disease onset in 1987 was estimated to be 9.6 years on average. These estimates are similar to those of Etzioni et al [119], who developed estimates of preclinical incidence of disease based on age-specific prevalence rates from autopsy studies [120]. By comparing the calculated incidence with the published prevalences, Etzioni et al [119] estimated the preclinical duration to be between 11 and 12 years on average.

A recently published study that promises to provide new insights into disease natural history is the Prostate Cancer Prevention Trial (PCPT) [121]. The PCPT enrolled 18,000 men who were randomized to receive finasteride or placebo for seven years. Study

Figure 5. Posterior medians of subject-specific PSA change points versus posterior medians of subject-specific post-change point slopes by study and disease stage from meta-analysis of PSA results of three studies Baltimore Longitudinal Study of Aging (BLSA), Beta Carotene and Retinol Study (CARET), and the Nutritional Prevention of Cancer Trial (NPCT). Filled symbols denote local stage while open symbols denote metastasis. [114]
participants were screened annually with PSA and digital rectal exam (DRE), with a biopsy recommended in the case of a positive result on either test. The design of the trial is unique in that even men with negative screening test results were scheduled for a prostate biopsy at its conclusion. Results published to date have shown a prostate cancer prevalence of approximately 15% among screen-negative subjects in the placebo arm, with approximately 15% of these cases being high-grade (Gleason score 8-10) [122]. A recent statistical analysis [123], of disease latency in the placebo arm estimated the average preclinical duration to be approximately 16 years in this group; 17 years among low to moderate-grade cases (Gleason 7 or less) and 9 years among high-grade cases (Gleason 8-10).

An aspect of natural history that is related to tumor latency but that has been rather less well studied is the stage- and grade-specific duration of disease. Of particular interest is the length of the early (localized) stage, or the interval from preclinical onset to metastasis. Cowen et al [124] developed a Markov model of stage-specific progression in the absence of treatment or other-cause mortality, based on observed progression times among localized prostate cancer cases managed conservatively. They estimated the duration of stage A1 disease to be 50 years on average. Inoue et al [125] used retrospective, stored-serum data from the BLSA to derive an estimate of the median years from onset to metastasis in this cohort, with a result of 6.07 years. While these estimates are quite divergent, they are based on very different datasets – the higher estimate is derived from progression rates in conservatively managed cohorts, which may be subject to selection biases, and the lower estimate is based on a relatively small group of advanced cases (n=8) diagnosed prior to the PSA era. Draisma et al [117] considered nine different disease stages defined by all combinations of three grade (low, moderate, and high) and three stage (localized, regional and distant) categories. Their model allowed both grade and stage to progress over time; resulting estimates of the length of the localized stage ranged from 6.95 years (low grade) to 5.25 years (high grade). The question of whether disease grade is progressive has not been conclusively answered. Choo et al [126], studied progression of histologic grade from radical prostatectomy to local recurrence in 43 patients with clinically isolated local recurrence following surgery. Their study found a trend towards higher Gleason score at the time of local recurrence; at the time of local recurrence (median 3.6 years after surgery), Gleason score was upgraded in 13, downgraded in 7, and remained the same in 23 patients. However, this study does not address whether Gleason score may progress within the primary tumor.

The above-referenced studies of tumor latency do not clearly indicate whether prostate cancer is primarily a disease with a long and relatively slow development phase or several diseases with less aggressive and more aggressive forms. However, the fact that the histological prevalence of prostate cancer far outweighs the number of clinically apparent tumors makes it critical to distinguish life-threatening tumors that require treatment from tumors that will not progress if left alone. This issue becomes particularly important in the context of the increasing use of the PSA test, which can lead to the detection of large numbers of prostate cancer cases, the vast majority of whom would never have known that they had the disease. It has been estimated that, in the absence of PSA, approximately 75% of men with prostate cancer would not have been diagnosed within their lifetimes[119], creating enormous potential for overdiagnosis and overtreatment. Reports of the extent of overdiagnosis vary, but range from 30% [118, 127] to 50% [117] of men detected by PSA screening, depending on the screening schedule and population.

To identify predictors of disease progression, a number of cohort studies of untreated, conservatively managed men with localized prostate cancer have been conducted. Both long-term and short-term studies are available. While results differ depending on study populations, era of diagnosis, and definition of progression, some broad inferences can be made. First, for cases diagnosed prior to the PSA era, disease histology is a key predictor, and perhaps the most important predictor of disease progression. In their long-term analysis of 767 men diagnosed between 1971 and 1984 in Connecticut, Albertsen et al [128] found that over the 20 years following diagnosis, prostate cancer death rates ranged from 6 per 100,000 person-years for men with Gleason scores between 2 and 4, to 121 per 100,000 person years for men with Gleason scores between 8 and 10. A second long-term study, that of Johansson et al [129], analyzed data from a Scandinavian population cohort diagnosed between 1977 and 1984, and also showed a strong correlation between Gleason score and the risk of prostate cancer death. However, the two studies differed in their assessment of the risk of late (beyond 15 years from diagnosis) disease-specific mortality. Johansson et al [129] reported a 3-fold increase in prostate cancer death rates after 15
years; this was not the case in the Albertsen study, which found the risk of late prostate cancer death to be similar to the risk observed within the first fifteen years. Reasons for the discrepancy are not clear, but could be due to differences in the way cause of death was ascertained [128].

A recent review by Martin et al [130] summarizes progression in five cohorts of patients with clinically localized prostate cancer diagnosed in the PSA era and who were actively monitored for disease recurrence and progression [131-135]. In all but one study, the men were followed up for less than 5 years. The studies were limited to participants with stage T1-T2 disease. The monitoring protocols varied, although all included serial PSA levels and DRE assessment. Three also included repeated transrectal ultrasound-guided biopsies [132, 135], and others a variety of clinical measures. As a consequence of these different protocols and definitions, reported progression rates differed with little clear relationship to median duration of follow up, mean age or median initial PSA level.

Several factors were found to be associated with cancer progression, although findings were not always consistent across all studies. In studies of men diagnosed before the PSA era, for example, grade and stage of cancer are consistently predictive of progression. However, in these five studies of men with localised prostate cancer, only three showed associations between clinical progression and baseline Gleason score [131], cancer stage [135], and prostate volume [133]. Further, two of these studies and another, larger study, found no associations between progression and age [132, 133], Gleason score [132, 135], or tumour stage [132]. These null findings are not simply explained by the studies being underpowered to detect an effect, since the largest study [132] found no associations, but are more likely to reflect the variable protocols and definitions of progression and, possibly, the relatively short period of follow up. Associations of baseline serum PSA with clinical progression were observed in some [133, 135], but not all studies.

The proportion of cancer cases progressing were 25% over a median of 44 months [135], 17% in 29 months [132], and 29% in 23 months [133]. Two of the studies followed up men using a combination of both clinical (DRE / radiological / clinical evidence of metastases) and biochemical (PSA) criteria, but did not include routine histological surveillance [131, 134]. The proportion of men progressing during follow-up varied: in the series of men with T1a disease, 8% of cancers progressed in 88 months [131]; in the series of men with T1c disease 33% were defined as having progressed in 23 months [134].

The short-term probability of metastasis was low. Actuarial probabilities of freedom from disease progression at 4-5 years of follow-up were 67%-72% [131, 132, 135]. In four studies, there was no evidence of metastatic progression after a median of between 23-44.1 months of follow-up [132-135]; in men with T1a cancer followed up for a median of 7.3 years, 1 man (2%) progressed to bony metastases after 12 years [131].

The lengthy interval from diagnosis to metastasis in the Martin review [130] confirms the findings of Pound et al [136], who studied the natural history of progression in a large surgical series. Although all cases in the Pound series underwent radical prostatectomy, they did not receive adjuvant or neoadjuvant hormone therapy, and they were not treated at the time of biochemical recurrence. The time from biochemical recurrence to clinical metastasis was eight years on average in this cohort, and once men developed metastases, the average time to prostate cancer death was 5 years. Furthermore, both the time to biochemical recurrence and the PSA doubling time were predictive of the time to metastasis.

Research in the area of prostate cancer progression is controversial and developing rapidly, focusing on molecular aspects that include genetic changes. Many molecular studies are being conducted in treated cohorts of patients, which may limit their utility for predicting progression in the absence of treatment. Greater understanding of the molecular and genetic basis of prostate cancer is expected to improve the ability to predict progression, but while there are promising developments [137, 138], no novel markers for predicting progression have yet made it to the clinic.

V. SPECIAL SECTION: GENETIC MARKERS OF RISK AND PROGRESSION FOR PROSTATE CANCER

1. Introduction
Prostate cancer is common among aging men. A better understanding of its risk factors is expected to be derived from the molecular epidemiological studies of this complex disease. Prostate cancer is multifactorial and heterogeneous in its potential causes,
which may include environmental, endocrine and genetic factors. The study of the interactions between genes and multifactorial diseases has expanded in recent years. DNA polymorphisms in genes involved in hormone synthesis, signaling and metabolism may be involved in the pathogenesis of prostate cancer. Furthermore, identification of the genetic determinants of age-related changes in the prostate could provide insights for risk assessment, prevention and new therapeutic targets.

2. PROSTATE CANCER SUSCEPTIBILITY GENES

Prostate cancer is the most frequent malignant tumor among men over 50 years old. Its incidence varies according to national origin and ethnic group. Known risk factors are race and positive family history of the disease. Familial aggregation (at least 2 cases in the family) is observed in about 20% of cases and a hereditary form of prostate cancer in 5%. This proportion increases with younger age of the proband at diagnosis. Eight putative loci (Table 1) are already identified for hereditary prostate cancer but undoubtedly, others will be found in forthcoming studies. The genetic heterogeneity observed in hereditary prostate cancer reflects the variety of origins of the studied families. In some families, aggregation of prostate cancer and other cancers suggests the involvement of common susceptibility genes. In other familial forms and in sporadic cases, the genetic component appears to be polygenic; prostate cancer wouldn’t result from segregation of a major gene mutation transmitted according to a monogenic inheritance, but rather to sharing of alleles at many loci, each contributing to a small increase in cancer risk. Indeed, several genetic polymorphisms have been associated with an increased risk of developing prostate cancer and could explain the variations of prostate cancer incidence observed between populations.

Abundant data suggest the existence of a common “latent” and nonprogressive form of prostate cancer that is not associated, unlike “clinical” cancer, to mesologic or ethno-geographic variations. These latent cancers suggest that there are different levels of genetic determinism: on one hand, those associated with the initiation phase of the cancer (carcinogenesis); and on the other hand, those associated with the promotion phase (progression) of prostate cancer [139].

In 1996, Smith and colleagues suggested that the 1q24-25 region from chromosome 1 should contain a susceptibility gene for prostate cancer (HPC1) [140]. In Europe in 1998, investigators identified a locus of predisposition, named PCaP, in the telomeric region of the long arm of the chromosome 1 (1q42.2-43) [141]. Since then, other loci for hereditary prostate cancer susceptibility genes have been identified: HPCX (Xq27-28), HPC20 (20q13), HPC2 (17p11),

<table>
<thead>
<tr>
<th>Affection</th>
<th>Gene (locus)</th>
<th>Mutations - Variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hereditary prostate cancer</td>
<td>HPC1/ RNASEL (1q24-25)</td>
<td>Mutations: E265X, Met 1Ile, 471delAAAG;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variants: Arg462Gln, Glu541Asp</td>
</tr>
<tr>
<td></td>
<td>PCaP (1q42-43)</td>
<td>Not identified</td>
</tr>
<tr>
<td></td>
<td>HPCX (Xq27-28)</td>
<td>Not identified</td>
</tr>
<tr>
<td></td>
<td>HPC20 (20q13)</td>
<td>Not identified</td>
</tr>
<tr>
<td></td>
<td>HPC2/ELAC2 (17p11)</td>
<td>Mutations: Arg781His, 1641insG, Glu216stop;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variants: Glu622Val, Ser217Leu, Ala541Thr</td>
</tr>
<tr>
<td></td>
<td>PG1/MSR1 (8p22-23)</td>
<td>Mutations: Arg293X, Asp174Tyr, Pro36Ala,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ser41Tyr, Val113Ala, Gly369Ser, His441Arg;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variants: Pro275Ala, PRO3, INDEL1, IVS5-59,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>INDEL7</td>
</tr>
<tr>
<td>Hereditary prostate and</td>
<td>BRCA2 (13q12-13)</td>
<td>6051delA (Exon11)</td>
</tr>
<tr>
<td>breast cancer</td>
<td></td>
<td>999del5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6174del5</td>
</tr>
<tr>
<td>Hereditary prostate and brain</td>
<td>CABP (1p36)</td>
<td>Not identified</td>
</tr>
<tr>
<td>cancer</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
PG1 (8p22-23), a locus at 7q11-21 and a locus at 17q22. Other loci, associated with a particularly aggressive familial form of the disease, were suggested in chromosome regions 5q31-33, 7q32 and 19q12. Several other genome wide scans were performed, leading to the identification of numerous potential candidate chromosomal regions [142]. These results confirm the heterogeneity of the genetic susceptibility to prostate cancer with at least two modes of inheritance, autosomal dominant and linked to the X chromosome (Figure 6); as well as a strong difference in the impact of the studied genes and the ethno-geographic origin of families. Moreover, in families with cancers of the breast and prostate before the age of 55 years, alterations of the BRCA2 gene are clearly identified. Germline alterations of BRCA2 are found in 2% of the men affected by prostate cancer before the age of 55 years. The proportion of hereditary prostate cancer related to BRCA2 remains small, however, explaining less than 5% of cases.

Some other candidate genes have been studied: RNASEL for HPC1, ELAC2 for HPC2, and MSRI for PG1. The small number of mutations observed in the analyzed families didn’t allow clear definition of the implications of the mutations of those genes for susceptibility to hereditary prostate cancer. Several variants (Single Nucleotide Polymorphisms) for the RNASEL, ELAC2, and MSRI genes were associated with prostate cancer risk in familial or case-control studies (Tables 1 & 2). Currently, highly penetrating genes involved in oligogenic predisposition to prostate cancer are likely to account for only a relatively small proportion of cases, and genetic polymorphisms which have been associated with an increased individual or familial prostate cancer risk would then be shared in a polygenic mode of heredity. They could explain on one hand, the important

![Clinical criteria for hereditary prostate cancers.](image-url)
<table>
<thead>
<tr>
<th>Gene (symbol)-(locus)</th>
<th>Polymorphism associated to an increase risk of developing prostate cancer</th>
</tr>
</thead>
</table>
| Androgen receptor (AR)-(Xq12) | CAG repeat (<18-23 repeats)  
GGC repeat (<16 repeats) |
| Steroid 5-alpha reductase 2 (SRD5A2)-(2p23) | TA repeat (18 repeats)  
A49T  
V89L (Protective effect of the Leu/Leu genotype) |
| (CYP3A4)-(7q22) | CYP3A4-V |
| Steroid 17-alpha-hydroxylase (CYP17A1)-(10q24) | -34bp C/T promoter (CYP17-A2 allele) |
| Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and  
steroid delta-isomerase 2 (HSD3B2)-(1p13) | B2-C759G |
| Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and  
steroid delta-isomerase 1 (HSD3B1)-(1p13) | B1-N367T |
| Aromatase (CYP19A1)-(15q21) | STRP- intron 4 (171bp allele)  
Exon 7 C/T polymorphism (CT and TT genotypes) |
| Estrogen receptor 1 (ESR1)-(6q25.1) | GGGA repeat– intron 1 (alleles with other than 5 repeats)  
C10T (C/C genotype)  
PvuII polymorphism (TT genotype) |
| Vitamin D receptor (VDR)-(12q12-14) | FokI (Allele f)  
Repetition polyA (>18 repetitions)  
Pomorphisms TaqI (Allele T) |
| Catechol-O-methyltransferase (COMT)-(22q11.21) | G/A genotype  
Ile105Val polymorphism (Val/Val genotype)  
MspI (m2 variant)  
3801T>C  
2455A>G |
| (CYP1B1)-(2p22) | Leu32Val (Val/Val genotype)  
Aa119Ser (T/T genotype)  
C-G-C-C-G haplotype of -1001C/T, -263G/A, -13C/T,  
+142C/G, +355G/T |
| (CYP2D6)-(22q13) | Allele B low activity |
| (CYP2E1)-(10q24.3-qter) | Dnal polymorphism (DD genotype) |
| Glutathione-S-transferase P1 (GSTP1)-(11q13) | Ile105Val (Val allele) |
| Glutathione-S-transferase M1 (GSTM1)-(1p13) | Null genotype |
| Glutathione-S-transferase M3 (GSTM3)-(1p13.3) | Null allele |
| Glutathione-S-transferase theta 1 (GSTT1)-(22q11.23) | Null allele |
| Glutathione-S-transferase A1 (GSTA1)-(6p12.1) | A*B* or B*B* genotypes (among smokers) |
| N-acetyltransferase 1 (NAT1)-(8p22) | NAT1*10 higher activity |
| N-acetyltransferase 2 (NAT2)-(8p22) | NAT2 slow acetylator |
| Interleukin 8 (IL8)-(4q12-13) | IL8-251 (Protective effect of TT genotype) |
| Interleukin 10 (IL10)-(1q31-32) | IL10-1082 (AA genotype) |
| Insulin-like growth factor-I (IGF1)- | CA repeat in the promoter region (19 allele) |
| Insulin-like growth factor-binding protein-3 (IGFBP-3)-(7p13-12) | 202bp A/C – promoter (C Allele is associated with a more aggressive form of the disease) |
| Vascular endothelial growth factor (VEGF)-(6p12) | VEGF-1154 (Protective effect of AA genotype)  
-460bp C/T – promoter (TT genotype) |
| Transforming growth factor-beta 1 (TGF-beta1)-(19q13.2) | L10P (TC and TT genotypes)  
T10P (TT allele) |
| Estrogen receptor 9 (ESR2)-(12q13) | Null allele |
| Cyclin-dependent kinase inhibitor 1A (CDKN1A)-(6p21.2) | 20bp 3'T/C (T allele) |
incidence variations observed between populations and on the other hand, some familial aggregations. These polymorphisms mainly affect genes involved in steroid hormonal regulation (Androgen, estrogen or vitamin D receptors; 17alpha-hydroxylase; 5-alpha reductase; aromatase; CYP1B1; 17 beta-hydroxysteroid dehydrogenase), as well as genes relating to the DNA repair system.

Some studies have also identified associations between increased prostate cancer risk and genes involved in different mechanisms of carcinogenesis such as inflammation, angiogenesis, or cell cycle regulation (Table 2). Some of these genetic variants could be associated with aggressive forms of prostate cancer and an increased relapse risk after radical prostatectomy.

### REFERENCES


110. Carter HB, Morrell CH, Pearson JD, Brant LJ, Plato CC, Metter


CHAPTER 3

Committee 3B

Epidemiology of Prostate Cancer in Asia

Chairman

M. Murai (Japan)

Members

Ch. Cheng (Singapore),
R. Khaulil (Lebanon),
E. Lee (Korea),
R.M. Sahabuddin (Malaysia),
K. Sasidharan (India),
T. Tsukamoto (Japan),
L. Zhou (China)
CONTENTS

I. INTRODUCTION

II. RECENT TRENDS IN PROSTATE CANCER IN ASIA

III. FACTORS INFLUENCING THE EPIDEMIOLOGY OF PROSTATE CANCER IN ASIANS

1. AGEING POPULATION

2. DIAGNOSTIC INTENSITY AND SCREENING BIAS

3. GENETIC POLYMORPHISM

4. HORMONAL FACTORS

5. ENVIRONMENTAL AND DIETARY FACTORS

IV. CONCLUSION

REFERENCES
Epidemiology of Prostate Cancer in Asia

M. Murai


I. INTRODUCTION

Prostate cancer remains one of the most common cancer afflicting men today. It is the third most common cancer in the world and the most frequently diagnosed male cancer in the United States, with a world age-standardised rate of 104 per 100,000 [1]. Prostate cancer rates are the highest in Western countries and lowest in Asian countries. With the aging population and increasing use of PSA (prostate specific antigen), the incidence of prostate cancer in the high-risk countries has risen sharply in the past decade [2-4]. In Asia, however, the incidence of prostate cancer is significantly lower and it often plays second fiddle to lung, stomach and colon cancer. Recent data from Asia, however, have shown a general trend towards increasing incidence of prostate cancer, with some low-risk regions, such as Japan and Singapore, reporting a more rapid increase than some high-risk countries [5]. Understanding the genetic and environmental basis for this difference and research into the changing demographics in Asian prostate cancer has emerged as an important field of study. We present the changing epidemiological trends in Asian prostate cancer and discuss the possible reasons behind this trend.

II. RECENT TRENDS IN PROSTATE CANCER IN ASIA

1. Overview

National and regional cancer registries are pivotal in providing an in-depth look at the epidemiological map of prostate cancer across Asia. The number of cases of prostate cancer reported in the listed Asian countries (Table 1) ranged from 24-558 during 1978-1982 to 146-1,654 during 1993-1997. Age-standardised incidence rates per 100,000 man-years for prostate cancer from Asian countries over a 20-year period from 1978 to 1997 confirmed that the incidence of prostate cancer has risen between 5% to 118% in most of the indexed Asian countries [6-9].

Centres in Japan rose as much as 102% (Miyagi 6.3 to 12.7 per 100,000 person-years) whilst the incidence in Singaporean Chinese surged 118% from 6.6 to 14.4 per 100,000 person-years. The lowest rate was in Shanghai, China and the highest incidence rates were found in Rizal Province in Philippines, although their rate is still much lower than the incidence in United States and many European countries. Whilst the absolute value of the increase is not comparable to North American and European populations, the percentage change in incidence rates (incidence ratio) in many Asian centers is quite similar to the high-risk countries. The rising incidence trend is well reflected in Figure 1.

The mortality data for prostate cancer showed a similarly rising trend [10] (Table 2). The number of reported mortality from prostate cancer ranged from 99-1,894 during 1978-1982 to 55-26,651 during 1993-1997. The increases in age-adjusted mortality rates per 100,000 person-years, adjusted to the world standard, ranged from 50% in Thailand to 260% in Korea. Unfortunately, complete mortality data on prostate cancer was not available from India, Indonesia and China, three of the largest Asian countries. The trend in prostate cancer mortality is illustrated in Figure 2.

2. Recent Trends in Japan

The incidence rate of prostate cancer in Japan showed a gradual increase in the last 2 decades. Nakata et al. investigated 1,411 prostate cancer
Table 1. Age-adjusted incidence rates of prostate cancer in 7 Asian countries.

<table>
<thead>
<tr>
<th>Countries</th>
<th>1978-1982</th>
<th>1993-1997</th>
<th>% change b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of cases</td>
<td>Incidence a</td>
<td>Rank</td>
</tr>
<tr>
<td>Philippines, Rizal</td>
<td>230</td>
<td>11.1</td>
<td>1</td>
</tr>
<tr>
<td>Singapore, Chinese</td>
<td>179</td>
<td>6.6</td>
<td>4</td>
</tr>
<tr>
<td>Japan, Miyagi</td>
<td>277</td>
<td>6.7</td>
<td>3</td>
</tr>
<tr>
<td>China, Hong Kong</td>
<td>558</td>
<td>6.2</td>
<td>5</td>
</tr>
<tr>
<td>India, Mumbai</td>
<td>414</td>
<td>8.2</td>
<td>2</td>
</tr>
<tr>
<td>Thailand, Chiang Mai</td>
<td>60</td>
<td>4.0#</td>
<td>6</td>
</tr>
<tr>
<td>China, Shanghai</td>
<td>265</td>
<td>1.8</td>
<td>7</td>
</tr>
</tbody>
</table>

- a: Per 100,000 person years, age-adjusted using the world standard. 
- #: data from 1983-1987

Figure 1. Plot of incidence rate of prostate cancer over time in 9 selected Asian regions.

Patients newly diagnosed between 1985 and 1992, and 656 patients who died from prostate cancer between 1981 and 1992 in Gunma Prefecture, Japan [11]. The yearly incidence rate showed an increase but the mortality rate showed no marked fluctuations. Both incidence and mortality rates showed a marked increase with age. The age-specific incidence showed a double logarithmic relationship to age. The incidence and mortality rates in districts with a history of manganese mining were higher compared to those in districts without mining. In contrast, the incidence and mortality rates in districts with a history of zinc mining were comparatively lower. The authors suggested that manganese and zinc might be factors influencing prostatic carcinogenesis.

Mortality due to prostate cancer has increased in recent birth cohorts especially in older age groups [12]. Sasagawa et al reported that age-adjusted prostate cancer mortality rate in Japan rose from 4.4 to 8.6 per 100,000 between 1980 and 1998 [13]. There was some evidence of geographical clustering at the prefecture level of high and low rates. Imaizumi has evaluated data regarding 77,492 prostate cancer deaths reported during the period 1955 to 1996 obtained from death certificate records in Japan [14]. Age-adjusted prostate cancer mortality rates increased 6.4-fold during that period. Imaizumi has suggested that the changing patterns in the mortality rate was explained by a gradual rise in the aged population as well as rapidly changing lifestyles among the Japanese.
Nakata et al. calculated the age-adjusted death rates, age-specific death rates and standardized mortality ratio (SMR) for prostate cancer in Japan and analyzed their features [15]. The respective number of deaths and the age-adjusted death rate was 1107 and 2.29 in 1973 and 6251 and 5.15 in 1997. The age-specific death rates showed an exponential increase with age and the rate of increase was higher in older age groups. They concluded that the prostate cancer death rate is increasing rapidly in Japan. However, the age-adjusted death rate has remained stable from 1996 to 1997. This trend mirrors that in North America, where there was a rise in the incidence following widespread use of PSA and a gradual decrease in mortality of prostate cancer in the 1990s following the introduction of retropubic radical prostatectomy.

In 2001, the Japanese Urological Association initiated computer-based registration of prostate cancer patients in Japan to better study the changing epidemiological trends in aetiological risk factors, diagnostic methods, tumour pathology and clinical outcomes[16]. A total of 173 institutions responded and 4529 patients diagnosed in 2000 were registered.

### Recent Trends in Korea

The incidence rate of prostate cancer per 100,000 persons adjusted for the world population in Korea was reported to be only 2.98 in 1989. The percentage of prostate cancer amongst all cancers occurring annually in Korean men subsequently increased from 1.2% in 1989 to 3% in 2002 (Figure 3) [18]. According to the reports from Korean Urological Association, the annual number of patients newly diagnosed with prostate cancer surged from 588 in 1991 to 2445 in 2002 [19]. In 1996, prostate cancer first became one of the top ten cancers with respect to cancer incidences in Korean males, rising to the sixth commonest cancer in 2002 with an age stan-
dardized incidence rate of 7.71 per 100,000 [20]. From 1995 to 2002, prostate cancer showed the highest rate of increase (standard incidence ratio of 2.11) among all cancers in Korean males [18]. Unlike the situation in US where the mortality from prostate cancer is said to be on the decline currently, the mortality rate from prostate cancer among Koreans is still increasing gradually from 0.5 in 1991 to 2.7 in 2001 according to the Korea National Statistics Office [21,22]. Despite this recent increase, the age-adjusted mortality rates from prostate cancer in Korea is still considerably lower when compared to many western countries.

4. RECENT TRENDS IN THE MIDDLE EAST

The incidence of prostate cancer in the countries of the Middle East is much lower than that of western countries, although the reported incidence from several countries increased in the 1990's after the introduction of PSA screening.

Regional data from Saudi Arabia showed that prostate cancer ranked sixth in men in the country in 1999-2000 [23]. The ASR is 3.5 / 100,000 of population. The data from Saudi Arabia National Cancer Registry revealed a clear difference between urban parts of the country (ASR 8 / 100,000 in the capital city of Riyadh) and rural areas (ASR 0.6 / 100,000 in Baha).

In Lebanon, the absence of a national cancer registry until recently makes it difficult to have an accurate assessment of the prevalence of prostate cancer. According to Shamseddine et al [24], prostate cancer ranked third among cancers diagnosed in Lebanese men with ASR 21.5 / 100,000. This high frequency in contrast to neighboring countries is attributed to surveillance and public awareness campaigns promoting screening for prostate cancer in recent years, and the fact that most of the Lebanese population is concentrated in urban areas that are accessible for screening and health care.

According to the National Cancer Registry in Kuwait for 2003, prostate cancer ranked fourth among cancers diagnosed in men. ASIR was 12.8 / 100,000. The Kuwaiti population is composed of native Kuwaitis and a large proportion of immigrants coming to work in the country. Most of the immigrant population is composed of young people and this may explain the low prevalence of prostate cancer in the immigrant population (ASIR 5.3 / 100,000).

The apparent dramatic increases in incidence rates need to be analysed in the context of several possible confounding factors. The denominator for the incidence and mortality rates is the study population and this is dependant on the accuracy and comprehensiveness of the population census. In certain areas, such as parts of India, China, Indonesia or Philippines, obtaining an accurate population census is a challenging task. In other areas, such as Hong Kong and Singapore, migration patterns may dilute or
increase the incidence rates of the native population. In some countries, cancer notification is voluntary or by administrative order and year-to-year changes can be changed due to differences in reporting patterns. In addition, there can be startling differences in incidence rates between different provinces and between rural and urban populations within the same country. A prime example would be China, where the difference can be up to 7.8 times between Hong Kong and Qidong County (Table 3).

Table 3. Age-adjusted incidence rates of prostate cancer in China.

<table>
<thead>
<tr>
<th>Region</th>
<th>Number of cases</th>
<th>Incidence(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hong Kong</td>
<td>1654</td>
<td>8.6</td>
</tr>
<tr>
<td>Shanghai</td>
<td>940</td>
<td>3.0</td>
</tr>
<tr>
<td>Beijing</td>
<td>266</td>
<td>2.9</td>
</tr>
<tr>
<td>Tianjin</td>
<td>224</td>
<td>2.0</td>
</tr>
<tr>
<td>Wuhan</td>
<td>178</td>
<td>2.0</td>
</tr>
<tr>
<td>Jiashan</td>
<td>16</td>
<td>1.9</td>
</tr>
<tr>
<td>Qidong County</td>
<td>33</td>
<td>1.1</td>
</tr>
</tbody>
</table>

\(^a\) : Per 100,000 person years, age-adjusted using the world standard.

III. FACTORS INFLUENCING THE EPIDEMIOLOGY OF PROSTATE CANCER IN ASIANS

1. AGEING POPULATION

Age is a well-known risk factor for prostate cancer. With increasing sophistication of medical care and better nutrition, more Asians have longer life expectancy. More men therefore live long enough for prostate cancer to be diagnosed.

2. DIAGNOSTIC INTENSITY AND SCREENING BIAS

Widespread availability of serum PSA, screening programmes, prostate cancer awareness programmes, news highlight in the media and testimonies from celebrity patients have increased public awareness and attention to prostate cancer and led to increased screening and detection of clinically asymptomatic cancers. In Asian countries, the availability of transrectal ultrasound and extended systematic biopsies in the late 1990s increased the number of cancers detected, from a positive predictive value (PPV) of 8.9% with sextant biopsies in the early 1990s to between 24.6% and 29.3% with 10-core biopsies in 1999-2002 [25-27].

3. GENETIC POLYMORPHISM

The ethnic differences in the CAG repeat sequence of Exon 1 in the androgen receptor gene at Xq11.2-q12 chromosome have been reported to be an important risk factor for prostate cancer. This CAG repeat sequence encodes for a polyglutamine chain in the region of the AR associated with DNA transcription. Men with exceptionally long CAG repeat lengths experience clinical androgen insensitivity because of reduced transcriptional activity of the AR. Irvine proposed that men with shorter repeat lengths are therefore at a higher risk of prostate cancer [28]. Sartor et al showed that CAG repeat length varies with different ethnic groups; African-americans have the highest incidence of prostate cancer but a shorter median CAG repeat length of 19 whilst non-hispanic white men have a longer median CAG repeat lengths of 21 [29]. In a study of 190 Chinese men with prostate cancer, the median CAG repeat length was 23. Chinese men with CAG repeat lengths shorter than 23 had a 65% increased risk of prostate cancer [30]. This was further confirmed by an analysis of 116 Chinese men in Northern China which showed that the frequency of CAG repeats greater than or equal to 22 was higher in the Chinese population (80.4%) compared to American white men (52.1%) and African-american men (25%) [31]. However, two other studies showed no difference in CAG repeat lengths between Chinese and Caucasian men [32,33].

Vitamin D modulates prostate cancer growth via the vitamin D receptor (VDR). Several studies on VDR gene polymorphism have shed conflicting results, with some researchers in Taiwan and Japan showing higher risks with vitamin D BsmI genotype [34,35] and others showing no difference with BsmI, Apal, TaqI, or FokI genotypes [36].

The CYP17 gene, which encodes for the enzyme P450c17\(_{\alpha}\) that catalyses the conversion of progesterone and pregnenolone into precursors of potent androgens, was postulated to affect prostate cancer risk. The A2/A2 allele, which contains a T-to-C polymorphism in the 5’ promoter region that creates a Sp1-type (CCACC box) promoter site, was shown to occur in higher frequencies than the A1/A2 and
A1/A1 allele in Caucasians with prostate cancer (70%) than in controls (57%) [37]. In Japanese populations, Yamada et al [38] found that the A2 allele conferred a higher risk of prostate cancer but Habuchi et al [39] found that the A1 allele posed a higher risk. Madigan et al [40], however, found no difference in prostate cancer risks in Shanghaiese patients in China with the CYP17 genotype. This was supported by a similar study in Taiwan by Lin et al [41].

The SRD5A2 gene encodes for the steroid 5α-reductase II which is involved in the conversion of testosterone to dihydrotestosterone (DHT), the active metabolite associated with androgen activity that leads to prostate cell division and prostate cancer. Four types of polymorphism have been documented: A49T (a substitution of threonine for alanine at codon 49), V89L (a substitution of leucine for valine at codon 89), R227Q (a substitution of glutamine for arginine at codon 227), and a (TA)n dinucleotide repeat. The V89L leucine (L) allele, which involves substitution of Valine with Leucine at codon 89, was found to be associated with lower DHT levels and lower prostate cancer risk than the Valine (V) allele. In a nested, matched, case-control study involving 320 Caucasian men without prostate cancer who underwent prostate biopsy, Nam et al [42] found that the adjusted odds ratio for having prostate cancer for patients with at least one V allele was 2.53 compared with patients with the L/L genotype. Hsing et al [43] showed in a study involving 191 Chinese men that the frequency of the lower risk L/L allele was higher at 35% compared to 10% in the high risk Caucasian population in Nam et al’s study.

4. Hormonal factors

Ross et al found that African-Americans had a 15% higher total testosterone level, which may explain a twofold increase in prostate cancer risk when compared to Caucasians [44]. They also noted that these white and black men had significantly higher values of AAG and androsterone glucuronide than Japanese subjects. As these androgens are downstream products of 5α-reductase activity, there was a suggestion that reduced 5α-reductase activity may have resulted in the lower prostate cancer incidence rates among Japanese [45]. Wu et al found that the DHT:testosterone ratio was highest in African-Americans, intermediate in whites, and lowest in Asian-Americans, corresponding to the respective incidence rates in these groups and providing indirect evidence for ethnic differences in 5α-reductase enzyme activity [46].

Insulin-like Growth Factors (IGF-I, IGF-II) are polypeptides functioning both as growth factors and endocrine hormones. IGF-I has been found to have strong mitogenic and anti-apoptotic effects on normal and transformed prostate cells in vitro and in vivo, suggesting that it may play a role in the development of prostate cancer in human [47-50]. It is produced in the liver, together with at least six IGF Binding Proteins (IGFBP). In the circulation, more than 90% of IGF-I are bound to IGFBP-3.

Chokkalingam et al reported a population-based case-control study in 128 men with prostate cancer and 306 population controls in Shanghai, China and demonstrated that men in the highest quartile of IGF-I levels had a 2.6-fold higher risk of developing prostate cancer and men in the highest quartile of IGFBP-3 had a 46% decrease risk relative to the lowest quartile [51]. Men in the highest quartile for IGF-I:IGFBP-3 molar ratio had a 2.5-fold higher risk of developing prostate cancer. Unfortunately, there are currently no studies comparing IGF-I and IGFBP-3 levels between Caucasian and Asian populations.

5. Environmental and dietary factors

Dunn et al first reported that the risk of developing prostate cancer in successive generations of early Japanese migrants to the United States gradually approaches that of white men [52]. Cook et al reviewed data from the Surveillance, Epidemiology and End Results Program in the United States and observed that Chinese and Japanese men aged 45 to 69 years born in China and Japan respectively had half the annual incidence rates of their counterparts who were born in the United States [53]. These findings suggest that lifestyle or dietary factors may have contributed to the difference in androgen production and incidence of prostate cancer and that Asian-American men may have retained some of these characteristics that continue to make their prostate cancer risk less than white residents in United States.

a) Obesity

The association between obesity and prostate cancer stems from its influence on increasing circulating oestrogen level and decreasing androgen levels as androgens are aromatized to form oestrogens in adipose tissues. This may increase the prostate cell sensitivity to androgens and increase the risk of prostate cancer, following observations that androgen receptors in canine prostates were upregulated by oestra-diol [54].

Hsing et al interviewed 238 Chinese men with
prostate cancer and 471 controls in Shanghai during 1993-1995 and measured their height, weight, BMI, waist, hip, right upper arm circumferences and waist-to-hip ratio (WHR). They found that men with the highest quartile of WHR, an indicator of abdominal adiposity, were conferred a 2.71 times risk compared to men in the lowest quartile. BMI was not associated with excess risk in this study [55].

b) Dietary factors

Many dietary factors have been studied with regards to prostate cancer risks but their role may be summarized into two contributing factors: (1) as an influence on circulating androgens or oestrogens, and (2) as a general protective effect against mitogens. Dietary factors that have been cited as a possible contributing factor to the low incidence in Asians included low dietary fat, isoflavonoids in soybeans and polyphenols in green tea.

1. Dietary fat

Early epidemiological studies suggested a possible causal association between dietary animal fat and prostate cancer [56-60]. However, the evidence was less obvious in later studies. Questionnaire-based studies showed a tendency for weak positive association between total fat consumption and prostate cancer risk but not serum-based studies. There was inconsistent correlation for saturated fat but unsaturated fat showed an increased risk in questionnaire-based studies. Linoleic acid showed no risk association whereas α-linolenic acid showed inconsistent correlation. Only oleic acid showed some positive association with prostate cancer risk [61].

Lee et al interviewed 133 cases and 265 controls in 12 cities in China and observed that the daily fat intake and percentage energy from fat was 3.6 times higher in cases than control [62]. Marine fatty acid consumption, particularly the long-chain eicosapentaenoic and docosahexaenoic acids, have been found to be associated with decreased risk of prostate cancer in several studies and were thought to be an important factor in communities with high fish consumption, such as the Japanese [63].

2. Isoflavonoids

Dietary phyto-oestrogens are broadly grouped into 2 types: lignants and isoflavonoids. Lignants occur in seeds, berries, whole grain bread, vegetables and tea, whereas isoflavonoids are found in soybean and related products.

Soybean products are commonly found in traditional Chinese and Japanese diet. Lee et al assessed 133 prostate cancer cases and 265 controls in China on consumption of soy foods and isoflavones using a food frequency questionnaire and observed a reduced risk of prostate cancer associated with consumption of soy foods and isoflavones (such as tofu and soybean) [64]. Sonada et al observed decreased prostate cancer risks in Japanese men who consumed bean curds (tofu) and natto (fermented beans) in a case-control study of 140 men with prostate cancer and 140 age-matched controls in 4 different geographical areas in Japan (Ibaraki, Fukuoka, Nara, and Hokkaido) [63]. The exact mechanism of the protective effect of isoflavonoid is not clear but Onozawa noted that genistein (a type of isoflavonoid in soybean) suppressed DNA synthesis and inhibit apoptosis in LNCaP cell lines [65]. PSA expression was also noted to be suppressed. Other investigators observed that isoflavonoids decreased circulating oestrone concentrations and plasma DHT levels in human and rat models respectively but further work is needed to clarify the mechanism of action [66,67].

3. Green tea

Green tea is a popular beverage consumed in China and Japan, and has been postulated to be a factor in reducing prostate cancer risks in these populations. Specifically, epigallocatechin-3-gallate (EGCG), the main polyphenol present in green tea, is a potent antioxidant and has been shown to induce apoptosis in human prostate carcinoma cells by concurrent effect on two important transcription factors, p53 and NF-kappaB, and shift the balance between pro- and anti-apoptotic proteins (Bax and Bcl-2) in favour of apoptosis [68,69]. Jian et al conducted a questionnaire-based case-control study in Hangzhou, a South-eastern Chinese city famous for production of Long Jin green tea, involving 130 cases of prostate cancer and 274 controls [70]. He observed that prostate cancer risk declined with increasing frequency (OR 0.27, 95% CI 0.15-0.48 for those drinking more than 3 cups per day), duration (OR 0.12, 95% CI 0.06-0.26 for those who drank tea over 40 years) and quantity of green tea consumption (OR 0.09, 95% CI 0.04-0.21 for those consuming more than 1.5 kg of tea leaves per year).

IV. CONCLUSION

Prostate cancer incidence and mortality rates showed a rising trend over the last two decades but still remained lower in Asia relative to Western countries.
The difference may be the result of genetic factors and certain protective lifestyle, dietary or environmental factors. However, with globalisation and gradual westernization of many Asian cultures, Asian countries may be losing their protective factors and acquiring high-risk ones. A better understanding of how these factors interact to cause prostate cancer through further studies with a multi-ethnic perspective will facilitate appropriate public health strategies to minimize the high risk factors and maintain the protective factors to keep prostate cancer at bay.

REFERENCES


Committee 1

New Developments in the Pathobiology of Prostate Disease

Chairman

J. A Schalken (The Netherlands)

Members

F. Algaba (Spain),
D. Bostwick (USA),
J. Isaacs (USA),
T. Shiraiishi (Japan),
R. Umbas (Indonesia),
B. Watson (Ireland)
In this chapter the committee decided to focus on several research areas that are considered to be novel or in which rapid progress was made. Particularly, the role of stem cells in cancer (re) gained interest since evidence for this working hypothesis has been achieved for several tumor types, most recently also for prostate cancer [1]. The field of determinants of prostate cancer aggressiveness has a long standing track record and is therefore also included in this chapter. Finally, we have focussed on the results from high throughput target discovery efforts that have led to a series of candidate genes that can be used as diagnostic/prognostic parameter or as ‘druggable’ target. The recent discovery of the activation of two oncogenes in prostate cancer (erg and ETV1) [2] was considered to be so relevant that we included it in the chapter even though it was not discussed at the meeting in Paris.

II. CANCER STEM CELLS

Solid tumours are heterogeneous, typically containing varied populations of cells that differ in the specific proteins -phenotypic markers-, they express. The cancer stem cell (CSC) hypothesis suggests that neoplastic clones are maintained exclusively by a rare fraction of cells with stem cell properties [3,4]. As early as in 1976 Fialkow [5-7] identified the cancer stem cell for chronic myelocytic leukaemia and further evidence was obtained independently by others [7]. For leukaemia’s the cancer stem cell hypothesis is now generally accepted [8]. For solid tumours dramatic progress was achieved during the last years. A candidate cancer stem cell population was identified in breast cancer [9-11] and also brain tumour initiating cells are isolated and characterized [12,13]. Singh and colleagues [13] describe how they have isolated a minority population of human brain-cancer cells based on the expression of a cell-surface marker called CD133. They report that, when injected into the brains of mice, this subpopulation of CD133+ cells could by itself drive tumour growth and dissemination. As few as one hundred of the CD133+ cells formed tumours that could be serially transmitted from mouse to mouse, whereas tens of thousands of cancer cells lacking CD133 failed to do so. When tumours that arose from the injected CD133+ cells were examined, the cellular heterogeneity and architecture closely resembled that of the patients’ tumours from which the cells had originally been taken.

In the normal brain, neuronal stem cells as well as early progenitor cells, but not their fully mature progeny, express the CD133 marker (Figure 1a). In the brain tumours examined, Singh et al. [13] found distinct subpopulations of cells that expressed either CD133 or various markers of mature brain cells. Thus, the cellular architecture of the brain tumours may be a caricature of that of the normal brain, with brain-cancer stem cells, probably derived from normal CD133+ stem or progenitor cells, giving rise to aberrantly differentiated progeny (Figure 1b).

Stem cells have two unique properties that make it likely that they are involved in cancer development. First, they are often the only long-lived cells in a tissue that have the ability to replicate. Multiple muta-
tions, occurring over many years, are necessary before a cell becomes cancerous. So the implication is that cancer-inducing mutations accumulate in the long-lived, normal stem cells. Second, through a process called self-renewal, stem cells generate new stem cells with similar proliferation and differentiation capacities to their parental cell. By contrast, with each round of replication, progenitor cells become progressively more differentiated and are eventually destined to stop proliferating. Predictably, self-renewal is an essential property of some cancer cells, and at least some genes that regulate normal stem-cell self-renewal also do so in cancer cells [14,15]. There appear to be common signalling pathways implicated in stem cell expansion and cancer stem cell growth such as wingless [16,17], and hedgehog [18]; for a review see Rizvi [19]. Interestingly Beachy and colleagues have recently provided evidence for hedgehog being involved in normal and malignant prostate development [20,21] This suggests that cancers arise either from normal stem cells or from progenitor cells in which self-renewal pathways have become activated (Figure 1B).

These observations provide a mechanism by which breast- and prostate cancer patients with cancer cells in their marrow can remain for prolonged time progression free [22,23] One explanation for this
tumour dormancy is that the microscopic clusters of cancer cells did not contain cancer stem cells and so, like the CD133+ brain-cancer cells, were unable to grow further. Taken together with the observation that circulating cancer cells in the blood are an indicator of prognosis in breast-cancer patients [24,25], this suggests that the use of markers to reveal cancer stem cells could help in making decisions about treatments.

The identification of cancer stem cells is a significant step in the fight against these dreaded diseases: because self-renewal is essential if tumours are to grow, agents that target such cells may be effective treatments. A possible complication is that the mechanisms known to regulate cancer-stem-cell self-renewal also regulate the process in normal stem cells. Unlike normal stem cells [26,27], however, the expansion of cancer stem cells is not tightly regulated, implying that there are significant differences between the normal and the cancerous self-renewal pathways. This gives hope that the isolation of cancer stem cells, coupled with our knowledge of the mutations causing cancer, will result in ways to eliminate cancer cells while sparing normal tissues.

Table 1. Immunophenotype of the cell types in non-malignant prostate acini; K14, -5, -18= keratin 14, -5, -18; AR=androgen receptor; NE=neuroendocrine markers e.g. serotonin, chromagraninA)

Thus, the identification and functional characterization of cancer stem cells, can contribute significantly to improved methods for prognosis/treatment decision and treatment of cancer.

1. Prostate epithelial stem cells

The existence of prostate epithelial stem cells and their putative role in prostate cancer development was proposed by Isaacs and Coffey [28-30]. The stem model described in this paper is very similar to the one described above. Prostate cancers arise from prostate secretory acini; these acini are characterized by two cell layers that can be discriminated morphologically, i.e. the undifferentiated basal cells, and the luminal cells primarily composed of terminally differentiated exocrine (PSA producing) cells. The neuroendocrine cells are found 'supra' basally with protrusions through the epithelium. The first evidence for a hierarchical relation between the basal cell and the luminal cells was provided by our group [31,32] using keratin antibodies as differentiation markers. We and others found further indications that the neuroendocrine and the exocrine cells have a common progenitor termed the transiently amplifying/TraN-SiT cell [33-36]. Clearly, most of these studies are descriptive and enable discrimination between the various cell types based on specific immunophenotypes. The location of the cells, as well as, the hormone manipulation studies [32] suggest a hierarchical relation between the basal cells and the luminal cells. The early and late progenitors are characterized by ‘intermediate’ immunophenotypes (Figure 2 - Table 1). The first evidence for a hierarchical relation using primary epithelial cell cultures were described by our group [37]. More recently Collins and colleagues succeeded in the isolation of a more
pure candidate prostate epithelial stem cell, using CD133 selection [38]. Thus a very specific immunophenotype of the stem cell, the early and late progenitor cell populations and the terminally differentiated exocrine and neuroendocrine cells emerges (see Table 1).

The hierarchical relation between the cell types is schematically illustrated in Figure 2. The exocrine lineage, resulting in the tall columnar PSA producing cells, is critically dependent on the hormone DHT. Upon castration more than 90% of epithelial cells die through apoptosis [39,40]. The remaining cells have renewal capacity, since the kinetics of regrowth after implanting testosterone containing slow release devices is independent of the time interval between castration and implanting the silastic testosterone containing devices [28;32]. The remaining population of cells after castration are thought to represent the stem- and early progenitor cells. In the developing prostate (embryogenesis and peri-pubertal) there is a relative enrichment in the progenitor cell populations [33]. The early- and late progenitors are thought to play a pivotal role in the development of benign and malignant prostate neoplasms [41-43].

The implicit evidence that the remaining cell population after castration has renewal ability is provided by the early experiments by Coffey and Isaacs [28]; they show that the kinetics of prostate regrowth is independent of the time interval between castration and testosterone re-administration. Long term culture experiments using CD133 selected cells proliferate and can be maintained for > 140 days. SCs are known to reside in a ‘niche’ and our understanding of the regulation of the expansion into the various epithelial lineages of differentiation is growing steadily through the pioneering work of Fuchs and colleagues [44-49]. Unfortunately, relatively little is known on the location/niche of the prostate epithelial stem cell. Collins and colleagues show that there appears to be niche in which the stem cells are more firmly attached to the basement membrane [50]. The branching morphology of the secretory ductal system is rather complex and 3D reconstruction is difficult for the human prostate secretory system. The combined data indicate that the branching points and the tips of the acini are the ‘candidate’ niche for the stem cell [33,41,51]. The stroma plays an essential role in the induction of branching morphogenesis and essential mediators appear to be be HGF, FGF10 [52-55]. In general, despite the unique characteristics of the various specialized epithelial, common signalling mechanisms appear to play a role such as wingless-, notch- hedgehog and BMP signalling (for a recent review see [19].

Figure 2. Prostate epithelial cell hierarchy. The stem cells divide, give rise to a new stem cell -self renewal- and more committed progenitor cells (early & late) for the functional exocrine and neuroendocrine cell lineages; the exocrine lineage is critically dependent on DHT, and in fact this population represents > 90 % of all epithelial cells in the adult prostate gland.
2. **Prostate Cancer Stem Cells**

Most studies so far shed light on the candidate prostate cancer stem cell through phenotyping with the earlier mentioned markers; whereas a number of similarities between the brain stem cell model and the prostate are apparent, there are also clear differences. The most striking feature is that the stem cell marker K14 and CD133 are so far not identified in primary- or metastatic prostate cancer [38;56-60], suggesting that the prostate cancer stem cell is most similar to the early- or late progenitors.

The ontogeny of prostate cancer cells from early or late progenitors, whereby genetic changes have accumulated in the stem cell or early progenitor is schematically outlined in Figure 3.

On basis of the cancer stem cell phenotype at least three different types of prostate cancers can be discriminated on basis of their progenitor phenotype is illustrated in Figure 3 [59-61]. This minority population of cells in these specimens are the candidate cancer stem cells(1). Indeed comparative analysis of primary cancers before and after endocrine therapy resulting in impaired exocrine differentiation show a strong relative increase in cells with the progenitor phenotype-[59;62;63].

These data suggest that also for prostate cancer a cancer stem cell population can be identified most similar to the early and late progenitors of the exocrine and neuroendocrine differentiation lineages.

The unique ability of self renewal of these cells make them the most challenging target for therapeutic intervention. With the growing number of targeted therapies for prostate cancer, functional validation of the relevant target in the stem cell model is critical.

---

**Figure 3. Ontogeny of prostate cancer stem cells.** In primary human prostate tumours three classes can be discriminated; those with a minority of cells (candidate stem cells) similar to the early- or late progenitors, and the pre-terminally differentiated phenotype.
Neoplasia are malignant due to their metastatic ability. Malignant cells are able to move from their origin and metastasise following a sophisticated modification of the inter-cellular adhesion molecules, stroma cells and endothelial cells. Understanding these changes may, consequently, help us predict the state of aggressiveness at the time of the study, and perhaps find ways of treatment in the future.

1. Mechanisms of intercellular junction

Epithelial cells inter-relate through the tight junctions, the adherens junctions, the desmosomes and the gap junctions. Among all these inter-cellular junctions, adherens junctions and desmosomes are the most significant ones, and their structure grant a fundamental role to cadherins which, through the most significant ones, and their structure grant a junctions. Among all these inter-cellular interactions, the adherens junctions, the desmosomes and the gap junctions. Among all these inter-cellular interactions, the adherens junctions, the desmosomes and the gap junctions.

2. Cadherin switching in prostate cancer

E-cadherin is coded at chromosome 16q21/22 and inter-relates with MUC-1 (EMA) (episialin). It is expressed in the prostate normal secreting cells.

N-cadherin is coded at 18q 11.2 and it is not expressed in the normal prostate [65]. The loss of E-cadherin seems to play a quite important role in the metastatic ability of prostate carcinoma [66]. This loss of expression of E-cadherin is accompanied by a progressive N-cadherin expression, which in turn evolves from a membrane pattern towards a dotted pattern, with intermediate stages of co-expression of both cadherins in a same cell [65]. These changes correlate with the Gleason score. The progressive appearance of N-cadherin in the prostate cancer cell membrane brings about a mesenchymal-like transformation of the malignant cells, as if such mimesis favoured the metastatic ability by means of adherence to the stromal cells. We may thus consider E-cadherin a tumor-suppressing gene, and its cellular recovery could have great significance as a treatment of cancer, a fact that looks quite possible.

In addition to the inter-relation with N-cadherin, other possible mechanisms of action on the neoplastic cells have been observed, and so the E-cadherin fragments have been reported to possibly be involved in the activity of metalloproteinases, consequently favouring the neoplasia progression.

3. Integrins

Integrins are the major metazoan receptors for extracellular matrix proteins and, in vertebrates, also play important roles in certain cell-cell adhesions. Integrins are heterodimeric cell surface receptors that mediate heterophilic cell-cell interactions and interactions between cells and the extra cellular matrix. As such, they are involved in morphogenetic processes during development, as well as in the maintenance of normal tissue architecture in fully developed organs. The interaction with the ECM via transmembrane connections to the cytoskeleton results in the activation of many intracellular signalling pathways. Since the recognition of the integrin receptor family around 15 years ago [67], they have become the best-understood cell adhesion receptors. 8 β sub-units can assort with 18 α subunits to form 24 distinct integrins. These can be considered in several subfamilies based on evolutionary relationships. Integrins and their ligands play key roles in development, immune responses, and (for a review see [68].

Despite the tremendous advances in this field including an array of integrin knock out mice (~27), relatively little is known on the role of integrins in prostate development and cancer.

One of the most remarkable changes in integrin expression in prostate cancer is the loss of α4β6 the receptor for LN5, marking the hemidesmosomal contact of epithelial cells with the basemembrane [69]. Considering that prostate cancer is characterized by the absence of true basal cells, the loss of α4β6 can be interpreted as a loss of this cell population, similarly to P-cadherin [70] and keratin 14. The progressive loss of α4β6 from non-malignant prostate epithelium, to PIN (71) to cancer supports this interpretation. Human prostate cancer is devoid of expression of α6, β2 and β3 integrin [72]. The integrin matrix receptors that are most consistently reported to be expressed in prostate cancer are the ‘RGD’ receptors α5β1 and α3β1 [73] associated with fibronectin dependent migration. Whereas, rather little is know on changes in integrin expression in prostate cancer the lack of attachment with the basement membrane (loss of α4β6) and increased propensity to migrate over FN based matrices seem
to emerge as a common pathway. Initial evidence for the activation of integrin based signalling through FAK is in agreement with such a model [74].

4. IMMUNOGLOBULIN SUPERFAMILY

The common characteristic of these proteins are the subdomains resembling immunoglobulin repeats. N-CAM, is found to be upregulated in PrCa and might be associated with neural invasion [75]. The C-CAM cell adhesion molecule was identified as an androgen regulated suppressor of prostate growth [76;77]. The down regulation of C-Cam has been confirmed in a number of studies most recently by Busch and colleagues [78], and is even pursued as therapeutic target [79-81].

Some adhesion molecules can as of yet not be classified into one the large families and will discussed separately.

5. CD44

As mentioned in the introduction, cell-adhesion molecules were once believed to function primarily in tethering cells to extracellular ligands. Cadherins and integrins have now well recognized functions in cell signalling, and also the CD44 transmembrane glycoprotein family adds new aspects to these roles by participating in signal-transduction processes — not only by establishing specific transmembrane complexes, but also by organizing signalling cascades through association with the actin cytoskeleton [82]. CD44 and its associated partner proteins monitor changes in the extracellular matrix that influence cell growth, survival and differentiation. The designation CD44 describes a group of type I transmembrane proteins which share N-terminal and C-terminal sequences. These molecules differ in the central extracellular domain by the use of sequences encoded by ten variant exons which may be completely absent or included in various combinations and by cell type specific addition of glycosaminoglycan and carbohydrate moieties [83]. CD44 was thought to contribute to the metastatic phenotype by acquiring certain properties through alternative splicing [84]. Certain splice variants (CD44v) can promote the metastatic behaviour of cancer cells. In human colon and breast cancer the presence of epitopes encoded by exon v6 on primary resected tumour material indicates poor prognosis. Metastasis-promoting splice variants differ from those that seem not to have a role in the induction of metastasis by the formation of homomultimeric complexes in the plasma membrane of cells. This may increase their affinity to ligands such as hyaluronate. The affinity can be further regulated over a range from low to very high by cell-specific modification. The fact that CD44v epitopes are found on normal epithelial cells such as skin, cervical epithelium and bladder enforces cautious evaluation of the significance of CD44v expression in human cancer. Nevertheless, certain epitopes can serve as tools in early diagnosis of certain cancers and will facilitate the development of specific targeted therapy.

It is now recognized that cancer cells can recruit characteristics of CD44 through variable mechanisms [85;86]. CD 44 is intensely studied in prostate cancer. One of the first indications that CD44 can have pleiotropic effects in cancer progression came from studies on human PrCa specimens. Initial studies using PrCa cell lines suggested a relation between increased CD44 expression and prostate cancer progression [87;88]. However, the first report analyzing human PrCa tissue showed a paradoxical down regulation of CD44 in cancer and expression of variant CD 44 isoforms (v3 & v6) in prostate basal cells [89]. Also in PrCa metastases CD44 expression was reduced or absent [90].

Gao and colleagues even suggested that CD44 could in prostate cancer act as a tumor suppressor gene [91;92]. The reduced expression of CD44 appeared to be associated with a poor prognosis [93;94]. The only relation between a CD44 isoform and cancer progression is the report of Aaltoma [95], who conclude that CD44v3 expression is associated with PrCa progression. Surprisingly, in a more recent study they do not come supoprt this conclusion [96]. Thus the literature to date is quit consistent that in PrCa and its metastasis CD44 expression is lost.

6. Ep-CAM/ALCAM-CD166

Ep-CAM is a 40 KD antigen recognized by antibody 19A1, and is over expressed in a number of carcinoma’s including prostate cancer [97]. The new generation antibody against EpCAM, ING1 is now being pursued for therapeutic purposes [98]. In the future the combination with taxol or navelbine might be considered since this upregulates EpCAM expression [99].

ALCAM (CD166) is involved with homophylic and heterophylic cell-adhesion through yet unknown mechanisms. It can be recruited to the cell membrane coordinately with E-cadherin by α-catenin [100].
ALCAM is upregulated in low grade PrCa [101], whereas expression is lost in high grade cancer. Like cadherins and integrins, ALCAM function is dependent on an intact actin filament network [02]. Although may questions remain to be resolved a common picture in which coordinated interactions in the subcortical cytoskeleton are triggered emerges [103].

7. SUMMARY AND PERSPECTIVES

In the last 15 years cell adhesion molecules have been studied extensively in prostate carcinogenesis. A summary of changes in expression of adhesion molecules is presented in table 2. Whereas the clinical implications so far seem to be rather limited, the most intriguing insight obtained is that cell adhesion molecules are integral part of ‘outside-in & inside-out’ signalling. The wnt signal seems to initiate a coordinate switch towards proliferation via the canonical wnt/Wg pathway, activating proliferation associated genes such as c-myc and p21 via ? catenin/tcf and epithelial to mesenchymal transition by regulating snail, a transcription factor controlling repression of E-cadherin and MMPs. Thus this pathway is in the limelight as target for therapy. Considering that both APC and ? catenin mutations are frequently found in human cancers, leading to constitutive activation of the ? catenin/tcf induced transcrip-

tion, suggest that for interfering with this pathway the target need to be chosen down stream of APC/B catenin/GSK3ß. Significant advances have already been made. The exact molecular interaction between wnt signalling GSK3ß and snail/slug is not yet know, but again intervention with the EMT switch provides a challenge for drug development.

All of these findings show, once again, the great inter-relation existing between the various cell-cycle regulating systems, but it is probable that an approach to this interweaving through regulation and de-regulation of the adhesion molecule expression turns to be one of the most useful pathways in future.

8. APOPTOSIS AS A MARKER OF AGGRESSIVENESS

Growth and maintaining homeostasis in the normal prostate gland is regulated by a delicate balance between cell proliferation via mitosis and cell death by apoptosis. The cellular events that occur during apoptosis in the prostatic epithelium have yet to be fully elucidated. However, it is clear that apoptosis in the glandular epithelial cells of the human prostate share many features of apoptosis seen in other cell types.

Table 2. Summary of changes in expression of adhesion molecules

<table>
<thead>
<tr>
<th></th>
<th>Up</th>
<th>Down</th>
<th>Mutated</th>
<th>SNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadherins</td>
<td>Cadherin 11-OB&lt;br&gt;Cadherin 2-N&lt;br&gt;Protocadherin</td>
<td>E-Cadherin/α&lt;br&gt;D-catenin/P-cadherin</td>
<td>β-catenin</td>
<td>-160CA/E-cadherin promoter</td>
</tr>
<tr>
<td>Integrons</td>
<td>α5β1 &lt;br&gt;α5β3</td>
<td>α6β4 &lt;br&gt;β3, β3, β4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG like</td>
<td>N-CAM</td>
<td>C-CAM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Misc</td>
<td>EpCAM</td>
<td>CD44</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
During the development of prostate cancer it is clear that resistance to apoptotic cell death plays a role in the accumulation of cells and their survival against different treatment strategies such as hormone ablation and chemotherapeutic approaches. However, in the early development of prostate cancer there is in fact an increase in the apoptotic rate of the rapidly proliferating prostate cancer cells. Early studies by Tu et al demonstrate that there was a significant increase in the proliferative index of localised and metastatic prostate tumours compared with normal tissue, which was associated with a corresponding higher apoptotic index [104]. They also demonstrated that these metastatic cancer cells have little or no Bcl-2 expression. However, it is clear that hormone resistant prostate cancer cells display a significant resistance to apoptosis via a range of alterations of the apoptotic signalling pathway. These studies indicate that on the treatment of prostate cancer there is a selection or stimulation of apoptotic resistant cancer cells (via mechanisms to be discussed later) which emerges and leads to a more aggressive phenotype.

The mechanisms associated with the development of and contribution to aggressiveness of prostate cancer represents important targets in it ultimate treatment. Thus the identification of the processes involved in this altered apoptotic process emerges as a significant therapeutic target for the effective elimination of prostate cancer. However, the identification of the altered apoptotic pathobiology and getting consensus of their importance represents the first step in this process.

Apoptosis at the level of the cellular pathology can be regulated by two general pathways: the over expression of anti-apoptotic proteins or loss of expression of pro-apoptotic proteins. This review will specifically focus on apoptotic related proteins that are conclusively altered in clinically relevant prostate cancer samples. Of significant interest is that the majority of regulated proteins identified by this review mediate their effects specifically around the disruption and stabilisation of the mitochondria. The mitochondria houses several factors that can trigger apoptosis. These include cytochrome c, SMAC/DIABLO, Omi, apoptosis inducing factors (AIF), and endonuclease G [105]. These factors are located in the outer and inner mitochondrial membrane. Two pathways have been demonstrated to mediate the release of these factors: The first been the formation of autonomous channels by Bax or Bak allowing the release of factors from the intermembrane space. The second is facilitated by the specific interaction of Bax or Bcl-2 with components of the permeability transition pore which results in the swelling of the mitochondrial matrix and rupture of the outer mitochondrial membrane. The release of the mitochondrial content triggers apoptosis via both caspase dependent and independent pathways which eventually lead to the classical characteristic of apoptosis which include nuclear and chromatin condensation, cell shrinkage and blebbing eventually leading to their ingestion by surrounding phagocytes in a controlled and non toxic way [106].

A number of genes and proteins which contribute to the stability of the mitochondrial membrane have been shown to be up regulated in prostate cancer. Most notably these include Bcl-2 which has been shown to be up regulated by various degrees up to 70% depending on the grade of prostate cancer. There is some debate as to the usefulness of Bcl-2 expression as a marker of outcome, where some studies [107] demonstrated that treatment decisions should not be based on the primary tumour but rather on tissues from recurrent or metastatic lesions, as Bcl-2 expression was significantly higher in the tissue from patients that had salvage radical prostatectomy after radiotherapy [108]. Others have shown that Bcl-2 expression in the primary tumour can predict disease recurrence following radical prostatectomy [109] and biochemical failure after radiotherapy [110]. Other members of the anti-apoptotic Bcl-2 family have also been shown to be up regulated including Bcl-xL but to date no correlation has been shown between its expression and biochemical failure [111].

Heat Shock Proteins (HSP) also contribute to mitochondrial stability either directly or indirectly. Their ability to maintain cell survival is not only related to their role as protein chaperones but is dependent on their ability to interact with proteins or polypeptide substrates. They can prevent the permeabilisation of the outer mitochondrial membrane by inhibiting the activity of pro-apoptotic Bcl-2 proteins. The disruption of apoptosome formation represents another mechanism by which HSP can prevent apoptosis which is required for the activation of the caspase cascade [112]. The expression of HSP in prostate cancer tissue is however controversial with early studies showing that HSP 27 did not correlate with degree of histological differentiation, T-stage, nodal status, local recurrence, metastases or survival and in fact there was decreased expression in some tumours [113;114]. However, more recent studies have shown
that HSP is initially lost but with the emergence of an advanced cancer there is an increase in HSP 27 which is associated with poor clinical outcome [115].

The Inhibitors of Apoptosis (IAP) family of proteins, specifically cIAP-1, cIAP-2, XIAP and survivin have been shown to be increased as a frequent and early event in the etiology of prostate cancer [116], with expression often evident in PIN. Specific studies examining survivin have again shown mixed results. Kaur et al demonstrated that despite expression in the majority of samples survivin was not a prognosis-related marker and did not correlate with grade or stage [117].

However, Shariat et al clearly shows that survivin is expressed in normal tissue but its expression increases with Gleason score and increased risk of biochemical progression on univariate analysis [118;119]. These proteins have been shown to interact and inhibit caspase activation triggered by both the mitochondrial and death receptors and therefore mediate apoptotic resistance [120].

Where clusterin has also been proposed as an anti-apoptotic protein, its mechanism is unknown, but it is known to mediate resistance to androgen ablation, cytotoxic chemotherapy and TNF treatment. It is clear that clusterin may not be expressed in the primary tumours [121] and in fact Scaltriti et al have shown a down regulation in prostate cancer in comparison to matched benign controls [122]. However, on androgen withdrawal clusterin is significantly increased up to 17-fold, representing a time interval for therapeutic manipulation preventing the emergence of an apoptotic resistance phenotype [123].

Another new survival factor is TWIST which has recently been shown to have a positive correlated with Gleason grade [124]. The anti-apoptotic effects of TWIST are unknown but siRNA to TWIST results in increased sensitivity to taxol induced apoptosis which was associated with decreased Bcl/Bax ratio, leading to the activation of the death pathway [125]. TWIST has been proposed as a novel oncogene that induces tumourigenesis in non-malignant cells and promotes tumour progression in malignant cells.

The loss of pro-apoptotic factors have also been associated with the development of prostate cancer. The proteolytic caspases represent the central mediators of the caspase dependent apoptotic pathway and result in many of the characteristic of apoptosis from actin cleavage resulting in cell shrinkage to lack of DNA repair due to cleavage of PARP [126]. It has been shown that there is a Gleason grade dependent loss in the protein expression of both caspase 1 and 3 [127;128]. The mechanism for this loss has yet to be determined but would contribute to the ability of cancer cells to survive a caspase dependent mechanism of cell death.

Further to the stability of the mitochondrial membrane decreased expression of both Bid and Bax have also been shown in prostate cancer. Clinical outcome data has revealed an association of higher levels of Bid with longer recurrence-free survival in locally advanced prostate cancer [129].

Decreased Bax expression was also shown to occur in 23% of patients, but some patients did demonstrate increased expression and others expressing both an increased and decreased expression, which supports the concept of prostate cancer being a heterogenous disease [130]. Other studies have also shown increased expression of Bax which was correlated with stage of carcinoma, but not with the apoptotic index and it was suggested that this was associated with a non-functional Bax protein [131].

With this in mind it may not just be the expression of Bax that is important but the expression of proteins that regulate its activity, causing mitochondrial membrane disruption. Bax inhibitor-1 protein has been shown to be increased in prostate carcinoma as assessed by real-time PRC using laser-captured microdissected epithelial tissue samples. This represents a protein that functionally prevents Bax activity and a mechanism for apoptotic resistance [132].

Where the gain and loss of expression of a number of pro- and anti-apoptotic proteins is associated with the development of prostate cancer. It is evident that no one single protein is responsible for the development of an apoptotic resistant phenotype that will eventually result in these diseases of the prostate.

As has been clearly demonstrated in the recent paper by Shab et al, prostate cancer is a heterogenous group of diseases [133], that will not only require individual treatment but also a multiple of treatments per patients if a number of different phenotypes of disease are shown. Understanding the pathobiology of prostate cancer represent the single most difficult step, without which targeting therapy is not achievable.
In this chapter we will focus on hypothesis driven genetic studies; there is a wealth of target discovery studies using state of the art ‘omics’ technology platforms (Genomics, transcriptomics, proteomics etc).

1. Methylation, SNPs and Polymorphisms in Genes Involved in Endocrine Metabolism

Androgens play an important role in the development of prostate cancer. Prostate cancer is initially androgen dependent, but most cases eventually relapse to an androgen-independent state after androgen deprivation therapy. A key component of the androgen transduction cascade in responsive tissues is the androgen receptor (AR), and alterations in its structure and expression are thought to be responsible for the progression to androgen-independence in prostate cancers.

This article summarizes current perspectives on epigenetic regulation of AR gene, and polymorphisms of genes involved in AR and androgen metabolism among prostate cancer patients including Japanese subjects.

a) AR methylation

Epigenetic mechanisms including DNA methylation and histone deacetylation are thought to play important roles in gene transcriptional inactivation. Heterogeneous expression of androgen receptor (AR), which appears to be related to variable responses to endocrine therapy in prostate cancer (PCa) may also be due to epigenetic factors. The methylation status of the 5’CpG island of the AR in 3 prostate cancer cell lines and clinical PCa samples from Japanese was determined using the bisulfate PCR methods (Nakayama et al). In DU145, CpG-rich regions of the AR were hypermethylated. By an immunohistochemical analysis, only one PCa sample had no AR expression, the others being heterogeneous. Bisulfite sequencing and methylation specific PCR analysis showed aberrant methylation of AR 5'-regulatory region in 20% of primary and 28% of hormone-refractory PCa samples. The DU145 cell line, treated with a demethylating agent, 5-aza-2'-deoxycytidine (azaC), and a histone deacetylase inhibitor, Trichostatin A (TSA), re-expressed of AR mRNA. The results suggest that epigenetic regulations including CpG methylation and histone acetylation may play important roles in the regulation of the AR.

b) AR polymorphism

The N-terminal transcriptional activation domain of the AR gene contains polymorphic CAG repeats encoding polyglutamine. Several reports showed a higher frequency of short CAG repeat lengths in African-American men compared with whites and Asians, but no difference in CAG repeat lengths between whites and Asians.

Since the length of this trinucleotid e repeat is inversely correlated with the transactivation function of the AR, the clinical significance of the CAG repeat length in the pathogenesis of prostate cancer have been investigated.

The length of the CAG repeat did not significantly differ between prostate cancer and benign prostatic disease. Although not statistically different with regard to clinical stage and serum PSA level, the CAG repeat length was associated with histological grade and age at diagnosis. In addition, the CAG repeat length in CR and in non CR patients significantly differed, suggesting that the CAG repeat length can act as a molecular marker with which to predict response to endocrine therapy (Suzuki et al).

c) CYP19(Aromatase)

CYP19 catalyzes the conversion of C19 androgens to C18 estrogens. Polymorphisms in introns 4, 5, 6 and 7, the 5’ regulatory area and 3’UTR have been. With reference to prostate cancer, an association between the 171 and 187 bp alleles of CYP19 and prostate cancer risk in White French has been described. In Modugno’s study a C to T substitution in exon 7 showed an increased in risk of borderline significance in prostate cancer among Caucasian patients (OR 2.50; 95% CI, 0.99-6.28) and combined effects of short androgen receptor CAG repeats (OR 1.77; 95%CI, 1.00-3.14).

However, Japanese subjects does not reveal significant relationship between the Arg264Cys (exon 7) substitution and prostate cancer risk (Fukatsu et al). The genetic polymorphism does not correlate with clinicopathological factors including the staging or grading of the prostate cancer. These results are not in line with Modugno’s findings, possibly due to ethnicity.

d) 5α Reductase type 2 (SRD5A2)

Steroid 5α-reductase type II (SRD5A2), which converts testosterone to the metabolically more active dihydrotestosterone 2 polymorphisms: V89L, which substitutes leucine for valine at codon 89, and A49T, which substitutes threonine for alanine at codon 49.
Several case-control studies of the association between the SRD5A2 V89L polymorphism and prostate-cancer risk have been reported. The hypothesis was that the L allele should be protective. However, Lunn et al. reported genotypes including an L allele to be somewhat more common in prostate-cancer cases (56%) than in controls (48%) among Caucasians, albeit without significance (OR = 1.4, 95% CI 0.8-2.4, p = 0.24).

Febbo et al. also reported no significant association between the genotype and prostate-cancer risk. The results among Japanese subjects are in line with these 2 studies (Yamada et al). Putting these results together, the V89L polymorphism in SRD5A2 appears to have little association with prostate-cancer risk, though it is difficult to make statements concerning this association because most of the studies covered only a small number of cases and controls.

e) CYP17

The human CYP17 gene encodes the cytochrome P-450c17, which mediates both steroid 17-hydroxylase and 17,20-lyase in the steroid biosynthesis pathway. There is a polymorphic T(A1)-to-C(A2) substitution in the 5-untranslated region of CYP17. The base pair change creates an SP1-type (CCACC box) promoter site, which may enhance transcriptional activity.

There have been several documented case-control studies of the association between the CYP17 polymorphism and prostate-cancer risk. Lunn et al. reported that the CYP17 A2 allele (combined A1/A2 and A2/A2 genotypes) occurred at a higher frequency in Caucasian patients with prostate cancer (70%) than in clinical urology patients (57%), suggesting that it conveys increased prostate-cancer risk (OR = 1.7, 95% CI 1.0-3.1, p = 0.05).

However, Wadelius et al. reported that significantly more male Caucasians heterozygous for the CYP17 A1 allele were prostate-cancer patients compared with control individuals (OR = 1.61, 95% CI 1.02-2.53, p = 0.04). The Japanese subjects revealed that the frequency of the CYP17 A2/A2 genotype in cases (18.8%) was higher than in controls (14.5%). Compared with the A1/A1 genotype, the odds ratio for the A2/A2 genotype was 2.39 (95% confidence interval 1.04-5.46, p = 0.04)(Yamada et al). Thus, there was significant association between CYP17 genotype and prostate-cancer risk, though contradictory data have been reported.

2. DIFFERENTIALLY EXPRESSED GENES

A comprehensive summary will be provided by committee 2. We have focussed on genes for which clinical implementation is foreseen in the near future. Also prostate cancer research took advantage of the high throughput target discovery ‘omics’ technologies. In a ‘record’ time genes identified by expression profiling proved to be ready for clinical implementation. The best example is probably AMACR (‘racemase’) a marker for prostate cancer that is used almost routinely in US pathology labs and which is penetrating into pathology laboratories rapidly worldwide. Another gene identified by differential gene expression analysis, PCA3, is as test available for research use only (RUO) to detect prostate cancer by analysis of urinary sediments after DRE [134;135] and will most become available as in vitro diagnostic (IVD) kit in 2006! Other examples of genes identified by expression profiling that may be used clinically soon are erg1, cyclinD1, pim1, c-myc and EZH2. The latter one is particularly interesting since it can be associated with cancer stem cell survival and expansion [136;137]. Recently, through the original (COPA) use of bioinformatics the ets related oncogenes erg and ETV1 were shown to be activated in the majority of prostate cancers by fusion to the androgen regulated TMPRSS2 gene(2). Thus the use of a combination of a panel of genes comprising PCA3, EZH2, TMPRSS2-ets/etv fusion genes and AMACR could well be the basis for a gene based diagnostics panel [138]. Clearly the limited number of parameters circumvents the bio statistical concerns that hamper interpretation of large panels of genes.

REFERENCES


Committee 2

Advances in Biomarkers for Prostate Diseases

Chairman

R. H. Getzenberg (USA)

Members

P.A. Abrahamsson (Sweden),
E. I. Canto (USA),
A.M. Chinnaian (USA),
B. Djavan (Austria),
B. Laxman (USA),
O. Ogawa (Japan),
K. Slawin (USA),
S. A. Tomlins (USA),
J. Yu (USA),
CONTENTS

I. CURRENT CLINICAL UTILIZATION OF BIOMARKERS FOR PROSTATE DISEASES

1. PSA LEVEL CUT-OFFS FOR SCREENING
2. COMPLEXED PSA

II. PSA AND PSA ISOFORMS

1. IS THE TOTAL PSA ERA COMING TO AN END?
2. FREE PSA, A COMPLEX MIXTURE OF MOLECULES
3. CLINICAL APPLICATION OF FREE PSA AND COMPLEXED PSA
4. CLINICAL APPLICATION OF FREE PSA MOLECULAR FORMS
5. FREE PSA MOLECULAR FORMS IN THE MANAGEMENT OF PROSTATE CANCER

III. BIOMARKERS IDENTIFIED BY DIFFERENTIAL GENE EXPRESSION ANALYSIS AND THEIR APPLICATION

1. INTRODUCTION
2. EXPRESSED SEQUENCE TAG (EST) DATABASE MINING
3. SERIAL ANALYSIS OF GENE EXPRESSION (SAGE) PROFILING
4. SUBTRACTIVE HYBRIDIZATION AND DIFFERENTIAL DISPLAY
5. DNA MICROARRAYS
6. HEPSIN
7. AMACR
8. EZH2
9. IDENTIFICATION OF BIOMARKERS OF BPH FROM DNA MICROARRAYS
10. IDENTIFICATION OF BIOMARKERS OF AGGRESSIVE PCA FROM DNA MICROARRAYS
11. BIOMARKERS FOR DRIVING GENETIC CHANGES IN PCA
12. CONCLUSION

IV. BIOMARKERS IDENTIFIED BY PROTEOMIC ANALYSIS AND THEIR APPLICATION

1. PROTEOMICS
2. DIAGNOSING PROSTATE DISEASES BASED ON PROTEIN PATTERNS
3. 2D ELECTROPHORESIS
4. HIGH THROUGHPUT MEANS TO ANALYZE LARGE AMOUNTS OF DATA
5. FOCUSED PROTEOMICS
6. BPH
7. SUMMARY

V. GENETIC POLYMORPHISMS

1. INTRODUCTION
2. GENETIC POLYMORPHISMS AND PROSTATE CANCER
3. GENETIC POLYMORPHISMS AND BPH OR OTHER PATHOLOGICAL CONDITIONS OF THE PROSTATE
4. CONCLUSIONS

VI. NEUROENDOCRINE MARKERS

1. INTRODUCTION
2. NEUROENDOCRINE CELLS OF THE NORMAL PROSTATE
3. NEUROENDOCRINE DIFFERENTIATION, TUMOUR PROGRESSION AND HORMONE-INDEPENDENCE IN PROSTATIC CARCINOMA
4. PROGNOSTIC SIGNIFICANCE OF NEUROENDOCRINE DIFFERENTIATION IN PROSTATIC CARCINOMA
5. NEUROENDOCRINE DIFFERENTIATION AND NEW TREATMENT MODALITIES
6. SUMMARY

RECOMMENDATIONS and CONCLUSION

REFERENCES
I. CURRENT CLINICAL UTILIZATION OF BIOMARKERS FOR PROSTATE DISEASES

Prostate cancer represents a significant problem for men’s health, especially in Western populations. In the United States, prostate cancer is the most common malignancy among adults and the second most common cause of cancer death in men. In fact, prostate cancer alone accounts for approximately one third of new cancer cases in men [1]. It is estimated that 1 in 6 men will be diagnosed with prostate cancer at some point in their lives, and that approximately 30,000 men will die of the disease each year.

The advent of prostate specific antigen (PSA) testing revolutionized the diagnosis of prostate cancer. Initiated in the early 1990s, PSA testing produced a surge in the number of newly diagnosed prostate cancer cases. Since the early 1990s, annual PSA testing has been recommended for men in the United States over the age of 50 years. However, these recommendations are not supported by a number of physician groups and national organizations, nor are they followed by a large number of practicing clinicians.

The main reasons cited for the lack of annual PSA screening include concerns about over diagnosis and over treatment. Although more cases of early prostate cancer are detected, there is no clear evidence that survival rates have improved as a result of population screening.

In the United States, the most complete information on the epidemiology of prostate cancer has been assembled by the Surveillance, Epidemiology, and End Results (SEER) database of the National Cancer Institute [2]. The SEER incidence data for years 1973-1999 can be divided into the pre-PSA and PSA eras, with the PSA era beginning in late 1980s. During the pre-PSA era, there was a gradual rise in the incidence of prostate cancer, likely due to the increasing number of TURPs (transurethral resection of the prostate) being performed. Once TURPs became a routine part of prostate management, prostate cancer rates stabilized in the late 1980s until the advent of the PSA era. At that time, an abrupt rise in prostate cancer incidence was observed (with no links to environmental or other risk factors). The incidence of newly diagnosed prostate cancer peaked in 1992 with 237 cases per 100,000 person-years. Thereafter, the annual incidence rate declined until 1995, likely due to the cull effect (i.e., removal of detectable cases in prior years resulting in fewer available cases for repeated screening). A relatively stable incidence rate was observed from 1995 to 1999, but the rates were higher than those observed in the pre-screening era.

A review of the age-specific incidence of prostate cancer shows that most countries report few cases for men younger than 50 years of age, with the incidence rising exponentially with advancing age and reaching a maximum after age 80. The incidence rate in men over the age of 75 is 20- to 83-times higher than for men ages 50-54 [3]. As indicated by the SEER data, the rise in prostate cancer incidence in PSA era has been most prominent in men between 50-59, whereas the incidence in men above 60 years has gradually declined since 1992 [4]. These trends are characteristic of a screening effect.

The role of tumor markers in prostate cancer began...
with the identification of acid phosphatase a half century ago. Shortly thereafter, prostatic acid phosphatase (PAP) became the gold standard for tracking prostate cancer. For many reasons, PAP was associated with a large number of false-negative results, limiting the usefulness of this marker even after a radioimmune assay was developed. PAP has essentially been replaced by another prostatic enzyme marker, prostatic-specific antigen (PSA), which is much more specific to prostate tissue. During the past 15 years, PSA has become indispensable for the management of prostate cancer. While PSA remains the most clinically relevant tumor marker in human oncology, recent evidence now refutes this premise in the early diagnosis of prostate cancer.

Prostate cancer is one of the few malignancies for which the incidence varies widely across various parts of the world. Hsing and colleagues classified 15 countries according to their level of prostate cancer risk. High-risk countries included the US, Canada, Sweden, Australia, and France. Medium-risk countries included most other Western European countries, and low-risk countries included most of Asia [3]. The same group of investigators also examined trends in the incidence rates from 1973 to 1992. From 1988–1992, when PSA testing became widespread, the incidence rates in the high-risk countries ranged from 48.1 to 137 per 100,000 person-years, while the incidence in low-risk countries ranged from 2.3 to 9.8 per 100,000 person-years (see Table 1). In general, the prostate cancer incidence rates rose in all countries during these years, with percent increments ranging from 16.2% to 113.3%.

Advantages of PSA testing as a screening tool can be summarized as follows:

- Earlier stage at diagnosis—more localized disease and less advanced/metastatic disease (stage migration)
- Earlier age at diagnosis
- Lower PSA level at diagnosis
- Improved prostate cancer survival rates

The disadvantages of PSA screening can be summarized as follows:

- Increased detection and treatment of indolent tumors

Table 1. Age-Adjusted Incidence Rates of Prostate Cancer in 15 Countries, 1973-1977 and 1988-1992

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n *</td>
<td>Age-adjusted Incidence</td>
<td>n *</td>
<td>Age-adjusted Incidence</td>
<td></td>
</tr>
<tr>
<td><strong>High risk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>430</td>
<td>23.0</td>
<td>1502</td>
<td>48.1</td>
<td>109.1</td>
</tr>
<tr>
<td>US blacks</td>
<td>2664</td>
<td>79.9</td>
<td>7129</td>
<td>137.0</td>
<td>71.5</td>
</tr>
<tr>
<td>US whites</td>
<td>24,192</td>
<td>47.9</td>
<td>66,227</td>
<td>100.8</td>
<td>110.4</td>
</tr>
<tr>
<td>Australia</td>
<td>3661</td>
<td>28.4</td>
<td>10,870</td>
<td>53.5</td>
<td>88.4</td>
</tr>
<tr>
<td>Canada</td>
<td>3126</td>
<td>39.8</td>
<td>10,473</td>
<td>84.9</td>
<td>113.3</td>
</tr>
<tr>
<td>Sweden</td>
<td>16,556</td>
<td>44.4</td>
<td>25,253</td>
<td>55.3</td>
<td>24.5</td>
</tr>
<tr>
<td><strong>Medium risk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>England</td>
<td>5461</td>
<td>20.1</td>
<td>9529</td>
<td>29.3</td>
<td>45.8</td>
</tr>
<tr>
<td>Italy</td>
<td>219</td>
<td>22.8</td>
<td>884</td>
<td>28.2</td>
<td>23.7</td>
</tr>
<tr>
<td>Spain</td>
<td>291</td>
<td>17.6</td>
<td>641</td>
<td>27.2</td>
<td>54.5</td>
</tr>
<tr>
<td>Denmark</td>
<td>3932</td>
<td>23.6</td>
<td>7392</td>
<td>31.0</td>
<td>31.4</td>
</tr>
<tr>
<td>Israel</td>
<td>1238</td>
<td>15.5</td>
<td>3147</td>
<td>23.9</td>
<td>54.2</td>
</tr>
<tr>
<td><strong>Low risk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>India</td>
<td>193</td>
<td>6.8</td>
<td>764</td>
<td>7.9</td>
<td>16.2</td>
</tr>
<tr>
<td>Singapore</td>
<td>100</td>
<td>4.8</td>
<td>415</td>
<td>9.8</td>
<td>104.2</td>
</tr>
<tr>
<td>China</td>
<td>219</td>
<td>1.6</td>
<td>539</td>
<td>2.3</td>
<td>43.8</td>
</tr>
<tr>
<td>Japan</td>
<td>222</td>
<td>4.9</td>
<td>737</td>
<td>9.0</td>
<td>83.7</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>268</td>
<td>5.1</td>
<td>1185</td>
<td>7.9</td>
<td>54.9</td>
</tr>
</tbody>
</table>

Adapted with permission from Hsing et al.
* Incidence/100,000 person years.
• Overdiagnosis and overtreatment
• Unclear PSA screening cutoff levels
• Unclear data on prostate cancer survival rates due to lead- and length-time bias

1. PSA LEVEL CUT-OFFS FOR SCREENING

With increasing numbers of prostate cancer cases being detected in men with PSA below 4.0 ng/mL [5,6], the suitability of retaining a PSA cutoff of 4.0 ng/mL is being questioned. A recent study conducted by Thompson and colleagues [7] among 2950 men with PSA levels < 4.0 ng/mL, identified prostate cancer in 15.2%, of which 14.9% had a Gleason score > 7. In addition, 26.9% of the men with PSA values between 3.1 to 4.0 ng/mL had prostate cancer. The introduction of age-specific reference ranges for PSA screening by Oesterling and colleagues [8] and race- and age-specific ranges by Morgan and colleagues [9] has demonstrated that the detection and treatment of prostate cancer are affected by both age and race.

The sensitivity and specificity of complexed PSA for prostate cancer detection makes it another potential candidate for screening.

2. COMPLEXED PSA

Complexed PSA includes PSA that forms stable covalent complexes with major extracellular antiproteases such as a1-antichymotrypsin, a2-macroglobulin, protein C inhibitor, and to a much lesser extent, a1-protease inhibitor. Complexed PSA is a more stable test than percent free PSA, and its levels are not affected by prostatic manipulation. Furthermore, increased complexed PSA levels are strongly associated with the presence of prostate cancer, such that complexed PSA testing may eventually replace total PSA in the near future.

The positive predictive value for a PSA between 4.0-10.0 ng/mL in a patient with a normal DRE is about 30%. This suggests a relatively low specificity. To improve performance, other “modifications” of PSA have been implemented, including PSA velocity (PSAV), PSA density (PSAD), and free-to-total PSA ratio (Table 2).

a) New Biomarkers other than PSA

Potential new markers for the diagnosis and outcome of prostate cancer have also been investigated. Molecular markers have the potential to serve not only as prognostic factors but may be targets for new therapeutic strategies and predictors of response in a range of cancers. The molecular biology of prostate cancer and its progression is characterized by aberrant activity of several regulatory pathways: apoptosis, androgen receptor signaling, signal transduction, cell cycle regulation, cell adhesion, and angiogenesis. Variations at the DNA, RNA and/or protein levels of molecules involved in these pathways are potential candidate markers of prognosis and therapeutic response.

<table>
<thead>
<tr>
<th>Table 2. The Usefulness of Various Indicators for Biopsy of the Prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indication for Biopsy</td>
</tr>
<tr>
<td>Total PSA (tPSA)</td>
</tr>
<tr>
<td>PSA velocity</td>
</tr>
<tr>
<td>PSAD</td>
</tr>
<tr>
<td>Age-adjusted PSA</td>
</tr>
<tr>
<td>Atypical, small acinar proliferation on prior biopsy</td>
</tr>
<tr>
<td>High-grade PIN on prior biopsy</td>
</tr>
<tr>
<td>Free/total PSA (percent free PSA)</td>
</tr>
</tbody>
</table>
b) Requirements for prognostic markers

Any new prognostic marker must be measured in the context of accepted predictors of prostate cancer recurrence and death (clinical or pathological disease stage; surgical margin involvement, Gleason score or grade, serum PSA concentration at diagnosis) [10,11]. For a useful prognostic marker, it must provide value additional to and possibly independent of, that are provided by these factors.

However, molecular markers are not only important because of potential relationships with outcome, they might also become new targets for molecular-based intervention therapy. Thus, an association with adverse outcome might suggest a key role for a molecule in the disease state, but it does not mean that markers that are not prognostic are of no use. For example, a marker that is present in a large number of prostate cancers and might be targeted therapeutically is likely to be of considerable interest and utility.

There are different approaches to study cancer outcome. One can take a candidate gene approach in which important genes are assessed in a series of tumor samples and compared with clinical-pathological factors including outcome. As an alternative, one can use a variety of techniques in an attempt to discover new genes that may be important in the cancer concerned. Techniques designed to detect chromosomal abnormalities in prostate cancer identified a number of potential candidate molecules for evaluation in prostate cancer. The ability to assay tumor tissue using cDNA and oligonucleotide arrays with identified sequences for a large number of molecules has expanded the number of such markers inestimably [12].

Candidate molecules identified in these ways can then be evaluated in tumor samples. The construction of tumor tissue microarrays in which cores of multiple different cancers are assembled in one paraffin block and can be stained for protein expression on a single slide allows rapid assessment and validation of these markers. [13] Selection of overexpressed molecules by cellular localization and function can lead to the development of new markers for cancer in blood [14]. The search for prognostic tissue markers provides information on outcome but may also lead to advances in diagnostic methods, elucidate new therapeutic targets and identify other related molecules important in cancer development and progression.

c) Cancer Progression

The essential elements in initiation and progression of hormone-dependent cancer are deregulated cell proliferation, avoidance of apoptosis, resistance to hormonal control and metastasis (Table 3).

<table>
<thead>
<tr>
<th>Process</th>
<th>Key molecules/markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androgen receptor signaling</td>
<td>AR, possible alternate signal transduction pathways</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>Bcl-2, p53</td>
</tr>
<tr>
<td>Cell cycle regulation</td>
<td>c-Myc, p16INK4A, p27kip1, pRb, apoptotic index, Ki67</td>
</tr>
<tr>
<td>Signal transduction</td>
<td>Epidermal growth receptors</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>VEGF, VEGF receptors, nitric oxide</td>
</tr>
<tr>
<td>Cell adhesion and cohesion</td>
<td>E-cadherin, α-catenin, metalloproteinases, chondroitinsulfate</td>
</tr>
</tbody>
</table>

d) Cell proliferation

Increased proliferation index measured by Ki67 or proliferating cell nuclear antigen correlates with the presence of advanced stage disease and increased tumor grade. Ki67 index is independently predictive of outcome in patients with clinically localized disease. Recurrent tumors have Ki67 indices approximately double that of the primary tumor. Several studies identified increased apoptotic index (number of apoptosing cells within a prostate cancer) as adversely prognostic.

e) Apoptosis

The major apoptotic regulators are p53 and Bcl-2. They both demonstrate abnormal function and expression as prostate cancer progresses. Following therapy with androgen ablation, p53 and Bcl-2 expression as well as the apoptotic index increase in many cases. Failure of apoptotic response as measured by the apoptotic index correlates with relapse.

f) p53

The effects of p53 in cancer initiation and progression can be summarized as following: cell cycle regulation, apoptosis and angiogenesis/metastasis. It regulates the transcription of genes involved in the G1-phase growth arrest of cells in response to DANN damage. P53 also is involved in the regula-
tion of the spindle checkpoint, centrosome homeso-
tasis and G2-M phase transition and it regulates
apoptosis and tumor angiogensis in malignant cells.
Nuclear accumulation of p53 is a prognostic indica-
tor in several human cancers. The value of p53 nuclear
accumulation as a prognostic factor in localised
prostate cancer has been debated. A number of studies
have shown that p53 nuclear accumulation is
prognostic at a variety of dichotomising cutoff points, based on the number of p53 positive nuclei.
These studies either describe a poor prognosis group of patients with >20% p53 positive nuclei or a group of patients with lower percentages of positive cells in a heterogeneous focal staining pattern where either the presence of any nuclear accumulation or the presence of clusters of cells showing nuclear accumulation is adversely prognostic.

However, other studies comparing p53 nuclear accumulation have failed to provide conclusive evidence for the importance of p53 in localised prostate cancer or a strong correlation between nuclear accumulation and p53 gene mutation. Borre et al reported on a pop-
ulation of patients observed with no treatment after prostate diagnosis and found p53 nuclear accumulation to be predictive of prostate cancer related death
[16]. Quinn et al demonstrated the adverse prognostic effect of an increased percentage of cells with p53 nuclear accumulation that was independent of PSA, Gleason score and pathological stage [17].

However, there are more than 100 studies reporting series of patients with prostate cancer evaluated for p53 nuclear accumulation. Essentially, they demon-
strate increasing p53 expression with increasing grade and stage with a prognostic effect that may or may not be independent of these two variables. How-
ever, the clinical use of p53 as prognostic marker in prostate cancer still needs further studies.

g) Bcl-2

Bcl-2 is the prototype of a novel type of oncogenes that inhibit apoptosis [18]. It is part of an expanding family of apoptosis-regulatory molecules, which
may act as either death antagonists (Bcl-2, Bcl-xL, and Mcl-1) or death agonists (Bax, Bak, Bcl-xS, Bad and Bid). The selective and competitive dimerisation between pairs of antagonists and agonists determines how a cell will respond to a given signal [19]. Several studies [20,21] demonstrate that increased expression of Bcl-2 in prostate cancer confers androgen resistance, particularly in advanced disease, and facilitate progression to androgen independence. Recent work suggests that Bcl-2 overexpression has a role in resistance to radiotherapy in prostate cancer [22] and is adversely prognostic in localized prostate cancer. These studies also suggest that Bcl-2 expression increases with grade and stage. This is one optional reason that Bcl-2 expression on biopsy may be independently prognostic in radiotherapy cohorts and not those treated with RP. Another is that patients with Bcl-2 overexpression do better with radiotherapy because the cells are more sensitive to its effects. It could be interesting to test this hypoth-

sism in a prospective trial of radiotherapy stratified
for normal against Bcl-2 overexpression in prostate
cancer on biopsy, matched for clinical stage and Gleason score.

h) Androgen receptor

Androgen receptor (AR) overexpression is a more obvious potential marker of prognosis and thus, also a feature of progression, recurrence, lymph node (LN) metastasis and/or anti-androgen resistance in human prostate cancer [23]. Recent studies failed to prove an association between AR overexpression in primary prostate cancer and outcome, but did find an AR-expression in >70% of LN metastases [24]. It is suggested in several studies that overexpression is correlated with mutation and/or amplification and with androgen resistance [25]. The Garvan Institute showed that in case of high AR expression in the epitheilum and reduced expression in the sur-
rrounding stroma the prognostic outcome is more likely to be adverse. Growth factors interacting in epithelial-stromal tissue, such as TGFbeta [26], are progres-
sively overexpressed in epithelial cells with prostate cancer progression, because of loss of TGF-beta cell cycle inhibition [27].

i) Human Kallikrein 2

hK2 belongs to the same family of serine proteases as PSA (hK3) and shares approximately 80% sequence homology with PSA [28]. It is a glycoproteine with a molecular mass of 31-33 kDa and trypsin like activity [29]. It has been shown that hK2 can convert the zymogene from PSA into enzymati-

cally active protein. [30] Thus, hK2 might probably serve as physiological activator of PSA, but the sig-
ificance of these findings in vivo is not yet known. Several investigators have evaluated the utility of hK2 in combination with serum PSA and/or fPSA. While some reports have assessed total hK2 [31,32], the majority measure the hK2:fPSA ratio or hK2 multiplied by the fPSA:tPSA ratio [33,34,35]. Two studies evaluated a combined total of 604 men with PSA > 3.0 ng/mL who underwent prostate cancer
screening [36]. In these studies, hK2 values multiplied by the tPSA/fPSA ratio improved sensitivity and specificity as compared with tPSA or percent fPSA. Likewise, Nam and colleagues [37] reported that mean hK2 and hK2 to fPSA ratio values were significantly higher in men with prostate cancer than in men with benign disease. These studies suggest that hK2 may be used as an adjunct to PSA; however, to date, no prospective multi-institutional studies have confirmed these reports. Interestingly, some investigators have examined the role of the CâT polymorphism in hK2 and its relation to prostate cancer risk. One study examined 1287 men undergoing prostate biopsy and found that the odds ratios for prostate cancer were significantly higher in men with ≥ 1 T allele than in those with no T allele (wild-type CC). Furthermore, when this information was combined with serum hK2 levels, the combined adjusted odds ratio for having prostate cancer was 14 for patients with high hK2 levels and at least one T allele relative to those with low levels and with at least one T allele [38].

j) Human Kallikrein 11

This is another member of the kallikrein gene family that might become an additional marker in the diagnosis of prostate cancer. Nakamura et al [39] investigated hK11 serum levels that could be used to distinguish between prostate cancer and benign prostatic hyperplasia. They found that serum hK11 levels and the hK11:total PSA ratio were both significantly lower in prostate cancer patients than in BPH patients. ROC curve analysis demonstrated that the hK11:total PSA ratio curve (AUC = 0.83) and percentage of free PSA (AUC = 0.83) were much stronger predictors of prostate cancer than total PSA (AUC = 0.69). In addition, in the group of patients with percentage of free PSA less than 20, an additional 54% of BPH patients could have avoided biopsies by using the hK11:total PSA ratio. These data suggest that the hK11:total PSA ratio could be a useful tumor marker for prostate cancer and could be combined with percentage of PSA to further reduce the number of unnecessary prostate biopsies.

k) New Markers

A number of comprehensive reviews describing prostate cancer markers have been published [40,41,42]. Below is a discussion of the most promising markers that may be relevant to prostate cancer diagnosis (Table 4).

AMACR, alpha-methylacyl coenzyme A racemase; DD3/PCA-3, differential display code 3/prostate cancer 3; GRN-A, chromogranin-A; GSTP-1, glutathione-S-transferase P1; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; PIN, prostatic intraepithelial neoplasia; PSA, prostate-specific antigen; PSCA, prostate stem cell antigen; PSMA, prostate-specific membrane antigen.

Table 4. A Partial List of Potential Markers for Prostate Cancer

<table>
<thead>
<tr>
<th>Marker</th>
<th>Characteristics</th>
<th>Potential Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTP-1</td>
<td>Hypermethylation of this gene or its promoter in its CpG islands.</td>
<td>May distinguish PIN from prostate cancer. GSTP1 expression seems to be independent of PSA expression.</td>
</tr>
<tr>
<td>GRN-A</td>
<td>Found in the secretory granules of neuroendocrine cells.</td>
<td>May be a marker for advanced hormone-refractory disease.</td>
</tr>
<tr>
<td>PSMA</td>
<td>Elevated in both primary and metastatic disease.</td>
<td>Under evaluation as a target for diagnosis and radioimmunotherapy.</td>
</tr>
<tr>
<td>IGFs and IGFBP</td>
<td>Molecules involved in regulating growth, cellular proliferation, cellular transformation, and apoptosis.</td>
<td>Tissue expression of IGFs and IGFBPs associated with established parameters of disease progression.</td>
</tr>
<tr>
<td>DD3/PCA-3</td>
<td>A gene that does not appear to code for a protein, but is markedly upregulated in prostate cancer.</td>
<td>Potentially better positive and negative predictive values than PSA.</td>
</tr>
<tr>
<td>PSCA</td>
<td>Expressed primarily in prostate cancer cells.</td>
<td>PSCA levels and disease severity appear to correspond. Unclear whether it has more utility than PSA levels.</td>
</tr>
</tbody>
</table>
l) Prostate-Specific Membrane Antigen

Prostate-specific membrane antigen (PSMA) is probably the most well-studied of non-PSA markers. It is present in normal prostate tissue and in prostate cancer, but in the latter, it tends to be found within the plasma membrane rather than in the cytosol. Many studies have shown PSMA levels to be elevated in both primary and metastatic disease [43,44]. In metastatic tissue from patients with hormone refractory disease, the expression of PSMA is enhanced rather than diminished, as is the case with PSA, so there is hope that PSMA will be a better marker for advanced disease and progression. PSMA is also being tested as a diagnostic tool and a target for radioimmunotherapy. At present, the exact value of this marker remains unclear.

m) Chromogranin-A

Chromogranin-A (GRN-A) is another rather well-studied, potential tumor marker. It is present in the secretory granules of neuroendocrine cells, and may signify the presence of advanced hormone-refractory disease. Indeed, elevated levels of GRN-A appear to predict for poor prognosis; however, since many advanced prostate cancers do not seem to harbor neuroendocrine cells, the value of this marker is uncertain [40].

n) Glutathione-S-Transferase P1

Glutathione-S-transferase P1 is a part of a large family of enzymes that act as chemical detoxifiers. In prostate cancer, the gene or its promoter is often hypermethylated, especially in its CpG islands, which appears to increase the expression of GSTP1. The detection of this defect seems to distinguish between prostatic intraepithelial neoplasia (PIN), prostate cancer, and normal/benign tissue. More importantly, GSTP1 expression seems to be independent of PSA expression, further increasing its potential as a marker of early disease. GSTP1 may also be useful because it is detected in both blood and urine. A search for drugs that can reverse methylation in prostate cancer and other malignancies is now underway [45].

o) Prostate Stem Cell Antigen

Prostate stem cell antigen (PSCA) is related to prostate stem cell function, but its mechanism of action is poorly understood. However, this protein is expressed primarily in prostate cancer cells. The majority of prostate cancer patients overexpress this protein, and PSCA levels and disease severity appear to correspond. In hormone refractory and/or metastatic disease, PSCA is strongly expressed, prompting investigators to seek out therapies (e.g., monoclonal antibodies) that target this protein. Whether this marker adds significant additional information beyond that provided by PSA levels, is still uncertain [46,47].

p) IGF and its Binding Proteins and DD3

The insulin-like growth factors (IGFs) and their cognate binding proteins (IGFBPs) are complex cell signaling molecules involved in regulating growth, cellular proliferation, cellular transformation, and apoptosis. The tissue expression of IGFs and IGFBPs has been associated with established parameters of disease progression, such as tumor grade, pathologic stage, and clinical recurrence in a variety of cancers, including prostate cancer [48,49]. High circulating IGF-1 and low IGFBP-3 levels have been shown to correlate with increased risk of prostate cancer development and progression [50,51]. These findings were recently confirmed in a prospective study that found higher levels of acid-labile subunit (ALS) – which modulated IGF-1 levels – associated with a 40-60% increased risk of prostate cancer (Capsule Summary) [52].

Higher levels of ALS also correlated with a 2-fold risk of advanced stage PC that persisted for more than 9 years after blood testing. Still, the origin and role of circulating levels of IGFs and IGFBPs remain unclear and under intense investigation.

The differential display code 3/prostate cancer (DD3/PCA3) gene was identified by differential display analysis. DD3 does not appear to code for a protein, but it is markedly upregulated in and specific to prostate cancer. Recent technological advances have allowed this gene to be measured quantitatively, and preliminary data from measured urine samples after prostatic massage showed better positive and negative predictive values than those reported with PSA. Obviously, these results will require confirmation, but this opportunity has sparked interest among investigators and commercial companies [53,54].

q) Alpha-Methylacyl Coenzyme A Racemase

Alpha-methylacyl coenzyme A racemase (AMACR) is an enzyme involved in the oxidation of fatty acids. AMACR is over expressed in PIN and prostate cancer, and some investigators have suggested that its detection could be useful in tissue diagnosis. Currently, the enzyme cannot be measured directly, but quantitation of anti-AMACR antibodies appears to
discriminate between normal and malignant prostate cells with better sensitivity and specificity than PSA [55,56]. Emerging reports evaluating the detection of AMACR transcripts or protein in urine specimens immediately following prostastic massage or post-TRUS/biopsy demonstrate the utility of AMACR as an adjunct to PSA. AMACR antibody quantitation also appears to be useful in stratifying low- vs high-risk patients (Capsule Summary) [57].

A recent study [58] explored the use of AMACR as a biomarker for aggressive prostate cancer. They determined AMACR protein expression by immunohistochemistry using an image analysis system on two localized prostate cancer cohorts, with 204 men treated by radical prostatectomy and 188 men followed expectantly. The end points were time to PSA failure and time to prostate cancer death in the watchful waiting cohort. They found that lower AMACR tissue expression was associated with worse prostate cancer outcome, independent of clinical variables (HR 3.7 for PSA failure; P=0.018; HR 4.1 for prostate cancer death, P=0.0006). Among those with both low AMACR expression and high Gleason score the risk of prostate cancer death was 18-fold higher. This study shows that AMACR expression is significantly associated with prostate cancer progression.

u) Early Prostate Cancer Antigen

PSA only has low specificity for prostate cancer, so identification of a unique blood-based marker would provide a more accurate diagnosis. A novel biomarker for prostate cancer is early prostate cancer antigen (EPCA). EPCA antibodies were shown to positively stain negative biopsies of men who developed prostate cancer 5 years later.

Recently, Getzenberg et al.[59] evaluated whether EPCA antibodies could be used in a clinically applicable plasma-based immunoassay to detect prostate cancer. With a cutoff value of 1.7, only the prostate cancer population expressed plasma-EPCA levels above the cutoff. Sensitivity of the EPCA assay for prostate cancer patients was 92% whereas the overall specificity was 94%. This initial study shows the potential of EPCA to serve as a highly specific blood-based marker for prostate cancer.

r) Fas-associated death domain containing protein (FADD)

It has been demonstrated that phosphorylation of FADD at serine 194 plays an important role in the induction of apoptosis by anticancer drugs in human prostate cancer cells. Shimada et al. [60] evaluated if this phosphorylation status is valuable as a marker for prostate cancer progression. Immunohistochemical studies revealed higher phosphorylation of FADD at serine 194 in normal epithelial cells than in cancer cells although FADD was highly expressed to the same extent in both cases. The positivity for phosphorylated FADD was significantly lower for patients with a Gleason score greater than or equal to 7, a positive surgical margin, extracapsular or seminal vesicle invasion. Moreover, a relationship between cancer cells refractory to neoadjuvant hormonal therapy and FADD was found.

In addition, they found that in Gleason score 3+4 tumors the positivity for FADD phosphorylation was increased by neoadjuvant hormonal therapy. In vitro data showed that overexpression of a phosphorylation mimicking mutant FADD caused G2/M cell cycle arrest, while a non phosphorylation mimicking mutant had no effect, whereas S194A overexpression resulted in cell cycle progression.

The results clearly show that transition from phosphorylated FADD to the non phosphorylated form might be associated with carcinogenesis and that induction of FADD phosphorylation could therefore be a target for chemohormonal therapy of human prostate cancer. Moreover, assessment of FADD phosphorylation may be useful as new biomarker for the prediction of cancer progression.

s) Cathepsins

Tumor cell invasion and metastasis are associated with the proteolytic activity of various types of proteinases. Cathepsins, which are lysosomal proteinases, have been reported to be elevated in several human cancers. Cathepsins have different roles in cancer progression. Cathepsin D has a mitogenic activity independent of its proteolytic activity. Cathepsins B and L have been shown to play an important role in matrix degradation and cell invasion. The administration of their inhibitors prevents the invsion and metastasis of cancer cells. Thus, cathepsins might not only be useful biomarkers in the detection and progression of prostate cancer but might also act as potential target for cancer therapy. [61].

Miyake et al. evaluated the usefulness of combined systematic prostate biopsy with the serum level of cathepsin D to predict disease extension. The incidence of extraprostatic disease in patients with more than half of the biopsy cores positive or > or = 15 ng/mL cathepsin D was significantly higher than that
in patients with less than half of the biopsy cores positive or $< 15\text{ng/mL}$ cathepsin D, respectively. Furthermore, in patients with more than half the biopsy cores positive and $> or = 15\text{ng/mL}$ cathepsin D or those with more than half of the biopsy cores positive and $> or = 10 \text{ ng/mL}$ PSA, extraprostatic disease was significantly more common than in those with less than half the biopsy cores positive and $< 10 \text{ ng/mL}$ PSA respectively. The authors concluded that patients with more than half the biopsy cores positive, $> or = 15 \text{ ng/mL}$ cathepsin D and $> or = 10 \text{ ng/mL}$ PSA could avoid prostatectomy because there is a significantly high probability that they already have extraprostatic disease.

The cysteine protease cathepsin X maps to the chromosomal region 20q13, a locus which is of ten amplified in prostate cancer. Nagler et al [62] could demonstrate that PIN and prostate cancer stained highly positive for cathepsin X showing a significant difference to the staining of normal prostate glands. In contrast, only weak staining was observed for cathepsins F, B and L. Those results suggest that cathepsin X may play a role in early tumorigenesis of prostate cancer. Further studies are underway to define the utility of this protease as a diagnostic marker for early detection of prostate cancer.

Cathepsin B has been shown to play an important role in invasion and metastasis of prostate cancer. A recent study compared the serum levels of cathepsin B in healthy controls, in BPH patients and in prostate cancer patients [63]. Cathepsin B-density was calculated by dividing the serum levels of cathepsin B by the prostate volume. The mean values of cathepsin B and cathepsin B-density in prostate cancer were significantly higher than those in healthy controls and BPH patients. Moreover, the cathepsin B and cathepsin B-density levels in patients with metastatic disease were significantly elevated compared with those in patients without metastasis. However, there was no significant association between the elevation of cathepsin B and cathepsin B-density levels and cause specific survival in prostate cancer patients.

1) E-Cadherin

This is a cell adhesion molecule fragment (80kDa) with possible predictive value for prostate cancer. A recent study found a significant difference in the expression level of E-Cadherin in the serum of healthy individuals versus patients with BPH and between BPH versus localized prostate cancer and metastatic prostate cancer. ($P = 0.001$). Highest expression levels are observed in advanced metatstat-ic disease. The results showed in addition, that patients with an E-cadherin level of $>7.9 \mu g (l^{-1})$ at the time of diagnosis have a 55-fold higher risk of biochemical failure after surgery compared to those with lower levels. Using this cutoff, high expression at the time of diagnosis is associated with significantly increased risk of biochemical failure [64] A different study evaluated that the A-160 C -> A polymorphism in the promoter region of E-cadherin can decrease gene transcription, thus resulting in decreased E-cadherin expression in human prostate cancer. This finding is associated with low tumor grade and advanced clinical stage. They did not find any association between E-cadherin polymorphism and poor differentiation or invasiveness of prostate cancer.

Wu et al [65] evaluated the role of p53, bcl-2 and E-cadherin expression in the prediction of biochemical relapse for organ confined prostate cancer. P53 immunoreactivity was identified in 22.9%, only 4.3% expressed bcl-2. Aberrant E-cadherin expression was noted in 55.7%. At a median follow-up 30% experienced biochemical relapse. They noted that the biochemical failure rate in patients with abnormal bcl-2 and E-cadherin expression was significantly higher and the multivariate analysis showed that the parameters contributed independently to the prediction of PSA relapse ($P=0.017$ and 0.005, respectively). They concluded that bcl-2 and aberrant E-cadherin expression are independent factors predicting biochemical relapse in pT2 prostate cancers [66].

II. PSA AND PSA ISOFORMS

1. IS THE TOTAL PSA ERA COMING TO AN END?

The introduction of prostate-specific antigen (PSA) in the late eighties revolutionized the diagnosis, staging, and management of prostate cancer. No other technology has had such a profound effect on the diagnosis and management of prostate cancer. Only the development of the grading system devised by Donald Gleason, M.D. Ph.D. in the early seventies, has had a comparable impact on prostate cancer staging [1]. The widespread use of PSA as a screening tool has resulted in a marked stage migration over the last two decades [2, 3]. Because there have been no major breakthroughs in the treatment of prostate cancer over the last twenty years, most authors attribute the recent decline in prostate cancer-specif-
ic mortality to the use of PSA both as a screening and as a management tool for prostate cancer. Over the last two decades, an increasing amount of data has accumulated to support this hypothesis [2-4].

Although far from perfect, serum PSA testing in conjunction with digital rectal examination of the prostate (DRE) compares favorably with other cancer screening modalities. The positive predictive value (PPV) of a PSA above 4ng/mL is 30 to 40% [5]. An abnormal mammogram for example, has a PPV of 10 to 20% and is an order of magnitude more expensive than PSA [6].

Nevertheless, the widespread acceptance of PSA paradoxically has led to a marked decrease in its utility for both prostate cancer screening and staging. The average yearly rise in serum PSA concentration in men with either prostate cancer or benign prostatic hyperplasia (BPH) is low (i.e. 0.75ng/mL/year for cancer and 0.1-0.5ng/mL/year for BPH). Therefore, as a larger percentage of the population enters the pool of men being screened on a yearly basis, the average PSA of men above the commonly used cutoff of 4ng/mL moves ever closer to that cut off point [7, 8]. Unfortunately, because of the complex relationship between PSA, prostate cancer, and BPH, the clinical utility of PSA in this range for both staging and screening is modest at best. Therefore, PSA’s unprecedented impact and widespread adoption for the screening of prostate cancer has severely taxed its performance for both screening and staging. Nevertheless, the PSA era will not come to an end until a more specific screening marker and a more sensitive staging and management tool become commercially available.

2. Free PSA, A Complex Mixture of Molecules

PSA, also known as seminogelase, seminin, P-30, human kallikrein-3, and gamma-semiprotein, is a 33-kDA serine protease of the kallikrein family. It was first isolated from human serum in 1971, from human semen in 1978, and from prostate tissue in 1979 [9-11]. PSA is produced in clinically significant quantities by prostate luminal epithelial cells only. These cells secrete PSA into the seminal fluid where it degrades semenogelin I and II and fibronectin, the major gel-forming proteins of the human ejaculate, to allow the release of spermatozoa [12, 13]. PSA enters the circulation through an unknown mechanism reaching ng/mL concentrations 10^6 times less than its concentration in the seminal fluid [13]. Because it is produced by normal, hyperplastic, and neoplastic prostate epithelial cells, it is an organ-specific rather than a tumor-specific marker. Moreover, the presence or absence of prostate cancer is only one of the biological variables that influence the release of PSA into the circulation [14]. Because of these and other inherent, biologically determined drawbacks, PSA loses its specificity as a screening marker and its sensitivity as a staging tool when its serum concentration falls within 4 to 10ng/mL.

The quest for a better prostate cancer marker and the improvements in the technologies available for the study of proteins resulted in the identification of a growing number of distinct molecular forms of PSA. Shortly after the complementary DNA for PSA was cloned in 1987, it was observed that PSA isolated from human serum exists in either a bound (i.e. to α-1 antichymotrypsin) or unbound form [15, 16]. Serum concentrations of protease inhibitor-bound PSA, also known as complexed PSA (cPSA), were found to have a positive correlation with the presence of prostate cancer [16]. Conversely, serum concentrations of unbound, or free PSA (fPSA), as a fraction of total PSA, were found to have a negative correlation with the presence of prostate cancer [17]. Although the biological explanation for this phenomenon eludes researchers, it was theorized that the loss of tissue architecture that results from disorganized cancer growth facilitates the binding of inhibitors delivered by the circulatory system or produced locally within prostate tissue. The result is that the amount of cPSA released into the bloodstream is greater than that released by benign prostate tissue. In contrast, PSA produced by BPH is secreted into the seminal plasma and must leak back through the intercellular space, where it is exposed to proteases, before reaching the circulation. Because cleaved, inactive forms for PSA are unable to bind protease inhibitors, they remain unbound in the blood, comprising the fPSA fraction. Nevertheless, we now have evidence to prove that this hypothesis is not completely true because fPSA forms have been isolated that are released preferentially by prostate cancer [18].

Approximately 75% of measurable serum PSA is irreversibly bound to the protease inhibitor α-1 antitrypsin (PSA-ACT). A lesser fraction binds to α-2 macroglobulin (PSA-AMG) or α-1-protease inhibitor (PSA-API). PSA-AMG may retain part of its enzymatic activity, but neither PSA-ACT nor fPSA is enzymatically active [16]. α-1 antitrypsin has been shown to bind the active site of PSA and form a stable acyl-enzyme bond [19].
Like many other secreted proteases, PSA is produced in an inactive form as a “pre-pro” peptide. The amino-terminal-most 17-amino acids target the protein upon translation into the endoplasmic reticulum for secretion into the seminal spaces. These 17-amino acids define the “pre” peptide. The following 7 amino acids of the amino terminus define the “pro” peptide and maintain the enzyme in inactive form until it reaches the seminal spaces. Here, one or more enzyme, cleaves the pro-peptide sequence off, activating the now mature PSA [15] (Figure 1). Surprisingly, studies examining the actual amino terminal sequences of the various inactive forms of fPSA in the seminal plasma, serum, and normal, hyperplastic, and cancerous prostate tissue have found that various alternatively processed forms of fPSA have retained some or all of the “pro” sequence of amino acids. In addition, mature inactive forms of PSA with and without internal cleavages have also been identified. (Figure 2)

The following fPSA molecular forms have been identified in human serum: mature but inactive non-cleaved PSA, inactive zymogen forms, including -2, -4, -5, and -7 proPSA, and cleaved, also inactive, forms of otherwise mature PSA, including cleavages at Ile 1, Hist 54, Phen 157, Lys 145, and Lys 182 (Figure 2). Zymogen forms of PSA were first noted when PSA was expressed using recombinant DNA in otherwise non-PSA-expressing mammalian and insect cells [20-22]. The first two studies that examined human serum reported conflicting results. Noldus et al., who were the first to purify PSA from pooled human serum in search of PSA isoforms, found both inactive mature PSA and cleaved forms, but not zymogen forms [23]. In contrast, using immunoadsorption rather than gel filtration, Mikolajczyk et al. did find various zymogen forms of PSA, also known as proPSA, molecular forms [24] (Figure 2). The absence of proPSA in the Noldus study probably was caused by contamination with enzymatically active hK2. hK2 has been shown to cleave proPSA forms to generate mature PSA. The purified PSA fraction of fPSA in the Noldus study displayed both trypsin and chymotrypsin-like activity. PSA has only chymotrypsin-like activity, whereas hK2 has trypsin-like activity. Subsequent studies have confirmed the presence of proPSA molecular forms containing 2, 4, 5, or all 7 of the zymogen amino acids in the serum of patients with prostate cancer (18, 25). Recently, Mikolajczyk et al. showed that zymogen forms of PSA are more abundant in peripheral than in transition zone tissue and in prostate cancer than in normal peripheral zone [18] (Table 1).

Inactive fPSA isolated from BPH tissue and seminal plasma has only a limited amount of proPSA molecular forms. Conversely, inactive fPSA isolated from normal peripheral or normal transition zone tissue has only a limited amount of internally cleaved, mature fPSA [26] (Table 2). Inactivation of the fPSA in BPH tissue and seminal plasma is primarily the

### Table 1. Zone & Pathology–Specific Markers as a Fraction of Total Tissue PSA (18)

<table>
<thead>
<tr>
<th>Zone/Pathology</th>
<th>TZ-N (PV&lt;25cc)</th>
<th>TZ-BPH (PV&gt;50cc)</th>
<th>PZ-N</th>
<th>PZ-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPSA</td>
<td>4.1 (mean %) N=8</td>
<td>11.4 (mean %) N=10</td>
<td>3.7 (mean %) N=18</td>
<td>4.3 (mean %) N=18</td>
</tr>
<tr>
<td>-2,-4proPSA</td>
<td>0.4 (mean %) N=8</td>
<td>1.7 (mean %) N=10</td>
<td>3.9 (mean %) N=18</td>
<td>5.6 (mean %) N=18</td>
</tr>
<tr>
<td>Inactive PSA</td>
<td>40.1 (mean %) N=8</td>
<td>38.0 (mean %) N=10</td>
<td>36.6 (mean %) N=18</td>
<td>36.2 (mean %) N=18</td>
</tr>
</tbody>
</table>

TZ-N = normal transition zone tissue, TZ-BPH = transition zone with BPH, PZ-N = normal peripheral zone, PZ-C = peripheral zone with cancer

### Table 2. BPSA as a fraction of total protein (26)

<table>
<thead>
<tr>
<th>Zone</th>
<th>TZ-N (median ug/mg of total protein)</th>
<th>TZ-BPH (median ug/mg of total protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPSA</td>
<td>0.17 N=8</td>
<td>2.0 N=10 P&lt;0.005</td>
</tr>
</tbody>
</table>
| TZ-N = normal transition zone tissue, TZ-BPH = transition zone with BPH

97
Figure 1. Production of Complzx PSA PSA is produced as a prepropeptide. The leader, "pre" amino acid sequence directs the nascent protein into the endoplasmic reticulum (ER) where a signal peptidase cleaves off the pre amino acids to produce -7proPSA. An unknown enzyme cleaves the pro amino acids to produce mature PSA. Once it reaches the circulation, mature active PSA will bind α-1-antichymotrypsin.

Figure 2. Molecular forms of free PSA. -7proPSA can be processed by an unidentified enzyme(s) to -2proPSA, -4proPSA, or -5proPSA. Inactive mature PSA (without pre or pro amino acids) is incapable of binding α-1-antichymotrypsin. Mature, inactive free PSA can have a variety of internal cleavages or be in intact form. BPSA is a form of mature inactive free PSA that is cleaved at Lys 145 and Lys 182.
result of internal cleavages [18, 26-28]. Although a variety of internal cleavage sites have been noted, the most abundant cleaved form of fPSA from BPH tissue is cleaved at Lys 145 and Lys 182 [26]. This mature, enzymatically inactive form of fPSA has been dubbed BPSA. BPSA comprises about one third of the absolute fPSA in serum [29].

3. Clinical application of Free PSA and Complexed PSA

PSA has at least five epitopes. Binding to all five epitopes is hindered by the interaction of PSA with AMG. Conventional total PSA assays, therefore, detect both fPSA and cPSA (i.e. PSA-ACT) but not PSA-AMG. Two of PSA’s five epitopes are inaccessible to antibodies when the molecule is bound to α-1 antichymotrypsin. Commercial fPSA assays are based on antibodies that specifically bind to the epitopes that are hidden when PSA is bound to ACT. The only cPSA assay approved by the U.S. Food and Drug Administration is based on antibody-mediated elimination of all fPSA followed by measurement of the remaining ACT-bound PSA (Bayer Innumo1 cPSA Assay, Bayer Diagnostics, Tarrytown, NY). There are at least three %fPSA assays approved for prostate cancer screening. Because the performance of these assays varies, the established cutoffs for each specific assay should be used.

a) Screening.

The use of fPSA, expressed as a percent of total PSA, %fPSA (i.e. 100 x fPSA/total PSA), as an adjunct to PSA and DRE-based prostate cancer screening has gained significant acceptance since its FDA approval. %fPSA is approved for the risk stratification of patients with a normal DRE and PSA in the diagnostic grey zone of 4-10ng/mL. The pivotal study that lead to FDA approval was published in 2000 by a multicenter group. Using the Tandem-E fPSA assay, Catalona et al. found that a fPSA cutoff of 25% could reduce the number of unnecessary biopsies by 20% while still detecting 95% of the cancers that would have been diagnosed had all patients with a PSA between 4 and 10ng/mL been biopsied. This study showed conclusively that the risk of prostate cancer decreases as the %fPSA rises and that the use of %fPSA could improve the specificity of a PSA-based prostate cancer-screening program [30].

With the recognition that up to 25% of men with a negative DRE and a PSA between 2.6 and 4ng/mL may have prostate cancer, studies were carried out to evaluate %fPSA for its ability to improve the specificity of PSA-based prostate cancer screening in this setting. Using the Tandem-E fPSA assay, Catalona et al. reported an 18% reduction in the number of unnecessary biopsies while maintaining a sensitivity of 90% when patients with a %fPSA below 27% and a total PSA between 2.6 and 4ng/mL were biopsied (31). In this study, 83% of the cancers diagnosed met criteria for clinical significance. Another large study using the Abbott AXSYM free PSA assay also found that %fPSA can improve the performance of PSA-based screening in men with PSA between 3 and 4ng/mL. When a %fPSA cutoff of 19% was applied, 90% of cancers were detected and 44% of the biopsies performed were positive for cancer [32].

The cPSA assay was developed with the goal of replacing both PSA and %fPSA. Even though early data suggested that cPSA by itself (not in a ratio to total PSA) could perform as well as %fPSA, large studies failed to confirm this finding. The largest multicenter study of cPSA showed that only when the range of total PSA was restricted to below 6ng/mL did cPSA perform as well as %fPSA. For the 2 to 10ng/mL range, only in a ratio to total PSA did cPSA perform as well as %fPSA [33]. Because it offers no significant improvement over %fPSA even when in a ratio to total PSA, cPSA has failed to gain widespread use.

b) Staging.

The data available on the use of %fPSA for prostate cancer staging, unlike that on its use for prostate cancer screening, is inconclusive. Three large studies have evaluated the potential of %fPSA in prostate cancer staging. Only one of the three studies found %fPSA to be a stronger predictor than commonly used parameters such as Gleason grade and PSA. This large multicenter study evaluated 268 men with no palpable tumors and total PSA between 4 and 10ng/mL who underwent radical prostatectomy. A value of 15% or less was found to correlate with unfavorable final pathology [34]. Nevertheless, two later studies found that %fPSA had a predictive value comparable to that of serum PSA and biopsy Gleason score in univariate analyses, although it failed to provide additional staging or prognostic information in multivariate analyses that included clinical stage, Gleason score, and PSA [35, 36].

Analysis of three recent studies evaluating cPSA as a staging tool for prostate cancer shows that in none of the studies did cPSA remain an independent predictor of final pathologic stage when Gleason score and PSA were included as predictors. In all three studies, cPSA provided the same staging information as did PSA. When evaluated in a ratio to total PSA, the performance of cPSA actually declined [37-39].
Although the use of %fPSA is far more common than the use of cPSA, %fPSA is not without drawbacks. The performance of %fPSA decreases when prostate size exceeds 40cc. In these larger prostates, %fPSA has been shown to increase even in the presence of cancer [40]. fPSA is less stable than cPSA under the commonly used storage conditions of 4 to –20°C. For this reason, it is recommended that specimens that will not be processed within 8 hours for fPSA be stored at –70°C. In contrast, loss of immunoreactivity of cPSA can be prevented by storage at –20°C. [41]. Finally, compared to fPSA, cPSA concentration has been shown to be less susceptible to changes associated with manipulation of the prostate such as DRE and cystoscopy [42].

Although the molecular mechanisms behind the production of free and bound forms of PSA still are not completely understood, it has been observed that, at least in practical terms, the utility of %fPSA depends on the correlation between fPSA and prostate volume. Large studies have clearly shown that, in patients without prostate cancer, serum PSA concentration correlates with prostate volume. This correlation has been shown to be age dependent [43]. In the presence of prostate cancer, however, this correlation no longer holds true, presumably because of the significant impact that prostate cancer has on serum PSA levels. In contrast, recent studies demonstrate that serum PSA (the absolute fPSA value, not in a ratio to PSA) concentration is proportional to prostate size, independent of age, and is maintained even in patients with prostate cancer [29]. In light of these observations, a simplistic, yet probably accurate, understanding of %fPSA is that it represents a serologic equivalent of PSA density (PSA/prostate volume), albeit inverted. In the absence of a serum marker known to be expressed only by prostate cancer, it is likely that the next generation of PSA-based screening strategies will continue to depend on ratios of markers that correlate with prostate volume and markers that correlate with the presence of prostate cancer. Indeed, as we review recent discoveries relating to the various molecular forms of fPSA, the concept of serologic equivalent of PSA density will take center stage.

4. CLINICAL APPLICATION OF FREE PSA MOLECULAR FORMS

Although the introduction of %fPSA has only modestly improved our effectiveness in screening prostate cancer, the clinical success of this assay renewed the urologic community’s interest in the study of molecular forms of PSA. Recently, fluorogenic immunoassays have been developed that specifically recognize the various proPSA forms as well as the major cleaved fPSA form, known as BPSA. Using recombinant fusion protein products containing the various amino-terminal sequences of the known proPSA forms fused to hK2, Mikolajczyk et al. developed four proPSA assays currently available for research use only. These include a –2 proPSA assay, a –4 proPSA assay, a combined –5 and –7 proPSA assay and a pan-proPSA assay. The combined –5 and –7 proPSA assay detects both –5 and –7zymogen forms. The pan-proPSA assay recognizes all proPSA forms [24].

BPSA consists of mature PSA that has been inactivated by cleavages at Lys 145 and Lys 182. The cleavage at Lys 182 generates a conformational change in this form of fPSA allowing for the selection of antibodies specific for fPSA forms with this internal cleavage. Unfortunately, the antibodies generated against BPSA detect fPSA molecular forms with a cleavage at Lys 182 only, as well as those with cleavages at both Lys 182 and Lys 145. Nevertheless, tissue studies have demonstrated that the ratio of true BPSA (cleaved at both Lys 182 and Lys 145) to fPSA cleaved only at Lys 182 is relatively fixed. Therefore, it is expected that the available assay for BPSA (which measures both fPSA cleaved at Lys 182 only and fPSA cleaved at Lys 182 and Lys 145) overestimates by a fixed proportion the true concentration of BPSA [44].

Understanding the ratios of fPSA molecular forms currently under study requires that the relationship between these various forms and both prostate cancer and BPH be made very clear. As noted above, the absolute concentration of fPSA correlates with prostate volume in both biopsy-negative and biopsy-positive patients. Nevertheless, we now know that fPSA is composed of both relatively cancer-specific and BPH-specific molecular forms. (Figure 3 & 4) The cancer-specific forms arezymogen forms of PSA and are, therefore, known as –2, –4, –5 and –7proPSA. The BPH–specific fPSA forms are exemplified by BPSA. If fPSA is composed of both cancer-specific and BPH-specific molecular forms and %fPSA is, indeed, a serologic equivalent of PSA density, then subtraction of the cancer-specific molecular forms from fPSA should improve the performance of %fPSA. On the other hand, subtraction of the BPH-specific form from fPSA should negatively affect its performance. Both of these hypotheses have been proven correct [45].
Early data showed that absolute values of the zymogen forms of PSA could not discriminate between patients with and without cancer, probably because proPSA forms are made by both normal and neoplastic peripheral zone tissue (Table 1). Given the success of %fPSA, it would be reasonable to evaluate ratios of relatively cancer-specific markers such as proPSA, PSA and cPSA to markers that correlate with prostate volume, such as fPSA and BPSA, in order to generate new serologic PSA density equivalents. Both our data and that of others support –2proPSA/fPSA as the best marker for prostate cancer in men with PSA below 10ng/mL [45].

a) Screening

The clinical evaluation of these recently discovered molecular forms of fPSA has been facilitated by the availability of stored banks of serum generated for studies evaluating both PSA and %fPSA. It has been shown, using these stored serum samples, that proPSA forms, particularly –2proPSA/fPSA, modestly, yet consistently, outperform %fPSA in the diagnostic grey zone of 4-10ng/mL PSA. In a study involving just over 1000 archived specimens, Catalona et al. found that either –2proPSA or pan-proPSA in a ratio to fPSA outperformed both %fPSA and cPSA in the 4-10ng/mL total PSA range. In this range of PSA, while maintaining a 90% sensitivity, pan-proPSA/fPSA spared 21% of unnecessary biopsies while %fPSA spared only 13% and cPSA spared 9% (P<0.0001), a modest, yet statistically significant improvement [46]. Mikolajczyk et al. studied 380 serum samples and also found that –2proPSA/fPSA modestly outperformed both %fPSA and cPSA. At a 90% sensitivity, -2proPSA/fPSA maintained a specificity of 21% compared with 20 and 19% respectively for %fPSA and cPSA [47].

Studies focusing on the 2-4ng/mL total PSA range have found a more robust improvement in the performance of PSA-based screening when including proPSA molecular forms of fPSA in the screening strategy. A small, initial study, including only 119 men with PSA between 2.5 and 4ng/mL, found that the AUC for proPSA/fPSA was larger than that of %fPSA (0.688 vs 0.567) [48]. This trend was confirmed in the previously mentioned, much larger study by Catalona and associates. In the 2-4ng/mL PSA range, -2proPSA/fPSA had a specificity of 19% at 90% sensitivity compared to 10 and 11% respectively for %fPSA and cPSA [46].
With the goal of further improving the specificity of PSA and fPSA-based prostate cancer screening, attention has been extended from the diagnostic grey zone of total PSA between 4 and 10ng/mL to the low-specificity range defined by %fPSA of less than 15%. Patients with a %fPSA of less than 15% have a probability of a positive biopsy of between 30 and 50%. Khan et al. showed that the use of proPSA/BPSA can further reduce by about half the number of unnecessary biopsies in patients with %fPSA below 15% and PSA between 4 and 10ng/mL while maintaining a 90% sensitivity [49]. Comparable results were noted by Mikolajczyk et al. using pan-proPSA/fPSA in patients with %fPSA below 15%. A 90% sensitivity was maintained and about 1/3 of the unnecessary biopsies could have been avoided [47].

b) Staging.

Data on prostate cancer staging using the various molecular forms of fPSA are less abundant than those for screening. This is due primarily to the need for surgery in order to obtain a pathologic stage. Nevertheless, an initial exploratory study involving a cohort of 62 consecutive prostatectomies from Baylor showed that the absolute pre-operative levels of proPSA forms correlated with tumor volume and percent positive biopsy, and predicted extracapsular extension as well as risk of recurrence based on the stratification method defined by D’Amico et al. These associations remained significant after adjustment for the effects of total PSA, clinical stage, and biopsy Gleason score. (50)

In a larger study, Catalona et al. confirmed these findings. They showed that -2proPSA/fPSA outperformed both cPSA and %fPSA in the prediction of final pathological Gleason score greater or equal to 7 and/or extracapsular extension or a cancerous surgical margin. This finding held true for pre-operative PSA values between 2 and 4ng/mL and between 4 and 10ng/mL. In the total PSA of 2-4ng/mL group, -2proPSA/fPSA had a specificity of 19% for detection of aggressive tumors at 90% sensitivity, while cPSA and %fPSA had 11 and 10% specificity, respectively. In the total PSA of 4-10ng/mL group, -2proPSA/fPSA had a specificity of 31% for detection of aggressive tumors at 90% sensitivity while cPSA and %fPSA had 19 and 20% specificity respectively [51].

5. FREE PSA MOLECULAR FORMS IN THE MANAGEMENT OF PROSTATE CANCER

In addition to screening and staging, molecular forms of fPSA may be useful in management and detection of recurrence after definitive therapy for prostate cancer. Both radiation and radical prostatectomy are associated with a low, yet significant, risk of recurrence over the 10-15 year period after primary therapy even in the best of circumstances. Recurrence after either radiation or prostatectomy raises a number of important clinical questions. With either therapy, it may be important to determine if the recurrence is local or due to metastatic disease. From the patient’s point of view, prognosis at the time of recurrence is of paramount importance. Although the clinical situation may vary greatly, a number of clinical and pathologic parameters may be used to direct further care and determine prognosis. However, there is still a need for serum markers capable of distinguishing between aggressive, likely metastatic, recurrence and indolent, likely local, recurrence.

The study of serum markers for evaluation of prostate cancer recurring after primary therapy is hindered by the need for long periods of follow-up and the involvement of additional practitioners other than the primary urologist in the patient’s care. Nevertheless, although data are not yet available, studies are ongoing at Baylor to evaluate the use of molecular forms of fPSA and their ratios in the setting of post-prostatectomy PSA recurrence.

6. CONCLUSION

Serum PSA has become the most commonly used cancer test. Its success has fueled intense research in the field of molecular forms of PSA in both academia and industry. As a result, our understanding of the various molecular forms of PSA, particularly fPSA, has grown exponentially over the last two decades. The specific role in the screening, staging, and management of prostate cancer that one or more of the fPSA molecular forms will play in the near future is yet to be defined. Nevertheless, it is very likely that one or more of these new molecular forms of fPSA will improve our ability to screen, stage, and/or manage prostate cancer and thereby prolong the PSA era.
1. INTRODUCTION

Prostate cancer (PCA) is the most common non-cutaneous cancer of men in the United States, and an estimated 232,090 new cases of PCA will be diagnosed in 2005 [1]. In the benign prostate, the glandular epithelium consists of two cell layers, the basal layer and the differentiated luminal secretory layer. Prostatic intraepithelial neoplasia (PIN), characterized by nuclear and architectural changes in luminal epithelial cells with maintenance of the basal epithelium and basement membrane, is an accepted precursor lesion of frank adenocarcinoma. Retrospective studies have suggested that by the age of 80, greater than 50% of American men have some cancer in their prostate[2], yet most men shown no symptoms and clinical treatment is not needed. However, some prostate tumors are highly aggressive and quickly spread locally and metastasize throughout the body. While surgical resection of clinically localized PCA is often curative, no effective therapeutics exist for metastatic disease which becomes refractory to anti-androgen treatments. The introduction of testing for prostate specific antigen (PSA) in serum has impacted the diagnosis and management of PCA by leading to an increased number of cancers being diagnosed at an early stage and it is the only FDA approved biomarker for the detection of PCA. However, increased PSA levels are not specific for PCA and recently the value of PSA testing has been questioned [3]. Thus, sensitive and specific biomarkers that could predict response to these two pharmaceutical classes would be valuable as many patients do not respond to specific treatments and at present they cannot be prospectively identified [13].

In an effort to identify biomarkers of prostatic disease, several techniques have been utilized, including techniques characterizing proteomic, genomic and transcriptomic changes during disease progression. Techniques used to identify biomarkers through analyses of differential gene expression, including mining of the expressed sequence tag (EST) database, serial analysis of gene expression (SAGE), subtractive hybridization, differential display and DNA microarrays, will be the focus of this review. After introducing each of these technologies, specific candidate biomarkers identified by the technique will be discussed. Critical analysis of these candidates suggests that several can be developed as biomarkers to influence diagnostic and prognostic treatment decisions. The use of DNA microarrays to identify biomarkers of prostatic disease will be highlighted.

2. EXPRESSED SEQUENCE TAG (EST) DATABASE MINING

Several studies have mined the expressed sequence tag (EST) database (http://www.ncbi.nlm.nih.gov/dbEST/index.html) in an effort to identify genes specifically expressed in the prostate or differentially expressed between benign and cancerous prostate tissue [14-20]. ESTs are short (approximately 500 bp) single-pass sequencing reads from mRNA converted into complementary DNA (cDNA) clones. The collection of ESTs from a cDNA library is a static representation of genes expressed in a specific tissue or disease state. For example, by examining the cellular abnormalities that contribute to the pathogenesis of this disease remain unknown. To date, the precise etiology of BPH is unclear and a number of factors have been implicated its pathophysiology. Although BPH is a benign neoplastic process, it results in urinary obstruction in nearly 50% of patients [7] and has a major impact on the quality of life of most aging men [8]. Thus, identification of biomarkers that could identify which patients are likely to develop symptomatic BPH would help to guide treatment decisions. Besides surgery, men with symptomatic BPH are also commonly treated with two pharmacological treatments, a-adrenergic-receptor antagonists (i.e., tamsulosin and alfuzosin) or 5-a-reductase inhibitors (i.e. finasteride and dutasteride) [9-12]. Thus, sensitive and specific biomarkers that could predict response to these two pharmaceutical classes would be valuable as many patients do not respond to specific treatments and at present they cannot be prospectively identified [13].
EST database for transcripts specifically and highly expressed in the prostate, Essand and colleagues identified the T-cell receptor gamma chain (TRG?) as being highly expressed in prostatic epithelial cells [14]. Several subsequent microarray profiling studies have confirmed the over-expression of TRG? or TARP [21], which encodes a protein from an alternative reading frame within the TCR? locus, in PCA compared to normal prostate tissue. Cysteine rich secretory protein-3 (CRISP-3) was also identified in a separate analysis of the EST database by Asmann and colleagues as a transcript over-expressed in cancerous compared to normal prostate tissue [19]. In subsequent work, they found CRISP-3 to be secreted after transient transfection in HEK293 cells and confirmed CRISP-3 over-expression in PCA using quantitative real-time PCR (QPCR) [22]. Despite these promising results, there has not been a report of CRISP-3 expression in tissue sections comparing benign and cancerous prostate or in serum from PCA and control patients. A number of other transcripts with varying degrees of specificity for the prostate and prostate tissue in the EST database have also been described, including PATE20, PRAC18, PRAC217 and GDEP16.

3. SERIAL ANALYSIS OF GENE EXPRESSION (SAGE) PROFILING

Serial analysis of gene expression [23], a technique where short (10-15mer) tags from cDNA transcripts are linked into concatemers which when sequenced give a quantitative relationship of the expression profile of the given tissue, has also been used to identify potential biomarkers of prostatic disease [24-26]. For example, Waghry and colleagues performed SAGE analysis from matched normal and tumor samples pooled from four patients [24]. They identified 156 differentially expressed genes (P < 0.05), including over-expression of E2F4 in PCA, which they validated using immunohistochemistry (IHC) of E2F4 protein expression on five tissue sections.

4. SUBtractive HYBRIDIZATION AND DIFFERENTIAL DISPLAY

Several potential biomarkers of prostatic disease have been identified through application of two techniques, subtractive hybridization and differential display, that were developed to identify genes differentially expressed between two samples, conditions, or disease states. Differential display uses a limited number of short arbitrary primers in combination with anchored oligo-dT primers that bind to a subset of mRNA transcripts to convert mRNA to cDNA and amplify the products. These amplified products from two different tissues or conditions are then separated by electrophoreses and bands that are differentially displayed are further characterized [27]. Although there are numerous variations, subtractive hybridization relies on competitive hybridization of nucleic acids from two samples to subtract transcripts present in both samples. For example, from mRNA isolated from two samples to be compared, one population can be converted to cDNA which is subsequently hybridized to the mRNA from the first sample. The hybridized cDNA/mRNA is removed, resulting in cDNA for characterization that is only present in one of the samples.

Although several genes identified through differential display have been proposed as biomarkers of prostatic disease, including POVI28, PTOV-129, HPG-130, PTI-131, and PCGEM132, this review will highlight PCA3 (DD3) which shows great promise as a biomarker of PCA. One of the most prostate specific transcripts described to date, PCA3, which also shows over-expression in PCA compared to benign tissue, was initially identified through differential display analysis [33]. Interestingly, the authors noted a high density of stop codons in all reading frames, suggesting that PCA3 is a noncoding mRNA transcript. During the initial identification, a 10-100 fold over-expression of PCA3 in cancerous areas compared to adjacent benign tissue was observed in 53 of 56 tissues as assessed by Northern blot analysis [33]. In follow up work using QPCR to assess PCA3 expression, a median 34-fold over-expression in cancerous tissue compared to benign tissue was determined. Although low levels of PCA3 were expressed in normal prostate tissue, no expression could be determined in 21 other normal tissues, blood, or 39 non-prostate tissues [34]. In an effort to assess PCA3 as a biomarker of PCA, Hessel and colleagues assessed PCA3 expression using QPCR from urine sediments obtained after prostatic massage from 108 men with a serum PSA value > 3ng/ml. Of the 24 men with PCA upon biopsy, 16 were PCA3 positive by QPCR (67% sensitivity, 90% negative predictive value) [35].

Using an alternative assay, uPM3, which simultaneously detects the relative expression of PCA3 and PSA as a marker for prostate cells in urine, Tinzl and colleagues analyzed urine samples obtained from prostatic massage before biopsy from 201 patients. Out of 201 urine samples analyzed, 158 contained enough prostate cells sufficient for PCA3 analysis by
PSA expression (79% adequacy rate). PCA was found in 62 (39%) of the evaluable patients, and the uPM3 assay at a cut-off of 0.5 probability had a sensitivity of 82%, a specificity of 76%, a positive predictive value of 67% and a negative predictive value of 87% [36]. The uPM3 assay was further validated in a multi-center study on urine sediment obtained after prostate massage from 517 patients undergoing biopsy. 443 of the 517 sediments (86%) had an assessable sample. In this study, the overall uPM3 sensitivity, specificity, positive predictive value and negative predictive value was 66%, 89%, 75% and 84% respectively, with an overall accuracy of 81%, compared with 43% and 47% for total PSA at 2.5 and 4.0 ng/mL cutoffs, respectively [37]. A pilot study has also demonstrated that PCA3 mRNA can be obtained inconsistently from blood after invasive treatment of the prostate in patients with PCA, however no blood samples obtained before treatment contained PCA3 mRNA [38].

In summary, multiple studies using different techniques to quantify PCA3 expression in urine sediments obtained after prostatic massage have obtained sensitivities of 67%, 82% and 66%, with negative predictive values of 90%, 87% and 84%, superior to total PSA testing. As PCA3 appears to be a noncoding transcript, these results can not be translated to antibody based tests for assessing protein expression, and obtaining adequate mRNA from urine can be problematic. In an effort to circumvent this difficulty, a second generation test measuring PCA3 mRNA relative to PSA mRNA is being developed. Results from two unpublished studies presented at the American Association for Clinical Chemistry’s Oak Ridge Conference in 2005 suggest that 100% of urine samples obtained after prostatic massage contained adequate PCA mRNA for analysis, and the sensitivity and negative predictive value of the test in whole urine samples was 66% and 73% respectively. Although these results should be interpreted cautiously until publication, the potential to use PCA3 expression in combination with PSA expression from urine as a biomarker for PCA looks promising.

Studies using subtractive hybridization techniques have also identified genes which have been proposed as candidate biomarkers for prostatic disease including Annexin II (ANXA2) [39], STEAP [40], STEAP2 [41], and KLK4 (prostate) [42-44]. For example, KLK4, a member of the same kallikrein family as PSA, was identified as a prostate specific marker through subtractive hybridization [42]. As an initial investigation into the potential of KLK4 as a biomarker, Day and colleagues analyzed serum for the presence of antibodies to KLK4, with 7 of 20 patients with PCA but 0 of 13 normal donors having antibodies present [43]. In addition to larger studies being needed to fully evaluate the potential of KLK4 as a biomarker, as a member of the same family as PSA, KLK4 may face similar problems as a biomarker and there is controversy over whether the mature KLK4 protein is secreted [44-45]. Nell2, a potential biomarker of BPH, was first associated with BPH through cDNA subtraction hybridization [46] and has subsequently been identified from DNA microarray studies profiling BPH and will be discussed below. ?-methylacyl-CoA racemase (AMACR, also known as P504S), which has demonstrated enormous potential as biomarker of PCA in different body compartments, was also initially identified through a combination of subtractive hybridization and DNA microarrays [47]. and will be described below.

5. DNA MICROARRAYS

The introduction of DNA microarrays, which allow for the monitoring of expression changes across the entire transcriptome of samples of interest, has revolutionized cancer research. DNA microarrays are based on the arrangement of thousands of cDNA clones or oligonucleotides (“probes”) on a solid support, with features commonly identified by their location. Isolated RNA from samples of interest are converted to complementary DNA (cDNA) or antisense RNA (aRNA), or “targets”, that are labeled with a fluorescent tag. The target is allowed to hybridize to the array and the microarray is scanned, with the fluorescence of each spot proportional to the amount of target hybridized. By profiling a large number of samples (i.e., PCA and benign prostate tissue samples) expression patterns across the groups can be identified. Due to the massive amounts of data obtained during microarray experiments, numerous bioinformatic tools have been developed for data storage, normalization and analysis, which can profoundly influence the final conclusions drawn from a microarray study and need to be carefully considered [48-50].

Different microarray technologies have emerged, which have important effects on experimental design and complicate comparisons between profiling studies. On the first microarrays developed, cDNA microarrays, each probe is a cDNA clone representing an individual transcript amplified by PCR [51-52]. These probes are much longer than those on
oligonucleotide arrays and importantly, two samples are usually hybridized competitively to the array. For each experiment, cDNA from an experimental sample is fluorescently labeled with one tag and cDNA from a control sample is labeled with a different tag. The array is scanned to excite both fluorescent tags, and analysis programs quantify the ratio of experimental to control fluorescence for each feature, giving a measure of transcript abundance in the experimental sample compared to the control sample. An alternative microarray platform was developed by Affymetrix, whose GeneChip system uses millions of 25-mer oligonucleotide probes, each designed to hybridize to a particular part of a transcript [53-54]. These probes are synthesized in situ using photolithography onto silicone wafers. Importantly, aRNA from a single sample is hybridized to each oligonucleotide array. Other commercial suppliers offer microarrays in a variety of formats with distinct advantages and limitations [55]. As an example, Agilent offers 60-mer oligonucleotide arrays printed using inkjet technology, which can be used for hybridizations with or without a control sample [56]. Because of the differences in technology and experimental design, output profiles obtained from different arrays are often difficult to compare directly [57]. This is a crucial process for selecting potential biomarker candidates for validation since hundreds to thousands of probes are often dysregulated between samples or groups of sample. Our group has developed a meta-analysis method to compare microarray studies from different platforms, which we applied to four of the earliest prostate profiling studies from cDNA and oligonucleotide microarrays to identify genes consistently dysregulated across the data sets [58]. Several of the genes identified in the meta-analysis as being consistently dysregulated in PCA, such as TCR?, AMACR and Hepsin (HPN), have been identified in almost every subsequent study or were identified through other techniques as described above and likely represent potential biomarkers as well as genes involved in PCA biology. We have also developed a web-based resource, Oncomine (www.oncomine.org), to catalogue expression profiling studies and allow data mining across multiple studies [59]. To demonstrate the power of Oncomine for identifying candidate biomarkers, we provide a meta-analysis of genes over-expressed in cancerous prostate tissues compared to benign prostate across seven large scale profiling studies in Figure 1 that was generated by Oncomine.

In this review, we will focus on several aspects of DNA microarray studies as they relate to the identification of biomarkers of prostatic disease. Three genes which have been consistently identified as being dysregulated across profiling studies comparing PCA to benign prostate tissue or studies comparing localized PCA to metastatic PCA and subjected to follow up investigations as potential biomarkers, AMACR, HPN and EZH2 will be described in detail. In addition, both individual genes and genetic signatures that have potential as biomarkers will be
described in the context of the individual profiling studies that demonstrate important aspects of experimental design for biomarker identification. An overview for the identification of biomarkers of prostatic disease from DNA microarray profiling studies is demonstrated in Figure 2.

6. HEPsin

Marked over-expression of the type II serine protease hepsin (HPN) has been demonstrated in almost every DNA microarray study profiling PCA [58]. HPN mRNA is up-regulated in ~90% of prostate tumors, with expression confined to epithelial cells. HPN protein expression is strongest at the PIN stage and decreases during the transition to metastatic cancer. Importantly, the intensity of HPN protein expression in localized tumors has been inversely correlated with PSA recurrence after surgical treatment [60]. However other studies have found that HPN mRNA is highest in high grade and stage tumors, although metastatic tissues were not examined [61,62]. Whether HPN or antibodies to HPN can be identified in serum or urine from patients with PCA is under investigation. Nevertheless, the consistency and magnitude of HPN over-expression in PCA suggest that HPN may be incorporated into different biomarker assays for PCA.

7. AMACR

The enzyme α-methylacyl-CoA racemase (AMACR), which is involved in peroxisomal α-oxidation of dietary branched-chain fatty acids, was originally identified in the context of PCA through subtractive hybridization and microarray analysis [47]. The functionally active protein is specifically over-expressed in PCA epithelium compared to benign epithelium and DNA microarrays consistently reveal AMACR to be one of the most up-regulated transcripts from normal to cancerous tissue [58,63,64]. Results from the initial microarray studies identifying AMACR have led to great interest in the utility of AMACR as a biomarker of PCA. Several large scale studies from multiple institutions have demonstrated sensitivities and specificities for diagnosing PCA by AMACR protein expression on tissue sections between 90-100%, demonstrating the usefulness of this marker in the workup of PCA [65-70]. Several reports have extended these results demonstrating that the addition of AMACR staining is useful for difficult to diagnose needle biopsy samples [71-76]. The specific staining of PCA compared to benign adjacent prostate tissue is demonstrated in Figure 3.

In addition to use as a diagnostic marker, several groups have analyzed AMACR as a potential biomarker for PCA from different body compartments. Although attempts to detect AMACR specifically in serum from PCA patients have been unsuccessful, Sreekumar and colleagues demonstrated that an immune response to AMACR has the potential to be used as a serum biomarker for PCA. Sera from patients with biopsy-proven PCA and from control patients were screened for a humoral immune response to AMACR using protein microarrays (46 patients, 28 controls), high-throughput immunoblot analysis (151 patients, 259 controls) and ELISA (54 patients, 55 controls). All three assays showed significantly higher immunoreactivity against AMACR in serum from patients with PCA than in control patients. In subjects with intermediate PSA levels (4-10 ng/mL), the immune response as assayed by high-throughput immunoblot was more sensitive and specific than PSA in distinguishing PCA patients than controls (sensitivity and specificity of 77.8% and 80.6% versus 45.6% and 50%, respectively). Using receiver operating characteristic curves, the area under the curve was 0.789 for AMACR immunore-
Figure 2. Schema of the utility of DNA microarrays for identifying biomarkers of prostate cancer (PCA), from discovery to candidate selection to validation. Careful selection of tissues representing different stages of PCA progression (benign epithelium (NOR), PIN, localized PCA and metastatic PCA (MET)) is crucial for successful biomarker identification. Expression signatures of selected tissue classes are generated using DNA microarrays analysis. As microarray profiling studies often generate hundreds to thousands of dysregulated genes, careful selection of candidate biomarkers is paramount. One technique to select candidates that show consistent dysregulation across multiple studies is through meta-analysis. Identified candidates can then be evaluated as biomarkers across numerous biocompartments including prostate tissue, urine and blood using techniques such as immunohistochemistry, Western blotting and QPCR.
activity versus 0.492 for PSA [77]. Rogers and colleagues used Western blot analysis to assay for AMACR in voided urine obtained after prostate biopsy from 26 consecutive patients. AMACR was detected in the urine in all 12 patients with biopsy confirmed PCA, in 5 of 12 with no evidence of cancer on biopsy and in 1 of 2 with atypia on biopsy. Overall, AMACR detection was associated with PCA status by biopsy in 86% of patients [78]. Ziele and colleagues performed an analysis of AMACR transcript expression relative to PSA transcript expression by QPCR in urine obtained after prostatic massage from 21 patients, similar to the techniques described above for PCA3. After imposing a diagnostic cutoff of 2 standard deviations above the median AMACR/PSA level, 9 of 9 patients without PCA (7 healthy men and 2 with BPH) were below the cutoff and 7 of 10 with PCA and 2 of 2 with high grade PIN were above the cutoff. Additionally, 2 of the 3 false-negative patients showed clinically insignificant disease [79]. All of these studies require large scale confirmation and their clinical usefulness has yet to be demonstrated. Nevertheless, the case of AMACR demonstrates the power of DNA microarrays to identify potential biomarkers of PCA, as AMACR represents the first biomarker identified through DNA microarray studies to improve the diagnosis of PCA.

8. EZH2

Although most of the early PCA profiling studies focused on identifying genes differentially expressed between benign prostate tissue and PCA, two studies from our group also profiled 20 metastatic PCA samples in addition to 22 benign prostate tissues (BPH and normal adjacent tissue) and 59 localized tumors [60,80]. Using significance of microarray (SAM) [81] analysis, we identified EZH2 as the most over-expressed gene between metastatic PCA compared to localized PCA samples. In addition to acting as a histone methyltransferase responsible for gene silencing, EZH2 has also been shown to be regulated by the pRB/E2F pathway and is essential for proliferation in several cell types [80,82-85]. Using IHC on tissue microarrays (TMAs), we demonstrated that EZH2 protein expression increased from benign prostate tissue to localized PCA to metastatic PCA samples. Importantly, in a multivariate Cox model including surgical margin status, tumor size, Gleason score and pre-operative PSA, increased EZH2 protein expression was the best predictor of recurrence (P = 0.02) [80]. Representative protein expression of EZH2 across prostate tissues is shown in Figure 4. EZH2 has also been identified as being significantly over-expressed in metastatic PCA compared to localized PCA in other profiling studies [86,87]. In a follow-up study, combined EZH2:E-cadherin expression (high EZH2, low E-cadherin) was found to be significantly associated with PSA recurrence in localized PCA across a training set of 103 patients (relative risk [RR] = 2.52, P = 0.021) and in a validation set of 80 patients (RR = 3.72, P = 0.009). Importantly, the EZH2:E-cadherin expression remained significant (RR = 3.19, P = 0.003) in a multivariate model incorporating other known risk factors [88]. In addition to being over-expressed in PCA, EZH2 has been found to be over-expressed or amplified in other cancers, suggesting a role as a general oncogene [85]. A recent study also demonstrated that IgG reactive to three EZH2 peptides could be detected in the plasma of almost half of PCA patients [89]. Taken together, these results suggest EZH2 may be a candidate biomarker for aggressive localized PCA as well as metastatic PCA.

9. Identification of Biomarkers of BPH from DNA Microarrays

In addition to providing candidate biomarkers for PCA, DNA microarrays have also provided substantial insight into gene expression patterns associated with BPH and identified possible biomarker candidates [90-92]. Luo and colleagues profiled 9 BPH specimens from men with extensive hyperplasia and 12 normal prostate tissue samples. They identified 76 differentially expressed genes with role in cellular growth, metabolism, differentiation, immune regulation and inflammatory response, including IGF-1 and -2, TGFβ-3, BMP5, NELL2, MMP2, α2-macroglobulin, COX2, and LUM (lumican), which were all over-expressed in BPH compared to benign prostate tissue. Eight transcripts were validated by RT-PCR from tissue samples of BPH and benign prostate tissue.

Prakash and colleagues analyzed expression profiles of different categories of patients with BPH by profiling 10 benign tissue samples from patients with no prostate pathology, 5 samples from patients with asymptomatic BPH, 8 samples from patients with symptomatic BPH and 8 samples from patients with BPH and PCA [91]. Using an ANOVA model, they identified 511 differentially expressed genes, which discriminated the four groups. Genes associated with cell proliferation such as calcium/calmodulin-dependent serine kinase, phosphoserine phosphatase and
S-phase kinase-associated protein 2 were notably upregulated in the symptomatic BPH group compared to the other three groups. Symptomatic BPH and BPH with cancer were distinct from the normal and asymptomatic BPH in their expression of inflammatory mediators (lymphotoxin beta, immunoglobulins and chemokine receptors), cytokines (RANTES) and extracellular matrix associated proteins (osteonectin and LUM). The intriguing correlation between symptomatic BPH and inflammation suggests development of therapeutic approaches using anti-inflammatory agents to target BPH symptoms and possible exploitation for biomarker development. Interestingly, JM27, a gene previously identified as being overexpressed in prostate cancer [93], was overexpressed in symptomatic BPH compared to benign prostate tissue and asymptomatic BPH. The authors validated this finding by IHC on TMAs and Western blot analysis. JM27 was later shown to be androgen regulated, implicating it in prostatic growth regulation [94]. These observations suggest JM27 as a potential candidate marker for symptomatic BPH. In addition, CYR61, an extracellular matrix signaling protein, was found to be over-expressed in all classes of BPH compared to benign prostate and was associated with the development and progression of BPH [91,95].

In our group’s recent study, we identified genes that shared expression patterns between BPH and benign pubertal prostate tissues compared to benign adult prostate tissue, PCA and MET tissue by DNA microarray analysis [92]. Genes over-expressed in BPH and pubertal prostate included TGFßR-2 and -3, IGF-2, laminin α4 and α1, a-2-macroglobulin and epidermal growth factor receptor substrate 8. The
observed overlap of expression patterns between pubertal prostate tissue and BPH supports the “reawakening” of the fetal processes in BPH that has been suggested previously [96].

As described previously, candidate selection from microarray studies can be aided by meta-analysis to identify potential biomarkers of BPH. Thus, we developed a BPH gene signature by meta-analysis of the gene expression profiling data of the three studies described above [90-92] as shown in Figure 5. These genes, consistently dysregulated across all three studies, can serve as a focused set of potential candidates. For example, NELL2, a transcript over-expressed in BPH in all the three independent studies, is a neuron specific thrombospondin-1-like extracellular protein containing six epidermal growth factor-like domains [97]. Although, the biological function of Nell2 remains unknown, it has been implicated in neural growth based on its expression and biochemical profiles [98,99]. Di Lella and colleagues also identified Nell2 as being over-expressed in BPH by cDNA subtraction and suggested that Nell2 could have a role in epithelial-stromal homeostasis [46]. Taken together, these studies demonstrate the ability of DNA microarrays to identify transcripts specifically over-expressed in BPH that can be evaluated as potential biomarkers.

10. IDENTIFICATION OF BIOMARKERS OF AGGRESSIVE PCA FROM DNA MICROARRAYS

The first studies profiling PCA using large scale DNA microarrays, published in [200160,93,100-102], and subsequent meta-analysis of four of these studies58, identified candidate biomarkers for PCA such as AMACR and HPN as described above. However, recent attention has focused on studies attempting to profile progression, in an effort to identify gene signatures of metastatic PCA and aggressive localized disease. The ability of a biomarker, whether a gene signature or a single candidate, to specifically identify localized PCA at a high risk of recurrence irrespective of other clinical parameters would have enormous clinical impact. The ability of DNA microarray studies to profile large numbers of samples on a consistent platform make it ideally suited to these studies as opposed to the differential gene expression techniques described above. Thus, in addition to the valuable insight into the biology of PCA progression, results from these profiling studies, such as the identification of EZH2 as described above, should help in the identification of gene signatures or individual candidates that can be assessed using alternative techniques as possible biomarkers. In line with this, several groups have tried to identify expression changes characteristic of aggressive tumors using different experimental strategies. Some groups have chosen to profile advanced and metastatic tumors directly and identify expression patterns that distinguish these samples from localized cancer, while others have focused on identifying expression patterns that predict biochemical or clinical recurrence after treatment. A list of these and other large scale studies profiling human PCA samples is provided in Table 1.

A study by LaTulippe and colleagues profiled 3 benign prostate samples, 23 localized PCA samples, and 9 metastatic PCAs [87]. The authors chose to focus on genes differentially expressed between the subset of primary cancers without recurrence and the metastatic samples. 3,436 probesets were identified that demonstrated at least 3 fold mean differential expression between the two groups. JAG1, a notch signaling ligand, demonstrated marked over-expression in the metastases compared to the localized PCA samples without PSA recurrence (mean 32.8 fold difference). JAG1 was also over-expressed in metastatic samples compared to localized PCA in our group’s profiling study [80] and subsequent investigation of JAG1 protein expression on TMAs confirmed significantly higher expression in metastatic cancer compared to localized cancer and benign tissues was observed. Similar to EZH2, increased JAG1 expression was found to be significantly associated with recurrence in localized PCA samples, independent of other clinical parameters [103].

Vajana and colleagues profiled 23 localized PCA samples (12 Gleason score 9 and 11 Gleason score 6), 5 metastatic PCA samples and 8 benign adjacent prostate tissues [104]. Although the authors did not report on expression patterns that could differentiate between the metastatic and localized cancers, they validated several genes by QPCR that showed progressive dysregulation through localized and metastatic PCA samples. The authors performed focused validation studies on ZNF185, a gene they identified as being down-regulated in PCA and metastatic PCA, demonstrating that ZNF185 is epigenetically silenced in both localized and metastatic PCA.

As an alternative to studies profiling aggressive and metastatic samples in an attempt to understand PCA progression, several groups have attempted to use
Figure 5. Meta-analysis of genes dysregulated in benign prostatic hyperplasia (BPH, purple) compared to benign prostate tissue (NOR, blue) from three independent profiling studies [90-92], indicated by the last name of the first author. The raw data from each array was log2 transformed and median-centered per array. A one-sided t-test was performed between the NOR group and the BPH group for each gene and the p value was calculated by shuffling sample labels and running 10,000 permutations. No adjustment for multiple hypothesis testing was performed. The genes in each of the dataset were normalized such that the mean gene expression of the normal group equaled zero and the standard deviation equaled 1. The 20 genes with significant differential expression (p < 0.05) in each of the three datasets are shown. Columns represent individual arrays and rows represent individual genes. Red and green saturation of cells represent relative over and under-expression, respectively, according to the color scale. Gray cells represent technically inadequate features on the indicated array.

Table 1. DNA microarray studies profiling human prostate tissues. Studies are identified by the last name of the first author from the appropriate reference. For each study, the type of array (Affymetrix or cDNA), the number of probes or genes monitored on the array (as reported by the authors) and the number of benign samples (NOR, including BPH), PIN, localized PCA, and metastatic samples (MET) are indicated.

<table>
<thead>
<tr>
<th>Author</th>
<th>Array Type</th>
<th>Probes</th>
<th>Profiled Samples</th>
<th>NOR</th>
<th>PIN</th>
<th>PCA</th>
<th>MET</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ashida</td>
<td>cDNA</td>
<td>23,040</td>
<td></td>
<td>10</td>
<td>20</td>
<td></td>
<td></td>
<td>130</td>
</tr>
<tr>
<td>Best</td>
<td>cDNA</td>
<td>6,400</td>
<td></td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td>131</td>
</tr>
<tr>
<td>Bull</td>
<td>cDNA</td>
<td>1,877</td>
<td></td>
<td>11</td>
<td>2</td>
<td>16</td>
<td></td>
<td>93</td>
</tr>
<tr>
<td>Chen</td>
<td>Affymetrix</td>
<td>6,800</td>
<td></td>
<td>3</td>
<td>1</td>
<td>4</td>
<td></td>
<td>61</td>
</tr>
<tr>
<td>Dhanasekaran</td>
<td>cDNA</td>
<td>9,984</td>
<td></td>
<td>22</td>
<td>59</td>
<td>20</td>
<td></td>
<td>60</td>
</tr>
<tr>
<td>Ernst</td>
<td>Affymetrix</td>
<td>12,625</td>
<td></td>
<td>9</td>
<td></td>
<td>17</td>
<td></td>
<td>132</td>
</tr>
<tr>
<td>Glinsky</td>
<td>Affymetrix</td>
<td>12,625</td>
<td></td>
<td></td>
<td>79</td>
<td></td>
<td></td>
<td>118</td>
</tr>
<tr>
<td>Henshall</td>
<td>Affymetrix</td>
<td>59,619</td>
<td></td>
<td>72</td>
<td></td>
<td></td>
<td></td>
<td>116</td>
</tr>
<tr>
<td>Kristiansen</td>
<td>Affymetrix</td>
<td>3,950</td>
<td></td>
<td>42</td>
<td></td>
<td>42</td>
<td></td>
<td>133</td>
</tr>
<tr>
<td>Lapointe</td>
<td>cDNA</td>
<td>26,000</td>
<td></td>
<td>41</td>
<td>62</td>
<td>9</td>
<td></td>
<td>86</td>
</tr>
<tr>
<td>TULIPPE</td>
<td>Affymetrix</td>
<td>63,175</td>
<td></td>
<td>3</td>
<td>23</td>
<td>9</td>
<td></td>
<td>87</td>
</tr>
<tr>
<td>Luo</td>
<td>cDNA</td>
<td>6,500</td>
<td></td>
<td>9</td>
<td>16</td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Luo</td>
<td>Affymetrix</td>
<td>&gt;47,000</td>
<td></td>
<td>15</td>
<td></td>
<td>15</td>
<td></td>
<td>134</td>
</tr>
<tr>
<td>Luo</td>
<td>cDNA</td>
<td>12,000</td>
<td></td>
<td>25</td>
<td>25</td>
<td></td>
<td></td>
<td>135</td>
</tr>
<tr>
<td>Magee</td>
<td>Affymetrix</td>
<td>6,800</td>
<td></td>
<td>4</td>
<td>9</td>
<td>2</td>
<td></td>
<td>101</td>
</tr>
<tr>
<td>Moore</td>
<td>cDNA</td>
<td>6,200</td>
<td></td>
<td>12</td>
<td></td>
<td>12</td>
<td></td>
<td>136</td>
</tr>
<tr>
<td>Ramachandran</td>
<td>Affymetrix</td>
<td>39,000</td>
<td></td>
<td>12</td>
<td>28</td>
<td></td>
<td></td>
<td>114</td>
</tr>
<tr>
<td>Rossi</td>
<td>Affymetrix</td>
<td>12,625</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>137</td>
</tr>
<tr>
<td>Singh</td>
<td>Affymetrix</td>
<td>12,625</td>
<td></td>
<td>50</td>
<td>52</td>
<td></td>
<td></td>
<td>112</td>
</tr>
<tr>
<td>Stamey</td>
<td>Affymetrix</td>
<td>6,800</td>
<td></td>
<td>8</td>
<td>9</td>
<td></td>
<td></td>
<td>138</td>
</tr>
<tr>
<td>Stamey</td>
<td>Affymetrix</td>
<td>6,800</td>
<td></td>
<td>26</td>
<td></td>
<td>12</td>
<td></td>
<td>139</td>
</tr>
<tr>
<td>Stuart</td>
<td>Affymetrix</td>
<td>12,625</td>
<td></td>
<td>50</td>
<td>38</td>
<td></td>
<td></td>
<td>140</td>
</tr>
<tr>
<td>Vanaja</td>
<td>Affymetrix</td>
<td>12,625</td>
<td></td>
<td>8</td>
<td>23</td>
<td>5</td>
<td></td>
<td>104</td>
</tr>
<tr>
<td>Welsh</td>
<td>Affymetrix</td>
<td>12,625</td>
<td></td>
<td>9</td>
<td>23</td>
<td>1</td>
<td></td>
<td>102</td>
</tr>
<tr>
<td>Yu</td>
<td>Affymetrix</td>
<td>37,777</td>
<td></td>
<td>83</td>
<td>66</td>
<td></td>
<td></td>
<td>117</td>
</tr>
</tbody>
</table>
expression patterns to predict which patients will demonstrate biochemical or clinical recurrence after treatment. These studies have been encouraged by demonstrations that microarray profiling can stratify patients into “good prognosis” vs “bad prognosis” groups in other cancers, including lymphomas [105,106], leukemias [107-109] and breast cancer [110,111]. In a study by Singh and colleagues profiling 52 PCA samples and 50 benign prostate specimens, the authors attempted to identify genes whose expression correlated with outcome [112]. Unfortunately, only 21 of the 52 patients with localized PCA were evaluable at the time of publication with respect to recurrence during at least 4 years of follow-up, and only 8 patients had PSA recurrence. The authors reported that although they were not able to identify a single gene that significantly associated with recurrence, they developed a 5-gene model using k-nearest neighbor classification that reached 90% accuracy in predicting recurrence during leave-one-out cross validation. Five genes were identified as being used in over half the prediction models, including chromogranin A, PDGFRB, HOXC6, ITPR3 and SIAT1. In multiple profiling studies, HOXC6 has been identified as over-expressed in recurrent localized or metastatic PCA samples [80,87,112]. A recent study validated confirmed increased HOXC6 expression by RT-PCR in metastatic PCA compared to benign tissues samples [113], while another functional study demonstrated that silencing of HOXC6 resulted in increased apoptosis in LnCaP PCA cells [114], suggesting a possible functional role as well as being a candidate biomarker.

Similarly, Lapointe and colleagues also analyzed their profiling study of 62 primary PCA samples, 41 benign adjacent tissues and 9 lymph node metastases in an attempt to identify genes associated with recurrence [86]. Unfortunately, this cohort only contained seven tumors with PSA recurrence and the authors reported that recurrence information was missing for 31 of the 62 tumors. However, the authors identified 23 genes associated with early recurrence, although the false discovery rate (FDR) [115] was 16%, which they attributed to the short clinical follow-up period. Although no genes overlapped with Singh and colleagues’ five outcome predictor genes, Lapointe and colleagues demonstrated that their 23 gene signature was able to accurately predicted recurrence in 71% (15 of 21) of the samples in Singh and colleagues’ study (although only 9 of the 23 genes were present on the microarrays used in Singh and colleagues’ study). Importantly, using unsupervised hierarchical clustering, Lapointe and colleagues identified three clusters of tumor samples, with high Gleason grade samples, advanced stage tumors, and tumors with PSA recurrence or clinical metastasis disproportionately represented in two of the three clusters. These two clusters also contained all of the lymph node metastases. To investigate the clinical relevance of the tumor subtypes observed, the authors focused on two genes, AZGP1 and MUC1 that demonstrated differential expression between the observed tumor clusters. Analyzing protein expression on TMAs composed of 225 primary tumors, increased MUC1 expression (P = 0.0005) and decreased AZGP1 expression (P = 0.002) were predictors of recurrence independent of grade, stage or preoperative PSA in a multivariate proportional hazards analysis [86].

Three recent profiling studies were designed with a more focused objective of identifying genes associated with recurrence or aggressive cancers, with the possibility of identified gene signatures serving as biomarkers of aggressive disease. Henshall et al. profiled 72 localized PCA samples, with 17 of the 72 patients having PSA recurrence after resection [116]. The authors identified 266 probes that were significant predictors of PSA recurrence at p < 0.01, although the FDR97 was 23%. The authors focused on one gene, TRPM8 (also known as trp-p8), encoding a putative Ca2+ channel, which showed down-regulation in samples with recurrence compared to non-recurrence samples. The loss of TRPM8 remained a significant predictor of PSA relapse in a multivariate Cox proportional hazards model (P = 0.0008). As the authors had also profiled normal tissue from various locations, they determined that normal liver showed only low-level expression while no detectable expression was seen from samples derived from 32 other normal tissues. Unfortunately, TRPM8 had not been monitored in any other published microarray studies, so it could not be validated in independent data sets; however the authors noted that none of the eleven genes commonly appearing in Singh and colleagues’ 5 gene models appeared in their list of 266 probes.

Yu and colleagues profiled 23 prostate samples from patients with no prostate pathology, 60 normal prostate tissues adjacent to PCA and 66 prostate tumors [117]. The authors divided the PCA samples into two groups for predicting tumor aggressiveness, with tumors demonstrating invasion into seminal vesicles or adjacent organs, PSA recurrence, or distant metastasis classified as aggressive, and the
remainder were classified as non-aggressive. The authors identified 72 genes as being differentially expressed between the two groups (P < 0.002). In an effort to classify the samples, the 5 most differentially expressed genes were identified and groups of 5 genes were added sequentially, with the classification rate evaluated by leave-one-out cross validation. A 70-gene model was found to perform the best, correctly classifying 89% of the samples. This 70-gene model was then validated on an independent data set of 23 cases of aggressive and non-aggressive cases PCA, with 78% overall accuracy, although no information was provided about this data set. Although the authors noted that 60% of the 70 gene model were ESTs or genes with an unknown function, it was unclear if any genes in the model overlapped with other predictors.

Glinsky and colleagues attempted to identify expression signatures predictive of prognosis using a unique approach [118]. The authors first analyzed Singh and colleagues’ data set, identifying 218 genes differentially regulated in the 8 samples with PSA recurrence and the 13 samples without recurrence events (p < 0.05). These genes were then compared to expression profiles from three xenograft models of highly aggressive human PCA cells and Glinsky and colleagues developed an algorithm that combined small gene clusters exhibiting highly concordant expression patterns across the clinical and model system samples. This algorithm, composed of 14 genes, was able to classify 90% of the samples correctly with respect to recurrence. Importantly, this algorithm was then validated on Glinsky and colleagues’ own data set containing 79 tumor specimens (with 37 samples with PSA recurrence). This algorithm was found to be an independent predictor of disease recurrence in the Cox multivariate analysis (P = 0.0001) and was able to correctly classify 75% (59 of 79) of the samples.

An evaluation of these studies attempting to identify a gene signature as a biomarker of PSA recurrence does not reveal the presence of a single gene capable of distinguishing recurrent from non-recurrent localized PCA samples with the sensitivity and specificity of AMACR or PCA3 in the context of benign prostate tissue versus PCA. While it is important to consider that the magnitude of expression changes between benign and cancerous prostate tissue is far greater than those between prostate tumors which will or will not recur after resection, there are numerous other reasons for the low observed overlap between the gene signatures identified in the different studies. These reasons include the different analytical methods used to determine signatures, the lack of overlap between probes on the different arrays used across the studies and different inclusion criteria. Each group also used a unique combination of data processing, algorithms and significance cut-offs to define their classifiers, each of which have been shown to profoundly influence classification signatures and success rates [119-120]. Perhaps the biggest limitation of many of these studies is the limited number of samples used in the analysis, particularly with regard to the number of recurrence events. The issue of sample number also impacted many of the earliest studies profiling PCA versus benign prostate samples. Recent analysis of several published studies attempting to predict outcome in other cancers suggests that the number of samples profoundly influences the accuracy and stability of the classifier [121-122].

In addition, the use of expression signatures from the described studies as biomarkers of aggressive disease faces several pitfalls. Robust validation across multiple large scale data sets is still lacking for most signatures and very few candidates have been validated using any methodology, including QPCR or IHC on independent samples. Further, although some groups demonstrated that their signatures can predict outcome independent of other known risk factors using multivariate models, they have not assessed how accurate the best currently available classifier is without the new signature relative to the classifier with the signature. Only the study by Glinsky and colleagues directly addressed this question and while they found that their classifier alone or incorporated with a Kattan nomogram [123] was able to classify 59 of 79 (75%) samples, the nomogram alone was able to classify 56 of 79 (71%) samples.

Although large profiling studies may be able to develop signatures that can serve as biomarkers of aggressive disease, samples that can currently be utilized for microarray analysis are limited to frozen tissues, as formalin-fixed, paraffin embedded (FFPE) tissues yield degraded RNA which is incompatible with current microarray techniques. This presents a serious problem, as samples with considerable clinical follow up, which are essential for developing robust and clinically usable classifiers, are poorly represented in most frozen tissue archives. Alternative techniques, such as QPCR, allow for analysis of gene expression from FFPE tissues with increased sensitivity compared to DNA microarrays.
A recent study by Paik and colleagues [124] demonstrates the enormous power of combining bioinformatic analysis of DNA microarray data with QPCR. In this study, the authors mined breast cancer microarray data sets to select a panel of genes to assess by QPCR in an effort to predict recurrence of tamoxifen-treated breast cancer. After testing 250 candidate genes on three independent tissue sets containing 447 tumors in total, the authors created an algorithm with 21 genes for use in a prospective study with 675 tissues. The sensitivity of QPCR allowed the algorithm, which calculates a "recurrence score", to stratify patients into three risk groups: low, intermediate, or high. Importantly, using a multivariate Cox model, the recurrence score provided significant predictive power independent of the known risk factors age and tumor size (P < 0.001). An important feature of this study was the prospective selection of the 21 gene panel, which included genes representing a proliferation cluster, an estrogen cluster, a HER2 cluster, an invasion cluster, three individual genes and 5 reference genes. These genes and clusters may be representative of hundreds or thousands of genes which show similar expression patterns by microarray analysis. Importantly, this study demonstrates that accurate monitoring of representative genes from important biological processes in breast cancer and metastasis in general, along with only a few "outcome" genes, can effectively predict clinical behavior based on global gene expression. A similar study for identifying aggressive PCA can be easily imagined. Several functional groups with a large number of dysregulated genes have been identified in the development of PCA as shown in Figure 6 and could serve as a starting point for genes to include in a QPCR biomarker study.

The ability to use FFPE tissues would also allow for focused studies with controlled clinical and pathological parameters, such as identifying a poor prognosis signature for aggressive tumors with a low Gleason score, which are very poorly represented in most frozen tissue archives. Nevertheless, these results suggest that future expression profiling studies in combination with other techniques such as QPCR may be able to guide treatment options for PCA through the identification of gene signatures as a biomarker of aggressive disease. Further, gene signatures identified in the studies described above can be mined to identify candidates, such as those described above, which may be used in combination as biomarkers of aggressive disease.

![Figure 6. Functional classes of dysregulated genes in prostate cancer (PCA). Functional classes of genes with several dysregulated members during PCA progression are indicated. Gene names annotated with a ↑or ↓indicate genes over or underexpressed, respectively, during prostate cancer progression from benign epithelium to PIN to localized PCA to metastatic (MET) disease.](image-url)
11. BIOMARKERS FOR DRIVING GENETIC CHANGES IN PCA

Expression profiling using DNA microarrays may also have a role in identifying signatures that can act as biomarkers of the driving genetic changes underlying individual prostate tumors. For example, despite its importance in terms of therapeutics and biological behavior, in breast cancer profiling studies ERBB2 does not appear high on the list of the most up-regulated genes between normal breast tissue and breast cancer. Most commonly, studies attempt to identify the genes that are most differentially expressed (often using a t-test) between two classes of samples. In these analyses, ERBB2 is not highly significant, because it is not over-expressed in all cases of cancer, even though it is very highly expressed when it is over-expressed. A similar situation has recently been discovered during trials evaluating the EGFR inhibitor gefitinib in the treatment of non-small cell lung cancer, where approximately 20% of patients had major objective responses, yet almost all of the responders had tumors with mutations in the ATP-binding site of EGFR, the target of gefitinib [125,126]. Thus, if researchers were mining lung cancer microarray data for genes or signatures differentiating between lung cancer and benign lung tissue, the downstream targets of activated EGFR would be missed. Almost all PCA profiling studies have analyzed profiles in a similar fashion, however recent work has confirmed that signatures in individual tumors can reveal oncogene activation or amplification when compared to mouse models [127,128]. For example, a study by Ellwood-Yen and colleagues used expression signatures from mouse tumors driven by prostate specific expression of Myc to identify a subset of human PCAs that have a “Myc-like” signature [129]. One gene present in both the mouse and human tumors with the Myc-like signature was PIM-1. Previously, our group had shown that increased expression of PIM-1 was significantly associated with PSA recurrence in patients with localized PCA [60]. These results suggest that focused analysis of profiling studies may be able to genetic signatures as biomarkers of genetic alterations that drive PCA which could be exploited as therapeutic targets.

12. CONCLUSION

Biomarkers for numerous aspects of prostatic disease are urgently needed. Several potential candidates have been identified through techniques that analyze differential gene expression including PCA3, AMACR, EZH2 and NELL2 (Table 2). The recent explosion of DNA microarray studies profiling PCA as well as BPH have provided several candidates and holds the potential to identify gene signatures as biomarkers of disease processes. Although validation is lacking for many candidates, DNA microarrays can provide focused lists for further validation studies using alternative techniques and several aspects of prostatic disease have not been adequately analyzed.

IV. BIOMARKERS IDENTIFIED BY PROTEOMIC ANALYSIS AND THEIR APPLICATION

1. PROTEOMICS

As discussed earlier in this chapter, proteomics has a long history in prostate cancer. PSA, a marker identified more than 25 years ago, is a protein expressed in the prostate which has revolutionized the treatment of this disease. Since that time, the protein composition of the prostate, prostate cancer, BPH, serum and urine has been extensively characterized. The goal of these studies is to identify novel diagnostic, prognostic and theranostic markers for prostate diseases. The question could be asked what is proteomics, and why should it be studied. Proteomics simply is the study of the protein components of whatever you are analyzing. The reason that the study of the proteins is so critical to our understanding diseases is that the proteins are truly the readout of the cell. Despite extensive knowledge of genomic and other changes, the proteins are the direct units that carry out cellular functions. Proteins are dynamic. They change frequently depending on cell or tissue response to hormonal changes, environmental changes, etc. In addition, they can be modified in many ways with post-translational modifications including phosphorylation, glycosolation, methylation and other types of modifications. Each of these changes really results in a different type of protein being formed which may have different recognition by receptors, antibodies, etc. For the past four or five years, the field of proteomics has had its ups and downs as related to prostate diseases. There have been a number of encouraging studies. The current state of the field has not been as optimistic as many had originally hoped (Petricoin, E. F., et al, 2004 and Posadas, E. M., et al, 2005).

As previously described, PSA has been in clinical
Table 2. Selected candidate biomarkers identified through differential gene expression studies. For each candidate, alternative gene names, the primary technique used to associate the gene with prostate disease, the type of prostatic disease associated with the biomarker, and the types and bio compartments of studies performed to evaluate the candidate as a biomarker are listed. Candidates are listed alphabetically and references are provided in the text.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alternative names</th>
<th>Technique</th>
<th>Prostatic Disease</th>
<th>Validation studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMACR</td>
<td>a-methylacyl-coA racemase, P504S</td>
<td>Subtractive hybridization / microarray</td>
<td>PCA</td>
<td>mRNA, protein, humoral response, tissue, serum, urine</td>
</tr>
<tr>
<td>ANXA2</td>
<td>Annexin II</td>
<td>Subtractive hybridization</td>
<td>PCA</td>
<td>mRNA and protein, tissue</td>
</tr>
<tr>
<td>AZGP1</td>
<td>Alpha-2-glycoprotein 1, zinc</td>
<td>Microarray</td>
<td>Aggressive PCA</td>
<td>protein, tissue</td>
</tr>
<tr>
<td>BMP5</td>
<td>Bone morphogenetic protein 5</td>
<td>Microarray</td>
<td>BPH</td>
<td>mRNA, tissue</td>
</tr>
<tr>
<td>CRISP-3</td>
<td>Cysteine-rich secretory protein 3</td>
<td>EST mining</td>
<td>PCA</td>
<td>mRNA and protein, tissue</td>
</tr>
<tr>
<td>CYR61</td>
<td>Cysteine-rich, angiogenic inducer, 61</td>
<td>Microarray</td>
<td>BPH</td>
<td>mRNA and protein, tissue</td>
</tr>
<tr>
<td>E2F4</td>
<td>E2 transcription factor 4</td>
<td>SAGE</td>
<td>PCA</td>
<td>mRNA and protein, tissue</td>
</tr>
<tr>
<td>EEF1A1</td>
<td>PTL-1</td>
<td>Differential display</td>
<td>PCA</td>
<td>mRNA and protein, tissue</td>
</tr>
<tr>
<td>EZH2</td>
<td>Enhancer of zest homolog 2</td>
<td>Microarray</td>
<td>Aggressive / metastatic PCA</td>
<td>mRNA and protein, tissue</td>
</tr>
<tr>
<td>GDFP</td>
<td>Gene differentially expressed in prostate</td>
<td>EST mining</td>
<td>Prostatic specific</td>
<td>mRNA, tissue</td>
</tr>
<tr>
<td>HOXC6</td>
<td>Homeobox C6</td>
<td>Microarray</td>
<td>Aggressive / metastatic PCA</td>
<td>mRNA, tissue</td>
</tr>
<tr>
<td>HPG-1</td>
<td>Human prostate-specific gene-1</td>
<td>Differential display</td>
<td>Prostate specific</td>
<td>mRNA, tissue</td>
</tr>
<tr>
<td>HPN</td>
<td>Hopsin</td>
<td>Microarray</td>
<td>PIN / PCA</td>
<td>mRNA and protein, tissue</td>
</tr>
<tr>
<td>JAG1</td>
<td>Jagged 1</td>
<td>Microarray</td>
<td>Aggressive / metastatic PCA</td>
<td>mRNA and protein, tissue</td>
</tr>
<tr>
<td>JM27</td>
<td>PAGE4</td>
<td>Microarray</td>
<td>Symptomatic BPH</td>
<td>mRNA and protein, tissue</td>
</tr>
<tr>
<td>KLK4</td>
<td>Prostate</td>
<td>Subtractive hybridization</td>
<td>PCA</td>
<td>mRNA, humoral response, tissue, serum</td>
</tr>
<tr>
<td>MUC1</td>
<td>Mucin 1</td>
<td>Microarray</td>
<td>Aggressive PCA</td>
<td>protein, tissue</td>
</tr>
<tr>
<td>NELL2</td>
<td>Neural epithelial growth factor-like 2</td>
<td>Subtractive hybridization / microarray</td>
<td>BPH</td>
<td>mRNA, tissue</td>
</tr>
<tr>
<td>P63</td>
<td>Expressed in prostate and skin</td>
<td>EST mining</td>
<td>Prostate specific</td>
<td>mRNA, tissue</td>
</tr>
<tr>
<td>PCA3</td>
<td>DD3</td>
<td>Differential display</td>
<td>PCA</td>
<td>mRNA, tissue and urine</td>
</tr>
<tr>
<td>PCANAP6</td>
<td>Protein</td>
<td>Subtractive hybridization</td>
<td>Prostate specific</td>
<td>mRNA and protein, tissue</td>
</tr>
<tr>
<td>PCGEM1</td>
<td>Prostate-specific non-coding gene</td>
<td>Differential display</td>
<td>PCA</td>
<td>mRNA, tissue</td>
</tr>
<tr>
<td>PIM1</td>
<td>Pim-1 oncogene</td>
<td>Microarray</td>
<td>Aggressive PCA</td>
<td>protein, tissue</td>
</tr>
<tr>
<td>PRAC</td>
<td>Small nuclear protein prac</td>
<td>EST mining</td>
<td>Prostate specific</td>
<td>mRNA, tissue</td>
</tr>
<tr>
<td>PRAC2</td>
<td>Prostate/rectum and colon protein 2</td>
<td>EST mining</td>
<td>Prostate specific</td>
<td>mRNA, tissue</td>
</tr>
<tr>
<td>PTEN</td>
<td>Prostate tumor overexpressed gene 1</td>
<td>Differential display</td>
<td>PCA</td>
<td>mRNA and protein, tissue</td>
</tr>
<tr>
<td>SLC43A1</td>
<td>SPOV1</td>
<td>Differential display</td>
<td>PCA</td>
<td>mRNA, tissue</td>
</tr>
<tr>
<td>STEAP</td>
<td>Six transmembrane epithelial antigen of the prostate</td>
<td>Subtractive hybridization</td>
<td>PCA</td>
<td>mRNA and protein, tissue</td>
</tr>
<tr>
<td>STEAP2</td>
<td>Six transmembrane epithelial antigen of the prostate 2</td>
<td>Subtractive hybridization</td>
<td>PCA</td>
<td>mRNA, tissue</td>
</tr>
<tr>
<td>TARP</td>
<td>TCR alternate reading frame protein</td>
<td>EST mining / microarray</td>
<td>Prostate specific / aggressive PCA</td>
<td>mRNA and protein, tissue</td>
</tr>
<tr>
<td>TRPM8</td>
<td>Trp-p8</td>
<td>Microarray</td>
<td>Prostate specific / aggressive PCA</td>
<td>mRNA, tissue</td>
</tr>
<tr>
<td>ZNF185</td>
<td>Zinc finger protein 185</td>
<td>Microarray</td>
<td>PCA / metastatic PCA</td>
<td>mRNA, tissue</td>
</tr>
</tbody>
</table>
utility for the past 25 years. During this time, many studies have examined the characteristics of this protein and the antibodies raised against it to serve as markers of prostate disease. One of the limitations that PSA will always suffer from is that it is not a, “cancer specific marker”. PSA is a protein that is normally expressed in the prostate and, in fact, is found at equal levels in the prostate of men with prostate cancer. It appears that PSA ends up being secreted into the wrong areas of the prostate in prostate cancer. Instead of being secreted apically into the acini of the prostate, PSA is miss-sorted and ends up getting into the vasculature of the prostate and subsequently being able to be detected in the serum. While several groups have now questioned the utility of PSA in the diagnosis of prostate cancer (Stamey, T. A., et al, 2004) it is clear that PSA, using appropriate cutoffs, has a role in the treatment of prostatic diseases (Baillargeon, J., et al, 2005 and Thompson, I. M., et al, 2004). With the limitation of PSA, there have been a number of studies that have revealed novel molecular markers for prostate cancer (Rubin, M.A. and De Marzo, A. M., 2004 and Sartor, O., 2004).

2. Diagnosing Prostate Diseases Based on Protein Patterns

Over the past several years, a good bit of work has focused on utilizing mass spectrometry to identify patterns existing within prostatic tissues, as well as within the serum and urine of individuals with prostatic diseases, to develop protein signatures for these diseases. These signatures are similar to a fingerprint of the disease in that a spectral pattern results which when matched to a reference pattern can identify whether an individual has a disease or not. While this may be an overly simplistic view of this approach, this type of technique is being applied for many different diseases. In several diseases, including lung, breast and other cancers, it has been quite successful and resulted in patterns that appear to have predictive information related to the patients. Much of this work is focused on using protein chip arrays based upon surface enhanced laser desorption/ionization (SELDI) time of flight mass spectrometry to profile these samples. Several studies have now been conducted in prostate tissues in comparison to prostate cancer samples revealing patterns that differentiate these diseases (Liu, A. Y., et al, 2005 and Zheng, Y., et al, 2003). One of the potential drawbacks to this approach is that tissues are actually complex mixtures of various cell types. Even in reasonably pure tumor samples, for example, a certain amount of the material contained within that sample will be normal. Several investigators have tried to enhance this technique by using laser capture microdissection (LCM) as a means to focus only on the desired samples. This permits the isolation only of the prostate cancer cells without utilizing normal areas for the proteomic analysis (Kunz, G. M. and Chan, D. W., 2004 and Ahram, M., et al, 2003). While this approach is reasonably straightforward and would seem primed for success, a major drawback has been the lack of reproducibility of the instruments utilized to generate the protein patterns. It has been unclear whether representative patterns can be obtained from running the same sample from one machine to another much less from one institution to another. This limitation has been greatly eliminated recently by the utilization of more complex mass spectrometry techniques which have permitted a more reproducible description of these patterns. The other limitation to this approach is that tissues need to be obtained to examine this type of pattern. Therefore, this type of study would only be appropriate in individuals from whom biopsies or other samples were obtained. This has led to the analysis of these types of approaches in serum samples as the next horizon.

As described for the tissue samples above, considerable effort has been focused on discerning specific proteomic patterns that exist within the serum of individuals with prostate diseases. The goal here again is to identify a protein pattern that exists within the blood that might be able to serve as a, “fingerprint” of the disease in that it would allow us to identify individuals, for example, with BPH, prostate cancer, etc. Again, much of the early work on this revolved around the use of SELDII analysis (Semmes, O. J., et al, 2005). As expected, protein patterns have been identified which appear to be representative of prostate cancer and may actually have some prognostic implications as well (Chin, J. L. and Reiter, R. E., 2004). More recent approaches have focused on using more high resolution instruments as has been done for the tissue samples which have resulted in patterns, for example, that may be able to discriminate prostate cancer from benign prostates in the PSA range of 2.5 and 15.5 nanograms per milliliter (Ornstein, D. K., et al, 2004). The current state of affairs for proteomic patterns is that preliminary data is suggestive that there may, indeed, be patterns that can provide us both diagnostic and prognostic information particularly related to prostate
cancer. Little work on this has been done in the field of single of BPH which is a much more complex disease that may or may not be able to have a reflective pattern. Despite the excitement raised for these types of patterns, it is clear that large validation studies still need to be performed. It may be that these approaches are best suited for reference laboratories rather than everyday clinical chemistry laboratories as many clinical tests are currently being performed. We should know in the next couple of years how useful these types of patterns are at identifying men with these diseases.

3. 2D Electrophoresis

An approach that has been around since the early 1970’s involves separating proteins by two dimensions. 2D Electrophoresis (O’Farrell, P. H., 1974) utilizes separation of proteins by isoelectric point in the first dimension and then by size using SDS/PAGE in the second dimension to identify protein spots and is still an essential element of proteomics. While this technique has evolved over the past several years with the introduction of differential approaches whereby different proteins, after being labeled with substrates, can be applied to the same gel and differences in colors detected. It still is a very valuable tool for our identification of proteins which may be altered in prostate diseases. Currently this is a discovery technique whereby we can examine tissue, urine or serum to identify particular markers which are subsequently characterized. After characterization, antibodies are generated and more standardized forms of detection developed. A number of markers have been identified utilizing this technique which is currently being validated, so laboratories are combining both 2D electrophoresis and mass spectrometry to identify novel markers (Lee, S.W., et al, 2005).

4. High Throughput Means to Analyze Large Amounts of Data

One of the challenges that we have in utilizing proteomic approaches is similar to that found for the genomic approaches. There is a tremendous amount of complex data being generated by these techniques. This complex data requires us to rethink how we analyze information. A number of groups have now developed informative tools for analyzing the large amounts of data specifically generated by proteomic approaches. Algorithms have been developed for looking at data obtained from mass spectrometry of serum samples (Lilien, R. H., et al, 2003). Other approaches have utilized pattern recognition to develop tools to match patterns between samples (Yasui, Y., et al, 2003). More importantly techniques have been utilized to take into account the abundant proteins that are frequently found within samples. It is important to control for these abundant proteins in order to find the less abundant proteins that exist. Utilizing an abundance ratio tool which takes into account how abundant a protein might be in this analysis has further allowed us to decipher this information (Griffin, T. J., et al, 2003). Other algorithms are currently in the process of being developed, and this information will certainly allow us to make better use of the complex information that is being generated by modern proteomics.

In addition to finding better ways to handle the data, a number of groups are now combining markers to take advantage of the added value that these markers can sometimes bring. The difficulty of examining too many markers at one time is that this makes what already might be a complex series of experiments much more complicated. To handle this, high throughput techniques are required that permit the analysis of a large number of markers and a high number of samples quickly (Gannot, G., et al, 2005). It is clear that looking at multiple markers may, indeed, be one way which we can solve some of the limitations that many of the markers that currently are available have (Mikolajczyk, S. D., et al, 2004). On the cutting edge of this type of approach, several groups are now combining proteomic and genomic approaches to allow us to really look at the full spectrum of available potential markers (Gelmann, E. P. and Semmes, O. J., 2004 and Kumar-Sinha, C. and Chinnaiyan, A. M., 2003).

A number of groups have also attempted to simplify the analysis by focusing on a more defined subset of starting proteins. These have consisted of proteins that have resulted from tissue culture analysis of cancer versus, “normal” cell types utilizing some of the same techniques described above (Dvorzhiski, D., et al, 2004). This work can even be applied to using proteomic approaches to help identify alterations associated with metastatic prostate cancer (Gretzer, M. B., et al, 2004). As an intermediate step, people are also looking at the use of animal models of these diseases to decipher important new model markers (Bok, R. A., et al, 2003).

5. Focused Proteomics

In an effort to minimize the limitations introduced during typical proteomic analysis, a number of
approaches analyze a functional subset of the part that is being analyzed. This, in principal, eliminates the issues related to abundant proteins that can often mask the signals observed as well as “sticky” proteins and other proteins which aggregate with others which can often complicate, for example, serum analysis of proteins. Several studies have focused on examining the proteomics of nuclear structure as a means to develop tools to identify biomarkers for the disease. Alterations in nuclear structure are a hallmark of the cancer process. The protein components of the nucleus, the nuclear matrix, comprise less than 10% of the nuclear proteins and less than 1% of the total cellular proteins. As such, there are low abundant proteins that will not be detected based on many of the approaches outlined above. By focusing on this hypothesis driven subset of proteins, novel potential markers, which may play a central role in the disease process may be elucidated. Utilizing this approach for prostate cancer, a number of markers have been identified specific to the disease. One of these markers, early prostate cancer antigen, EPCA, was identified as a marker found in nuclear structure of prostate cancer cells that was not found in that of the normal prostate. This protein was originally identified by high resolution three dimensional electrophoresis and then was sequenced. Antibodies released which resulted in a very interesting pattern of staining. The early prostate cancer antigen was found to be expressed everywhere in the prostates of individuals with prostate cancer but was not found in the prostates of individuals without the disease, including those with BPH. The question was then raised related to the utility of this marker. The idea was developed that if this marker could, indeed, stain anywhere in the prostate of someone with prostate cancer, a biopsy anywhere in the prostate would reveal the presence of the disease. In several studies that have been performed, these results have shown that, indeed, EPCA can identify individuals with prostate cancer even when analyzing the normal areas of the prostate (Dhir, et al, 2004 and Kakehi, et al, 2005). Further validation studies have revealed that by examining individuals with repeat negative biopsies in comparison to individuals that have had biopsy proven prostate cancer; by examining the normal cores from each of these, EPCA can reliably identify those with prostate cancer even in the absence of the disease within the biopsy sample. EPCA is an immunohistochemical tool that is, therefore, now being applied in pathology laboratories as a way to help the many men that have elevated PSA’s but yet have not had prostate cancer identified on biopsy discerned whether they have the disease or not. With more than 1.8 million biopsies being performed in the U.S. alone each year, this is an increasingly important problem where many millions of men exist in a limbo state unsure whether they have the disease or not. As described above, PSA has many limitations. The principal limitation is the fact that it is not specific for the disease. EPCA has now been applied as a serum test for prostate cancer. In this analysis, it has shown to have high specificity (greater than 90%) for prostate cancer in comparison with a number of other benign conditions (Paul, et al, 2005). Further validation studies are currently being performed, but from this initial work, it appears that EPCA may indeed serve as a novel marker for prostate cancer that can aid PSA’s ability to identify those with the disease.

6. BPH

While most of this section is focused on prostate cancer, some proteomic studies have been done related to the most prevalent prostate disease – BPH. Although prostate cancer analyses are quite complex, those related to BPH are even more so. The problem with BPH is that it represents more than one disease. While it is a disease of the prostate, it also affects the bladder, and not all BPH’s are created the same. A number of both proteomic and genomic studies have been done to help understand more about the biology of BPH. Nuclear matrix protein analysis has been performed in BPH and identified a pattern of proteins that are reflective of the disease (Getzenberg, et al, 1990??). Similarly, gene expression analyses have been performed which have identified a number of interesting markers. These have now been validated at the protein level. One of these markers JM-27 has been developed into both the tissue and serum assay for the disease. At the tissue level, JM-27 can differentiate between symptomatic and asymptomatic BPH. Therefore, JM-27 can, for the first time, delineate between an aggressive form of BPH which is resulting in high AUA symptom scores and the need for more invasive approaches to treat it from the more histologic form of the disease. Therefore, not all BPH’s are created the same. JM-27 has recently been developed into a serum assay whereby men with the aggressive form of the disease can, indeed, be differentiated from those with histologic disease as well as those with prostate cancer. This is the first a serum marker exists for BPH which has an apparent high level of clinical application. It is clear that further validation studies are necessary before this is applied to the clinic, and these studies are currently ongoing.
7. SUMMARY

We have attempted in this section to describe the state of the art of the proteomic approaches as applied to prostate diseases. By doing so, we clearly have missed many important studies that have been conducted. The goal here was to try to give a flavor for these types of approaches as well as an understanding for the rationale being used for the study of proteins and their potential use in prostate diseases. Proteomics is clearly at the forefront of our fight to develop novel markers for prostate diseases. It is apparent that novel markers for these diseases will be used in the clinic based on these approaches in the very near future.

2. GENETIC POLYMORPHISMS AND PROSTATE CANCER

Studies comparing the concordant development of prostate cancer in twins have demonstrated that the genetic component in the risk of prostate cancer is more significant than those in any other type of human cancer [4]. In terms of the genetic component, prostate cancer can be divided into two categories: hereditary and sporadic. Although several candidate genes for hereditary prostate cancer (HPC) susceptibility have been identified [5], these high-penetrance susceptibility genes account for only 5-10% of prostate cancers in the general population. On the other hand, genetic polymorphisms that may be associated with sporadic prostate cancer are much more common in the general population than the high-penetrance genes. Cumulative lifetime exposure to particular environmental factors could play an important role in the development of prostate cancer in genetically predisposed men. Racial differences in genetic polymorphisms that have roles in the development or progression of prostate cancer may partly account for the racial differences in the risk of prostate cancer.

Assessment of inherited genetic predisposition may be important in terms of prevention of prostate cancer. Identification of genetic variants that influence susceptibility to prostate cancer allows more precise risk assessment, thereby facilitating cost-effective cancer screening and chemoprevention. In addition, prediction of prostate cancer outcomes may be possible using the inherited genotype, since the genotype may influence the biological nature of the tumor by acting in tumor etiology or the response of an individual to pharmacological treatments. For example, genotypes involved in androgen metabolism could be associated with different tumor characteristics, if they modulate the bioavailability of androgens to prostate cancer. Genotypes involved in DNA repair or carcinogen metabolism may affect the tumor characteristics if they influence the accumulation of somatic genetic damage associated with malignant progression.

a) Polymorphisms on candidate HPC genes

At least seven gene loci, namely 1p36 (CAPB), 1q24-25 (HPC1), 1q42 (PCAP), 16q23, 17p11 (HPC2), 20q13 (HPC20) and Xq27-28 (HPCX), are involved in the development of HPC, and several candidate HPC genes have been cloned [6]. In addition, a recent study presented evidence for linkage to a new locus at 8p22-23.
1. HPC2/ELAC2

The HPC2/ELAC2 gene was identified by linkage analysis from familial prostate cancer patients in USA. Although the function of this gene has yet to be identified, 2 polymorphisms (Ser217Leu and Ala541Thr) have been reported to be associated with prostate cancer risk [7]. Recently, Yokomizo et al. reported that the Thr allele at 541 has strong significance for the predisposition of Japanese men to sporadic prostate cancer [8]. However, several other studies did not confirm this association [9,10]. To elucidate the controversial observations on the association between prostate cancer risk and these two polymorphisms, Severi et al. conducted a meta-analysis combining their data in Australia with those from 7 published studies [11]. The results of this meta-analysis showed no significant associations of either of these ELAC2 polymorphisms and prostate cancer risk.

2. HPC1/RNase L

The RNase L gene, which is linked to the HPC1 locus on chromosome 1q24-25, encodes a widely expressed latent endoribonuclease that participates in an interferon-inducible RNA-decay pathway thought to degrade viral and cellular RNA [12]. Casey et al. evaluated a common missense variant of RNase L, Arg462Gln, using 423 cases and 454 sibling controls in the USA [13]. They estimated that heterozygous carriers had an odds ratio (OR) of 1.46, while homozygous carriers had an OR of 2.12, giving a population attributable fraction for this variant of 13%. However, another study found the opposite trend, with ORs of 0.83 for heterozygotes and 0.54 for homozygotes [14]. Furthermore, a study from Japan based on 101 familial prostate cancers and 105 controls did not find any cases with the Arg462Gln variant, although 7.6% of the controls carried it [15].

3. Macrophage-scavenger receptor 1 (MSR1)

The MSR1 gene at 8p22-23, which encodes subunits of a macrophage-scavenger receptor, has emerged as a candidate gene for prostate cancer susceptibility [16]. Xu et al. studied 5 common polymorphisms on the MSR1 gene (PRO3, INDEL1, IVS5-59, P275A and INDEL7) in 301 patients with sporadic prostate cancer and 250 controls, and found significantly different allele frequencies between cases and controls for each of the polymorphisms [17]. Recently, Lindmark et al. identified 18 variants in the MSR1 gene, including 2 exonic changes, 7 intronic changes and 9 changes in the 5'- or 3'-noncoding regions [18]. In contrast to the results of Xu et al., they found no associations between any of the 5 polymorphisms and prostate cancer.

b) Genes associated with androgen metabolism

Androgens play important roles in normal and hyperplastic prostate growth, and prostate cancer has proven to be the most androgen-sensitive cancer upon hormonal manipulation. Thus, it is not surprising that genes encoding key proteins involved in the androgen metabolic pathway have been analyzed as possible candidates for genetic susceptibility to prostate cancer.

1. ANDROGEN RECEPTOR (AR)

The AR gene, located at chromosome Xq, encodes a ligand-activated transcription factor that mediates the androgen response. The first exon of the AR gene contains 2 polymorphic microsatellite trinucleotide repeats, namely a CAG repeat and a GGC repeat which encode variable length polyglutamine and polyglycine stretches, respectively. Due to its possible functional significance, the CAG repeat polymorphism has been more extensively examined for an association with prostate cancer risk. Inherited CAG expansions to over 40 repeats result in an X-linked spinal and bulbar muscular atrophy with partial androgen insensitivity, which is known as Kennedy’s syndrome [19,20]. This observation indicates that the length of the CAG repeats may influence the androgen-associated responses, and that this polymorphism is functionally significant. Indeed, several in vitro studies have demonstrated that shorter CAG repeats elevated the transactivity and transregulatory functions [21].

Despite several previous molecular epidemiological studies, the effect of the CAG repeats on prostate cancer risk remains controversial, although many, but not all, reports showed that shorter CAG repeats were associated with increased risk of prostate cancer [21, 22, 23, 24, 25]. Recently, Freedman et al. conducted a systematic evaluation of the AR locus, including the CAG polymorphic site, for its prostate cancer risk in a large multiethnic cohort of patients with sporadic prostate cancer [26]. Their study involving 2,036 patients and 2,160 ethically matched controls failed to detect a significant association between the CAG repeats and prostate cancer risk, even when the different cut-off points reported in previous positive studies were used.

CAG repeats have also been shown to be associated with tumor stage, grade and, most importantly, the
response to endocrine therapy [27]. Recently, investigators at the M.D. Anderson Cancer Center reported a significant association between the length of the CAG repeats and biochemical failure after radical surgery [28]. Their study involving 354 patients with relatively homogenous backgrounds showed that patients with higher CAG repeats (≥24) had a significantly longer biochemical-free survival than those with fewer repeats (≤23). A clinical survey of ethnicity-specific outcomes of prostate cancer showed that the general and stage-specific survival rates for men with prostate cancer are poorer for African-American men than for white men [29]. Considering that black men tend to have shorter CAG repeats with an average of 18 repeats, whereas white men and Japanese men tend to have longer repeats with averages of 20 and 23 repeats, respectively [30, 31], the CAG repeat polymorphism may be associated with the outcome of prostate cancer patients. Further confirmatory studies in different ethnic groups are mandatory.

The functionality of the length of the GGC repeats on AR transactivation is more controversial. Previous studies have reported contradictory results where both long and short repeats have been associated with prostate cancer risk [32], while other studies found no association [33, 34].

2. CYP17

The CYP17 gene encodes a key enzyme, cytochrome P-450c17a, which is involved in androgen biosynthesis and catalyzes the activities of 17a-hydroxylase and 17,20-lyase. The CYP17 gene has a single nucleotide polymorphism, A (A1 allele) to C (A2 allele), at position -34 from the translation initiation site. Since the polymorphism, designated the A2 allele, creates an additional Sp1 binding site, this polymorphism is hypothesized to be associated with altered transcriptional activities.

Several case-control studies have investigated the association between this CYP17 polymorphism and prostate cancer risk and produced contradictory findings. Wadelius et al. and Habuchi et al. found that the CYP17 A1 allele was associated with an increased risk of prostate cancer in Swedish and Japanese men, respectively [35, 36]. However, these results are in contrast to those in other studies demonstrating that the A2 allele was associated with prostate cancer risk in both white and black men [37, 38]. Recently, Ntais et al. conducted a comprehensive meta-analysis of 10 studies involving 2,404 patients and 2,755 controls from different ethnic groups [39]. This meta-analysis showed no significant association between the CYP17 variants and prostate cancer risk, and the authors concluded that this CYP17 polymorphism may not have a strong effect on susceptibility to prostate cancer. However, they did report a significant effect for the A2 allele on prostate cancer risk when the analysis was only conducted in a subgroup of African descent.

3. CYP19

The CYP19 gene, located on chromosome 15, encodes the enzyme aromatase that catalyzes the irreversible conversions of androstenedione to estrone and testosterone to estradiol.

Latil et al. reported that the distribution of a tetranucleotide simple tandem repeat polymorphism in intron 4 of CYP19 differed significantly among controls and patients with prostate cancer [40]. They suggested that the 171 allele and 187 allele were associated with prostate carcinoma risk. Another study also demonstrated an increased risk associated with the Arg264Cy5 polymorphism, although the result was of borderline significance [41]. However, Li et al. recently reported a null result for an association between the CYP19 polymorphism and prostate cancer using a sibling-based analysis, since they failed to show any significant association of the tetranucleotide repeat polymorphism in intron 4 and the risk or aggressiveness of prostate cancer [42].

4. SRD5A2

In the prostate, the conversion of testosterone to its more active metabolic form dihydrotestosterone (DHT) is catalyzed by the enzyme 5-alpha-reductase. The SRD5A1 and SRD5A2 genes, which encode the two isoforms of 5-alpha-reductase, have been cloned on chromosomes 5p15 and 2p23, respectively. The type I isoform is predominant in the skin and liver, while the type II isoform is predominant in the prostate. Hence, the SRD5A2 gene is considered to play a crucial role in androgen regulation in the prostate.

There are several polymorphisms in the SRD5A2 gene. Among them, a dinucleotide TA repeat polymorphism at the transcribed 3′ untranslated region, an A49T polymorphism (substitution of threonine (T) for alanine (A) at codon 49) and a V89L polymorphism (substitution of valine (V) to leucine (L) at codon 89) have been extensively investigated to clarify their associations with prostate cancer risk.

Reichardt et al. reported a statistically significant finding that SRD5A2 TA alleles are only present in
They also reported that the strongest associations for prostate cancer risk via an elevated intra-prostatic level of DHT.

Makridakis et al. reported that the V/V genotype of V89L was associated with a 39% higher level of serum androstenediol glucuronide, a surrogate marker for 5-alpha-reductase activity, compared with L/L individuals in an Asian cohort [44]. In accordance with this observation, Nam et al. reported that men with the V allele variant had a 2-fold increase in the risk of prostate cancer development when the age-adjusted OR was calculated with respect to the L/L genotype [45]. In addition, Li et al. reported that the V allele of the V89L polymorphism in the SRD5A2 gene dominantly increased the risk of prostate cancer in Japanese men [46]. Regarding the A49T polymorphism, a large prospective cohort study by Makridakis et al. showed that the T variant was the risk allele for prostate cancer [47]. Using an in vitro kinetic assay, they confirmed that the T variant had a higher in vitro V_max than the A variant, suggesting a functional consequence of this polymorphic site. However, other molecular epidemiological studies failed to show statistically significant associations between these polymorphisms and prostate cancer [48, 49]. It is of interest that finasteride showed a reduced affinity for the A49T variant enzyme in vitro [50]. Further studies are necessary to clarify the influence of SRD5A2 polymorphisms on the therapeutic response to 5-alpha-reductase in prostate cancer.

5. CYP3A4

The CYP3A4 gene encodes a member of the cytochrome p450 superfamily that is involved in oxidative deactivation of testosterone to biologically less active metabolites. The CYP3A4 gene, located on chromosome 7, has a genetic polymorphism consisting of an A-to-G transition in the 5′ regulatory region. Rebeck et al. identified an altered 5′ regulatory element, containing the A-to-G transition mutation, upstream of the CYP3A4 gene, and hypothesized that this polymorphism may affect the CYP3A4 protein activity, thereby leading to altered bioavailability of testosterone [51]. Paris et al. determined the association between the CYP3A4 genotype and prostate cancer in African-American men. They also reported that the strongest associations occurred between the homozygous variant (CYP3A4*1B) carriers and a high Gleason grade or grade/stage when the analysis was restricted to men >65 years of age [52].

It is also interesting to investigate whether the CYP3A4 genotype is associated with disease progression. Recently, Powell et al. showed there was no association between the CYP3A4 genotype and progression-free survival after prostatectomy [53]. However, based on the functional significance that CYP3A4 protein oxidizes finasteride, a 5-alpha-reductase inhibitor, the CYP3A4 genotype may influence the response of an individual to hormonal treatment, including chemoprevention, involving finasteride.

c) Genes involved in carcinogen metabolism

1. GLUTATHIONE-S-TRANSFERASES (GSTs)

GSTs are active in the detoxification of a wide variety of toxic and carcinogenic electrophiles by conjugating them to glutathione. In conjunction with this functional role, GSTs are thought to be involved in the intracellular transport of steroid hormones. Therefore, it is possible that polymorphisms of the genes encoding GSTs are associated with prostate cancer risk.

The GST gene superfamily consists of 4 gene classes based on sequence homology and substrate specificity (A, M, T and P), and most attention has been focused on GSTM1, GSTT1 and GSTP1 [54]. GSTM1 and GSTT1 are inactive in 50% and 10-25% of the Caucasian population, respectively, resulting from inheritance of two null alleles. In 1999, Rebeck et al. reported that the probability of prostate cancer development was increased in men who had non-deleted (functional) genotypes at GSTT1 (OR, 1.83; 95% confidence interval (CI), 1.19–2.80) but not at GSTM1 [55]. Based on evidence that GSTT1 is highly expressed in the prostate and can produce genotoxic effects upon exposure to specific carcinogens, the authors concluded that GSTT1 is associated with prostate cancer risk. On the contrary, in a case-control study conducted by Gsur et al., no significant effects on prostate cancer risk were detected for either GSTT1 or GSTM1 [56]. A Japanese study by Murata et al. and a Danish study by Autrup et al. also failed to demonstrate any significant associations between prostate cancer risk and GSTT1 or GSTM1 polymorphisms [57, 58].

GSTP1 is a major enzyme involved in the inactivation of cigarette smoke carcinogens. In addition, it is
interesting to note that decreased levels of this enzyme were found in malignant prostate tissue and high-grade PIN relative to the level in normal prostatic tissue [59]. Loss of GSTP1 gene expression is associated with hypermethylation of deoxycytidine residues (CG islands) in the 5'-regulatory region of the gene, and this epigenetic alteration appears to be a frequent and early event in prostate cancer development. GSTP1 has 2 polymorphic alleles, GSTP1*B and GSTP1*C, in addition to the wild-type allele, GSTP*A. Both polymorphic alleles have an A-to-G transition at nucleotide 313 (codon 104), which causes an isoleucine-to-valine change. The GSTP1*C allele has an additional C-to-T transition at nucleotide 341 (codon 113) that results in an alanine-to-valine change.

Wadelius et al. examined the associations of GSTP1*A, GSTP1*B and GSTP1*C in a study of 171 Swedish prostate cancer cases and 148 controls [60]. The allelic variants were equally distributed among the cases and controls and no significant associations were observed. However, Gsur et al. reported that the proportion of individuals homozygous for the GSTP1 variant alleles (GSTP1*B/*B, GSTP1*B/*C and GSTP1*C/*C) was significantly lower in prostate cancer patients (4.8%) than in benign prostate hypertrophy (BPH) controls (14.5%) [61]. They concluded that GSTP1 is the most interesting candidate biomarker for prostate cancer risk, since they found a 76% risk reduction in men homozygous for the polymorphic GSTP1 alleles compared to men with wild-type GSTP1.

Recently, Ntais et al. reported the results of a meta-analysis of more than 10 studies on GST genes [62]. This meta-analysis showed that the polymorphisms on these 3 GST genes are unlikely to be major determinants of susceptibility to prostate cancer.

2. N-acetyltransferase (NAT) 1 and NAT2

A variety of carcinogenic heterocyclic amines are produced during cooking of meat at high temperatures. These carcinogens are metabolized by NATs, which are polymorphic in the population. Although many molecular epidemiological studies have been conducted on NATs in bladder cancer and lung cancer, due to their etiological role in tobacco-associated human cancers, few case-control studies have been carried out on prostate cancer. Among these previous studies, only that reported by Fukutome et al. showed a positive association between prostate cancer risk and a NAT1 polymorphism [63]. They demonstrated that homozygosity for the NAT1*10 allele, a variant associated with the rapid acetylator phenotype, was associated with a higher risk than a single NAT1*10 allele or no NAT1*10 alleles.

d) Miscellaneous

1. E-cadherin

E-cadherin is a 120 kDa glycoprotein that plays a critical role in many aspects of cell adhesion and epithelial development, as well as in the establishment and maintenance of epithelial polarity. Loss of E-cadherin expression and the subsequent loss of homotypic cellular adhesiveness may be a critical step that allows epithelial tumor cells to invade and metastasize. In prostate cancer, it is possible that the loss of E-cadherin is related to tumor aggressiveness [64].

The E-cadherin gene, located at 16q22, has a polymorphism (A-to-C substitution) at position -160 from the transcriptional start site of its promoter that may influence the transcriptional efficacy [65]. Jonsson et al. analyzed the associations between this polymorphism and the risks of sporadic, familiar (2 close relatives) and hereditary (3 or more close relatives) prostate cancer using the genotypes of 1,036 prostate cancer patients and 669 controls [66]. Interestingly, they found that the A allele was significantly associated with HPC with a gene-dosage effect, whereas no significant associations were found for sporadic and familiar prostate cancers. Recently, Kamoto et al. reported a positive association between the A allele and an increased risk of cancer progression in a Japanese population [67]. Since HPC tends to be diagnosed at an advanced stage [68], these results suggest that the presence of one A allele is associated with tumor aggressiveness rather than tumorigenesis of prostate cancer.

2. Vitamin D Receptor (VDR)

The epidemiology of prostate cancer shows that prostate cancer mortality rates increase significantly with decreased ultraviolet radiation exposure. This observation, together with those in experimental studies showing the anti-proliferative effect of vitamin D on prostate cancer cells, led to the hypothesis that vitamin D deficiency is a risk factor for prostate cancer.

The primary effects of vitamin D are mediated through the VDR. There are 5 known polymorphisms within or near the human VDR gene, including 3 SNPs near the 3'-untranslated region that are recognized by the BsmI, ApaI and TaqI restriction enzymes. The BsmI polymorphism is in strong link-
age disequilibrium with the TaqI polymorphism. However, these polymorphisms are located on an intron or untranslated region, and their functional significances are unknown. Some case-control studies have indicated positive associations between prostate cancer risk and the VDR polymorphisms at these sites [67, 70]. Taylor et al. found a 3-fold increase in prostate cancer risk associated with the less active VDR allele (the T allele of TaqI polymorphism) in a Caucasian-American population. In addition, a 3-fold increased risk of prostate cancer was found to be associated with the bb genotype among 222 Japanese with prostate cancer compared to 209 men with benign prostate hypertrophy and 128 disease-free men [71]. However, other studies did not find such associations [72, 73]. Recently, Williams et al. analyzed BsmI and TaqI polymorphisms using archived specimens from a large series of radical prostatectomy patients at a single institution [74]. This study involving 428 white men and 310 African-American men showed little association between these genotypes and the extent of disease at diagnosis, Gleason score, preoperative prostate-specific antigen (PSA) or overall recurrence.

Another interesting polymorphism on VDR is the FokI polymorphism located near the 5' end of the gene. Since this polymorphism leads to a shorter VDR protein by affecting the start codon, its functional significance has been suggested by several investigators. A recent study by Xu et al. demonstrated that this polymorphism may be associated with the post-prostatectomy outcome [75]. They suggested that the presence of an F allele, which lacks the first ATG, increased the risk of being diagnosed with a more aggressive cancer, since subjects with the ff genotype had a lower mean percentage of Gleason grade 4/5 cancer than subjects with the FF or Ff genotypes. Consistent with these results, Oakley-Girvan et al. demonstrated that prostate cancer risk was associated with homozygosity for the F allele at the FokI site (OR, 1.9; 95% CI, 1.0-3.3), especially among African-Americans [76].

3. PSA
The PSA gene is androgen-regulated in the prostate, and the encoded serine protease is widely used as a tumor marker for early detection and monitoring of disease progression in prostate cancer. The PSA gene has 3 polymorphic sites at positions –158, -205 and –252 in the proximal promoter regions. Previous studies involving a relatively small number of Caucasian subjects have indicated that the polymorphism at position –158 is associated with the risk of advanced prostate cancer or an earlier onset of prostate cancer, but the results concerning the associations of the A or G alleles and the prostate cancer risk differed among the studies [77, 78]. Recently, Wang et al. evaluated the allelic frequencies of the two PSA polymorphisms at positions –158 and –252 in Japanese men. In their analysis involving 300 prostate cancer cases, 216 BPH cases and 266 controls, no significant associations were observed [79].

Besides the genes mentioned above, there are many other candidate genes for possible susceptibility to prostate cancer as follows: P53 [80], PTEN [81], Cyclin D1 (CCND1) [82], IGF-I [83], IGFBP-3 [84], Leptin [85], Macrophage-inhibitory cytokine-1 (MIC-1) [86] and so on.

3. Genetic Polymorphisms and BPH or Other Pathological Conditions of the Prostate
Similar to prostate cancer, the development of BPH is influenced by sex hormones. Although the specific pathophysiologic mechanisms of BPH are not clear, androgens have central roles in the development of epithelial nodules in BPH. Recent molecular epidemiological studies have demonstrated a significant association between prostate volume and genetic polymorphisms in certain genes important for prostate development. For example, a CAG repeat polymorphism in the AR gene has been reported to have a significant influence on the development of BPH, and several studies have shown that BPH patients with a short CAG repeat of the AR gene tend to have a large prostate [30, 87].

Similar to the molecular epidemiological studies on prostate cancer, the associations of AR polymorphisms with BPH are still controversial. Differences in the criteria used to define the BPH phenotype or a narrow disease spectrum among BPH study subjects may have contributed to these inconsistencies [88]. To exclude the potential biases in previous studies, Roberts et al. conducted an association study evaluating the associations between AR polymorphisms and surrogate measures of BPH assessed prospectively in a cohort of community-dwelling men (Roberts RO, 2004). In this study, they found that both CAG and GGN repeat polymorphisms are significantly associated with BPH-related measures, including the symptom score and BPH volume.

There have been many case-control studies on associations between BPH and the polymorphisms of prostate-related genes, but almost no consistent
results have been achieved [36, 71, 89, 90]. Recently, Klotsman et al. performed a case-based evaluation of the SRD5A1, SRD5A2, AR and ADRA1A genes as candidate genes for BPH severity [91]. They found significant associations between the polymorphisms on SRD5A1, but not on SRD5A2, and the severity of BPH.

In addition to the association between BPH development and inherited susceptibility, it is interesting to investigate whether we can enhance our ability to predict the response to various treatments, especially medical treatments, using genetic information. Two categories of drugs may provide effective treatment for BPH: alpha-1-adrenergic antagonists, which relax the smooth muscle in the prostate, and 5-alpha-reductase inhibitors, which shrink the prostate by blocking DHT formation from testosterone in the prostate. It is possible that the polymorphic variants of particular genes involved in the response machinery may modulate the response to these medical treatments.

Only one case-control study has reported results for an association between a polymorphism and a specific inflammatory or autoimmune disorder of the prostate, known as chronic prostatitis or chronic pelvic pain syndrome. Based on the observations that elevated cytokines in prostate fluid and semen are frequent findings in this unique disorder, Shoskes et al. studied genetic polymorphisms that can alter cytokine gene expression in men with chronic pelvic pain syndrome [92]. They determined the genotypes of the polymorphic sites in the promoters of various cytokines, namely tumor necrosis factor (TNF)-alpha 308, transforming growth factor (TGF)-beta 25, TGF-beta 10, interleukin (IL)-10 1082 and IL-6 174, and concluded that patients with chronic pelvic pain syndrome were more likely to have a low IL-10-producing genotype, suggesting autoimmunity as a potential etiology.

4. CONCLUSIONS

The development and progression of prostate diseases cannot be explained by genetic variation at a single locus, and it is difficult to demonstrate significant associations between specific polymorphisms on candidate genes and the diseases. Indeed, the reliability of results reported by many investigators remains questionable, and it is still unclear how genetic variations alone determine the susceptibility to prostate diseases. In order to clarify the role of genetic polymorphisms in the etiology of prostate diseases, association studies involving thousands of patients and ethnically matched controls with reasonable definition are required. In addition, we should take into account gene-gene and gene-environmental interactions.

Recent progress in determining the human genome has provided information for many thousands of potentially important polymorphisms. Furthermore, newly developed genotyping technology and high-throughput methodology are available for molecular epidemiological studies. In the future, inherited genetic variations could be useful markers to predict the risk and progression, and most importantly, the response to treatment in prostate diseases.

VI. NEUROENDOCRINE MARKERS

1. INTRODUCTION

The statistics for prostate cancer (PC) make alarming reading and set undoubted challenges for oncological research. In the Western world, where the incidence is highest, a man has a 10–11% chance of developing clinically apparent PC, and a 3–4% chance of dying from the disease [1]. Worldwide the incidence of PC is rising annually by 2–3% [2]. In many countries PC is now the second leading cause of cancer-related death and in Northern Europe it has already taken number one position as the leading cause of cancer-related death and in Northern Europe it has already taken number one position as the leading cause of cancer-related death in males [3]. The incidence has increased recently, largely due to better and earlier detection, but also because of the general aging of the world’s population and hence an increase in the proportion of men aged over 65 years old in whom the disease is known to be prevalent [4].

Despite its high incidence, knowledge and understanding of the pathophysiology of PC remains rudimentary. A clearer understanding of the biological nature of the disease could have a real impact on its management. The lack of known markers of tumour aggressiveness to help select patients for more or less aggressive management means that many patients are either overtreated or undertreated.

Much progress has been made towards better understanding of the development and progression of PC. Today the factors involved in the development of androgen independence, including neuroendocrine differentiation (NED), are still being elucidated and, therefore, it is unclear how to intervene, prevent or delay the process.

It is well known that the epithelium of the prostate
gland is under hormonal control of androgens. Most of the studies of hormone serum levels in different ethnic/racial groups prove that levels of androgens play a role in the incidence of PC. But besides the endocrine control, the homeostasis of the prostate is maintained by several regulatory factors, knowledge of which is necessary to arrive at an understanding of what causes prostate cells to become malignant and how to effectively treat the disease.

In the prostate, the interaction between the stroma and epithelial tissue appears to be an important aspect of growth regulation [5]. Epithelial cells are mostly secretory, with the basal component comprising less than 10% which are believed to contain a subset of stem cells. The wide variety of products contributing to seminal plasma as well as prostatespecific antigen (PSA) is produced by epithelial cells. Androgen ablation mostly affects these cells, reducing their number by up to 90% [6].

This review aims to elucidate the biological function of NED in prostatic carcinoma to improve our understanding of tumour progression and androgen independence. Such knowledge will lead to improved therapeutic protocols for treatment of PC.

2. NEUROENDOCRINE CELLS OF THE NORMAL PROSTATE

Neuroendocrine (NE) cells of the prostate were originally described by Pretl in 1944 [7]. NE cells with the dual properties of endocrine cells and neurons, i.e. acting in secretory and autocrine/paracrine fashions, are widely distributed in normal prostatic acini and ducts. Since NE cells do not contain cytokeratin, commonly found in the basal cell layer and urothelium, it has been suggested that these cells are of different origin from other prostatic epithelial cells [8-13]. In a recent study, human prostate NE cells were found to represent a cell lineage of their own, being of neurogenic origin and therefore distinct from the urogenital sinus-derived prostate secretory and basal cells [10]. The secretory epithelial and NE cells may interact in a paracrine fashion with the stroma [4, 14].

There are two types: the open cells with extensions at their apex that connect with the lumen, and closed cells with dendritic processes that extend between adjacent cells, resting on the basal lamina and in close topographical relationship with nerves. It is thought that via a variety of secretory products they form a communication network involved in cell regulation [6, 8, 15-17].

It has been suggested that paracrine and autocrine communications are localized versions of endocrine control. Whereas endocrine control, primarily via hormones, is effected by molecules secreted into the circulation and transported a considerable distance to the target tissue (e.g. testosterone), paracrine communication is essentially local and restricted to binding to the receptors of adjacent cells. Autocrine secretions, on the other hand, stimulate the very cells that secrete them. In normal circumstances, neither paracrine nor autocrine secretions enter into the general circulation [18].

It is now widely accepted that the main product chromogranin A (CgA), is an excellent marker of NE cell differentiation [9, 19-21] and it also serves as a generic marker of the NE cell population. Other commonly found secretory products include serotonin (5-HT), bombesin, neuron-specific enolase (NSE), calcitonin and other members of the calcitonin gene family, such as calcitonin-gene-related peptide, katacalcin, a thyroid-stimulating-like peptide, somatostatin and parathyroid hormone-related protein (PTHrP) [6, 22–29].

3. NEUROENDOCRINE DIFFERENTIATION, TUMOUR PROGRESSION AND HORMONE-INDEPENDENCE IN PROSTATIC CARCINOMA

It has been revealed that NED is more commonly expressed in prostatic carcinomas than in tumours arising in other organs of the urogenital tract. It may be explained by the fact that the largest population of NE cells is found in the prostate when a comparison is made with other organs in the male or female urogenital tract. Neuroendocrine differentiation is a common feature of prostatic adenocarcinomas and is usually determined by immunoreactivity for neuroendocrine markers, eg. CgA, NSE, or bioactive eutopic hormones such as somatostatin and 5-HT [15, 16]. In the literature, NED is reported in virtually all adenocarcinomas of the prostate, i.e. demonstrated in 30-100% of the tumours [15, 16]. However, there are other forms of NED associated with small cell carcinomas of the prostate. According to the new WHO classification system, these are entitled small cell neuroendocrine carcinoma. The malignant phenotype of NED is also found in certain carcinoid and carcinoid-like tumours. However, the most common histopathological pattern is focal NED in conventional adenocarcinomas of the prostate [13, 16].

NE tumour cells are found at all stages of PC and are
‘freely’ dispersed throughout the tumour. Independent groups of researchers have shown that NE cells lack or do not express the androgen receptors (AR) [16, 30-34]. The influence of NED in adenocarcinoma of the prostate on poor prognosis, tumour progression and androgen independence is extensively studied and reported in the literature. However, the exact role of NE tumour cells, their bioactive neuropeptides and biogenic amines in disease progression is still not clear and needs further investigations.

One of concepts of prostatic tumourgenesis is progression from normal prostate to PIN to adenocarcinoma, and finally to small-cell carcinoma of the prostate. It has been suggested that NED is a part of the oncogenic process. It is important to note that NE cells in malignant lesions are, at least to a certain extent, phenotypically similar to NE cells in normal epithelium in terms of expression of neuropeptides and biogenic amines. However, the malignant transformed NE phenotype of cancer cells differs morphologically from normal NE cells as they lack the characteristic cellular processes. Furthermore, they share the same morphologic feature as the surrounding cancer cells. Finally, the malignant phenotype of NE cells frequently expresses dual epithelial characteristics, i.e. prostatic acid phosphatase and/or PSA and NE markers, eg. CgA. Thus, the malignant NE differentiated cells should be distinguished from the normal NE cells of the prostate [13, 35-37].

The aggressive malignant potential of NE cancer cells associated with hormonal independence is partly due to the ability that most NE tumour cells escape apoptosis. Fixemer et al. showed that apoptosis is an extremely rare event in the NE phenotype of PC cells. This finding further substantiates why NED is associated with tumour progression and hormonal escape in adenocarcinomas of the prostate [38].

The overexpression of Bcl-2 proto-oncogene involved in apoptosis is highly correlated with cancer progression and androgen independence. Therefore, Bcl-2 proto-oncogene is frequently expressed at the hormone-refractory stage of the disease [39-41]. It has been revealed that malignant cells that express Bcl-2 are localised in close proximity to NE tumour cells. Recently, another gene encoding an apoptotic inhibitor designated survivin was expressed in normal NE epithelial cells and the NE phenotype of tumour cells [22, 42, 43]. Finally, Bernard et al. showed that the c-myc gene is required in androgen-independent growth acting downstream of AR through multiple growth factors and its expression in vitro allowed the growth of NE tumour cells during antiandrogen treatment [44]. Taken together, these data further support the increased resistance of NE differentiated tumour cells and surrounding non-NE cancer cells to programmed cell death.

It has been established that patients who develop resistance to androgen withdrawal therapies do so not because of loss of AR which are present in the majority of primary and metastatic sites [45]. It has been suggested that AR function may even play a major role in the proliferation of hormone-refractory PC cells. Activation of AR leads to a complex of proliferative, apoptotic and angiogenic events [46]. The mutations of AR leads to their supersensitivity to very low levels of androgens, interaction with other growth hormones, e.g. glucocorticosteroids and LHRRH, activation by growth factors, e.g. IGF-I or biogenic amines and, as a result, autocrine/paracrine modulation by NE cells products. Finally, this could be the reason why these tumour cells are able to multiply during incomplete androgen blockade [47-49].

In the study by Nakada et al. using double-labelling immunocytochemistry revealed that benign and malignant prostatic tissues contained both AR-positive and AR-negative NE tumour cells that may be also significant with regard to androgen–independent tumour growth and tumour progression [50].

Another interesting phenomenon is how cancer cells adapt to conditions of androgen deficiency that gives the ability of growth factors to functionally replace dihydrotestosteron and mimic the effects of androgens. It has been found that several growth factors and cytokines, e.g. IGF-1, keratinocyte growth factor (KGF), interleukin-6 (IL-6), forskolin, cyclin E, and butyrate, can activate the AR pathway in the absence of androgens through the MAPK pathway [13, 46, 51].

Culig et al. have reported that IGF-1, KGF and EGF directly activate the androgen receptor in the absence of androgens [52]. In the next study these authors also postulate that in androgen-independent tumours, autocrine stimulation may become more important which, via mitogen EGF, produced in the normal prostate and acting as a paracrine stimulator on stromal and epithelial cells, could also lead to unrestrained growth [53]. Interestingly, it has been reported that there is an overexpression of EGF receptors in the NE phenotype of tumour cells [28].

Studies have shown that regulation and function of normal and malignant NE cells is under the influence
of several NE hormones. For instance, somatostatin, 5-HT, bombesin, calcitonin and PTHrP are known to manifest tumour growth-promoting activity in vitro and in vivo and appear to be a potent mitogen associated with PC. It was shown that somatostatin is likely to counteract NE and other growth regulatory systems through somatostatin receptors (SSTR) present on secretory, NE, stromal and endothelial prostatic cells. The receptors were found to be up-regulated in carcinoma specimens while the potential mechanisms of SSTR antitumour effects include inhibition of angiogenesis, proliferation and promotion of apoptosis [54].

Serotonin (5-HT) is known to mediate diverse functions by binding to multiple receptor subtypes. Recently, 5-HT was found to show growth-promoting activity and functionally related to oncogenes. Thus, 5-HT is associated with tumour progression, androgen independence and poor prognosis [13, 55]. Earlier bombesin has been estimated as a potent mitogenic agent in lung cancer. Receptors for bombesin have been identified in prostatic carcinoma cell lines, and bombesin-like reactivity has been detected in prostate cancer tissue [13, 26]. Levine et al. demonstrated the role of bombesin and gastrin-releasing peptide receptors (GRP-R) in the NFkB-dependent up-regulation of proangiogenic gene expression, thus highlighting possible molecular mechanisms linking NE differentiation and the increased potential of androgen-insensitive PC [56].

An interesting aspect of recent research is the evaluation of androgen deprivation’s influence of NE-differentiated PC on in vitro and in vivo models. For example, Jongsmama et al. using PC-310 xenograft have revealed that androgen deprivation of NE-differentiated PC may induce the formation of both NE - and AR-positive dormant tumour residues, capable of actively producing NE growth factors via regulated secretory pathways, possibly leading to hormone refractory disease [57]. In another study using the human prostate primary xenograft model, it has been shown that the residual stem cell population that survives transplantation, or androgen deprivation, maintains significant pluripotentiality as demonstrated by the capacity to generate progeny that differentiate along the secretory epithelial lineages in response to androgen and along the NE lineage in response to androgen deprivation. The authors suggest that the identification of the mechanism and the source of NE cell differentiation could allow therapeutic intervention to inhibit the increase of NE compartment, prevent production of growth factors and inhibit the progression to recurrent disease [58].

4. Prognostic significance of neuroendocrine differentiation in prostatic carcinoma

There are conflicting data reported in the literature regarding the prognostic significance of NE tissue markers in PC. Some researchers have shown a significant correlation between NED, tumour grade and poor prognosis. In other words, in several studies an increased number of NE tumour cells in advanced tumour stages, high grade versus low-grade tumours and, especially after androgen suppression therapy during tumour progression, has been revealed [9, 59]. On the other hand, other groups of researchers did not find a correlation between the number of NE tumour cells, tumour grade and prognosis. Nevertheless, they suggest that the biological significance of NE tumour cells should not be minimized by the absence of strong correlation between NED, tumour grade and prognosis. Controversial data in terms of the prognostic value of NE tissue markers may be explained by different patient cohorts, various methodological approaches, and other difficulties associated with the studies, e.g. limited amount of obtained tissue samples and unequal distribution of NE tumour cells [32, 35, 60-63].

Serum measurement of NE markers does better reflect the entire NE tumour cell population, NED has been proven to correlate with tumour progression and androgen independence [13, 15, 60]. There are several studies that show a correlation between CgA and NSE serum levels, androgen independence, progression of the disease and prognosis. Some studies suggest that NED, as reflected by increased concentrations of NE secretory products in serum, was correlated with androgen independence, and poor prognosis. In addition, NED was not suppressed by androgen ablation treatment [9, 13, 35, 64, 65]. Kamiya et al. found higher NSE level in serum associated with poor prognosis in metastatic compared to non-metastatic patients with PC. They conclude that serum NSE measurements can predict prognosis in metastatic PC patients during androgen suppression therapy [66].

The data obtained in two studies of Hoosein et al. and Cussenot et al. demonstrated correlation between elevated NE serum markers and distant metastasis. On the other hand, local disease progression was not associated with elevated NE markers in serum [67, 68]. Angelsen at al. reported that the number of CgA-positive NE tumour cells correlated with serum CgA concentration [69].
Berutti et al. measured the two most commonly used NE markers, i.e. CgA and NSE in blood drawn from consecutive patients with PC or benign prostatic hyperplasia. They concluded that NE serum markers may be useful in terms of diagnosis and prognosis in PC patients. Moreover, serum measurement of NE markers may add complementary information with respect to PSA. In addition, CgA was superior to NSE and could be useful in the follow-up of patients with advanced disease [30]. In a second study, they showed a significant correlation between the extent of NE features as reflected by serum CgA, Gleason score and stage of the disease. However, no correlation was found between serum CgA and PSA, particularly in metastatic disease [70].

Recently, promising data using novel $^{99m}$Tc-labeled bombesin in diagnosis and staging of PC was demonstrated by Scarpinari and coworkers. $^{99m}$Tc-bombesin scintigraphy seems to be useful in detection of primary PC and local regional node involvement [71, 72].

In conclusion, at present time serum measurements of CgA is the matter of choice reflecting NED. Therefore, CgA is a valid serum marker, especially in hormone refractory PC, and adds important information in addition to PSA in terms of prognosis. Finally, CgA measurements in serum may be useful to monitor patients with the hormone refractory state of the disease.

5. NEUROENDOCRINE DIFFERENTIATION AND NEW TREATMENT MODALITIES

Despite initial success with androgen ablation therapies, it is still true for the vast majority of cancers that they will grow and progress. At hormone refractory stage, unfortunately, treatment with curative intent is not an option. Nowadays, once the patient no longer responds to any of the second-line hormonal alternatives then the patient is truly in a hormone-refractory condition. The only non-experimental option available now is chemotherapy, which is usually of limited benefit to patients at advanced stages of prostate cancer. Other drugs commonly used at this stage include established agents, such as estramustine and etoposide, as well as more recently developed drugs like the anti-microtubule taxanes (paclitaxel, docetaxel), and retinoid acid metabolism inhibitors (liarozole); many of these are undergoing comparative clinical trials [73–77].

Novel approaches currently being tested in early clinical trials include angiogenesis inhibitors, immunological therapies, gene therapy and differentiation therapies. Interference in growth-factor-mediated pathways is another new strategy in the treatment of cancer. For example, suramin can block the binding of several growth factors to their receptors.

a) Somatostatin analogues

Newly developed somatostatin analogues may also be useful agents in the treatment of prostate cancer [22, 78, 79]. Potential mechanisms of antitumour action include suppression of circulating levels of trophic hormones and growth factors as well as direct effects at the tumour level, potentially involving autocrine/paracrine mechanisms. More recently, the role of somatostatin in the pathophysiology and treatment of cancer has been explored. Somatostatin is a family of regulatory peptides produced by neuroendocrine, inflammatory and immune cells throughout the central nervous system and in most major peripheral organs. In addition, many tumour cells, when activated, produce somatostatin. Exogenously administered somatostatin produces a wide range of effects because it activates multiple target sites of action. Therefore, a number of selective peptide somatostatin analogues have been developed for clinical use. Selective non-peptide agonists have been developed for four of the SSTR subtypes.

Somatostatin prevents cell proliferation by inducing cell cycle arrest and apoptosis. These effects are believed to be mediated by SSTR on tumour cells and indirectly by receptors on non-tumour-cell targets which inhibit the secretion of hormones and growth factors involved in promoting tumour cell growth, inhibiting angiogenesis, promoting vasoconstriction and modulating immune cell function. Four receptor subtypes are involved in induction of cell cycle arrest via protein tyrosine phosphatase (PTP)-dependent modulation of MAPK associated with induction of retinoblastoma tumour suppressor protein and p21. One receptor subtype (SSTR3) is believed to trigger apoptosis and to activate p53 and the pro-apoptotic protein BAX. It is clear, therefore, that somatostatin has an important role in tumour development and in the future there may be a potential role for somatostatin analogues in the treatment of the disease [22, 54, 80].

b) Serotonin antagonists

NE cells produce and secrete 5-HT, a biogenic amine, neurotransmitter and potent mitogen associated with tumour growth. It has been demonstrated that 5-HT receptors (5-HTR), e.g. 5-HTR1 and 5-HTR4, are overexpressed in hormone refractory PC
tissues and in PC cell lines. Recent investigations show promising results using 5-HTR antagonists [16, 22, 24, 55, 81].

c) Bombesin antagonists

Bombesin induces androgen-dependent growth and invasiveness of PC cells. Bombesin also carries metastatic potential in androgen-insensitive PC. Therefore, bombesin-like antagonists could become an effective treatment option in the future [22, 56].

d) Cytokines

Recently, several studies are dedicated to IL-6, an inflammatory cytokine that not only regulates the immune response, but also modulates cancer cell growth, differentiation and survival. In prostate cancer LNCaP cells, IL-6 can regulate cell growth and NED by binding either to membrane or to soluble IL-6 receptors activating multiple signalling pathways, e.g. signal transducer and activator of transcription (STAT-3), mitogen activated protein kinases (MAPKs), cyclic AMP-dependent protein kinase (PKA) and phosphatidylinositol 3-kinase (PI3K) dependent signalling pathways. In vitro and in vivo experiments have demonstrated that IL-6-induced NE transdifferentiation of prostate cancer cells has a significant inhibitory effect on tumour growth. It means that agents, like IL-6, should also be considered as a new therapeutic approach for the treatment of PC [82-84].

In conclusion, recent progress in terms of PC research, especially the role of NED in prostatic carcinomas, has lead to the development of entirely new therapeutic modalities for hormone-refractory PC.

6. Summary

The biological nature of PC is a complex and difficult research subject which has seen the start of studies recently due to the achievements and possibilities of modern medical science. Much progress has been made towards better understanding of the development and progression of PC, and the factors which drive the development of androgen independence. The fact that most cancers eventually produce androgen-independent clones highlights the variety of genetic changes in the primary tumour which result in phenotypically distinct cells with different cellular capabilities, and their own characteristic response to the dynamic microenvironment.

NE cells may provide an intriguing link between NE cell differentiation and tumour progression in prostate cancer. This subset of androgen-independent cancer cells regulates the proliferation of neighbouring non-NE-phenotype cancer cells in a paracrine manner by secretion of NE products. In addition, various NE peptides stimulate proliferation of androgen-independent PC through transactivation of the androgen receptor being a key event in the development of androgen-independent tumour growth. Therefore, cancerous epithelial cells that increase their responsiveness to NE factors, or induce NE cells to release trophic factors may have a survival advantage over their siblings. This may be more important in the hormonal ablated state, since neuropetides and their intracellular signals may contribute to activation of the AR.

Thus, better understanding of the processes taking place in the prostate in the course of carcinogenesis and tumour progression with the formation of an androgen-independent state of PC will lead to the investigation and application of effective new methods of diagnosis and treatment of the disease. This review summarizes the pertinent literature in a way which is meaningful on a practical level to facilitate more rational decision-making in the treatment of the disease. Moreover, the theoretical basis is presented for the future appearance of new treatment modalities to better palliate and perhaps cure hormone-refractory carcinoma of the prostate.
1. PSA – most widely used marker of the prostate
   - Although total PSA still has clinical validity, stage migration and other factors are decreasing its utility
   - PSA isoforms are likely to improve upon the performance of % free PSA and cPSA
   - Despite its limitations, it is the best that we have and clearly has clinical utility

2. Some new tissue-based (pathologic) markers are in current clinical use and appear to have validity
   - AMACR
   - EPCA

3. Current Goals
   - To develop a biomarkers that can tell us the bad prostate cancers from those that are not as bad
   - To aid to the specificity of PSA in early detection
   - Tissue markers that are being applied
     1. EZH2, EPCA, GSTP1, AMACR
   - Serum markers
     1. proPSA, EPCA, D-2, hK2, humoral response to AMACR and others, protein fingerprints
   - Urine markers
     1. uPM3
   - Polymorphisms have not been demonstrated in a reproducible fashion - high throughput approaches are being applied

3. BPH
   1. PSA fulfills many of the criteria as an ideal BPH serum marker and has clinical utility for the disease
      - This currently is the only marker with sufficient clinical data to validate its use
   2. BPSA appears to be an independent predictor of BPH progression and is not related to prostate cancer
      - Available as a “research use only” test
   3. Significant differences exist between BPH with severe symptoms and those with no or mild symptoms
   4. New markers are being applied to clinical settings
      - Tissue markers
      - JM-27, contactin, other chemokines
      - Serum markers
      - JM-27
I. CURRENT CLINICAL UTILIZATION OF BIOMARKERS FOR PROSTATE DISEASES


## II. PSA AND PSA ISOFORMS


7. Bonilla J, Roehrborn CG, McConnel JD. 1995. Patterns of prostate growth observed in placebo treated patients in the PLESS trial over four years. J. of Urol 159: 301A


36. Graefen M, Karakiewicz PI, Cagnanos I, et al. 2002. Percent free prostate specific antigen is not an independent predictor of...
42. Lynn NN, Collins GN, O’Reilly PH. 2000. Prostatic manipulation has a minimal effect on complexed prostate-specific antigen levels. BJU Int 86: 65-7
49. Khan MA, Sokoll LJ, Chan DW, et al. 2004. Clinical utility of proPSA and "benign" PSA when percent free PSA is less than 15%. Urology 64; 1160-4

III. BIOMARKERS IDENTIFIED BY DIFFERENTIAL GENE EXPRESSION ANALYSIS AND THEIR APPLICATION


IV. BIOMARKERS IDENTIFIED BY PROTEOMIC ANALYSIS AND THEIR APPLICATION


Iwaki H, Kagayama S, Isono T, Wakabayashi Y, Okada Y, Yoshimura


V. GENETIC POLYMORPHISMS


37. Lunn RM, Bell DA, Mohler JL, Taylor JA. Prostate cancer risk and polymorphism in 17 hydroxylase (CYP17) and steroid reductase (SRD5A2). Carcinogenesis (Lond.), 20: 1727-1731, 1999


49. Lunn RM, Bell DA, Mohler JL, Taylor JA. Prostate cancer risk and polymorphism in 17 hydroxylase (CYP17) and steroid reductase (SRD5A2). Carcinogenesis. 20:1727-1731, 1999

50. Makridakis NM, di Salle E, Reichardt JK,. Biochemical and pharmacogenetic dissection of human steroid [alpha]-reductase type II. Pharmacogenetics, 10: 407-413, 2000


64. Kamoto at al, J J Clin Oncol, (in press)

VI. NEUROENDOCRINE MARKERS


Committee 4

New Developments in the Anatomical and Metabolic Imagery of the Prostate and Metastatic Sites

Chairman

*M. Resnick* (USA),

Members

*O. Akin* (USA),

*J. Braeckman* (Belgium),

*F. Coakley* (USA),

*D. Cochlin* (U.K),

*J. Descotes* (France),

*M. Igawa* (Japan),

*M. Terris* (USA)
NEW DEVELOPMENTS IN THE
ANATOMICAL AND METABOLIC IMAGERY
OF THE PROSTATE AND METASTATIC SITES

M. RESNICK,
O. AKIN, J. BRAECKMAN, F. COAKLEY, D. COCHLIN, J. DESCOTES, M. IGAWA,
M. TERRIS

I. TRANSRECTAL ULTRASONOGRAPHY

1. INTRODUCTION

The development of ultrasound technology originated with SONAR (Sound Navigation and Ranging), which made great strides in submarine navigation during World War II. Dussik is regarded as the first physician to have employed ultrasound in medical diagnosis. In 1942, he attempted to locate brain tumors and the cerebral ventricles by measuring the transmission of an ultrasound beam through the skull [1]. Nine years later, Wild and Reid invented A-mode transrectal ultrasonography (TRUS), which was initially described as a technique to evaluate rectal pathology [2]. TRUS was first used to evaluate the prostate in 1963 by Takahashi and Ouchi [3], and Watanabe et al. described the first clinically applicable images of the prostate obtained with TRUS in 1967 [4]. They used chair-mounted radial scanners with a 3.5 MHz transducer that was considered state of the art at the time. However, the images obtained with these early transducers provided information only about prostate size and shape. Over the years ultrasound technology has become more refined, such that visualization of the internal architecture of the prostate is now possible. By the mid-1980s, the 7 MHz ultrasound probe had been introduced. It produces a high-resolution image with a focal range from 1 to 4 cm from the transducer and high-resolution and handheld scanners revolutionized prostate biopsy techniques. Today, TRUS with high-frequency transducers is a standard diagnostic tool for evaluation of the prostate.

2. SONOGRAPHIC APPEARANCE OF THE PROSTATE

Advances in technology now allow visualization of the inner structure of the prostate, corresponding to McNeal’s concept of zonal anatomy [5, 6]. McNeal was the first to describe prostatic zonal anatomy, dividing the gland into peripheral zone (PZ), central zone (CZ), and transition zone (TZ) that have differing structural and functional characteristics. The anatomic distinction between the CZ and PZ is generally not appreciated by TRUS. In a normal man, these two zones are seen as a homogenous light- to medium-gray area in the posterior section of the prostate. Their normal echo pattern is used as a reference for defining other structures as hypoechoic or hyperechoic. The normal TZ in a young man comprises only a small percentage of the gland and exhibits heterogeneous hypoechogeticity relative to the other two zones.

The TZ surrounds the urethra and extends proximally from the ejaculatory ducts. It is the site of origin of benign prostatic hyperplasia (BPH). BPH nodules are most often hypoechoic, but can also be isoechoic or hyperechoic. Heterogeneity and hypoechogeticity are likely due to variations in the stroma and glands that comprise the BPH. In a man with increasing BPH, the TZ expands and compresses the CZ and PZ. The boundary between the TZ and the PZ is the “surgical capsule” of the prostate, a hyperechoic convex line and a sonographic landmark of zonal demarcation. Strongly reflecting objects are often seen in this region, which is consistent with the appearance of the corpora amylacea. Calcified deposits in this area interrupt the ultrasound waves, causing posterior shadowing that obscures the visualization of the
TZ. Approximately 20% of prostate cancer cases arise from this zone.

The PZ, occupying the posterolateral aspect of the prostate from the base to the apex, accounts for most of the volume (almost 75%) of the normal prostate. The majority (70%-80%) of prostate cancers arise from this zone.

The CZ is composed of tissue immediately surrounding the ejaculatory ducts, and it expands inferiorly. Approximately 5-10% of prostate cancer cases arise from this zone.

The seminal vesicles are visualized at the base of the bladder and are hyperechoic. The periprostatic fatty tissues are hyperechoic, while the neurovascular structures in the posterolateral prostate are generally hypoechoic.

3. **Volume Measurement**

Volume measurement of the prostate is useful and important in treatment planning for both benign prostatic hyperplasia and cancer, monitoring the response to therapies and improving the specificity of prostate specific antigen (PSA) levels for the presence of cancer. To estimate the size of the prostate using TRUS, either the step-section planimetric method [4] or one of several formulas is used. It is generally accepted that the step-section planimetric method is the most accurate [7]. However, because of its simplicity and ease of use, the most commonly used method is the elliptical volume calculation using three dimensions of the prostate. The formula is: (transverse diameter) x (cephalo-caudal diameter) x (anterior-posterior diameter) x (π/6). Though the prostate is not a perfect sphere, ellipse, or prolate spheroid, this formula correlates well with prostate specimen weights, with correlation coefficients greater than 0.90 [8].

4. **Appearance Of Prostate Cancer**

In the early 1980s, there was a debate as to whether prostate cancer is hyperechoic or hypoechoic. It is now accepted that most prostate cancers delineate as hypoechoic (Figure 1). However, the specificity is low (40-63%) [9-12], and the probability that hypoechoic areas are cancerous is less than 60% (Figures 2) [7, 9, 13, 14]. Moreover, 8-30% of palpable tumors were not visualized with TRUS[15]. Hypoechoic lesions also include inflammation, atrophy, hyperplasia, and even normal prostate tissue [11]. Greater than 80% of TZ cancers are isoechoic as are 30% to 50% of PZ tumors 9, and 1-2% of tumors are hyperechoic (Figure 3) [6, 17]. The positive predictive value of a hypoechoic lesion increases with the size of the lesion, the presence of a palpable nodule, and elevated PSA levels [10, 18, 19]. The number of “invisible” cancers is probably much higher today with stage migration during the PSA era. To improve lesion detection, the evaluation of secondary signs such as bulging and contour abnormalities has been advocated [13, 20].

Of 1,158 patients analyzed, who underwent prostate biopsies, three hundred and ninety-one were diagnosed with prostate cancer, and adenocarcinoma was detected in 63.1% of hypoechoic areas on a site-by-site basis (Table 1). The sensitivity of TRUS was 58.8% on a site-by-site basis (Table 2).

![Figure 1. A: TRUS showed a hypoechoic lesion in the left peripheral zone (white arrow). B: pT3a prostate cancer with Gleason score 3+3=6 corresponding to the lesion seen on TRUS was confirmed on examination of step-sections of the radical prostatectomy specimen.](image-url)
Figure 2. A 72-year-old man with prostate cancer. In the histopathological examination of the prostatectomized specimen, adenocarcinoma with Gleason score of 7 was seen in the left peripheral zone of the apex (not shown). A, Preoperative TRUS showed a hypoechoic area in the right peripheral zone of the mid gland (white arrow). B, The nodule corresponding to the lesion on the ultrasound (white arrow) was confirmed as benign prostatic hyperplasia.

Figure 3. A 65-year-old man with pT3a prostate cancer. A, Preoperative TRUS showed a hyperechoic area in the right lobe. B, Step-section analysis of the prostatectomized specimen showed adenocarcinoma of Gleason score 3+3=6 in the corresponding area.

Table 1. Comparison of different biopsy regimens and targeted biopsies on a site-by-site basis

<table>
<thead>
<tr>
<th>Biopsy regimens</th>
<th>No. of cores</th>
<th>Cancer</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>8,194</td>
<td>1,210</td>
<td>14.8</td>
</tr>
<tr>
<td>Sextant</td>
<td>6,948</td>
<td>999</td>
<td>14.4</td>
</tr>
<tr>
<td>Lateral</td>
<td>968</td>
<td>164</td>
<td>16.9</td>
</tr>
<tr>
<td>Transitional zone</td>
<td>86</td>
<td>8</td>
<td>9.3</td>
</tr>
<tr>
<td>Hypoechoic area</td>
<td>1,126</td>
<td>711</td>
<td>63.1</td>
</tr>
<tr>
<td>Hypervascular area</td>
<td>813</td>
<td>704</td>
<td>86.6</td>
</tr>
</tbody>
</table>

Of 1,158 men who underwent transrectal prostate biopsy for detecting prostate cancer in our institution between April 1998 and December 2004, 391 were diagnosed with prostate cancer. On histological examination of 8,194 specimens, adenocarcinoma was detected in 1,210. Lateral biopsies were performed by adding a biopsy from each side of the gland. A total of eight specimens have been obtained using our standard systematic biopsy method since September 2002. Targeted biopsies of hypoechoic lesions or abnormal Doppler signals were added except when the puncture line of systematic biopsy passed through the lesion.
Systematic biopsy of the prostate is the gold standard method of diagnosing prostate cancer. The more biopsies are obtained, the higher the positivity rate for a matched group. Patient tolerance is, however, a limiting factor so that, in practice, the number of biopsies has to be limited. A compromise is therefore necessary. In order to decide at what level to compromise, it is important to have accurate data on positivity.

Published comparisons of different biopsy regimes suppose that the number of biopsies obtained is the only important factor. There is evidence, however, that in matched populations who have the same number of biopsies that positivity rates still vary greatly. This is almost certainly due to differences in the technique of obtaining the biopsies and preparing them for histological analysis.

In some patients who have had a set of biopsies that are negative for cancer but who are still thought to be at high risk of cancer, it is common practice to advise re-biopsy. This group includes those with a persistent high serum PSA, a rising PSA, a suspicious or atypical core on the first biopsy or prostatic intraepithelial neoplasm (PIN) on the first biopsy. These groups are currently re-biopsied. The decision to re-biopsy, especially in the PIN groups may however need to be modified if the positivity rate on primary biopsy is high.

1. METHOD OF BIOPSY

Biopsy of the prostate guided by the finger during a digital rectal examination was, at one time, the accepted method. The needle may be directed to separate quadrants of the prostate or to a palpable abnormal area. This method is still appropriate for patients suspected, on digital rectal examination, of having clinically large advanced prostate cancer. It is quick, easy and cheap, and may be performed in the outpatient clinic. For the majority of patients, however, biopsy is performed under real-time guidance by transrectal ultrasound guidance.

The biopsies are best obtained using a core biopsy needle with an automated spring gun. A needle size of 18 gauge is sufficient for histological examination. Needles as large as 14 gauge were used at one time, but little is lost by using the smaller gauge, and the larger 14 gauge needle would, intuitively seem to be likely to cause more bleeding. This is not, however, proven. What is important is to use a needle that obtains maximal length of core. The largest commercially available needle gives a core length of 20mm.

Guidance is provided by advancing the needle through a needle guide. This is usually clipped to the side of the transrectal transducer, enabling easy removal for sterilisation or, preferably, the use of disposable guides. The predicted path of the needle is displayed by a line on the ultrasound screen. As the guide is long, the needle follows the predicted path fairly faithfully. Many systems use an end firing transducer with the guide lined up to one end of the tight curved linear transducer. This enables the needle to be visualised along its whole path. Other systems employ side-fire imaging with a needle guide traversing through the probe.

The prostate may be biopsied with image guidance in the axial or the sagittal plane. The axial plane enables better placement in the lateral plane, the sagittal enables better placement in the crano-caudal plane.

The plane used is used as a matter of personal choice and positivity rates for either technique appear to be similar. Many imaging systems allow simultaneous imaging in both planes which improves orientation but results in lower magnification with two smaller images displaced on the ultrasound monitor.
Patients are given antibiotic prophylaxis and informed consent is obtained. Preparation with a pre-procedure enema is of value in cleansing the rectum. Local anesthetic injected in various patterns around the prostate is administered by many. Rectal lidocaine jelly and oral analgesics may also be used. Details of these are, however, outside the scope of this article.

2. Biopsy Patterns

During the early years of prostate biopsy, it was customary to obtain four biopsies (quadrant biopsies), two each of the right and left lobes, one directed towards the base, one towards the apex. This was often performed under digital rectal guidance, in other cases by ultrasound guidance. With ultrasound guidance, a transrectal or transperineal route was available. The transperineal route had the advantage of being more sterile but guidance was less accurate and a general anaesthetic was often necessary. The transperineal route is used, exclusively in patients who have had an abdominal perineal (AP) resection, when the rectal route is not available. In these cases, guidance is also by transperineal ultrasound imaging. Transrectal ultrasound guidance with biopsy via the transrectal route is now the more commonly used method.

Very soon it was found that increasing the number of biopsies to six by adding biopsies midway between base and apex (sextant biopsies) improved positivity rates. This remained the standard biopsy pattern for several years, and it is on this pattern that much of the literature is based. Most later papers assessing positivity rates with different patterns use the sextant biopsy for comparison.

More recently, biopsy patterns have increased to 8, 10 and even up to 16 or more biopsies. Additional biopsies of abnormal areas may be obtained, guided by grey-scale ultrasound, Doppler studies, contrast ultrasound studies, elastography, MRI imaging or spectral MRI.

In addition to the biopsy pattern, the technique of the biopsy needle placement and subsequent preparation for histological examination is important. These topics will be presented in subsequent sections.

3. How to Measure the Sensitivity of Biopsies

a) Autopsy evidence

A measure of the sensitivity of different biopsy regimens would be extremely valuable. We do not, however, have a gold standard because it is not possible to know how many cancers are missed. A study of autopsy specimens in the USA has shown that a very high percentage of men have small histologically detectable foci of prostate cancer (Table 3). In this study [21] the prostate of men who died of trauma were examined histologically for evidence of prostate cancer. This showed that 47% of men between 50 and 59 years of age have foci of the prostate cancer. Between 60 and 69 the figure is 65%. Most men biopsied for suspected prostate cancer are aged between 50 and 70 years of age. We might, therefore, expect our positivity rate to be at least 56%.

<table>
<thead>
<tr>
<th>N</th>
<th>cores</th>
<th>Pick up rate</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>264</td>
<td>12</td>
<td>42.2%</td>
<td>Gore et al. [46]</td>
</tr>
<tr>
<td>303</td>
<td>10</td>
<td>38.9%</td>
<td>Ravery et al. [47]</td>
</tr>
<tr>
<td>244</td>
<td>12</td>
<td>27%</td>
<td>Naughton et al. [48]</td>
</tr>
<tr>
<td>483</td>
<td>10+D</td>
<td>42%</td>
<td>Presti et al. [49]</td>
</tr>
<tr>
<td>273</td>
<td>10+D</td>
<td>44%</td>
<td>Chang et al. [50]</td>
</tr>
<tr>
<td>119</td>
<td>13</td>
<td>40%</td>
<td>Eskew et al. [51]</td>
</tr>
<tr>
<td>202</td>
<td>12</td>
<td>41.5%</td>
<td>O'Connell et al [52]</td>
</tr>
<tr>
<td>187</td>
<td>12</td>
<td>38.5%</td>
<td>Lui et al. [53]</td>
</tr>
<tr>
<td>736</td>
<td>6</td>
<td>42%</td>
<td>Terris et al [54]</td>
</tr>
</tbody>
</table>

D= directed or targeted biopsies   Ref: 29, 46-54

Most normally biopsy a selected population who have elevated PSA levels and/or abnormal digital rectal examination, rather than the unselected population in the autopsy study. While the assumption that these indicate an increased probability of prostate cancer (This has been challenged [22], one would expect the positivity rates to be higher than those in the autopsy study. Whether true or not, the autopsy study would indicate that we miss many cancer foci. It may be argued that many of the cancer foci detected in the autopsy study were ‘clinically insignificant’ cancers. However, although an arbitrary distinction may be made in size, (a common figure being larger or smaller than 0.5 cc.), the definition of clinically insignificant cancers is unclear [23].

b) Positivity rates on repeat biopsies

A number of patients with negative biopsy studies,
who have a rising PSA level, will be offered repeat biopsies. Any positive results in the patients probably (though not necessarily) indicate false negatives (sampling error) in the original studies.

Patients whose biopsies show prostatic intraepithelial neoplasm (PIN) but no cancer also often have repeat biopsies. In this group, as there is only a short interval between the two procedures, any cancer detected on re-biopsy indicates false negatives on the original biopsy. Most papers quote positivity rates on re-biopsy following an initial sextant biopsy. In these positivity rates on re-biopsy are high –typically from 25 to 65% [24-26].

This suggests a high miss rate on the original biopsies. More recent studies with 8 or more biopsies first time show a far lower positivity rate on re-biopsy –typically less than 10% of the patients re-biopsied [27-28]. This represents approximately 1-2% of the original cohort of patients. This correlates with the higher positivity rate in the initial biopsies in this group, ie most of the cancers were detected on the first set of biopsies.

c) Studies using the same patients.

Another method of analyzing the subject is to study biopsy results of patients who have had extensive biopsies, all of which have been studied histologically and recorded separately. From this data, the positivity rates for the standard pattern of six, eight and ten biopsies may be calculated. One such series showed a significant increase in positivity from 6 to 8 biopsies of 5%, but a less marked increase of less than 2% from 8 to 10 [29-30]. Few centers however label their biopsy specimens separately, so few such studies are available.

4. COMPARISON OF PUBLISHED SERIES

A further method of comparing regimens is to study relative positivity rates in different series with similar populations. True matching of population is however difficult due to the multiple potential sources of bias: race is important because black men may have significantly higher incidence of ‘clinically significant’ (though probably not overall) prostate cancer than Caucasians and Hispanics.

Age may be a bias. Certainly the level of which serum PSA levels become significant appear to be age related.

Indication for biopsy may also introduce a bias. Most series use an elevated serum PSA and/or an abnormal digital rectal examination. A few, however, have studies only patients with both an elevated serum PSA and an abnormal digital rectal examination. Also the threshold of PSA level differs in different series.

From the published literature, despite differences in methodology and patient selection, a distinct pattern does emerge. Six biopsies give an unacceptably low positivity rate. Ten or more biopsies, or 8 in a gland under 40 grams, 10 over 40 grams is now common practice. What number above the figure of 8 to 10 is a matter of balancing a small increase in positivity against patient tolerance [31-45].

When large series, all with 8, 10 or more biopsies are studied positivity rates of between 39 and 43% are seen (Table 3) [29,46-54], with a few exceptions that are significantly lower. It seems on present evidence that this figure may be regarded as the gold standard.

Ultrasound-guided samples only from either hypoechoic lesions or palpable abnormalities is far superior to the previously used, digitally directed, blind biopsy. However, these targeted biopsies tend to miss many malignancies because of the limitations in cancer detection based on sonographic findings. Igawa and Shigeno report that 14.4% of cores obtained from sextant biopsies were positive, while 16.9% of cores were positive from lateral biopsies (Figure 4).

In 10 of 391 men with prostate cancer, tumors were detected only in the lateral biopsy specimens.

Figure 4. Correlation of detection rate and cumulative length of biopsy.
5. TECHNIQUE OF THE BIOPSY

Within matched series, with the same number of biopsies, there is a large variation in positivity rates [55]. The reason for this has not been fully validated, but it is certainly related to the total core length. Poor biopsy technique may produce some cores that are only part prostatic tissue, and others that contain no prostatic tissue.

Pathologists have found a positive correlation between positivity rates to total core length [56] studied. While the total core length is related to the number of cores obtained, it is also related to the biopsy system used, the technique of biopsy, and the preparation of the histology sample.

It is also probable that positivity rates may be related to accurate placement of the biopsies. A relatively even spacing throughout the gland would, intuitively, seem to increase the likelihood of detecting a cancerous nodule, while poor placement with perhaps two biopsies at or near the same site, and then a significant gap to the next, might reduce the chances, although this is likely, it is impossible to test the hypothesis.

Most cancers arise in the peripheral zone. In many patients that are studied, the peripheral zone is considerably thinned because of benign prostatic hyper trophy in the transitional zone. If the biopsy needle is advanced into the gland before firing a thin peripheral zone may be entirely missed. The operator must know the sampling area the biopsy gun employs. Some models advance 0.5 cm and sample the subsequent 1.5 cm when fired, whereas other models sample from the point of the needle tip prior to firing. It is important therefore to back the needle away from the prostate surface for the former biopsy gun and to fire the needle of the latter when its tip is just touching the gland. Whether this is achieved may be assessed on the pathology core by seeing the pseudocapsule. This has the added advantage of sometimes detecting extraprostatic spread. With the laterally placed (lateral horn) biopsies, placing the biopsy only slightly too medial may also miss a thin peripheral zone, while a slightly too lateral placement will miss the prostate gland completely.

Many operators with experience of imaging the prostate practice “informal targeting”. This is not overt targeting of a visible nodule with an extra biopsy but small alteration of the positions of the individual biopsies so that, while they remain within the overall pattern of systematic biopsies, they are subtly moved, within, for instance, the right apex to include any suspicious looking area of that sector of the gland. Again, this may well have an affect in positivity rates. The hypothesis has not been tested. It would be difficult (though not impossible) to test.

a) Simple mathematical model

Another way of looking at biopsies is to assume that the prostate gland is a cube, and that cancer may occur equally in different parts of the gland. Further assume that biopsies are taken at even intervals throughout the gland. If such a model gland is 20cc we can divide the cube up into 10 separate cubes of 1cc each. A 20mm biopsy needle will transverse 2 cubes. Then 10 biopsies evenly spaced across the gland will detect a 1cc cancer within that model gland.

If we reduce the size of the cancer to 0.625cc (a 5mm cube) then we increase the number of cubes in which the cancer may be situated by X8 (16 cubes). As the cubes are smaller, each biopsy will sample 4 cubes rather than 2. In this model we would only detect 25% of cancers.

The same would be true if we leave the cancer size the same, but increase the size of the gland to 80cc.

If we assume that a “clinically significant” tumour is one of less than 0.5cc then by the same calculation in a 20cc gland 16 biopsies are needed for certain detection, and in a 40 gram prostate 32 biopsies.

This is, of course, a grossly simplified model. Cancers do not occur randomly within the gland. Biopsy patterns are designed to concentrate the lateral peripheral zone where cancers are most likely to occur. Also cancers are not cubed, or even often not ovoid, but rather stellate, which increase the likelihood of biopsy detection. Nevertheless, this very simple model demonstrates that we are less likely to miss larger tumours than small tumours.

This may explain the discrepancy between the autopsy study and biopsy studies. In our simplified model, to be sure of detecting a 0.25cc tumour in a 20cc gland we would need 40 biopsies. To detect a 0.25cc tumour in an 80gram prostate, 120 biopsies would be needed! We know, however, that the vast majority of cancer foci found in the autopsy study will not develop into cancers that will affect the patient during their lifetime. We have no real way of knowing which will progress. One possible discriminator, however, is the size of the tumour – a significant size indicating that, as it has not already grown to that size, it is likely to progress further. A tumour that is likely to progress to a level that it is symptomatic or
fetal is loosely termed a “clinically significant” tumour. It is these tumours often defined as larger than 0.5cc that is important to detect. Assuming that size is one of the important parameters, we are less likely to miss these than smaller, perhaps “clinically significant” tumours.

The converse of this argument is that many of the tumours that we detected are “clinically significant” tumours. This may be suggested by a small length of tumour biopsy core. Unfortunately, however, this is not necessary accurate as a small foci could by found when the biopsy crosses a tenticle-like extension of a larger tumour.

There is evidence however that if sufficient (at least 8-10) biopsies are taken, that tumour volume (measured on radical prostatectomy specimens) correlates well with lengths of tumour in the biopsy cores and the number of cores involved. Based on this there is evidence that the increased positivity rate obtained when increasing from 6 to 8 or more cores does detect more clinically significant (>0.5cc) tumours, and that the percentage of tumours detected that are clinically insignificant is not increased.

In summary, therefore, there is no good measure of the sensitivity of biopsy detection of prostate cancer, nor is it clearly known what is being sought.

b) Histopathological preparation

Having obtained a set of biopsies, their careful preparation for histological analysis is important. The pathologist can only interpret that which he or she is finally presented with. A common practice is to place all the biopsies from one side of the prostate in to one container of formalin, and place these together into one wax block to be cut, stained and examined. It is difficult, using this technique, to embed the cores so that the maximum length of all the cores is cut. The practice of putting the cores into separate containers, and embedding them separately makes it easier to cut the cores level so that the maximum length is available for histological examination, but is prohibitively expensive at many facilities, even so, meticulous technique by the pathology technicians is essential [57-58].

6. ADDING TARGETED BIOPSIES

Abnormal areas of the prostate may also be detected by standard T2W, MRI and by MR spectroscopy [64-67]. MRI guided biopsy is not easy, though possible [68]. However, these abnormal areas, or at least the prostatic segment in which they are detected, may subsequently be biopsied by ultrasound guidance. Although they may not be visible on the ultrasound images, their position may be assessed for the MRI images. These techniques are discussed elsewhere.

a) When to re-biopsy

Another important question is how and when do we re-biopsy patients. Indications for re-biopsy are:

1. Suspicious or atypical small acinar hyperplasia (ASAP) or non-diagnostic cores
2. High grade prostatic intraepithelial neoplasia (PIN)
3. Rising PSA levels

Suspicious or atypical cores and the presence of ASAP are a definite indication for re-biopsy. The area of the atypical core or cores is biopsied at several sites. Whether the rest of the gland should also be re-biopsied is unclear. Positivity rates in this group of patients are high [25].

PIN is a histological change in the prostatic glandular epithelium. It is not prostate cancer, nor is it precancerous. The high grade variant of PIN is associated with prostate cancer in a large proportion, though not all cases. The cancers are not necessarily close to the foci of high grade PIN. It is customary therefore to re-biopsy patients whose initial biopsies show high grade PIN but no cancer. Most papers quote a positivity rate of 30-40% [69,70]. This indicates false negatives (geographic misses) on the first set of biopsies. This data is based on sextant biopsies. More recent data suggest that with extended biopsy regimes that achieve a higher positivity rate on the first set of biopsies, positivity rates for re-biopsy are correspondingly low, at about 2% [28,71]. It is therefore suggested that re-biopsy for high grade PIN alone is not necessary, if PSA remains stable.

A rising PSA level is an indication for re-biopsy. As re-biopsy is undertaken at varying time intervals after the first biopsy, sometimes quite long, it is difficult to tell how these relate to the first biopsy results. It is relevant however to discuss whether the pattern on repeat biopsies should be different to the first biopsies. Positivity rates in this group are high [24,25]. If however the first set of re-biopsies are negative, then subsequent sets of re-biopsies have a low positivity rate. This probably reflects the increased positivity rates of increasing numbers of biopsies. Two sets of sextant biopsies probably (though not necessarily) equate to a set of 12 biopsies.
b) Pattern of repeat biopsies

There is no consensus about biopsy patterns in re-biopsied patients. Some simply repeat the standard pattern. Some position the re-biopsies approximately between the first set ie the apical biopsies a little lower, the base a little higher, and so on. Others include the anterior gland, particularly in large glands in which standard biopsies do not reach the anterior gland. Some do this by placing appropriate biopsies more anteriorly by advancing the needle further before firing. Others add anterior biopsies to the standard set. Additional anterior biopsies aimed at the most anteriomedial aspect of the gland where transition. Zone tumors tend to arise would be expected to provide the highest yield. There is no evidence as to which if any biopsy pattern is superior.

7. Discussion

There is no way of accurately assessing the sensitivity of prostate biopsy. Further we are not certain what to measure with regard to so-called clinically significant and insignificant tumours. There is also the whole vexed question of whether we should be biopsying patients because of elevated PSA levels, at least at the lower end of the range, at all. What does emerge however is, that if we decide to biopsy patients, it is incumbent upon us to use the best methods available.

It appears that at least 8 or 10 biopsies are necessary to achieve a reasonable sensitivity. A good biopsy and histology preparation technique are also necessary. Such a regime should yield a positivity rate for a PSA elevated population of above 39%, at least in non oriental populations. There is a need to set some sort of benchmark standard. It is not clear how this can be achieved. Positivity rates are one possible solution. A record of the total length of biopsy cores at histology and the presence of pseudocapsule at the end of the cores is another. Positivity rates at re-biopsy should also be recorded.

8. Local Staging

Extracapsular extension can be characterized by an irregularity or interruption of the capsule, an irregular capsular bulge, or an obvious extension of a hypoechoic lesion in the surrounding fatty tissue. However, TRUS is limited in its ability to locally stage advanced cancer. The sensitivity and specificity of TRUS for detecting extracapsular extension was 48 – 86% and 50 – 90%, respectively [72-75]. Significant interobserver variability in the interpretation of extracapsular extension or seminal vesicle involvement is also a variable with TRUS analysis [76]. The inability of TRUS to detect microscopic extracapsular extension has been confirmed [72, 77].

When tumors are hypoechoic, increased length of contact between the lesion and the capsule correlated with the presence of extracapsular extension [78]. The loss of the triangle formed in the sagittal plane by the prostatic apex, urethra, and rectal wall was also a predictor of extracapsular extension [79]. Recent advances in Doppler TRUS, attempts at reconstructing a 3-D image of the prostate [80] and introduction of artificial neural network analysis [81] might improve accuracy at staging.

9. Doppler Ultrasonography

The use of Doppler ultrasound with targeted biopsy is expected to improve cancer diagnosis because of the increased detection of neovascularity found in pathological specimens of prostate cancer. Blood flow assessed by Doppler ultrasound may reflect the state of angiogenesis in prostate cancer (Figures 5, 6) [82]. Color and power Doppler ultrasonography have been shown to be an important adjunct to conventional gray-scale TRUS, improving the accuracy of cancer detection [20, 83-86]. Cancer has been detected in 86.6% of hypervascular areas (Table 1). Doppler TRUS has shown a sensitivity of 58.2%, similar to that of gray-scale TRUS, and a positive predictive value of 86.6%, much higher than that of gray-scale TRUS (Table 4).

<table>
<thead>
<tr>
<th>Doppler ultrasonography</th>
<th>CANCER (+)</th>
<th>CANCER (-)</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal signals (+)</td>
<td>704 (86.6)</td>
<td>109 (13.4)</td>
<td>813 (100)</td>
</tr>
<tr>
<td>Abnormal signals (-)</td>
<td>506 (6.9)</td>
<td>6,875 (93.1)</td>
<td>7,381 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>1,210 (14.8)</td>
<td>6,984 (85.2)</td>
<td>8,194 (100)</td>
</tr>
</tbody>
</table>

Sensitivity 58.2%, Specificity 98.4%, Positive predictive value 86.6%, Negative predictive value 93.1%
Figure 5. A 68-year-old man with pT2a prostate cancer. The preoperative PSA level was 23.4 ng/mL. A, An intense increase in Doppler signals was delineated in the right peripheral zone and the defined rectangle (20 mm²) for the measurement of pixel intensity (PI) is shown. B, A tumor with a Gleason score of 7 consistent with the lesion on ultrasonography was confirmed on examination of whole-mount sections of the radical prostatectomy specimen. C, Cytoplasmic staining for VEGF in tumor was intense, and the microvessel density was 110/mm²(D).

Figure 6. Correlation between the pixel intensity (PI) detected by color Doppler ultrasonography and microvessel density of the corresponding tumor area (p<0.001).
1. BACKGROUND

All cancers develop their own blood supply (neovascularity) which differs in pattern from the vascular bed of the tissue in which they develop [87-90]. If this vascular pattern can be imaged, then there is a potential for using such imaging to detect tumours. The recognition of tumours by their vascularity is well founded. Before the advance of CT and MRI, renal tumours were detected by angiography for the detection of their abnormal vascular pattern. The pattern of tumour neovascularity also has a potential for the study of other tumours. It is known from histological studies that tumour microvascular density has a correlation with aggressiveness. Also, the changes in vascularity may potentially be used to monitor tumour progression. This could be used in patients on an active surveillance regimen, or those who have been treated with high frequency focused ultrasound, or those on anti-tumour agents, particularly those designed to block neovascularity. (anti-neoangiogenesis agents).

In the case of prostate cancers, the use of Doppler technique has been studied for some time [91-103]. Doppler either ‘standard’ velocity domain or ‘power’ domain, may only detect vessels down to a fairly large size, typically arterioles. Tumour neovascularity is composed largely of vessels smaller than this. Nevertheless, Doppler studies had limited success in detecting prostate cancers by comparing the vascularity, as vessel density, on both sides of the prostate in each scan plane. Some information about staging and tumour aggressiveness was also obtained. In general tumour vessel density correlated to high Gleason stages, more aggressive tumours and poor prognosis, [90,104-111] hypervascular areas may then be target biopsied. The vessels shown on uncontrasted Doppler studies are probably largely the tumour feeding vessels rather than the intratumoral vessels.

The reason that smaller vessels are not visualised is that blood flow through them is of small volume and low velocity. It is this low volume, low power flow that can be shown by ultrasound contrast agents.

Ultrasound contrast agents are stabilized microbubbles of a size similar to red blood cells. Contrast specific imaging is a different way of visualising vessels after the intravenous administration of contrast. These bubbles are about 1000 x more reflective than red blood cells [112]. This enables Doppler systems to detect flow in small vessels down to 10? or perhaps less. These prompted further studies in the prostate using colour Doppler studies with ultrasound IV contrast.

The technique is well validated in liver tumours and to a lesser extent, in other sites [93-117]. Its use in the prostate has lagged behind these other areas. This is because the technique has technical difficulties, at the high frequencies normally used in the prostate. This difficulty has now been overcome and software is now available on a number of commercially available systems. Work in progress, however, would suggest that contrast studies using a contrast specific technique has a significantly more potential than the older Doppler techniques.

Microbubbles are extremely reflective and return a powerful ultrasound echoes. Within fine blood vessels, however, they are present in small numbers and are moving slowly. The ultrasound and the Doppler signals from them is therefore relatively low and are lost in the echoes from the surrounding tissues. A different imaging technique is therefore necessary.

As well as reflecting the ultrasound beam the bubbles vibrate. Their vibration is non-linear. They expand more than they contract. The result of this is that the returned echoes are not only at the frequency of insonation, but at frequencies above and below this frequency. The surrounding tissue, on the other hand, returns frequencies that are mainly at the fundamental frequency. Although other frequencies are produced (tissue harmonics) these are weak compared with the non-linear frequencies from the contrast bubbles.

A broad hand transducer is capable of detecting a wide range of frequencies. If the fundamental frequency is removed from the returned signal, the additional frequencies produced by the contrast bubbles produce the predominant images. The weak non-linear frequencies from the surrounding tissue produces a low intensity image that, while sufficient for localization does not interfere with the vascular map image.

The fundamental frequency may the removal in several ways; by filtration, by introducing a second pulse at 180 degrees to the first, this canceling out
the fundamental frequency (pulse inversion) or by more complex methods utilising pulse coding.

As well as causing vibration of the bubbles, the ultrasound beam, at the power normally used causes such violent expansion of the bubbles that they rupture. This has the effect of severely shortening the life of the contrast. This can be a severe disadvantage as it limits the time of imaging to one, or at most two passes. When the bubbles burst, however, they produce very high energies of ultrasound signal at a wide range of frequencies. This may be utilized to produce an image. There are thus two different methods of producing contrast images.

The first is a technique in which the power of the ultrasound beam (normally expressed as the mechanical index or MI) is kept low (low MI technique). This preserves the intravascular contrast agent, allowing continuous scanning for many minutes.

The second technique is to utilize a high power pulse to burst a large proportion of the bubbles in the slice being studied. The resultant high energy returned signal is then imaged (High MI technique). This may be repeated for other slices, after a period to allow new contrast to enter. Eventually a large proportion of the contrast is destroyed and no further images are possible. This may be counteracted by giving multiple smaller intravenous injections of contrast, or by continuous infusion.

The low MI technique is the easier technique to use. Present generation contrast agent have more robust bubbles than the previous agents and lend themselves to low MI imaging. The intermittent high MI technique is however better at detecting low concentrations of microbubbles. As the vessels in the prostate and also in prostate cancers are largely very small, the high MI technique may be superior.

Another method of imaging very fine vessels is a persistence technique in which consecutive frames are added together. With this technique as little as a single bubble passing slowly down a vessel may be enough to produce an image of that vessel. By its nature the technique produces blurring and artifacts with the slightest movement. With careful technique it is however useable in the prostate.

2. Technique

The prostate is first imaged by a conventional ultrasound technique. The chosen contrast imaging technique is then selected, the contrast is injected or perfused through an intravenous cannula. The pattern of uptake and washout may be studied, but this does not appear to be very useful in the prostate shortly after contrast is seen to enter the vascular tree of the prostate, a steady state is achieved. Multiple images are recorded of approximately 2mm spaced slices, usually in the axial plane, throughout the prostate.

With a real-time technique analysis at the time of scan enables targeted biopsies of abnormal areas to be performed at the same examination.

3. Results and Discussion

Early results using Doppler methods were encouraging despite the limitation of the techniques used.

Studies with enhanced conventional Doppler techniques showed that the technique could demonstrate prostate cancer [118-122].

Experience has shown that there is a recognizable pattern in the normal prostate. The central and transition zones have easily seen blood vessels that radiate from the midline outwards. The outer gland or peripheral zone appears relatively hypovascular (Figure 7). The peripheral zone probably has the same vessel density but has very fine vessels that run parallel to the surgical capsule and pseudocapsule. High resolution scans are necessity to demonstrate this pattern. One of these is persistence mode in which movement of individual bubbles may be tracked to outline the path of a vessel (Figure 8).

Benign prostatic hypertrophy (BPH) nodules have a variable vascular pattern. They tend to displace normal vessels that curve around them. The nodules themselves are mostly hypervascular but some are hypovascular (Figures 9, 10). This variability makes it difficult to differentiate them from inner gland cancers.

Cancers are detected by their hypervasculaarity (Figure 11), and it is this pattern that is described in most of the literature. A small number of hypovascular tumours have been seen (Figure 12). The fact that not all tumours are detected by their vascular density makes it likely that many are iso-vascular with the rest of the peripheral zone. Some tumours are seen as subtle alteration of the normal vascular pattern in the peripheral zone (Figures 11b and 12a). Appreciation of this sign requires a system that shows very fine vessels in great detail. At present such detail is sometimes achieved, but not in all patients.

It is too early to present any hard data that has been validated. Latest studies do suggest that there is potential for combining vascular targeted biopsies
with various systematic biopsy regimens to increase positivity or to maintain positivity while reducing the number of biopsies.

Contrast imaging of the fine vasculature of the prostate is a viable proposition with commercially available contrast agents and ultrasound equipment. Most sophisticated ultrasound machines are capable of contrast specific imaging, though extra software must be purchased. Not all machines offer the capability of contrast specific imaging via a transrectal prostate transducer, though more systems will have the capability probably in the near future.

The technique has low sensitivity in the detection of prostate tumours and cannot replace the standard tumour detection method of multiple ultrasound guided systematic biopsies. It is possible, however, that adding targeted biopsies of areas of suspected tumour vascularity and possibly areas of under perfusion may significantly increase relative positivity.

The downside is that it adds ten to fifteen minutes to the standard technique, as well as the added cost of the contrast agent.

The technique is relatively new. It relies on a complex interaction of transducer, software and the characteristics of the contrast agents used. Research and development ongoing to further optimise these factors and future improvements may make the technique more sensitive.

Imaging of tumour vascularity is a potentially useful tool in the study of tumours, as well as their detection. In those patients who opt for active surveillance of their tumours, change in vascularity may give important information about progression of the tumour and when it is appropriate to intervene.
Figure 9. Hypervascular BPH nodules. B in ‘persistence’ mode.

Figure 10. Hypovascular BPH nodules.

Figure 11. Prostate cancer. (A) A hypervascular cancer. (B) A slightly hypervascular cancer with altered vascular architecture. (C) A hypervascular cancer involving the inner gland. (D) A hypervascular cancer (arrowed). Compare the bilateral BPH nodules, hypovascular on the right, hypervascular on the left.
Contrast ultrasound may have a place in mapping tumour for high frequency focused ultrasound (HIFU) treatment and for monitoring the profit of treatment.

New drugs are being developed to prevent or delay the progress of prostate cancer. Contrast ultrasound, with its ability to study the neovascularity of tumour will have a place in the monitoring of such treatment. Finally, study of tumour neovascularity may have a place in assessing the aggressiveness of prostate cancer [123-127].

These are possible fields for further research, if contrast ultrasound proves to be sufficiently sensitive in mapping the tumour vessels.

4. CONCLUSION

TRUS is a versatile tool that is frequently used in urological practice. Its application covers many areas such as the assessment of prostatic size and volume, diagnosis of different prostatic diseases, detection and staging of prostate cancer, monitoring of the response to therapy and the guidance of prostate biopsy.

Concerning the detection of prostate cancer, however, targeted biopsies at lesions detected on ultrasound or digital rectal examination (DRE) are becoming less common with the stage migration seen in the current PSA era. Based on the lack of satisfactory sensitivity and specificity for detecting malignancy by TRUS, systematic biopsies are indispensable and it seems that the current concern is tending toward increasing the number of cores. However, when TRUS indicates the presence of a lesion, a targeted biopsy should be performed, since the specificity of an ultrasonographic abnormality is sufficiently high to justify the additional biopsy. Recent developments such as Doppler imaging, contrast-enhancement or 3-D imaging may provide higher specificity and positive predictive value for TRUS. Efforts need to be made to find any abnormalities in TRUS images in order to increase the sensitivity of cancer detection and decrease the number of unnecessary biopsies.

IV. PROSTATIC SONO-ELASTOGRAPHY

Elastography is a technique of mapping tissues by their elastic properties (soft or hard). As most cancers are harder than the surrounding tissue, elastography is a potential method of detecting cancers. Prostatic elastographs has been shown to be technically effective in many phantom studies, in vivo studies on resected specimens and animal studies. It has also been shown to be technically possible in a small number of in vivo human studies. It has low sensitivity as compared with cancer detection by multiple systematic biopsy and also a low specificity. The technique, in its present form at least, cannot replace multiple systematic biopsies. It may have a role in increasing relative positivity rates by detecting abnormal areas outside normally biopsied areas. It may have a similar role in patients who have had a negative set of biopsies but have a rising serum PSA
level. It may have a role in staging prostate cancer by accurately mapping tumour size.

In its present form, prostatic elastographs has poor reproducibility principally because of the lack of an accurate way of compressing the tissue uniformly to the same degree every time. Better methods are needed before the technique can become a useful clinical tool.

1. **BACKGROUND**

Elastography is a technique that measures the elasticity (stiffness hardness) of tissue by detecting the movement of individual elements in the tissue when it is vibrated by an external force, when it is compressed or, more usually, when it relaxes or vibrates following compression.

The physical principles of the technique have been known for many years and its potential application for medical use were first described in 1990 [128-130]. Tissue elastography may be presented in a number of ways; numerically, as an x-y graph or as a 2D image, the elastogram in which relative elasticity is represented by a grey-scale or a colour image map.

Potential clinical applications are mostly directed towards cancer detection. This is based on the principle that most cancers are harder, and less elastic than the surrounding normal tissue. It is this property that is the main reason that cancers are clinically palpable.

Early work in elastography was directed towards developing the technique. First experiments were done on gel phantoms then on in vitro tissue, muscle or liver in which an area had been hardened by heat (cooked) [131]. Early work on the prostate studied excised (radical prostatectomy) specimens [132]. These early experiments produced elastography techniques that could clearly differentiate tissues of differing elasticity.

In vivo work has been undertaken in the breast and in the liver as well as the prostate. Much of the work in the prostate has been performed on dogs [133] but increasingly studies are being performed on humans.

2. **TECHNIQUE**

The technique of elastography requires three steps:

1. The tissue studied must be stimulated – vibrated or compressed, preferably in an even and reproducible way.

2. The movement of individual elements within the tissue during vibration compression or relaxation must be detected and quantified.

3. The results must be displayed in a way that can be easily interpreted.

For the technique to be clinically useful, another step needs to be added.

4. An algorithm that decides how to use the information from the elastogram.

The four points will be discussed in turn.

3. **EXCITATION OF THE TISSUE**

There are a number of ways in which the tissue may be excited, not all applicable to the in vivo prostate.

1. Internal excitation by utilizing the ‘natural’ movement from cardiac pulsation, pulsation of blood vessels or muscle contraction. This method is not applicable to the prostate as the pulsation of the intraprostatic vessels is too weak.

2. External excitation by mechanical compression. This may be achieved in several ways. The easiest is simple mechanical compression. The probably better alternative is with alternate compressions and relaxation (vibration) by applying a modulated high power pressure wave or sound wave.

The simple compression method, in the prostate, is achieved by compressing the prostate with the transrectal transducer with a flicking motion. It has the benefit of being simple, but lacks good reproducibility [134,135].

Compression via a water filled balloon around the end of the transrectal transducer is another method that has been used [136].

External mechanical vibration has been used. The prostate lies deep within the pelvic cavity which makes this form of stimulation difficult. Success has nevertheless been achieved by applying the vibrational source via the pubic bone [134] though in this case MR elastography was used as the method of detection.

Stimulation by a source from the ultrasound transducer itself would seem intuitively to be a good solution and this has also been utilized, termed Acoustic Radiation Forse Impulse [137].

These methods all rely on simple stimulation and resultant vibration of the tissue. More complex methods are also possible. One such method that causes vibration at a small circumscribed point within the tissue is described later in this article [138].
4. MEASUREMENT OF TISSUE MOTION

Measurement of elasticity may be achieved by a number of different methods [139]. These include:

1. Parametric measurement in which the change in position of elements within the ultrasound image are measured by a variety of methods. [134, 140, 141]

2. Doppler tissue velocity measurements [135, 142].

3. Cross correlation and phase detection techniques that measure displacement of tissue [139].

4. MRI techniques may also be used for elastography measurements [137, 140].

The best method of detecting and quantifying the resultant tissue movement during compression or relaxation is by a frame by frame analysis of the ultrasound image, with the transducer held still. Computer analysis of the movement of individual speckles within the image detects and quantifies the tissue movement.

While this method produces the best results, it is, at present, not a real-time technique. While this does not discount its use, a real-time technique has distinct advantages for clinical use.

Doppler techniques may also be used to detect tissue motion. Such techniques are commonly used to measure cardiac wall motion. They produce real-time images of tissue movement, and are readily available. They do not, however, allow for any, other than very crude, quantifications of movement. They rely on setting the machine parameters so that normal tissues are displayed in colour, while less elastic tissues are displayed as a different colour or hue, or as areas of no colour.

Both techniques detect movement. This reflects elasticity because less elastic tissues move more slowly than more elastic ones. There are more sophisticated, potentially more successful methods of achieving images based on the elastic properties of tissue. One such method is briefly described [138].

Tissues may be compressed by insonating it with high power sound waves in the lower ultrasound frequency range. If the sound is made intermittent or amplitude modulated then the tissue will be alternately compressed and allowed to relax. The resultant alternate compression and relaxation of the tissue cause it to emit sound waves that may be detected and quantified. Harder, less elastic tissue will produce higher energies of sound.

This technique, when applied to a point source in tissue will quantify the elasticity of that point.

The simplest method is to direct a narrow amplitude modulated beam across the tissue to be studied. If this is moved in a line across the tissue, then a 2D graph of the average elastograph across this line may be constructed. With this technique, however, each point along the graph represents an average of the elasticity of a number of points at every depth within the tissue. It would be advantageous to confine the measurement to a point, or small volume (voxule) within the tissue. This may be achieved by using two unmodulated continuous wave beams of slightly different frequencies at different angles such that they converge at the desired point within the tissues. This achieves tissue vibration at the point of intersection.

The vibrating tissue emits a sound wave, the amplitude of which is related to its stiffness. This sound may be detected by a microphone. By mapping the sound intensities from many points, a 2D elastograph image may be produced.

This technique has obvious advantages. However, it requires a totally different equipment from ultrasound images, and would therefore need to be performed as a separate imaging technique.

5. DISPLAYING THE MOTION (ELASTICITY)

The tissue motion that reflects elasticity may be displayed as a numerical value for a particular volume of tissue or as a matrix of numerical values corresponding to voxules of tissue. Alternatively an x-y graph can be displayed for a given line across the tissue. Or a two dimensional image may be produced of a slice of tissue, with a grey scale or colour map corresponding to different numerical values of elasticity.

The absolute value of the numbers produced is hardly relevant, as it is a comparison of abnormal with normal tissue that is important.

It is the two dimensional image that is most appealing to most radiologists and clinicians as it may be directly related to the fundamental grey scale ultrasound image, as well as other cross sectional imaging techniques such as MRI. Numerical values are, however, valuable for scientific study of the technique.
**6. Tissue Harmonic Imaging**

While this is a different technique to sonoelastography there are some similarities, so it is worth mentioning here.

Tissue harmonic imaging is a technique that utilizes the non-linear echoes returned from insonated tissues rather than the fundamental reflected frequency. It relies on the fact that the transmitted ultrasound beam causes vibration of the tissues. These are at far higher frequencies that those used for elastography imaging, and so rely on different tissue parameters. The technique however does reflect partly the elastic properties of the tissue. Tissue harmonic imaging in the prostate produces broadly similar images to fundamental ultrasound imaging. There are however differences in the images. Some prostatic nodules are more hypoechoic on tissue harmonic imaging than on fundamental imaging, others are the same. This may reflect their elastic properties. At the moment, work in progress shows little correlation with biopsy results, but present numbers are too small to be definite.

**7. Algorithm for Acting on Results**

The principal reason for initial imaging of the prostate is to detect abnormal as suspicious areas for biopsy. Biopsy is necessary for definitive diagnosis and also for histological (Gleason) staging. Thus suspicious areas detected on elastography imaging are biopsied. With present results biopsying only these areas lacks sensitivity. Some method of systematic biopsy therefore still needs to be performed. Most suspicious areas will be included in the systematic biopsies. Any that are not should be biopsied. The size of the tumour should be recorded. Size of tumour is very important in deciding on treatment and for prognosis. At present however elastography estimation of tumour size has not been fully validated. It is important to compare results with radical prostatectomy specimens and also with MRI studies, the present pre-operative gold standard.

**8. Clinical Results**

It has been clearly shown that elastography techniques can demonstrate cancers in excised prostates [132]. Measurements have shown a large difference in elastic properties between normal prostate tissues and prostate cancer and importantly also between cancer and benign prostatic hypertrophy. (A) [137,143] (Table 5).

<table>
<thead>
<tr>
<th></th>
<th>Normal Glandular Prostate</th>
<th>Benign Prostatic Hypertrophy</th>
<th>Postate Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elasticity of Prostate Tissue in Kilo Pascals</td>
<td>64 ±17</td>
<td>36±9</td>
<td>100±20</td>
</tr>
</tbody>
</table>

(With permission from Ref 143)

It has been shown also that, using a variety of different methods of tissue excitation and detection of vibration, that some prostate cancers may be detected in vivo in humans. Cancers may be distinguished from normal prostate tissue and from benign prostatic hypertrophy [132,135,140,143] (Figures 13-16).

It is difficult from most of the literature to find the sensitivity and specificity of prostate elastography. By inference, and from our personal experience, it would appear to be significantly lower than multiple [135-139] systematic biopsies. Most papers state the possible benefits as adding extra positivity to systematic biopsies. Most, however, quote figures for sextant [133] biopsies, not 8 to 12, which is the current norm in most centres. Furthermore, most positivity figures for sextant plus elastography targeted biopsies are not significantly better than published figures for 8 – 12 systematic biopsies.

Another possible benefit of elastography imaging is in staging prostate cancers, by mapping the tumour more accurately than grey-scale ultrasound [140]. This may be useful in local staging and in assessing the volume of tumour. This aspect has not, however, been compared with MRI which is the correct gold standard for staging.

**9. Discussion**

Elastography is an emerging technology. It has been developed to a level that makes it useable in clinical practice. In the case of the more prognostic, simpler methods, their main drawback is lack of reproducibility. Some of the more sophisticated methods produce more reproducible results. The relative complexity of some methods however makes them difficult to use in clinical practice. Future improvements in the technology may overcome its present limitations.

Using available technology elastography has far too low a sensitivity to replace systematic ultrasound guided prostate biopsy. Given the heterogeneous growth pattern and histological of prostate cancer,
Figure 13. Protons in a magnetic field spin (or “precess”) at almost the exact same frequency of 42.6 MHz per Tesla. Slight differences in precessional frequency are the basis of MR spectroscopy, as shown in this spectrum (map of signal intensity versus frequency) showing the separate peaks of fat and water protons.

Figure 14. Normal prostate elastogram. The fibromuscular stroma (green arrows) is stiff and so is shown as a void.

Figure 15. Prostate cancer. (a) The grey-scale image shows some inhomogeneity of the outer gland but no definite tumour nodules. (b) The elastogram shows a large irregular void corresponding to the tumour confirmed by biopsy and prostatectomy.

Figure 16. Another prostate cancer. In this case a small nodule was seen on the grey-scale image but the elastogram more accurately mapped the extend of the tumour.
this is likely to remain so. The possible place of elastography therefore seems to be as an adjunct to systematic biopsy, adding extra biopsies of abnormal areas, with the aim of increasing the relative positivity rates of the technique, or possibly reducing the number of biopsies necessary while maintaining adequate positivity (sensitivity).

Both have been shown to be effective, but increased positivity in published series has been low, typically about 2%. Whether this figure justifies the use of the technique is debatable, but for most people, the figure needs to be substantially higher to justify introducing elastography into routine transrectal ultrasound and prostate biopsy lists. Improvements in technology may well achieve this in time.

Mapping of tumour size and local staging are also possibilities, but if this needs to be shown that elastography has significant benefits over MRI in the field for it to find a place.

V. COMPUTERIZED TOMOGRAPHY

Computerized Tomography (CT) is based on the x-ray principle with the computer displaying cross sectional images of the body. Since the eighties the upgrade to helical or spiral CT allows for fast and detailed image acquisition with minimal movement artefacts and high quality three-dimensional image reconstruction of virtually any organ in the body.

1. BENIGN PROSTATIC HYPERPLASIA

The diagnosis of symptomatic Benign Prostatic Hyperplasia (BPH) is based upon symptom analysis (LUTS, IPSS) and urodynamic findings like uroflow, residual urine and urethral pressure profile. Imaging is not part of the routine work up for BPH.

To rule out possible coexistent prostate cancer, PSA testing, digital rectal examination (DRE), transrectal ultrasound (TRUS) and ultrasonically guided biopsies are usually sufficient.

Patients with lower urinary tract symptoms may have complicated BPH or suffer from another pathological condition in the urinary tract. Imaging of the urinary tract is usually mandatory in such patients. Intravenous urography has long been the gold standard but today it seems that a CT urography adds more information with just an acceptable elevation of the radiation dose plus the advantage that there is no need for contrast injection [143].

Measurement of the volume of the prostate or of its transition zone is sometimes helpful for optimizing the choice of a surgical or non invasive treatment. Routine CT scan is hardly able to differentiate between the transition zone and the other anatomical zones and overestimates the prostate volume with approximately 50 % compared to transrectal ultrasound [144].

2. PROSTATITIS

The suspected diagnosis of acute or chronic bacterial prostatitis is confirmed by cyto-bacteriological tests. CT scan may be useful to rule out underlying pathology both in or outside the urinary tract. It is also indicated when prostatic abscesses are suspected [145].

Although transrectal ultrasound is at least as good to demonstrate the abscess, CT also permits to see the entire urinary tract and the adjacent structures.

In the chronic pelvic pain syndrome, historically related to prostatitis, CT scan can also be helpful to find related or other diseases.

3. PROSTATE CANCER

a) Primary diagnosis

For years CT scan did not appear to be of any use for early diagnosis of prostate cancer because it could not show a different X-ray absorption coefficient between benign and malignant prostatic tissue [146].

Besides this lack of soft tissue contrast resolution, it also has a low accuracy in the prediction of extracapsular extension (29 %) and of seminal vesicle invasion (69 %), according to Hricak et al. [147].

Recently it was demonstrated that contrast enhanced helical CT is able to distinguish prostate cancer from benign tissue in some instances [148]. This might be useful in patients with elevated PSA who have undergone abdominoperineal resection.

In normal circumstances however, TRUS remains the gold standard for initial imaging and guidance of the biopsies.

b) Biopsy

Over the eighties and nineties the blind finger-guided biopsies of the prostate were replaced for almost entirely by the technique of transrectal ultrasound guided punctures. The use of CT Scan for guidance of prostate biopsy is limited to patients after proctectomy [149].

168
c) Staging

For primary tumor staging purposes, provided DRE and TRUS are inconclusive, magnetic resonance imaging (MRI) using an endorectal coil in conjunction with a pelvic phased array is the best available technique today. Yu and Hricak report a 50% sensitivity and 95% specificity for the detection of extracapsular extension of prostate cancer [148]. This is consistent with other studies showing sensitivities between 51 and 89%, specificities between 67 and 87 % and overall accuracies between 54 and 88% [150,151] (Figures 17, 18).

Although it is still overused in clinical practice [152], the role of CT scan for locoregional staging of prostate cancer is actually limited to patients who would be candidates for pelvic lymphadenectomy [153].

Functional CT imaging, an established tool for measuring the microvasculature of prostate cancer [154], could assist in optimal treatment selection. The tumor microvasculature is a key element that influences its aggressiveness and its response to therapy. In their investigation, Henderson et al [155] showed that measurement of the blood flow in the prostate by functional CT was reliable, but other parameters of microvasculature like capillary permeability and blood volume could only be precisely measured in regions of elevated blood flow. Dynamic contrast enhanced magnetic resonance imaging has proven to be a better technique for measurement of the microvessel density [156].

d) External radiation therapy planning

CT has long been, and in many centers, still is the primary imaging modality for external radiotherapy planning [157].

Today it is challenged by MRI, said to be associated with less inter-observer variation in marking the contour of the prostate [158] and in defining its apex to accomplish potency-sparing radiotherapy [159].

On the other hand, with new techniques, developed to reduce movement artifacts, CT pre-planned external radiotherapy can be performed with markedly less local toxicity [158,160,161].

e) Brachytherapy

Transrectal ultrasound is the state of the art imaging tool for planning and guiding of brachytherapy in prostate cancer [162]. A large prostate size, interference of the pubic arch, urinary obstruction or defects from transurethral resection of the prostate may preclude its use. In these instances three-dimensional stereotactic posterior ischiorectal space computerized tomography offers an alternative for brachytherapy guidance [163]. In patients with possible or known invasion of the seminal vesicles, 3D CT scan might also be superior for direction of radioactive implants into these structures [164].

f) Local recurrence

CT does not seem to be a suitable method for diagnosis of locally recurrent prostate cancer after radical prostatectomy. Only 2 of 18 patients with biochemical relapse and local recurrence were correctly identified by CT in a study by Johnstone et al. [165] Kramer et al reported only a 36 % detection rate of residual cancer by CT in patients who all had recurrences larger than 2 cm [166].
Provided DRE and TRUS are negative, MRI is the best performing technique to demonstrate early local recurrence of prostate cancer after treatment with curative intent, especially after radical prostatectomy [167].

4. Conclusion

Since its introduction in the radiological clinics in 1974 CT quickly became and still is the state of the art technique for the evaluation of the chest, the abdomen and the pelvis. In prostatic disease however its usefulness is limited to prostate cancer in specific conditions.

CT can replace transrectal ultrasound in prostatectomized patients and it is especially appreciated for preoperative planning of external radiotherapy. In the future much is expected from techniques of imaging fusion with either Prostascint® or positron emission tomography with CT Scanning.

VI. MAGNETIC RESONANCE IMAGING

1. Introduction

The main problems for clinicians treating patients with locally prostate cancer include good staging and prediction of capsule involvement or seminal vesicle invasion by the tumour.

Different parameters are analysed by nomograms for assessing the probability of organ confined, like rectal examination, PSA level, Gleason score and the percentage of positive biopsies. According to these results, the preferred treatment (conformal radiotherapy, lymphadenectomy, radical prostatectomy) and the surgical approach may differ targeting neurovascular bundles preservation.

While transrectal ultrasound is used for guiding biopsies, MRI imaging is the most promising radiological examination for local staging.

Technique may differ from one center to another one and this explains considerable inter-reader variability in presurgical evaluation.

Endorectal MRI is commonly used but its low specificity reduces its use in clinical practice. So, its role in preoperative staging of prostate cancer remains controversial.

2. MR Endo Imaging of Prostate Cancer

Since MRI is used in the assessment of prostate cancer, endorectal coils have demonstrated their superiority to body coils for evaluation of the tumour because endorectal coils produce a higher resolution and a signal-to-noise ratio than body coils. Endorectal coil increases resolution of prostate gland and its capsule but has limitations.

Prostate cancer is classically based with the finding of low signal intensity foci in the peripheral zone of the gland on T2 sequences. However MRI images suffer from several disadvantages: it is a subjective and extremely operator dependent technique, the specificity is low, the technique depends on the performance of the equipment.

Several studies have shown the inaccuracy of T1-T2 images in determining tumour volume and tumour localisation in details because of false positive findings and changes induced by blood in tumoral areas after biopsy.

Despite the fact that the diagnostic criteria used by radiologists to evaluate extracapsular extension of the tumour are well defined in the literature (disruption of the prostatic capsule by low signal tumour, irregular capsular bulge, periprostatic fat infiltration, obliteration of rectoprostatic angle, asymmetry or direct involvement of neurovascular bundles), there is a wide range (50-92%) in staging accuracy.

But recently Wang showed that endorectal MRI adds incremental value for prediction of extracapsular extension if the images are analysed by GU radiologist [168].

To improve tumour margin definition, tumour volume, and “aggressivity” different approaches are used:

- Spectroscopy
- 3D MRI with multi planar slides
- 3 Teslas endorectal MRI
- Dynamic studies.

3. 3 Teslas External Phased-Array Coil

Imaging parameters depend on the type of machine used but optimal imaging sequences must include T1-weighted axial images including the entire pelvis and thin-section T2-weighted images with a small field of view in the axial, sagittal, and coronal
planes. The use of dynamic contrast material–
enhanced MR imaging is optional.

Prostate imaging studies were initially done with
derectal coil at 1.5 T, then combined external /
derectal coil at 1.5 T. It is supposed that the com-
bined use of endorectal coil with external phased
array coil at 3T will yield superior images.

Meanwhile, endorectal coil have some limitations
(deformation of the peripherical zone, artifacts
induced by prostate motion) and the low tolerance of
this radiologic examination is a limit for some
patients. Different authors hypothesised that for
prostate imaging, the image quality at 3 Teslas exter-
nal phased array coils would be comparable to that
obtained with an endorectal coil at 1.5 T [169]. The
signal to noise (SNR) of 3 T should be 2 times better
than comparable 1.5 system. Subsequently, endorec-
tal 3 T coils demonstrate excellent spatial resolution
and could reveal pathologic details not seen on
endorectal 1.5T or 3T external phased array coil.

Sosna and associates analysed in a prospective study
20 patients with prostate cancer (11 of them under-
went radical prostatectomy) with 3 T external
phased-array coil [169,170]. The results of 3 T
images were compared to other studies performed
during the same period with an 1.5 endorectal coil
and the quality of images were similar. They also
looked at the surgical specimens and concluded that
the in vivo volume determinations of the prostate
was very close to the ex vivo determinations con-
firming that this technique provides undistorted
images.

Staging and localizing prostate cancer using 3T
derectal coil MR imaging

It is recommended to perform MR imaging at least
4–6 weeks after prostate biopsy to avoid under- and
overestimation of tumor location and extent due to
post-biopsy changes.

Twenty-seven patients undergoing radical prostatec-
tomy were evaluated by Futterer et al with high res-
solution endorectal T2-weighted fast spin echo
images [171]. Minimal capsular detection was
detected; the localisation accuracy was 79% for the
experienced reader and 64% for inexperienced radi-
ologists.

4. DYNAMIC MRI OF THE PROSTATE (DCE-
MRI)

Generalities

The principle of dynamic imaging is to depict early
enhancement of hypervascular tumours (Fast
Dynamic Imaging). Intravenous injection of MR
contrast agent like Gadolinium (Gd-DTPA) is sug-
gested to improve the quality of images.

Recent developments allow fast multi slice contrast
enhanced MR and an acceptable spatial resolution,
and these modifications of the technique gives sig-
nificant advantage for tissue evaluation allowing
analysis of the entire prostate gland.

Gadolinium is used currently for prostate cancer
evaluation. After peripheral intravenous injection
with an injector, the contrast agent reaches the arteri-

al system as a bolus. During the first pass the differ-
ence in concentration between the intravascular and
extravascular compartment is maximal, and trans-
portation to the extravascular compartment occurs
and contributes to the increase of the signal intensity
on T1 weighted images. Thereafter the enhancement
gradually decreases [172]. This process of enhance-
ment and de-enhancement/washout can be graphically
displayed by a time signal intensity curve, [173]
with different characteristics: start of enhancement,
time to peak, slope, plateau and washout.

Tissue enhancement following contrast media
administration is multifactorial and dependent on
Physical factors: Sequence, parameter contrast medi-
um dose, machine gain setting, scaling factors. Phys-
iological factors: Tissue microvessels density, capil-
lary permeability, interstitial leakage space.

Several studies have shown that tumour neovascular-
ity is correlated with an increased risk of distant
metastasis, tumour recurrence after surgery, and
poorer overall survival. [174,175,176,177] (Table
6). However the limited experience in the use of
contrast enhancement explains why this technique is
still under evaluation.

DCE-MRI studies have two main goals:

• Find the best sequence after Gadolinium adminis-
   tration to detect ECE infiltration , or determine
tumour volume [190]; With Gd-DTPA strong
enhancement of the normal prostate, pronounced
in the central zone and inhomogeneous in case of
BPH limits the image interpretation. The use of
fast dynamic imaging enables quantification of
early contrast enhancement.

• Evaluate the variability inside the tumour of
   microvessels density, number and permeability
   which could limit the distribution of the contrast
media [191].

To achieve DCE-MRI images of diagnostic value,
### Table 6. Magnetic Resonance Imaging

<table>
<thead>
<tr>
<th>Author</th>
<th>Technique</th>
<th>Number of patients</th>
<th>DCE</th>
<th>Spectro</th>
<th>Results</th>
<th>Methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Engelbrecht M</td>
<td>3T combined pelvic phased array and endorectal coil at 3 T</td>
<td>6 volunteers</td>
<td>0.1 mmol/kg Magnevist</td>
<td>No</td>
<td>Distinction of intra and extracapsular enhancement</td>
<td>Metaanalyse 50 articles and 5 abstracts Total of 87 studies</td>
</tr>
<tr>
<td>Bloch 2004</td>
<td>Whole body 3 T coil compared with endorectal 1.5 T</td>
<td>20 patients</td>
<td>No</td>
<td>No</td>
<td>Equivalence of the quality of images</td>
<td></td>
</tr>
<tr>
<td>Sosna 2004</td>
<td>Whole body 3 T coil combined with 1.5 endorectal coil</td>
<td>22 cases</td>
<td>No</td>
<td>No</td>
<td>Better visualisation of posterior border, seminal vesicles an neurovascular bundles</td>
<td></td>
</tr>
<tr>
<td>Sosna 2004</td>
<td>Whole body 3 T coil</td>
<td>11 Specimens of Prostatectony</td>
<td>No</td>
<td>No</td>
<td>No distortion of prostate volume</td>
<td></td>
</tr>
<tr>
<td>Uematsu 2004</td>
<td>1.5 pelvic phase array coil</td>
<td>Necessary</td>
<td>No</td>
<td>No</td>
<td>Encouraging preliminary results</td>
<td></td>
</tr>
<tr>
<td>Tamada T 2004</td>
<td>3D proton MR with 1.5 T and 1.5 Endorectal coil</td>
<td>39 patients and 1 volunteer no</td>
<td>No</td>
<td>Multi shot planning is better than Fast spin echo (especially for zonal anatomy, and venous complex)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lichy MP 2004</td>
<td>Endorectal 1.5 MR</td>
<td>25 Prostate cancer and 3 BPH</td>
<td>No</td>
<td>Yes</td>
<td>Feasibility of 3D spectro MR, Interest of Spectro MR</td>
<td></td>
</tr>
<tr>
<td>Wang L 2004</td>
<td>Endorectal 1.5 MR</td>
<td>344 Patients before RP</td>
<td>No</td>
<td>Yes for 216 patients</td>
<td>Endorectal MRI adds incremental value in detection of ECE</td>
<td>Good</td>
</tr>
<tr>
<td>Mullerad M 2004</td>
<td>Endorectal 1.5 MR</td>
<td>344 Patients before RP</td>
<td>No</td>
<td></td>
<td>Roc curve in the GU radiologist group demonstrate high sensitivity and specificity for ECE detection</td>
<td>Good</td>
</tr>
<tr>
<td>Ogura K 2001</td>
<td>Endorectal 1.5 MR</td>
<td>38 patients before RP</td>
<td>Yes Gd-DTPA</td>
<td>No</td>
<td>Sensitivity for tumor detection: 81% Accuracy rate for ECE detection: 84%</td>
<td></td>
</tr>
<tr>
<td>Horiguchi A 2004</td>
<td>Endorectal 1.5T coil And pelvic coil</td>
<td>95 patients before RP</td>
<td>Yes O.1 mmol/Kg gadopentatate dimeglumine IV</td>
<td>No</td>
<td>PDAD is a good predictor of ECE MRI Sensitivity 57%, Specificity 82% for ECE (Use criteria of contact of 1 cm length of the tumor with the capsule) Good evaluation of Tumor volume (if diameter &gt; 1 cm)</td>
<td>Good</td>
</tr>
<tr>
<td>Author</td>
<td>Technique</td>
<td>Number of patients</td>
<td>DCE</td>
<td>Spectro</td>
<td>Results</td>
<td>Methodology</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------------------------------</td>
<td>--------------------</td>
<td>---------------------------</td>
<td>---------------</td>
<td>----------------------------------------------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Brassell SA</td>
<td>Whole body 1.5 MR and Endorectal Coil 1.5 T</td>
<td>40 patients before RP</td>
<td>No</td>
<td>No</td>
<td>Sensitivity 43% for ECE detection 89% Specificity and PPV (81%)</td>
<td></td>
</tr>
<tr>
<td>Padhani AR</td>
<td>Pelvic Coil 1.5 T</td>
<td>69 Patients with positive biopsy</td>
<td>Yes 0.1 mmol/Kg gadopentate dimeglumine IV</td>
<td>No</td>
<td>Good analyse of tissue enhancement Complete overlap of the enhancement patterns of central gland and tumor No correlation between gleason score and any enhancement parameter</td>
<td>Good</td>
</tr>
<tr>
<td>Preziosi P</td>
<td>1.5 Endorectal coil</td>
<td>11 patients before RP</td>
<td>Yes Gd-DTPA</td>
<td>No</td>
<td>DCE does not increase sensibility and specificity of cancer diagnosis DCE improves tumor margin definition Defines Signal intensity enhancement : s.i.% =pre contrast i-post contrast i / precontrast si X 100 Sequence 6 min</td>
<td></td>
</tr>
<tr>
<td>Rouviere</td>
<td>1.5 pelvic phased array coil</td>
<td>48 patients (42 before RP)</td>
<td>Yes Gd-DTPA</td>
<td></td>
<td>Prostate cancer enhances more and earlier than peripheral zone and adenoma Great interindividual variations No detection of prostate cancer within adenoma</td>
<td></td>
</tr>
<tr>
<td>Schlemmer H</td>
<td>Endorectal 1.5 coil</td>
<td>28 Patients before RP</td>
<td>Yes Gd-DTPA administration</td>
<td></td>
<td>MRI combined with DCE depicts 89% of tumors Contrasrt enhanced does not improve overall staging accuracy compared to T2 weighted MRI alone Useful for guiding biopsy ??</td>
<td>Good</td>
</tr>
<tr>
<td>Hevergagen J</td>
<td>1.5 standard head coil</td>
<td>20 beagle dogs with BPH</td>
<td>Yes 0.2 mmol/kg Gadoteridol</td>
<td></td>
<td>Evaluation of BPH by DCE MRI: periuretral zone is hypervascular; outer parenchymal zone is moderately vascularized Modifications with Finasteride (increases intensity of contrast enhancement, and maximal enhancement is reached later)</td>
<td></td>
</tr>
<tr>
<td>Buckley D</td>
<td>1.5 pelvic phased array coil</td>
<td>22 patients with Positive biopsy</td>
<td>Yes Gd-DTPA</td>
<td>No</td>
<td>Blood flow to tumor tissue exceeds to that prostatic normal tissue</td>
<td></td>
</tr>
</tbody>
</table>
Table 6. Magnetic Resonance Imaging (Ctd)

<table>
<thead>
<tr>
<th>Author</th>
<th>Technique</th>
<th>Number of patients</th>
<th>DCE</th>
<th>Spectro</th>
<th>Results</th>
<th>Methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larson B</td>
<td>2003 (194)</td>
<td>19 patients treated with minimally invasive treatment.(cryo) before RP</td>
<td>Yes</td>
<td>Gd-DTPA</td>
<td>No blood flow indicates necrosis MRJ gives a valid measurement of prostate necrosis Necrosis near the urethra is difficult to analyse</td>
<td></td>
</tr>
<tr>
<td>Donnelly S</td>
<td>2004 (195)</td>
<td>51 patients after cryo evaluation by biopsy (49 of them at 6 month)</td>
<td>Yes</td>
<td>Gd-DTPA</td>
<td>No A signal does not indicate an avascular gland and cell death</td>
<td></td>
</tr>
<tr>
<td>Oyen R</td>
<td>2003 (172)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Excellent Review article</td>
</tr>
<tr>
<td>Kiessling F</td>
<td>2004 (196)</td>
<td>27 patients before RP</td>
<td>Yes</td>
<td>Gd-DTPA</td>
<td>No The area under the curve was higher in tumor than in unaffected tissue Early intensity increase is best suited for discriminating prostate carcinoma The median of the slope value was higher as compared to non affected tissue (best parameter) No correlation between gleason score and dynamic parameters</td>
<td></td>
</tr>
<tr>
<td>Van Dorsten FA</td>
<td>(197)</td>
<td>23 Patients before RP</td>
<td>Yes</td>
<td></td>
<td>Combination of DCE and spectro improves localisation and characterisation of prostate cancer</td>
<td></td>
</tr>
<tr>
<td>Noworolski</td>
<td>2005 (198)</td>
<td>25 patients</td>
<td>Yes</td>
<td></td>
<td>Combination of technique Analysis of different patterns of prostate tissue (glandular or stromal BPH; Cancer )</td>
<td>++</td>
</tr>
</tbody>
</table>
high temporal resolution is needed to assess kinetic parameters relating to microvascular characteristics of the tissue. Therefore, compromises must be made between spatial and temporal resolution [178]. Typical malignant time intensity curve after bolus injection of Magnevist is described by Padhan et al. [173]. Today the improvement of overall staging accuracy of contrast-enhanced T1-weighted MRI after Gd-DTPA compared with T2 MRI sequences is still debated and contraversial [199].

5. MR Imaging Appearance of Prostate Cancer

T2-weighted MR images are essential in the evaluation of prostate cancer. On these images, prostate cancer is seen most commonly as a low signal intensity area within the high signal intensity normal peripheral zone. Benign prostatic hyperplasia nodules may obscure cancer in the transition zone. However, features such as homogeneously low signal intensity, ill defined margins, and lack of capsule help identify cancers in the transition zone. Post-biopsy changes, prostatitis, dystrophic changes, and post-treatment changes secondary to radiation or hormonal ablation therapy can be seen as low signal intensity areas on T2-weighted images and may mimic cancer [200].

In addition to T2-weighted images, dynamic contrast-enhanced T1-weighted imaging is sometimes used in prostate cancer localization and staging. Dynamic enhancement in prostate carcinoma differs from that in normal prostate tissue. These differences can be quantified and used to discriminate prostate carcinoma from normal tissue in both peripheral zone and transition zone and may provide slight improvement of cancer detection and staging performance in comparison with imaging protocols that rely on T2-weighted MR imaging alone [201]. However, further studies are necessary to improve temporal and spatial resolution of the dynamic contrast-enhanced MR sequences and to standardize the analysis of the signal-intensity-time curves.

6. Role of MR Imaging in Prostate Cancer Diagnosis and Staging

MR imaging is considered to be very useful in prostate cancer detection especially in the peripheral zone. Compared with digital rectal examination and other imaging methods, MR imaging has a higher accuracy in the assessment of local stage of the disease. However, there is a wide range (50%–92%) in the staging accuracy of MR imaging [178].

The primary role of MR imaging in the evaluation of prostate cancer is mainly to demonstrate extra-capsular extension and seminal vesicle invasion. MR imaging findings of extra-capsular extension include: irregular bulging of the prostatic capsule; contour deformity with step-off or angulated margin; overt disruption of the capsule with direct tumor extension; obliteration of recto-prostatic angle; and asymmetry of neurovascular bundles.

Seminal vesicle invasion is diagnosed when contiguous low signal-intensity tumor extension into and around seminal vesicles disrupting their architecture is demonstrated [202,203]. MR imaging is also helpful for diagnosing the invasion of adjacent organs such as the urinary bladder and rectum. In the evaluation of lymph node metastases, MR imaging and CT show similar performance.

7. Role of MR Imaging in Treatment Planning

MR imaging can provide important information about the size, location, and extent of the prostate cancer. This information may be useful for the surgeon to perform optimal resection minimizing the risks of urinary and sexual dysfunction and positive surgical margins. MR imaging can also be useful for predicting intraoperative blood loss during radical retropubic prostatectomy by showing the extent of periprostatic veins [204]. In addition, MR imaging can help predict urinary incontinence after radical retropubic prostatectomy by allowing assessment of the membranous urethral length [205].

Because MR imaging provides excellent anatomic images of the prostate, periprostatic tissues and adjacent organs, it is a very useful tool in radiation treatment planning. Tumor mapping with MR imaging optimizes dose delivery to the cancer foci and reduces the risk of normal tissue damage.

8. Role of MR Imaging in Post-treatment Follow-up

There is no consensus about the use of MR imaging in the evaluation of recurrent prostate cancer. MR imaging can provide valuable information in the evaluation of extent of local tumor recurrence and lymph node status after radical prostatectomy. MR imaging can also provide useful information after radiation treatment. However, the prostate demonstrates diffusely low T2 signal intensity and indistinct zonal anatomy due to radiation changes which may limit evaluation for recurrent tumor [206].
1. INTRODUCTION: MR SPECTROSCOPY AND MR SPECTROSCOPIC IMAGING EXPLAINED

MRI uses strong magnetic fields to induce coherent spinning of hydrogen protons, and then applies radiofrequency pulses (radiowaves) to generate an anatomic picture showing proton signal intensity by location. Each picture element (pixel) contains data from a corresponding small volume of tissue (voxel) in the patient. In routine MRI, the signal intensity of all hydrogen protons in each voxel is combined, although the signals from hydrogen protons in different molecules have slightly different frequencies (a property known as chemical shift). MR spectroscopy exploits this chemical shift property to produce a map of signal intensity versus frequency (i.e., a spectrum). At its simplest, MR spectroscopy can be used to compare the relative concentration of fat and water in the volume of tissue being interrogated, because fat protons process at a slightly slower frequency than water protons (Figure 19).

However, substantially more useful information can be generated using this technology. For example, protons in molecules other than fat and water also have distinct spectral peaks (albeit at much smaller levels, that can only be detected when the signal from protons in fat and water are entirely or largely suppressed). In addition, the information can be spatially encoded so that spectra from individual voxels are obtained, rather than from one large volume of tissue, and such anatomic localization of MR spectra is known as MR spectroscopic imaging (MRSI). MRSI generates spectra for multiple voxels, where each spectrum is a map of signal intensity versus frequency for the protons in the corresponding voxel. That is, the x and y axes of the spectral trace from an individual voxel represent frequency and intensity, respectively. The y-axis lacks absolute units. By convention, the x-axis is plotted as the downward frequency shift relative to water expressed in parts per million (this denominator adjusts for magnetic field strength, so the x axis units are fixed irrespective of the magnetic field strength of the MRI scanner used).

Chemicals with greater degrees of shift are plotted further to the left, and vice-versa. The metabolic peaks relevant to prostatic MRSI are choline, polyamines, creatine, and citrate, occurring at shifts of approximately 3.2, 3.1, 3.0, and 2.6 ppm, respectively (Figure 20). The peaks for choline, creatine and polyamines frequently overlap when MRSI is performed in vivo at 1.5T but can be distinguished at 3T and ex vivo. The areas under these peaks or resonances are proportional to the concentration of the respective metabolites, and changes in these concentrations can be used for tissue characterization and assessment. It is important to note that MRSI is always performed in conjunction with MRI, because MR spectra can only be fully interpreted when the source tissue for the spectra can be anatomically correlated and evaluated for MRI changes.

2. MRSI DATA ACQUISITION

Combined MRI and MRSI of the prostate can be performed in less than an hour using a standard clinical 1.5T MRI scanner and commercially available endorectal coils [207-209]. An endorectal coil is essential for performance of spectroscopy, and also significantly improves MRI staging accuracy [210]. The total examination time includes coil placement, patient positioning and both MRI and MRSI data acquisition. Several vendors are offering or are close to releasing product versions of this combined MRI and MRSI examination. No change in patient position or coil placement is required for performance of MRSI, which is essentially an additional MR sequence similar to the T1 or T2 weighted sequences that are routinely acquired. The major difference is that the MRSI sequence is slow, and requires some 15 to 20 minutes to perform. The failure rate for MRSI (due presumably to patient motion during the relatively long acquisition time) is approximately 5%.

VII. MAGNETIC RESONANCE SPECTROSCOPY
Three-dimensional MRSI data are acquired using a water and lipid suppressed double-spin echo PRESS (Point-Resolved Spectroscopy) sequence [180]. Water and lipid suppression is achieved using either BASING (BAnd S elective INversion with G gradient dephasing) pulses placed within the PRESS volume selection [211] or using spectral-spatial pulses capable of both volume selection and frequency selection [211,212]. Both of these approaches to water and lipid suppression allow for residual water to be left in the spectra to serve as a phase and frequency reference. Residual water also allows for assessment of technical success of the acquisition when there are no metabolite peaks present in prostate spectra due to successful therapy [209]. That is, if there are no metabolic peaks visible, the detection of residual water confirms that this reflects atrophy in the prostate rather than MRSI technical failure, since, in the latter case, residual water would not be detected. Axial T2 weighted images are typically used to graphically select the PRESS volume with the goal of maximizing coverage of the prostate, while minimizing the inclusion of periprostatic fat and rectal air. The PRESS volume is often obliqued in the z-axis since the long axis of the prostate is usually angled anteriorly (typically from zero to 25°) in this direction. The sharpness of the PRESS volume selection is enhanced through the use of high bandwidth spectral-spatial 180° pulses that also reduce chemical shift misregistration errors [211,213]. Even with the use of these optimized pulses, spectroscopic voxels at the edge of the PRESS volume can still be contaminated by residual signal arising in adjacent tissues. To further reduce contamination from tissues surrounding the prostate, recently developed very-selective outer volume saturation (VSS) pulses with very sharp transition bands are placed at the edges of the originally selected volume to better conform the rectangular PRESS volume to the shape of the prostate [214]. This often involves placing saturation bands across the corners of the PRESS volume to eliminate periprostatic lipids that normally occupy these regions.

Some of the technical challenges in obtaining high quality MR spectra of the prostate can be appreciated by considering the resolution required in the x and y-axes. Citrate protons spin with a frequency that is just 2.6 Hz per Tesla less than water protons, which spin with a frequency of 42.6 MHz per Tesla. The concentration of metabolites detected at MRSI is 1-10 mM, which is about 10,000 to 100,000 times less than the molar concentration of water protons. For these and other reasons, the possible sampling volume that can be interrogated with current MRSI technology is small, and the voxels are relatively large. For example, the standard endorectal MRSI protocol has a voxel size of 0.34 cc. A spherical tumor must be at least 0.66 cc in size in order to completely fill a 0.34 cc voxel, assuming the ideal scenario of both tumor and voxel having the same geometric center. Otherwise, incomplete filling of a voxel by tumor may result in partial voluming artifact. One of the prerequisites for good spectroscopy is a homogeneous magnetic field within the PRESS volume, since otherwise the spectral peaks cannot be properly resolved. Optimizing field homogeneity over the sample volume is known as “shimming the field”. The “sharpness” of the MR spectral peaks (i.e., linewidth) is a reflection of field homogeneity, and provides a measure of study technical quality.

3. MRSI DATA DISPLAY

MRSI produces arrays of spectra from contiguous voxels that are of approximately 0.3 cc in volume and cover most or the entire prostate. Because MRSI and MRI are acquired within the same exam, the data sets are already in alignment and can be directly overlaid (Figure 19). In this way, areas of anatomic abnormality (decreased signal intensity on T2-
weighted images) can be correlated with the corresponding area of metabolic abnormality (increased choline and decreased citrate and polyamines). Several different approaches have been used to display the combination of anatomic and metabolic information derived from simultaneous MRI and MRSI [215-219].

These include superimposing a grid on the MR image and plotting the corresponding arrays of spectra, and generating color-coded images of the spatial distribution of metabolites to overlay on the corresponding MR images. These formats provide an excellent summary of the spatial distribution of different metabolites enabling rapid identification of regions of suspected abnormal anatomy and metabolism. Additionally, since three-dimensional, volumetric MRI and MRSI data are collected, the data can be viewed in any plane (axial, coronal or sagittal), and the position of spectroscopic voxels can be retrospectively changed to better examine a region of abnormality on MRI after the data is acquired.

4. MRSI DATA INTERPRETATION

A number of general observations should be remembered when examining MR spectra. First, there is no absolute scale for the y axis, which is a “unit-less” dimension. The absence of an absolute scale requires use of internal denominators or ratios for objective quantification. In prostate MRSI, the ratio of choline and creatine to citrate and the ration of choline to creatine are frequently reported (see below). The interpretation of prostate MR spectra requires knowledge of the zonal location of the corresponding voxel, because the different zones of the prostate have differing metabolic profiles. High levels of citrate and intermediate levels of choline have been observed throughout the normal peripheral zone. The central and transition zones combined contain less citrate (Figure 21) [207].

The choline peak can be elevated in tissues surrounding the urethra and seminal vesicles due to the presence of high levels of glycerophosphocholine in the fluid within these structures, and may result in “overcalling” of tumor in these locations (Figure 22). With increasing age the glandular and stromal content of the transition zone changes due to the development of benign prostatic hyperplasia, which can be predominately glandular, stromal or most often a mixture of glandular and stromal proliferation. Predominately glandular benign prostatic hyperplasia demonstrates very high citrate levels similar to healthy peripheral zone tissue, while predominately stromal benign prostatic hyperplasia demonstrates dramatically reduced citrate [207].

Therefore, the first step in the analysis of the spectral data is to identify whether the corresponding voxels are in the peripheral zone or the transition zone. Since most prostate cancers arise in the peripheral zone, most MRI/MRSI research has focused on peripheral zone cancer. The interpretation of transition zone voxels is complicated by metabolic overlap between prostate cancer and predominately stromal benign prostatic hyperplasia that is almost always

Figure 21A. Axial T2-weighted image of the prostate. A grid has been overlaid on the image, and corresponds to the spectral array shown in Fig. 21B.

Figure 21B. Spectral array corresponding to the grid shown in Fig. 21A. Such an overlay and array illustrates one method of displaying and correlating MRI and MRSI data.
Figure 22A. Axial T2 weighted MR image of the mid prostate in a normal 35 year old volunteer. The spectra from the grid marked in the image are shown in Fig. 22B.

Figure 22B. Spectra from the normal prostate demonstrate three distinct metabolic patterns, shown in greater magnification and details in Figs. 22C-E.

Figure 22C. Spectrum from normal peripheral zone demonstrates high citrate and intermediate choline and creatine levels.

Figure 22D. Spectrum from normal central gland demonstrates lower citrate levels than the peripheral zone, but similar choline and creatine levels.

Figure 22E. Spectrum from normal periurethral tissue demonstrates low citrate and mildly elevated choline. The latter presumably reflects choline within the muscular layer of the distal prostatic urethra.
indicating a greater likelihood of malignancy. Creatine for tissue characterization, with a higher number was examined in early studies as a quantitative measure for tissue characterization, with a higher number of choline and creatine to citrate peaks [233].

Given that prostate cancer is characterized at MRSI by raised choline (a normal cell membrane constituent, which is elevated in many tumors) reduced citrate (a constituent of normal prostatic tissue) or both [234], the ratio of choline and creatine to citrate was examined in early studies as a quantitative measure for tissue characterization, with a higher number indicating a greater likelihood of malignancy. Creatine is included with choline because the spectral peaks of these two compounds often overlap, and may be inseparable. Inclusion of creatine in this ratio is not considered a potential source of error, since creatine appears to remain at a relatively constant level in both healthy and cancerous prostatic tissue.

Healthy prostate epithelial cells possess the unique ability to synthesize and secrete enormous quantities of citrate. The decrease in citrate with prostate cancer (Figure 23) is due both to changes in cellular function [222,223] and changes in the organization of the tissue, resulting in a loss of its characteristic ductal morphology [224,225]. Malignant prostatic epithelial cells demonstrate a diminished capacity for net citrate production and secretion [226,227]. Unfortunately, citrate can also be reduced by prostatitis or post-biopsy hemorrhage or any condition that causes a reduction in prostatic ductal morphology and the associated citrate rich fluids.

As in other human cancers, the elevation of the choline peak in prostate cancer is associated with changes in cell membrane synthesis and degradation that occur with the evolution and progression of cancer [228,229] and changes in epithelial cell density and altered phospholipid metabolism likely contribute to the observed increase in choline seen in prostate cancer [224,230]. Recent high-resolution NMR studies of ex vivo prostatic tissues have identified several new metabolic markers for prostate cancer including polyamines, which appear very elevated in spectra of healthy prostatic peripheral zone tissues and predominantly glandular benign prostatic hyperplasia and dramatically reduced in prostate cancer. Similar to changes in choline-containing compounds, changes in cellular polyamine levels have been associated with cellular differentiation and proliferation [231,232]. Moreover, it has recently been demonstrated that the loss of polyamines in regions of cancer can be detected by MRSI as an improvement in the resolution of the choline and creatine peaks [233].

The ratio of choline and creatine to citrate in normal prostatic tissue has been established as 0.22 +/- 0.13 [John Kurhanewicz, personal communication], while the ratio frequently exceeds 0.5 in malignant voxels (more than two standard deviations above normal). More recently, choline elevation has been recognized to be more specific for cancer than citrate reduction, so we also determine the choline to creatine ratio (suspicious if greater than 2). Based on metabolic changes in choline, polyamines and citrate in regions of prostate cancer a standardized five-point scale for the interpretation of peripheral zone metabolism in the pre-therapy prostate was recently developed and validated (Figure 24) [235].

Representative spectra illustrating this scoring system are shown in Figure 25. This scoring system has proved to be highly accurate (approximately 88% accuracy) in distinguishing benign and malignant tissue with excellent inter-observer agreement (kappa statistic = 0.80).

5. APPLICATIONS OF PROSTATE MRSI

a) Tumor diagnosis:

Only a few studies have investigated the accuracy of MRI in the diagnosis of prostate cancer, because MRI is generally reserved as a staging study in patients with biopsy-proven prostate cancer. That said, patients with an elevated PSA and one or more negative transrectal prostate biopsies are frequently encountered in clinical practice, and form a population in whom MRI/MRSI could potentially be of interest as a diagnostic test (Figure 26). In order to determine the diagnostic accuracy of endorectal MRI/MRSI in the diagnosis of prostate cancer, 40 patients were retrospectively identified who were referred for endorectal MRI/MRSI prior to biopsy [236]. All patients had an elevated serum PSA level. None of the patients had a histological diagnosis of prostate cancer at the time of imaging; 36 patients had a previous negative biopsy and 4 patients had never undergone biopsy. Based on MRI alone and then based on the combination of MRI/MRSI, the presence or absence of prostate cancer in each side of the prostate was rated on a 5 point scale (1 = definitely absent, 5 = definitely present) by a single
Figure 23A. Axial T2-weighted image of the prostate in a patient with prostate cancer.

Figure 23C. Axial T2-weighted image of the prostate at a level just superior to the image shown in Fig. 17C. The seminal vesicles are seen at this level, strongly suggesting that the spectral findings in Fig. 17B represented “pseudotumor” secondary to downward contamination or “leakage” of choline signal from the very high choline content in the ejaculatory fluid.

Figure 24A. Axial T2-weighted image of the prostate in a patient with Gleason 9 prostate cancer in multiple biopsy specimens.

Figure 24B. Corresponding MRSI grid shows widespread elevation of choline in virtually all the peripheral zone voxels, especially on the left side, consistent with extensive and aggressive tumor.
experienced reader. Data were analyzed for each side of the prostate, using the presence or absence of cancer on transrectal US guided biopsy performed after MR as the standard of reference. Transrectal US guided biopsy demonstrated no cancer in 24 patients, bilateral cancer in 11 patients, and unilateral cancer in 5 patients. The area under the receiver operating characteristic curve for the diagnosis of prostate cancer was 0.70 for MRI and 0.63 for combined MRI/MRSI. These values were not significantly different. These results suggest that (using current technology) MRI/MRSI has high specificity but low sensitivity for the diagnosis of prostate cancer in patients with an elevated serum prostatic specific antigen level. Also, the addition of MRSI does not appear to have a significant impact when compared to evaluation by MRI alone.

b) Tumor localization

In a study with two readers using step-section histopathology as the standard of reference in 53 patients [212], MRI alone had a sensitivity of 77-81% and a specificity of 46-61% for the sextant localization of prostate cancer. With the addition of MRSI, sensitivity fell slightly to 68-73%, but specificity increased substantially to 70-80%. These data suggest that MRSI is particularly helpful in preventing “overcalls” of tumor by demonstrating normal metabolism in areas of equivocally reduced T2 signal intensity (Figure 27). It should be emphasized that the results described above refer to the sextant localization of prostate cancer, which is not synonymous with volumetric localization. In a recent study [237] MRI and MRSI were performed in 37 patients prior to radical prostatectomy. Two independent readers recorded peripheral zone tumor nodule location and volume. Results were analyzed using step-section histopathological tumor volumetry as the standard of reference. The mean volume of all peripheral zone tumor nodules (n = 51) was 0.79 cc
Readers detected 20 (65%) and 23 (74%) of the 31 peripheral zone tumor nodules greater than 0.5 cc. For these nodules, tumor volume measurements by MRI alone and combined MRI and MRSI were all positively correlated with histopathological volume (Pearson’s correlation coefficients of 0.49 and 0.55, respectively), but only measurements by combined MRI and MRSI reached statistical significance (p < 0.05). These results for prostate cancer tumor volume measurement may appear disappointing, particularly in the context of other studies indicating high accuracy for sextant localization. Two factors probably account for this discrepancy. First, per sextant rather than per nodule analysis does not require size concordance between imaging and pathology, so a very small imaging abnormality counts as a true positive even if the tumor is pathologically much larger, and vice versa. Second, there has been a general downward stage migration of prostate cancer in the era of widespread PSA testing.

c) Tumor staging

Multivariate feature analysis has shown the MR imaging findings that are most predictive of extracapsular extension are a focal irregular capsular bulge, asymmetry or invasion of the neurovascular bundles, and obliteration of the rectoprostatic angle [238]. The addition of MRSI to MRI has been shown to increase staging accuracy for less experienced readers and to reduce interobserver variability [239]. The role of MRSI is not to directly depict extracapsular tumor, but rather to indicate whether a tumor is metabolically present and aggressive in an area where a questionable finding of extracapsular extension is present (Figure 28).

d) Treatment planning

Several groups have reported the use of MRSI to increase the brachytherapy radiation dose in prostatic locations considered suspicious for cancer [240,241]. Such studies, which suggest technically successful dose escalation in spectroscopically suspicious locations implies improved clinical outcome, must be viewed with caution, given the limited ability of MRI and MRSI to assess tumor volume, as discussed above. More recently, MRI/MRSI has been shown to improve preoperative surgical planning with respect to the decision to preserve or resect the neurovascular bundle, although this study did not separate the relative contribution of MRI versus MRSI [242].

e) Post-treatment follow-up

In a recent study [243], endorectal MRSI was performed at 1.5T in 21 patients with biochemical failure after EBRT for prostate cancer. Spectroscopic voxels were considered suspicious for malignancy if choline was elevated and citrate was absent (compared to pre-treatment studies, spectroscopic evaluation after therapy is simplified by radiation-induced metabolic atrophy). Receiver operating characteristic
Curve analysis was used to analyze cancer detection in each side of the prostate by MRSI at different thresholds based on the number of suspicious voxels in each hemiprostate, respectively.

The presence or absence of cancer on subsequent transrectal biopsy was used as the standard of reference. Biopsy demonstrated locally recurrent prostate cancer in 9 hemiprostates of 6 patients. The area under the receiver operating characteristic curve for MRSI was 0.81. In particular, the presence of three or more suspicious voxels in a hemiprostate showed a sensitivity and specificity of 87% and 72%, respectively, for the diagnosis of local recurrence (Figure 29). Of note, 7 hemiprostates demonstrated complete metabolic atrophy (i.e., no metabolic peaks) on MRSI, and showed only post-radiation atrophy on biopsy (Figure 30).

These preliminary data suggest MRSI can accurately detect locally recurrent prostate cancer after EBRT. In particular, complete metabolic atrophy appears to have high negative predictive value and may indicate that local salvage therapy will be unhelpful. Recent work investigating the role of MRSI for the evaluation of local control after EBRT is analogous to the primary indication for MRSI in neuroradiology, i.e., the distinction of post-radiation necrosis from recurrent tumor [244].

6. CONCLUSIONS

Combined endorectal MRI/MRSI provide combined anatomic and metabolic data and arguably represents the single best modality for local evaluation of prostate cancer extent and aggressiveness. The addition of MRSI to MRI has proven benefit in tumor localization, volume estimation and staging. MRSI may be particularly useful in the evaluation of suspected local recurrence after radiation therapy. It is also important to remember that this is a technology that remains in evolution (e.g., results for MRI/MRSI performed at 3T are beginning to emerge) [245] and it may be some time before the true roles and benefits of endorectal MRI/MRSI are clear.
Figure 29. Sixty-year-old man with a rising PSA 3 years after EBRT for prostate cancer. Photomontage showing an axial T2-weighted MR image of the prostate. A grid overlaid on the image corresponds to the adjacent MR spectral array, which demonstrates several suspicious voxels (arrows) with elevated choline in the left side of the gland. Transrectal ultrasound-guided biopsy confirmed the presence of locally recurrent prostate cancer in the left gland.

Figure 30. Sixty-nine year-old man with a rising PSA (to 1.0) 2 years after EBRT for prostate cancer. Photomontage showing an axial T2-weighted MR image of the prostate. The adjacent MR spectral array, which demonstrates complete metabolic atrophy, with no detectable metabolic peaks in any of the peripheral zone voxels. Subsequent biopsy showed no evidence of locally recurrent prostate cancer in the gland, and his PSA has stabilized spontaneously.
Positron emission tomography (PET) images in vivo biological processes three-dimensionally. A specific radiopharmaceutical, labelled with a radioactive isotope is injected. In the tissue the unstable isotope is transformed by a physical process called Beta Decay. Basically a proton turns into a neutron and a positron, and the positron is emitted. When this positron, in fact a positively charged electron, fuses with a true electron their mass is transformed into energy. This process is known as annihilation and the released energy is emitted as a photon. With photon detectors and photomultiplier tubes the process can be localized and translated into images.

Depending upon the radiopharmaceutical injected a number of metabolic processes in the body, varying from glucose metabolism, amino acid transport, DNA synthesis and membrane synthesis, can be studied.

Although PET has been in existence since the 1960s, it did not gain complete clinical acceptance until the 1990’s after Di Chiro, et al., demonstrated the ability of PET to differentiate between recurrent brain tumor and radiation necrosis, prompting the recognition of PET as a valid method of tumor imaging [246]. Researchers and clinicians have been able to detect a wide variety of malignancies with PET including lymphoma, melanoma, sarcoma, lung cancer, colon cancer, and squamous cell carcinoma [247-252]. Since current radiographic techniques such as magnetic resonance imaging (MRI) and computed tomography (CT) scans are often unable to accurately stage the extent of prostatic malignancies, [253,254] the utilization of PET for assessing this disease is an attractive alternative.

Some centres, equipped with a cyclotron, have the possibility to prepare positron emitters with short half life times. This almost instant availability of tracers with the possibility of short acquisition times was the start of a spectacular application of the PET scanners in the last years. It is already routine clinical practice in cardiology, neurology and some domains of oncology. PET is now the reference for the detection of viable myocardial tissue, for the evaluation of dementia and for localisation of epileptic foci. It is also used in case of fever of unknown origin in search for an infected or inflammatory process. Tumour imaging and management is another attractive possibility of this young imaging modality and it is already commonly accepted in the oncological work up for tumours of brain, lung, colon, breast, ovaries, thyroid, the musculoskeletal system, lymphoma and squamous cell carcinoma of head and neck [255].

1. **POSITRON EMITTERS**

The most commonly used PET tracer is 18-Fluoro-deoxy-Glucose (18-FDG). It allows to study the glucose metabolism in vivo and has a half life time of 110 minutes, which is very useful for transport to other places or centres. Other positron emitters than 18-FDG (Table 7) have rapid decays and can only be used if they can be prepared on site. 18-FDG is in general very useful for imaging of tumour pathology because most malignant cells have a markedly increased glycolisation activity.

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Half live time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-C</td>
<td>20.4</td>
</tr>
<tr>
<td>13-N</td>
<td>10.0</td>
</tr>
<tr>
<td>15-O</td>
<td>2.1</td>
</tr>
<tr>
<td>18-F</td>
<td>110</td>
</tr>
</tbody>
</table>

2. **PET TRACERS AND RELATED METABOLIC PROCESS**

Today’s most commonly used and studied tracers for PET scanning and the metabolic or physiologic process they interfere in. (Table 8)

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Metabolic process</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-F-deoxyglucose (18-FDG)</td>
<td>glucose consumption</td>
</tr>
<tr>
<td>18-F-thymidine (18-FLT)</td>
<td>nucleic acid consumption</td>
</tr>
<tr>
<td>11-C-methionine</td>
<td>amino acid transport</td>
</tr>
<tr>
<td>H^2 (15)O</td>
<td>perfusion</td>
</tr>
<tr>
<td>82-Rubidium</td>
<td>perfusion</td>
</tr>
<tr>
<td>13-NH^3</td>
<td>perfusion</td>
</tr>
<tr>
<td>18-F-misonidazole</td>
<td>hypoxia</td>
</tr>
<tr>
<td>18-F-DOPA</td>
<td>dopamine receptor binding</td>
</tr>
</tbody>
</table>
3. PREPARATION FOR 18-FDG PET IN ONCOLOGICAL PATIENTS

Precise planning is necessary because of the half life of the tracer. Patients are typically asked to fast for 6 hours prior to the examination to minimize glycemia and insulinemia that may limit the sensitivity of the study. Diabetic patients may be given more detailed preparatory instructions and may undergo spot glucose testing before the test is performed; those with hyperglycemia are re-scheduled for the examination once their glucose levels are better controlled. Images are acquired 60 minutes after injection of 12-17 mCi of F-18 deoxyglucose. During the time between injection and tissue acquisition, the patient is asked to avoid muscular activity. Emission images of the abdomen are acquired, followed by a Ga-68 transmission scan of the same area. Images are corrected for signal attenuation and reconstructed using filtered back projection in the transaxial, coronal, and sagittal planes.

5. NORMAL BIODISTRIBUTION OF 18-FDG 60 MINUTES AFTER THE INJECTION

The normal biodistribution of 18-FDG is characterized by an important cerebral activity and a good visualization of the tonsilla, the sublingual salivary glands and the vocal cords. The tracer accumulates moderately in the liver, the spleen and the bone marrow whereas it is variable in the heart and the gastrointestinal system. The renal clearance of the radiopharmaceutical permits good visualisation of the urinary tract.

6. POSSIBLE CLINICAL APPLICATIONS OF 18-FDG PET IN GENERAL ONCOLOGY

a) Primary diagnosis

Because of their rapid growth pattern and therefore increased need for energy and nutrition under hypoxic conditions malignant tumours preferably consume glucose. This avidity for glucose is well suited for 18-FDG PET and the intensity of the signal is to a certain extent a marker for the malignant potential.

b) Staging

T-staging

Because of their lower resolution and the absence of anatomical landmarks the PET images are less useful than the conventional imaging techniques for primary tumour (T) staging.

• N-staging

The malignant state of the loco regional and distant lymph nodes is not always proportional to their size, but certainly to their metabolic activity. This explains why 18-FDG PET is often more accurate than conventional imaging techniques for N-staging of malignant tumours [247].

• M-staging

PET is a very sensitive imaging tool for detection of organic metastases but for precise localisation of the lesions the conventional imaging techniques are more suitable. Combining the advantages of both tests might be the answer. Protocols for PET-CT and PET-MRI fusion imaging are currently under investigation.

c) Recurrent disease

The specificity of the conventional radiological techniques is significantly reduced after surgery, radiotherapy or chemotherapy because anatomical landmarks may have been disturbed and because of scar tissue formation. PET may be able to show the difference between cancer and scar, but the test should not be done within the first 3 months after surgery or radiotherapy because 18-FDG is also increasingly absorbed by inflammatory cells.

d) Monitoring therapy

Early re-evaluation of the target lesions by PET scan during chemotherapy can differentiate responders from non responders and help to avoid unnecessary prolonged toxic treatment in the latter.

e) Planning radiotherapy

Incorporation of PET images in the radiotherapeutical planning provides relevant adaptations of the target zone in 30 % of the cases.

7. LIMITATIONS OF 18-FDG PET

a) The resolution

The spatial resolution is limited to 4 – 5 mm for two adjacent hot spots, however single lesions smaller than 4 mm may be visualized.

b) The tracer

An important normal accumulation of 18-FDG precludes the diagnosis of hot spots in the brain and the urinary tract.

c) Absence of anatomical landmarks

A precise anatomical localization of the hot spots is difficult because there are no clear 18-FDG accumulating landmarks. This problem might be solved in the future with the possible availability of PET-CT or PET-MRI fused images.
d) Tumour associated limitations

The intensity of the signal depends upon the degree of hypermetabolic activity inside the tumour cells. Tumours with low proliferation rates are thus difficult to recognize. With the radioisotope accumulating both in malignant and in inflammatory cells, tumours from surgically or radiotherapeutically treated patients may produce false positive signals.

8. PET in urological tumours

The use of PET scan in urological oncology is still under investigation. As with other tumors, most experience is with 18 FDG. So far high grade renal cell and testicular cancer appear to be good indications [256]. Primary Bladder cancer is not a good indication but PET is currently under investigation for detection of pelvic recurrence or metastatic lesions.

Prostate cancer is another story, certainly in its early stages.

9. PET imaging of the prostate

Prostate imaging with PET scanning is currently studied with different tracers, not surprisingly mostly if not all aiming at prostate cancer. Experiments with the most commonly used tracer, 18-fluoro-2 deoxyglucose (FDG), are still disappointing and most investigators are now turning their attention and experiments to other tracers.

10. 18-Fluooro-2-Deoxyglucose (18-FDG)

a) Primary prostate cancer

The potential of PET for imaging prostate cancer was initially established in animal models of metastatic prostate tumors using the radiotracer N-3-18-fluoropropylputrecine [257,258].

Certainly in its early stages, prostate cancer is a slow growing neoplasm an this stands for lower uptakes of radioisotopes like 18-FDG (Figure 29). Image obscuring due to the adjacent bladder through which the radiopharmaceutical is eliminated and the relatively high uptake of 18-FDG by benign prostatic hyperplasia account for the varying reported amounts of FDG activity in prostate cancer cells and the altogether conflicting result [259,260,261].

b) Relapse after primary local treatment

FDG PET cannot reliably distinguish postoperative scar tissue from a local recurrence after radical prostatectomy [262]. Haseman et al [263] found only one positive FDG PET in 6 patients with biopsy proven local recurrence. Because of to many false positive and false negative findings this tracer is not recommended in post prostatectomy patients [264].

c) Monitoring treatment response

There appears to be a trend of decreased FDG accumulation both in primary and metastatic prostate cancer with positive biological response to hormonal ablative therapy, but the changes are of different orders of magnitude. This suggest the unreliability of the biological markers [265].

Inaba reported the first use of PET in imaging in humans for metastatic prostate cancer using 15-oxygen which demonstrates increased uptake in areas of hypervascularity [266]. Bares, et al, presented the initial experience with FDG for prostate cancer imaging in 1993 [267]. These authors, in a subsequent study found increased uptake and 100% of 7 patients with untreated metastatic disease [268]. Metastatic bone lesions are more consistently identified than nodal metastases in most series. Oyama et al found showed increased bone uptake by PET in 9 patients with metastases on bone scintigraphy [269]. Similarly, Kao et al reported PET detection of bone lesions in 11 patients with increased uptake in metastatic bone lesions but no increased uptake by PET in 20 patients with increased uptake on bone scintigraphy due to benign bone lesions [270].

Schielle et al evaluated the sensitivity of PET for detecting individual metastatic bone lesions [271]. Of 202 lesions in 22 patients, PET identified 131 lesions (sensitivity of 65%) with only 2 false positive lesions (positive predictive value of 98%). Sung et al found that in patients experiencing a favorable response to androgen deprivation therapy, PET did not reveal metastatic sites apparent on bone scintigraphy [272]. In untreated patients, however, PET identified lesions apparent on bone scintigraphy in 3 patients and detected a metastatic bone lesion in one patient with normal bone scintigraphy (Figure 31).

Although the detection of lymph nodes metastases is not as reliable as bone metastases, it remains superior to other imaging modalities (Figure 32). Heicap pel et al found increased PET uptake in 4 of 6 patients with lymph node metastases as small as 0.9cm which were not detected on CT scanning but were discovered pathologically following radical prostatectomy with pelvic lymph node dissection [273] (Figure 33).

There were 2 patients in that series with nodal metastases £0.5cm in the surgical specimen which were not detected by either CT or PET imaging. In a com-
Figure 31. Pelvic 18-FDG PET image in a 73 y/o with PSA 21.2ng/ml, 83 cc gland, and Gleason’s 3+4 in 4 of 12 biopsies. Image shows no increased prostatic uptake with diuretic PET imaging.

Figure 32. Bone scintigraphy (Panel A) and whole body 18-FDG PET image (Panel B) in the same patient showing bone lesions detected by PET but not bone scan.

Figure 33. Pelvic 18-FDG PET image in a 57 y/o man with PSA of 43.2 ng/ml and Gleason 4+5 prostate cancer in all 10 biopsy cores. Image shows increased uptake through entire prostate as well as in right seminal vesicle and left pelvic lymph node consistent with prostate cancer involvement of these structures.
parable study, Sanz et al found that PET revealed nodal metastases in 3 (27.3%) of 11 patients found to have metastases at radical prostatectomy and pelvic lymph node dissection [274]. In contrast, Selzer et al showed no difference in the detection of nodal metastases in 45 patients by CT and PET imaging, with both demonstrating a detection rate of 50% [275]. In the series published by Sung et al, PET identified nodal metastases in 3 patients, 2 of which (66.7%) had no evidence of adenopathy by CT imaging; no patients had adenopathy by CT that did not demonstrate increased uptake by PET [272].

Multiple authors have found that locally advanced and metastatic prostate cancer was best detected by FDG PET imaging in untreated and hormone refractory tumors [268-276]. In addition, Sung et al found variable tumor detection in patients with a partial biochemical response to hormone therapy and lack of FDG uptake in patients with an undetectable PSA levels in response to hormone therapy [272].

Base on these data it may be concluded that FDG PET performs inconsistently in the management of prostate cancer [256].

Application of PET imaging to prostate cancer has been problematic due to the relatively slow-growing nature of this tumor which is reflected as a concomitantly low rate of glycolysis [277]. Furthermore, local assessment of prostate cancer is inconsistent since FDG is excreted in the urine, accumulates in the bladder and prostatic urethra, (Figure 34) and can effectively mask any uptake in the prostatic parenchyma [277, 278] Liu et al utilized hydration, diuretic administration, and preprocedure bladder emptying to evacuate the non-specific isotope in the urine which allowed visualization of 8 (61.5%) of 13 locally advanced untreated or hormone refractory prostate tumors (Figure 35) [276] Effert et al, used urethral catheterization and bladder irrigation to clear the isotope from the bladder. [268]

But, with this technique, these investigators only found increased uptake in 2 of 14 (14.2%) untreated locally advanced, T3 or T4, tumors (Figure 36). This low detection rate may be due in part to their use of muscle as a measure of background intensity; they found that both benign prostates and primary prostate malignancy were not significantly different. Hara and colleagues suggested the use of 11-carbon-choline (11C-choline) PET over FDG PET in the imaging of prostate cancer because 11C-choline has negligible urinary excretion [279].

There are conflicting reports on the uptake of FDG by benign prostatic hyperplasia (BPH). Effert et al did not show increased uptake by clinically organ-confined prostate tumors, however, increased uptake was noted in patients with BPH which was theorized to be masking any small prostate tumors [268]. Laubenbacher et al found no significant difference in the FDG activity of primary prostatic adenocarcinoma compared to that of BPH [269]. Lui et al found no increased uptake by BPH or clinically organ-confined prostate cancers but noted increase uptake in prostate cancer patients with concomitant prostatitis [276].

The tendency of inflammatory processes, such as prostatitis, to demonstrate increased FDG uptake represents another limitation of this imaging technique (Figure 34) [280]. Inflammatory lesions resulting in false positive interpretation of FDG PET imaging has historically been problematic primarily in the detection of lung malignancies when granulomatous disease is present [281,282]. Most experienced nuclear medicine radiologists have learned to detect the sometimes subtle differences between the uptake demonstrated by granulomas and the uptake seen with malignancy. Inflammation from diverticular disease and prostatitis is less commonly recognized as a source of increased uptake in the pelvis that can simulate locally extensive urologic malignancies [276,283].

Most current investigations on the application of PET in prostate cancer are aimed at identification of alternative isotopes that, unlike 18-FDG, do not depend on a high rate of glycolysis or are not excreted in the urine [284-289].
11. 11-C-ACETATE

The uptake of acetate in tumor cells is related to their lipid synthesis. Acetate is metabolized and incorporated in phosphatidylcholine and in neutral lipids. Its lack of urinary excretion and its good tumor to background ratio, make this tracer more suitable for prostate cancer imaging than FDG. But acetate is not a cancer specific tracer, it also accumulates in normal and hyperplastic prostatic tissue [290].

This tracer has been investigated for preoperative staging of prostate cancer [291] and for the detection of local recurrent disease after radical prostatectomy, [292] resulting in moderate results. The biological uptake of 11-C-acetate in the normal prostate and especially in benign prostatic hyperplasia seems to be too high and restricts the applicability of this technique [293].

In an analysis, comparing 18-FDG and 11C-acetate, sensitivity was increased by approximately 10% using the latter pharmaceutical when evaluating patients for local recurrence and regional metastasis [270]. In that study, 20 out of 24 patients (83%) had detectable recurrent disease using 11C-acetate PET, with the majority being on hormonal therapy at the time of imaging.

As all C labelled radiotracers, 11 C acetate has a short half life time (20.4 min) and can only be used if prepared on site.

12. 18-F-ACETATE

Methods for safe and efficient synthesis of this radiotracer are still under investigation. Data from animal experiments suggest that it could be more useful than carbon labelled acetate for prostate cancer imaging [294].

13. C-METHIONINE

The accumulation of L-methyl-C-methionine in cancer cells is attributed to an increased amino acid transport and in part to proteine synthesis [295]. Nilsson et al described this high uptake in a considerable number of malignant lesions in patients with androgen resistant prostate cancer [296]. Recently, Toth et al [297], published results about 11-C-methionine PET detection of prostate cancer in 20 patients with a PSA between 3,49 and 28,6 ng/ml and one to five previous negative biopsies. In 15 patients 11-C-methionine accumulated suspiciously in the prostate and biopsies of these zones revealed prostate cancer in 7 patients. The prostatic biopsies in the
other 5 patients were negative. That still makes 8 (the patients with 11-C-methionine accumulation but negative biopsies) to 13 (plus the non accumulating patients) in whom there was no clear diagnosis.

14. Carbon-11-Choline

The metabolism of the cell membrane components of malignant tumours with high proliferation rates is also increased. This leads to a higher uptake of choline, one of the components of phosphatidylcholine, an essential element in the cell membrane. The intracellular choline is rapidly metabolized to phosphorylcholine (PC). Experiments with Magnetic Resonance Spectroscopy revealed that prostate cancer cells had a high uptake of PC, whereas normal tissue does not [298]. Unfortunately the uptake in BPH is also elevated and in individuals it is difficult to distinguish between cancer and BPH [299]. PET imaging with 11C-choline has an added benefit in the fact that the isotope is not excreted in the urine making diuretic administration or bladder irrigation unnecessary [284]. 11C-choline PET does not, however, avoid the problem of differentiating benign prostatic hyperplasia from prostate cancer. Despite the mean standard uptake value being 5.6 for prostate cancer and 3.5 for benign prostatic hyperplasia, crossover does occur, which makes it difficult to determine tissue type from this imaging alone [287]. Also, there is no correlation of the standard uptake value with Gleason score, prostate size, or PSA [287].

C-choline PET can show clear images of the prostate and the pelvic lymph nodes in the absence of bladder activity [279,300,301].

With all choline compounds an intensive bowel activity is observed and can cause false positive findings [302]. The blood clearance being approximately 7 minutes, imaging should be done as early as 3 to 5 minutes after the injection.

Currently it is still unclear whether the choline uptake in prostate cancer lesions can serve as a marker for their biologic aggressiveness. Studies so far showed no correlation [299].

The feasibility of 11-C-choline PET for the diagnosis of recurrent disease after radical prostatectomy or external beam radiation for localized prostate cancer was investigated by de Jong et al. [303]. Of 36 patients, 20 operated and 16 irradiated, 14 (7 in each group) had no biochemical evidence of disease and C-choline PET was negative in all. In the group of 22 patients with biochemical failure 12 had positive findings on 11-C-choline PET.

Histological confirmation of recurrent disease was obtained in 6 patients with local lesions and 4 patients with suspicious lymph nodes. Three patients had suspicious bone lesions on 11-C-choline PET, confirmed in 2 patients by a positive bone scan. However, a comparison of the PSA values in the biochemical failure group shows that only patients with PSA relapse above 4.3 ng/ml had positive findings on 11-C-choline PET (table 8).

Furthermore, in the group of operated patients, all lesions recognized on 11-C-choline PET were also identified by conventional transrectal ultrasound and CT scan. Not being able to identify tumour recurrence below a PSA of 4.3, 11-C-choline does not appear to add something to the conventional imaging techniques in this setting. But as stated by the authors and the comments, this group is too small and too heterogeneous to draw a final conclusion.

**Table 9**: PSA relapse in patients treated for localized prostate cancer by radical prostatectomy (R.P.) or external beam radiation (EBR). The values in bold go with positive findings on 11-C-choline PET [303].

Picchio et al [304] compared 11C choline PET with FDG PET in 100 men with biochemical relapse after radical prostatectomy (n=77) or external radiation (n=23). Besides a significantly better agreement with conventional imaging overall, 11-C choline also detected 10 of 16 local recurrences demonstrated by conventional imaging whereas 18-FDG only detected 6.

11 C choline has some advantages over 18-FDG and 11 C acetate, and this is reflected in the results of the clinical studies, but there are drawbacks like the rapid bowel excretion and a very short half life time.

15. 18-F-Choline

F-labelled choline could be a good alternative for C-labelled choline because of its longer half life time. Unfortunately this isotope is characterized by a rapid
16. 18-F-FLUORODIHYDROTESTOSTERONE

18 F Fluorodihydrotestosterone (FDHT) in the Form of 16-beta-18-Fluoro-5 alpha dihydrotestosterone is a radiolabeled analog of dihydrotestosterone, the primary ligand of the androgen receptor [307]. In a pilot study by Larson et al, 18F-DHT localized to tumor sites in patients with progressive clinically metastatic prostate cancers; [289] however, the appropriate use of this modality still remains to be determined. Ongoing studies are conducted to find out whether FDHT uptake in prostate cancer cells is indicative for well differentiated disease, likely to respond to androgen withdrawal.

17. 99mTcBOMBESIN

Another novel radiotracer under investigation is 99mTc-labeled bombesin. Bombesin is a neuropeptide, which is produced by several adenocarcinomas, including prostate cancer. De Vincentis et al demonstrated increased uptake when scanning with bombesin in 12 out of 12 patients with biopsy-confirmed primary prostate cancer, and identified four patients with positive lymph nodes, all of which were confirmed on pathological analysis after surgery [288].

PET imaging, either with FDG or one of the newer isotopes, can be further enhanced by fusion of the images with more traditional axial images. CT has been the most widely used for PET fusion imaging with encouraging initial results [308,309,310].

18. CONCLUSION

PET is an appealing new imaging technique for prostate cancer because of its ability to view the whole body with reduced irradiation, which is particularly interesting in a disease that may spread anywhere in it. The radiation dose for a PET scan is about equal to that of a conventional Computerized Tomography (CT), whereas the information obtained with on PET scan may demand for several CT Scans, e.g. Chest, Abdomen, Pelvis and Brain.

The basic biophysical principles of PET sustain its application for early diagnosis and for early local recurrence of prostate cancer. Clinical research with different radiotracers showed that there are still many obstacles to its introduction in the clinical setting: the availability of the isotopes, some with a short half life time, their applicability in the first region of interest being the small pelvis and the 4-5 mm limited spatial resolution of the images. But it is clear that based on the preliminary results PET deserves further attention with more clinical trials. F labelled tracers suffer from a rapid urinary excretion, C labelled tracers from a rapid intestinal excretion.

These problems might be solved in the future. Attempts have already been made to reduce the bladder activity by forced diuresis or by constant bladder irrigation with an indwelling catheter but did not improve the results. Problems to recognize the landmarks or the exact anatomical localization of PET hotspots can be solved my PET-CT or PE-MRI fusion technology.

Until further clinical research has shown much better results PET can not be regarded as a useful tool for initial detection or local staging of prostate cancer.

But the most interesting application in the future could be the identification of recurrent disease after treatment with curative intention.

**IX. INDIUM-111 CAPROMAB PENDETIDE-SCANNING (PROSTASCINT®)**

1. INTRODUCTION

The ProstaScint (In-111 capromab pendetide) scan (Cytogen Corporation, Princeton, NJ, USA) uses a radiolabeled murine monoclonal antibody targeted to the intracellular epitope of the prostate-specific membrane antigen (PSMA) molecule. PSMA is expressed on both benign and malignant prostate epithelial cells. Approximately 95% of prostatic malignancies express PSMA, including those that have lost the ability to express prostate specific antigen [311-313]. Prostascint imaging is approved by the United States Food and Drug Administration for the diagnostic imaging and staging of newly diagnosed prostate cancer patients who are deemed high risk for pelvic lymph node metastases. This indication is bolstered by the inability of standard cross-sectional imaging techniques such as computerized tomography (CT) and magnetic resonance imaging (MRI) to detect prostate cancer spread to lymph nodes that are not pathologically enlarged [314-321]. In addition, ProstaScint is approved for post-prostatectomy patients with a rising PSA, for whom there is a clinical suspicion of metastatic disease [322-
Uptake in the prostatic fossa on ProstaScint imaging can differentiate recurrent/residual disease from benign post-operative changes and obviates the need for transrectal ultrasound-guided biopsies of the prostatic fossa to confirm the presence of malignancy. ProstaScint does not, however, distinguish between recurrent malignancy and iatrogenic residual benign prostatic tissue remaining in the prostatic fossa.

2. Technique

Patients receive an intravenous infusion of 5 mCi of radiolabeled antibody followed by planar and cross-sectional single photon emission computerized tomography (SPECT) [311-313]. Normal biodistribution of capromab pendetide includes the most intense activity in the liver, spleen, bone marrow, and blood pool. Varying levels of activity are observed in the kidneys, nasopharynx, spermatic cord, and genitalia. Prostatic soft tissue metastases are typically located more often in pelvic lymph nodes but these tumor foci can be difficult to identify by ProstaScint scanning, due to masking by the bone marrow in the pelvis. As a result, detection of pelvic nodal disease requires careful evaluation with tomographic imaging and is further optimized by additional imaging adjustments. One adaptation employed to minimize false positive ProstaScint readings is the performance of dual isotope imaging. Dual imaging using both ProstaScint and radiotracelabeled red blood cells allows differentiation between true ProstaScint uptake and non-specific isotope collection in the vascular spaces and marrow [328]. More recently, delayed images have been employed to avoid the confounding blood pool activity. Delayed images are acquired three to five days following administration of the isotope in order to allow mobilization of the non-specifically bound isotope from blood vessels and bowel while persistent uptake due to malignancy remains [329].

3. Results

In most studies to date, the predictive ability of ProstaScint is superior to that of CT/MRI in detecting lymph node metastases prior to therapy. Rosenthal et al evaluated 152 men with high-risk disease (defined by Gleason score, PSA, and clinical stage) with ProstaScint prior to surgical staging [317]. Of 64 patients with positive lymph nodes, 40 were read as positive by ProstaScint scan (PPV = 62%). Of 88 patients without lymph node metastases, 63 were read as negative by ProstaScint (specificity = 72%). Overall, the sensitivity for detection of lymph node metastases was 62%. In this study, CT and MRI demonstrated PPV of only 4% and 15%, respectively [317].

PSA, Gleason grade, and other clinical data have been incorporated into algorithms or nomograms to aide in the prediction of lymph node metastases in prostate cancer. Polascik and colleagues [319] compared the ability of several clinical algorithms and ProstaScint scans to predict lymphatic metastases in 198 men with clinical T2-3 disease undergoing radical prostatectomy. A total of 39% of patients in this high risk cohort were found to have lymph node metastases at surgery. From 40.5% to 45.4% of lymph node positive patients were predicted by clinical algorithm compared to 66.7% by ProstaScint alone. When integrating ProstaScint with clinical algorithms based upon Gleason score, disease volume, and pre-operative PSA, a PPV of 72.1% could be achieved.

Several studies support the use of ProstaScint for prostatic fossa imaging [322-327]. In one of the largest series published by Raj et al, the authors found that of 255 men with PSA levels between 0.1 and 4.0 ng/ml after radical prostatectomy, uptake was noted in 72% (184 patients) [322]. A total of 31% (78 patients) were note to have local uptake (prostatic fossa) only. No minimum serum PSA value was needed to detect disease.

ProstaScint has also been evaluated for its role in demonstrating a durable response to salvage radiation for isolated uptake to the prostatic fossa but with variable results [327, 330-333]. Kahn and colleagues compiled the results of a multicenter study men who underwent salvage radiation therapy after radical prostatectomy [330].

Of the 32 patients evaluated, 70% demonstrated a durable response to salvage radiation with a normal extraprostatic scan compared with 22% with a scan positive outside the prostatic fossa. The median follow-up was 13 months after salvage radiation. In contrast, in a study of 30 men, Thomas et al found no significant difference in biochemical control, with a median follow-up of 34.5 months, between men who had a negative scan (31%) compared with men who had a positive scan in at least one location (either within or outside the prostate fossa) after salvage radiation therapy [330].

More recently, Wilkinson and Chodak found only seven of 15 men (46.7%) demonstrated a durable response to salvage radiation with positive Prostascint uptake to the prostatic fossa [326] (Figures 37, 38).
4. LIMITATIONS

As mentioned above, a major limitation of ProstaScint historically has been the collection of the isotope in blood pools and bowel. Recent improvements, particularly delayed imaging and fusion with cross-sectional imaging techniques have significantly improved the previously low positive predictive value of the technique [329,332].

Another fundamental limitation of the ProstaScint imaging is the necessity for an experienced interpreter [311-313]. As the findings of the study are often subtle, with a high risk of false positive due to bowel or blood vessels overlying the lymph nodes, there may be an improvement in interpretive accuracy as the reader becomes more experienced. The importance of reader experience in interpretation of ProstaScint scans is made evident by the reported data regarding staging. Initially, the test had a reported sensitivity, specificity, and overall accuracy of 62, 72, and 68%, respectively; [322] however, higher values have been reported in more recent studies (75, 86, and 81%, respectively)[321]. The discouraging initial results experienced at most facilities has limited the wide-spread use of this imaging technique in the past. To overcome the obstacle of the learning curve, Cytogen Corporation has developed a rigorous training program, called Partners In Excellence (PIE), and will not allow nuclear medicine specialists to purchase the isotope if they have not been trained through this program [334]. A panel of expert consultants has also been convened by the company to provide nuclear radiologists with rapid second opinions at no charge.

Fusion of capromab pendetide uptake with anatomically detailed CT or MR images provides information on risk factors that strongly influence the prog-
nosis and staging of CAP, which includes factors both within and beyond peripheral and transitional zone cancer, the implications of which have been discussed by Augustin et al. [335]. Similarly, capromab pendetide uptake can be used to identify whether extra-capsular extension (ECE) and perineural invasion [336] or involvement of the seminal vesicles [337,338] has occurred. Risk factors beyond the prostate include the FDA-approved application of identifying lymphatic metastases. In addition, two of the case reports that follow demonstrate that capromab pendetide uptake also identifies skeletal metastasis, which is one of the most prevalent sites of CAP metastasis [339] (Figures 39 A and B).

5. Future Study

Progress in optimizing techniques of fusing Prostascint images to CT and MRI has the potential to vastly improve the utility of this imaging technique [333]. Other monoclonal antibodies, such as human J591, are being investigated as a molecular-based imaging tool [340]. Human J591 targets the extracellular domain of prostate-specific membrane antigen and has been accurate (> 90%) in identifying metastatic prostate cancer in preliminary studies. Employing these PSMA antibodies for therapeutic purposes is another emerging application with wide-reaching implications [340].

X. LYMPHOTROPIC MAGNETIC NANOPARTICLE MRI

1. Introduction

The primary limitation of cross-sectional imaging techniques such as CT and MRI in the identification of lymph node metastases is the inability to identify disease within smaller (5 mm to 10 mm) lymph nodes. The infusion of lymphotropic magnetic nanoparticles prior to MRI provides a potential means of molecular imaging to discern normal lymphatic tissues from malignant deposits in lymph nodes that are not pathologically enlarged by standard imaging [341-343]. These nanoparticles have a monocristalline, inverse spiral, superparamagnetic iron oxide core, contain a dense packing of dextran to prolong their time in circulation and increase uptake by lymph nodes [342]. The nanoparticles themselves measure an average of 2 to 3 nm [344]. The mean overall particle size of the 10-kD dextran is 28 nm [344]. The nanoparticle-dextran comprise the agent ferumoxtran-10, manufactured by Advanced Magnetics, Incorporated (Cambridge, MA) and marketed as Combidex by Cytogen Corporation (Princeton, NJ) in the United States and as Sinerem by Guerbet Group (Aulnay-sous-Bois, France) in Europe. In the literature, these nanoparticles are also termed monocrystalline iron oxide nanoparticles (MION), lymphotropic superparamagnetic nanoparticles, lymphotropic superparamagnetic nanoparticles, ultrasmall superparamagnetic iron oxide (USPIO), superparamagnetic iron oxide (SPIO), and quantum dots [343-347].

2. Technique

After administration of ferumoxtran-10 (2.6 mg Fe/kg in 100ml saline infused intravenously over 30 minutes), the lymphotropic nanoparticles are slowly extravasated from the intravascular space into the interstitial space, from which they are transported by way of lymphatic vessels, through interstitial lymphatic fluid transport, into the lymph node tissue [348]. Within normal lymph nodes, nanoparticles are internalized by macrophages, and these intracellular iron-containing particles reduce the signal intensity (SI) of normally functioning nodes on post-contrast T2-weighted fast spin-echo or gradient-echo images through the magnetic susceptibility effects on iron oxide [343]. Metastatic nodes, in which macrophages are replaced by tumor cells, show no significant change in SI on postcontrast T2-weighted fast spin-echo or gradient-echo sequences. Even in lymph nodes with small foci of tumor, disturbances in lymph flow or in nodal architecture caused by the tiny metastases lead to abnormal patterns of accumulation of the nanoparticles that are detectable by MRI performed 24 to 36 hours after dosing [349].

By conventional MRI criteria, lymph nodes are classified as malignant if the short-axis diameter is elongated and exceeds 10 mm or is rounded and exceeds 8 mm. On MRI with lymphotropic magnetic nanoparticles, several criteria have been suggested by which nodes should be considered malignant on T2-weighted fast spin-echo or gradient-echo sequences after the administration of lymphotropic magnetic nanoparticles:

1. A decrease in signal intensity by at least 30 percent; [344] (Figure 40)

2. A heterogeneous signal (giving the entire node a mottled appearance), discrete focal defects (isolated islands of high signal intensity), or both; [344,345]
3. Nodes with a central area of hyperintensity (excluding a fatty hilum identified on T1 sequence) but a peripheral decrease in signal intensity; [344,349]

4. Partial decreased signal intensity in more than 50% of the node area [349].

3. **RESULTS**

The vast majority of studies investigating the clinic use of lyphotropic nanoparticles have grouped pelvic malignancies, including prostate cancer, into a single category for analysis [350-355]. In the only prostate cancer series to date, published by Harisinghani et al., 80 men with stage T1-3 prostate cancer had improved detection of nodal metastases by high resolution MRI following the administration of ferumoxtran-10 [344]. In this series, 334 lymph nodes were resected at surgery; 63 nodes in 33 men were found to contain metastatic disease on histologic analysis.

Only 15 of 33 patients with lymph node metastases were detected by conventional MRI size criteria, while all 33 were detected by MRI following ferumoxtran-10 infusion. Overall, 90.5% of all positive lymph nodes, and 96.4% of metastases in lymph nodes 5 mm to 10 mm in size were identified by ferumoxtran-10 infusion. Only a 5% false positive rate was observed. Unexpectedly, even very small metastases, less than 2 mm in diameter, were occasionally identified within normal-sized lymph nodes. Such microscopic tumor deposits are below the threshold of detection of any other imaging technique.
4. LIMITATIONS

The primary side effect of feromoxatran-10 is an anaphylactoid reaction described as being similar to iodinated contrast. The Advanced Magnetics’ January 2005 submission to the U.S. Food and Drug Administration states that of the initial 131 patients receiving a bolus injection of undiluted Combidex, three (2.3%) had serious adverse events in the form of anaphylactoid reactions; one of these patients died of the anaphylactic reaction [349].

The company has foregone the prior bolus injection method of administration and now recommends dilution of the standard 2.6 mg Fe/kg dose in 100ml saline to be infused over 30 minutes, stating that this technique not only significantly reduces the incidence of adverse events, but it also facilitates prompt intervention. The bolus technique was necessary to provide adequate liver/spleen imaging, but due to the adverse events, an indication for use of the agent in liver/spleen imaging is no longer being sought by the company. Since the introduction of the infusion technique, the rate of serious adverse events has dropped to 0.3% (5 of 1930 subjects), which is less than a third of the rate seen with administration of iodinated contrast agents [349]. The most frequent adverse events with the infusion technique were vasodilation (3.4%), rash (3.0%), back pain (2.4%), and pruritis (2.2%) [349]. In patients complaining of back pain during infusion, the discomfort was alleviated by cessation of the infusion and restarting at a slower rate [344]. Less common adverse reactions were urticaria, dyspnea, nausea, chest pain, sweating, and headache [349].

A dose-dependent sequestration of the particles in the liver has been demonstrated in rat models that persists for up to 63 days [356]. This has been suggested to be the result of particle breakdown products and the induction of ferritin and hemosiderin with increasing iron cores/loading factors. No long-term sequelae of this sequestration has been described.

5. FUTURE STUDIES

In the United States, a conditional approvable letter was received from the U.S. Food and Drug Administration (FDA) for Combidex on March 24, 2005. Final approval depends on publication of data for specific tumor sites rather than including all pelvic tumors into a single category. Ongoing studies are also taking place to optimize acquisition strategies, such as the timing of contrast material-enhanced imaging, the section thickness, the imaging plane, the imaging parameters for T2-weighted sequences, particle coating, and particle size [357,358]. Alternative agents, particularly anionic iron oxide nanoparticles are also being evaluated [359].

While there are great implications for improved imaging with this technology, there is an even greater amount of excitement in the development of fluorescent nanoparticles to aide in node dissection, antibody-conjugated nanoparticles, and nanoparticle-linked therapeutic agents [346,360].

Bone scintigraphy utilizing 99 m Tc labeled phosphates and phosphonates gradually accumulate in metabolically active bone with increased uptake in areas of greater metabolic activity. Not only does this include areas of malignancy but also inflammatory changes such as fracture healing. It is well recognized that these studies are useful in the assessment of patients with carcinoma of the prostate to determine the presence of metastatic disease but it is also well recognized that not all patients require this study either during their initial assessment or during treatment and monitoring. The study is sensitive for the detection of osteoblastic bone metastasis which commonly occurs in patients with metastatic prostate cancer (Figure 41).

Though bone surveys have been used in the past, studies have indicated that those with normal studies 23% demonstrate bone metastasis on scintigraphy [361]. Prior to the use of PSA, studies also demonstrated the value of routine use of bone scintigraphy in categorizing patients. Sixteen percent of patients having clinically localized disease were upstaged to having metastatic disease. With the widespread use of PSA in both the evaluation and staging of patients with carcinoma of the prostate, there has been a decreased utilization of bone scintigraphy in the assessment of these patients. In those with clinically localized disease based on PSA and biopsy characteristics (Gleason grade, tumor volume), it is evident that bone scintigraphy is not useful in those having a low likelihood of tumor metastases. Chybowski and associates demonstrated that those patients with positive bone scans have significantly higher PSAs (median – 158.0ng/ml) than those with negative studies (median 11.3ng/ml). Of those men with a PSA >20ng/ml, 1% had a positive bone scan [362].
Others have also tried to determine the indications for performing bone scan studies and Lee and associates demonstrated on a multivariate analysis that Gleason score, PSA and clinical stage were significant independent predictors for positive bone scintigraphy in patients with carcinoma of the prostate. For instance, of 308 men with Gleason 2-7 PSA of 50 or less, clinical stage T2b, only three were found to have a positive bone scan. The incidence of positive scans increase as these parameters increase and for those men with PSA >50, almost half had positive bone scans [363].

Though several decades ago, bone scans were used routinely in all patients with newly diagnosed prostate cancer, and these studies were also used in the follow up of patients, oftentimes on an annual basis, it is now recognized that they can be performed more selectively. Those patients in a low-risk category for presence of advanced disease do not require bone scans. Similarly in those patients with a stable PSA and lack of symptoms of bone pain or elevation of alkaline phosphatase, either at the time of diagnosis or during the periods of follow up, do not require these studies either. Should a rapid change occur in PSA with a short doubling time or the patient develops bone pain consistent with metastatic disease, bone scans are certainly indicated. Finally, bone scans are occasionally obtained during clinical trials or in those patients in whom a baseline evaluation is required.

Figure 41. Bone scan demonstrating metastatic disease from carcinoma of the prostate.
RECOMMENDATIONS

Random controlled trials assessing the value of each of the imaging modalities regarding diagnosis, staging and treatment assessment are limited to non-existent. Most reports are of individual or institutional experience. *The Committee is therefore unable to make definitive recommendation based on evidence-based medicine grading systems.*

ULTRASONOGRAPHY

- No evidence to demonstrate the role of ultrasound in diagnosis of prostate cancer.
- Transrectal prostate ultrasonography is not helpful for detection of microscopic involvement of capsule or seminal vesicles in patients diagnosed with carcinoma of the prostate.
- Transrectal ultrasound is helpful in assisting in prostate biopsy and permitting sampling of specific zones within the prostate or biopsyng ultrasound abnormalities. Costs need to be considered in the processing of biopsy cores. A specific number of cores to be obtained with each biopsy cannot be recommended.
- New developments in use of contrast agents and the role of color and power Doppler appear to be helpful in identification of malignancies but more studies correlating imaging and anatomical findings are needed.
- Transrectal prostate ultrasound is useful in assessment of total prostate and specific zonal size. These measurements are useful in determining PSA density.

COMPUTED TOMOGRAPHY

- Has a limited role for local and/or distant staging. The applications are based on biopsy information and prostate specific information.
- Internal architecture of the prostate is not seen and microscopic invasion cannot be visualized.

MAGNETIC RESONANCE IMAGING

- Provides excellent identification of prostate zonal anatomy.
- Microscopic invasion of the seminal vesicles and prostate capsule cannot be reliably identified.
- Magnetic Resonance Imaging has similar limitations for staging as computed tomography microscopic disease cannot be identified.

MAGNETIC RESONANCE SPECTROSCOPY

- Offers much potential for identification of specific abnormalities with the prostate.
- Magnetic Resonance Spectroscopy will potentially assist in biopsy and local therapy but greater experience is needed.

POSITRON EMISSION TOMOGRAPHY

- Is helpful in identifying local recurrence following local therapy, e.g., radical prostatectomy, radiation therapy.
- Offers potential in detection of metastatic disease particularly lymph node involvement.
- Positron Emission Tomography is useful in identifying abnormal areas within the prostate.

SPECT

- Offers potential in the detection of metastatic disease.
- SPECT is able to potentially identify abnormal areas within the prostate which has the potential for local therapy and assisting in biopsy.
- As with PET, offers the potential of identifying local recurrence and differentiating from metastatic disease in patients with PSA elevation following local therapy.

BONE SCINTIGRAPHY

- Useful in detecting bone metastases. It is helpful to select patients for the study with a reasonable likelihood of bone involvement based on clinical information, e.g., bone pain, elevation of PSA (.10ng/ml), grade and volume obtained of tumor on biopsy.
- The use of bone scintigraphy after treatment in asymptomatic patients with low/stable or undetectable PSA is not necessary unless participating in a research protocol.
Carcinoma of the Prostate

DIAGNOSIS

• Digital rectal examination abnormality (nodule, induration)
  and/or
• Abnormal total PSA
  and/or
• Abnormal PSA free/total ratios
  and/or
• Abnormal PSA velocity

Prostate Biopsy*

Positive

Further Evaluation And Therapy

Negative

Follow up: DRE, PSA

No Change

Follow up

Abnormal Change

Repeat Biopsy

* Number of cores (≥ 8).
Pathologic interpretation should also include laterality, number positive cores and percent tumor involvement, Gleason primary and secondary grade.
Carcinoma of the Prostate
STAGING after Diagnosis (DRE+PSA+Biopsy)

Life expectancy $\leq$ 5 years and asymptomatic
- Low or intermediate risk
  - No further workup observation
  - Bone scan
    - Positive
      - Systemic therapy
    - Negative
      - Systemic therapy (optional) or
      - Active monitoring

Life expectancy $\geq$ 5 years or symptomatic
- Low or intermediate risk
  - Pelvic lymph node Dissection (optional)
- High and very high risk
  - BONE SCAN
    - T1-T2 and PSA $>20$ ng and/or
    - Gleason $\geq 8$ or T3-T4
    - M+, or N+ or local extension
      - SUSPICIOUS NODES
        - FNA or RPLND
      - NEGATIVE
        - SYSTEMIC THERAPY
        - LOCAL THERAPY

POSITIVE
- M+, or N+ or local extension
  - Systemic Therapy
  - Local therapy in selected cases

NEGATIVE
- M0 and N0 and No local extension
  - LOCAL THERAPY

FNA: Fine needle aspiration
RPLND: Retroperitoneal lymph node dissection
REFERENCES

20. Alexander, A. A.: To color Doppler image the prostate or not: that is the question. Radiology, 1995; 195: 11.
22. Stamey TA. The era of serum prostate specific antigen as a marker for biopsy of the prostate and detecting prostate cancer is now over in the USA. BJU International 2004; 94:963-70.


54. Tauson K, Pham TQ, Issa MM, Kabalin JN. Routine transition zone and seminal vesicle biopsies in all patients undergoing transrectal ultrasound guided prostate biopsies are not indicated. J Urol, 1997; 157:204-206.

55. Trauner M, Varner M, Cochlin DL. Detection of prostate cancer by biopsy. Variations in positivity rates for matched populations using the same biopsy regime. Work in progress


netirezonance imaging for local staging and detection of ne- 
urovascular bundle involvement of prostate cancer: correlation 

185. Horiguchi A, nakashima J, Horiguchi Y, Nakagawa K, Oya M, 
Ohigashi T, Marumo K, Murai M.: Prediction of extraprostatic 
cancer by prostate specific antigen density, endorectal MRI, and 
biospy Gleason score in clinically localized prostate cancer. 

186. Brassell SA, Krueger WR, Choi JH, Taylor JA III: Correlation 
of endorectal coil magnetic resonance imaging of the prostate 

187. Padhani AR, Gajditski CJ, Macvicar DA, Parker GJ, Suckling J, 
Revell PB, Leach MO, Deanaley DP, Husband JE: Dynamic 
contrast enhanced MRI of prostate cancer: correlation with mor-
phology and tumour stage, histological grade and PSA. Clin 

188. Preziosi P, Orlacchio A, Di Giambattista G, Di Renzi P, Bor-
tolotti L, Fabiano A, Cruciani E, Pasqualetti P: Enhancement 
patters of prostate cancer in dynamic MRI. Eur Radiol. May 

189. Rouviere O, Baudrant A, Ecouched R, Socin-Pangaut C, 
Pasquiou C, Bouvier R, Marechal JM, Cyonnet D: Characteri-
zation of time enhancement curves of benign and malignant 

190. Schlemmer HP, Merkce J, Grobholz R, Jaeger T, Michel MS, 
Werner A, Rabe J, VanBaick G: Can preoperative contrast 
enhanced dynamic MR Imaging for prostate cancer depict microvessel density in prostatctomony specimen? Eur Radiology 
2004; 14:3409.

191. Neverhagen JT, von Teng-Koblik H, Baudenidstel KT, Jia G, 
Polzer H, Henry H, Levine AL, Rosol TJ, Knopp MV: Benign 
prostate hyperplasia: evaluation of treatment response with 

192. Buckley DL, Roberts C, Parker GJ, Logue JP, Hutchinson CE. 
Prostate cancer: evaluation of vascular characteristics with 
dynamic contrast-enhanced T1-weighted MR imaging—initial experience Radiology, 2004; 233:709-715.

193. Larson BT, Collins JM, Huidobro C, orica A, Vallejo S, Bost- 
wick DG. Gadolinium-enhanced MRI in the evaluation of min-

194. Donnelly SE, Donnelly BJ, Saliken JC, Raber EL, Vellet AD. 
Prostate cancer: gadolinium-enhanced MR imaging at 3 weeks 
compared with needle biopsy at 6 months after cryoablation. 

Michel MS, Trojan L, Ederle J, Abel U, Kauzcor HU, Semmler 
W, Delorme S. Simple models improve the discrimination of prostate cancers from the peripheral gland by T1-weighted 

196. van Dorsten FA, van der Graaf M, Engelbrecht MR, van Leen-
daerts GJ, Verhofstad A, Ripkema M, de la Rosette JJ, Barentsz 
JO, Heerschap A. Combined quantitative dynamic contrast-
enhanced MR imaging and (1) H MR spectroscopic imaging of 
20:279-87.

197. Noworolski SM, Henry RG, Vigner DB, Kurhanewicz J. Dynamic 
contrast—enhanced MRI in normal and abnormal prostate 
tissues as defined by biopsy, MRI, and 3D MRSI. Magn 

198. Quinn SF, Franzini DA, Demlow TA, Rosenkranz DR, Kim I, 
Hanna RM, Szumowski J: MR Imaging of prostate cancer with 


of prostate cancer from normal peripheral zone and central 
gland tissue by using dynamic contrast-enhanced MR imaging. 

staging with endorectal surface coil MR imaging. Radiology 
1991; 178: 797-802.

ar extension of prostate carcinoma with endorectal and phased- 
array coil MR imaging: multivariate feature analysis. Radiology 
1997; 202: 697-702.

203. Coakley FV, Eberhardt S, Wei DC, et al. Blood loss during rad-
ical retropubic prostatectomy: relationship to morphologic fea-
tures on preoperative endorectal magnetic resonance imaging. 
Urology 2002; 59:884-888.

204. Coakley FV, Eberhardt S, Kattan MW, Wei DC, Scardino PT, 
Hricak H. Urinary continence after radical retropubic prostatec-
tomy: relationship with membranous urethral length on preop-
erative endorectal magnetic resonance imaging. J Urol 2002; 
168:1032-1035.

205. Hricak, R., et al., Carcinoma of the prostate gland: MR imaging 

206. Males, R.G., et al., Clinical application of BASING and spec-
tral/spatial water and lipid suppression pulses for prostate cancer 
staging and localization by in vivo 3D H1 magnetic resonance 

207. Kurhanewicz, J., et al., Combined magnetic resonance imaging 
and spectroscopic imaging approach to molecular imaging of 

208. Hricak, H., et al., Carcinoma of the prostate gland: MR imaging 
with pelvic phased-array coils versus integrated endorectal— 

209. Star-Lack, J., et al., Improved solvent suppression and increased 
spatial excitation bandwidths for three-dimensional PRESS CSI 
using phase-compensating spectral/spatial spin-echo pulses. J 

210. Scheidler, J., et al., Prostate cancer: localization with three-
dimensional proton MR spectroscopic imaging—clinicopatho-

211. Schricker, A.A., et al., Dualband spectral-spatial RF pulses for 

212. Tran, T.K., et al., Very selective suppression pulses for clinical 
MRSI studies of brain and prostate cancer. Magn Reson Med, 

213. Tropp, J.S., et al., Characterization of MR spectroscopic imag-

214. Twieg, D.B., et al., Phosphorus-31 magnetic resonance spec-
troscopy in humans by spectroscopic imaging: localized spec-

215. Lenkinski, R.E., et al., Integrated MR imaging and spectroscopy 
with chemical shift imaging of P-31 at 1.5 T: initial clinical 

216. Lenkinski, R.E., et al., Combined MR imaging and spectroscopy 
with chemical shift imaging of P-31 at 1.5 T: initial clinical 

217. Maudsley, A.A., et al., In vivo MR spectroscopic imaging with 

218. Vigner, D.B., et al., Chemical shift imaging of human brain: 
axial, sagittal, and coronal P-31 metabolite images. Radiology, 


Committee 10

New Developments in Screening and Early Detection of Prostate Cancer

Chairman

F.H. Schröder (The Netherlands)

Members

P. Albertsen (USA),
L. Boccon Gibod (France),
O. Brawley (USA),
H.B. Carter (USA),
S. Ciatto (Italy),
J. Hugosson (Sweden),
I. Korfage (The Netherlands),
S. Machtens (Germany)
I. INTRODUCTION
1. REQUIREMENTS FOR SCREENING HAVE NOT BEEN MET
2. SCREENING FOR PROSTATE CANCER IN 2005

II. RESULTS OF OBSERVATIONAL STUDIES OF EARLY DETECTION

III. STATUS OF ONGOING RANDOMISED STUDIES
1. THE PROSTATE, LUNG, COLORECTAL, AND OVARIAN CANCER SCREENING TRIAL (PLCO TRIAL)
2. THE EUROPEAN RANDOMIZED STUDY OF SCREENING FOR PROSTATE CANCER (ERSPC)

IV. PSA AND THE TIMING OF EARLY DETECTION MEASURES
1. AGE TO BEGIN SCREENING
2. RESCREENING INTERVALS
3. AGE TO DISCONTINUE SCREENING

V. PROSTATE BIOPSY – WHAT IS CONTEMPORARY PRACTICE?
INTRODUCTION
1. INDICATIONS FOR PROSTATE BIOPSY – GENERAL INDICATIONS
2. RELATIVE INDICATIONS FOR BIOPSY
3. SUMMARY

VI. QUALITY OF LIFE WITH SCREENING – IMPACT OF TREATMENT
1. INTRODUCTION
2. PATIENTS AND METHODS
3. RESULTS
4. DISCUSSION AND CONCLUSION

VII. NEW DEVELOPMENTS IN SCREENING AND EARLY DETECTION OF PROSTATE CANCER. MODELLING OF OUTCOMES (LEAD TIME, OVERDIAGNOSIS, PC MORTALITY)
1. LEAD TIME
2. OVERDIAGNOSIS
3. MORTALITY

VIII. POPULATION-BASED SCREENING – ARGUMENTS FOR
1. DISCLAIMER
2. THE DECREASING PROSTATE CANCER MORTALITY IN THE US
3. THE INNSBRUCK STUDY
4. EVIDENCE FROM CASE CONTROL STUDIES
5. DECREASING RATES OF METASTATIC DISEASE
6. THE ROTTERDAM PILOT STUDIES

CONCLUSION

REFERENCES
Because of the absence of data from high quality randomized trials, the ICUD is unable to make Grade A recommendations concerning the value of prostate cancer screening defined as the application of a test to detect prostate cancer to the general population of healthy men. This view is supported by the Advisory Committee on Cancer Prevention of the European Union. They have recommended to the EU member states that “as long as randomised studies have not shown an advantage on prostate cancer mortality or related quality of life, screening for prostate cancer is not recommended as a healthcare policy” [1].

These recommendations are unchanged since the last consultation in 2000. Fortunately two studies, the Prostate, Lung, Colorectal, and Ovary (PLCO) cancer screening trial in the US and the European Randomized Study of Screening for Prostate Cancer (ERSPC) are approaching their endpoints and should provide a definitive statement. Until then, the Advisory Committee on Cancer Prevention has recommended to the European Commission and European Parliament that the European randomised trial should be completed [1].

1. REQUIREMENTS FOR SCREENING HAVE NOT BEEN MET

In 1968 Wilson and Jungner [2] outlined several requirements needed to validate a screening program. These included a) that the disease to be screened must be an important health problem, b) that the disease must be recognisable at an early stage when it can be treated, c) that a suitable test is available that is accepted by the general population, d) that an appropriate treatment is available that can alter the natural history of the disease, and e) the application of this treatment will lead to a decline in overall mortality from the disease. PSA testing clearly meets the first three criteria. Since 1993, when the ERSPC and PLCO trials were conceived, level one evidence has become available that demonstrates that the cause-specific survival and overall mortality of patients with clinically detected, localized prostate cancer is improved with radical prostatectomy as compared with the alternative treatment of delayed palliative treatment. In terms of prostate cancer and overall mortality [3]. Unfortunately, this level one evidence is based on a population of men whose prostate cancer was detected clinically, rather than by PSA testing. Therefore, the fifth criteria, that PSA testing leads to a decline in prostate cancer mortality among men tested for PSA has yet to be satisfied. While the natural history of clinically diagnosed prostate cancer is reasonably well-understood, the natural history of screen-detected cancer remains obscure. We are just beginning to understand the potential impact of lead time and over diagnosis. The criteria developed by Wilson and Jungner also require that a) screening should be repeated at intervals which are determined by the natural history of the disease, b) that the cost of screening programs should be balanced against their benefit, c) adequate facilities for diagnosis and treatment of abnormalities should be available and d) that the likelihood of physical or psychological harm to those screened should be less than the likelihood of benefit. Many of these issues have been debated, but they remain unresolved. All medical ethical committees and gov-
eral committees who have the reviewed protocols of ERSPC and PLCO agree that there is an equipoise between physical and psychological harm and the chance of benefit from PSA testing of the screened population.

2. SCREENING FOR PROSTATE CANCER IN 2005

This chapter summarises progress that has been made during the past several years to resolve questions surrounding the efficacy of screening for prostate cancer. Unfortunately, a definitive statement either in support or against prostate cancer screening with PSA cannot be made.

The chapter reviews observational evidence for and against an effect of screening on prostate cancer, the status of ongoing randomised studies, as well as important aspects of the application of screening tests in potential future screening policies. In addition the chapter addresses issues related to quality of life, specifically the impact of treatment on men undergoing screening. The chapter concludes with a summary of the arguments for and against prostate cancer screening with PSA.

II. RESULTS OF OBSERVATIONAL STUDIES OF EARLY DETECTION

Several key studies have shaped our understanding of the natural history of prostate cancer. Between 1989 and 2004, Johansson and colleagues published a series of four articles that documented the progression of untreated prostate cancer in a population based cohort of patients diagnosed with prostate cancer in Örebro Medical Center in Sweden.[4-7] Screening for prostate cancer was not performed while this study population of 648 consecutive cases was assembled. Initially the authors found relatively low 5 and 10 year mortality rates among men with clinically localized disease and challenged the use of aggressive initial treatment for all patients with early stage prostate cancer. Long term follow up of the study cohort, however, suggested a rising cause specific mortality rate for those men who survived 15 – 20 years following diagnosis. Johansson et al concluded that because of the relatively slow progression of prostate cancer, a majority of patients are treated without survival benefit. Their data also suggest, however, that the probability of developing lethal progression may increase after one or two decades.

Albertsen et al recently 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 [8]. The results of the 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. Conversely, those men with Gleason 7 and especially those men with Gleason 8-10 tumors had a high probability of dying from prostate cancer if managed with androgen deprivation therapy alone (42-70% and 60-87%, respectively). This was especially true for men under age 70 years. Men with Gleason score 5-6 tumors had an intermediate risk of disease progression (14% and 27% at 20 years respectively). The authors concluded that men with Gleason 7 tumors or higher are at greatest risk of dying from their disease and probably should consider active intervention. Men with lower grade tumors face a risk of disease progression over time, but this risk is much lower and plays out over a period of one to two decades.

The relatively slow progression of clinical T1 and T2 prostate cancers has also been confirmed in a recent report by Bill-Axelson et al who have followed 695 men with early stage prostate cancer in a Swedish randomized trial comparing watchful waiting versus radical prostatectomy [9]. After 10 years, 25.4% of the men undergoing surveillance had developed metastases and 14.9% had died from prostate cancer. Men undergoing surgery had a significantly better outcome, but still 15.2% developed metastases and 9.6% died from prostate cancer. Over two thirds of these patients were diagnosed with tumors having Gleason scores ≤ 6. No additional information was provided concerning the probability of disease progression based on Gleason score.

The recent report by Thompson et al concerning the high prevalence of prostate cancer among men with a PSA level less than 4.0 ng/ml has raised questions concerning what constitutes clinically significant prostate cancer [10]. As part of a large chemoprevention study comparing finasteride against placebo they performed prostate biopsies on 2950 men whose serum PSA never rose above 4.0 ng/ml during the seven year study period. They found that the prevalence of prostate cancer was 6.6% among men whose PSA was consistently below 0.5 ng/ml and as high as 26.9% among men whose PSA was between 3.1 – 4.0 ng/ml. The men ranged in age from 62 – 91 years, but more than half were below age 70 years at
Figure 1. Survival (white lower band) and cumulative mortality from prostate cancer (dark gray upper band) and other causes (light gray middle band) up to 20 years after diagnosis, stratified by age at diagnosis and Gleason score. Percentage of men alive can be read from the left-hand scale, and percentage of men who have died from prostate cancer can be read from the right-hand scale.
the time of biopsy. A majority of the men harbored tumors with a Gleason score of 6 or less, but 67 of the 449 men diagnosed with prostate cancer had tumors with a Gleason score of 7 or greater.

The lead time introduced by PSA testing makes it unclear how many of these men are destined to experience disease progression in the absence of treatment. Based on modeling performed by Draisma et al [11], these tumors have been identified anywhere from 5 to 10 years earlier in their natural history compared to the patients described by Johansson, Albertsen and Bill-Axelson.[7,8,9] Information concerning the natural progression of cancers identified by PSA testing is not readily available.

Limited information from observational trials suggests that prostate cancers diagnosed as a result of PSA testing also progress slowly. Choo et al reported results from a prospective phase II study of active surveillance with selective delayed intervention that was initiated in 1995 [12]. Patients were offered intervention if their PSA doubling times exceeded two years or if they were found to have grade progression on re-biopsy. The cohort consisted of 206 patients who had either low risk cancer and were < 70 years of age, or who had low or intermediate risk prostate cancer and were over age 70. The majority of these men remain on surveillance after a median follow up of 29 months. A total of 31 cases progressed according to the study criteria within a median follow up of 29.6 months yielding an actuarial progression-free probability of 81% and 67% at 2 and 4 years respectively. To date the disease specific survival is 99% and the overall actuarial survival is 85%.

Carter et al have also reported results from a case series of men undergoing expectant management [13]. They have followed 81 men with stage T1c prostate cancer for a period of 12-58 months. Their criteria for disease progression consisted of any evidence of tumor ≥ Gleason 7, any Gleason pattern 4 or 5 on repeat biopsy, more than two cores involved with cancer, or more than 50% involvement of one core. During the follow up period, 25 men (31%) had disease progression which occurred most often in the first two years of follow up. Of these, 13 underwent radical prostatectomy, and 12 were found to have disease localized to the prostate.

Patel et al performed a retrospective review of 88 men who were eligible for definitive therapy when diagnosed with prostate cancer between 1984 and 2001, but who elected active surveillance.[14] The mean age of the patients at diagnosis was 65.3 (range 44-79). The median Gleason score of the cohort was 5 (range 2-7). The actuarial progression-free probability at 5 and 10 years was 67% and 56% respectively. Only one patient in the cohort had a biochemical recurrence following radical surgery and no patients had evidence of metastases.

In summary, the natural progression of prostate cancer is variable and is best predicted by the Gleason score. Two large population based cohort studies have demonstrated that men harboring Gleason score tumors ≥ 7 have a high probability of dying from prostate cancer after a period of 5-15 years in the absence of definitive therapy. Men with Gleason score tumors ≤ 6 have a much lower probability of disease progression over this time period, but the risk never falls to zero. Radical prostatectomy can offer some protection for patients by reducing the probability of disease progression by 50%. Testing for PSA has advanced the date of diagnosis by 5 – 10 years. It is unclear whether contemporary cases identified by PSA testing progress in a fashion similar to those identified clinically. Small contemporary case series suggest that men with low risk T1c tumors also have a low probability of disease progression. Unfortunately, follow up in these studies remain relatively short.

III. STATUS OF ONGOING RANDOMISED STUDIES

Only two randomised controlled trials of screening for prostate cancer have been organized world-wide: the prostate arm of the Prostate, Lung, Colorectal, and Ovary (PLCO) randomised screening trial [15] and the European Randomized Study of Screening for Prostate Cancer (ERSPC) [16]. Both studies were initiated in 1994 after the completion of pilot studies. Investigators from these studies agreed to conduct these trials with several common features that should permit a combined analysis [17].

Both studies have been designed to:
• establish or disprove an effect of active screening on prostate cancer mortality,
• evaluate the efficiency of the screening tests: serum prostate-specific antigen (PSA), digital rectal examination (DRE), and transrectal ultrasonography (TRUS) in the early detection of prostate cancer,
1. **The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO Trial)**

The objectives of the Prostate, Lung, Colorectal, and Ovarian (PLCO) cancer screening trial were recently summarised in detail in the 2000 supplement of “Controlled Clinical Trials” [18]. The trial was designed to randomize at least 70,000 females and 37,000 males age 55-74 to each arm of the trial. The primary objective was to show whether screening with a) flexible sigmoidoscopy can reduce mortality from colorectal cancer, b) chest-X-ray can reduce the mortality from lung cancer, c) digital rectal examination (DRE) plus serum prostate-specific antigen (PSA) can reduce the mortality from prostate cancer in men and d) transvaginal ultrasound plus the serum marker CA125 can reduce the mortality from ovarian cancer in women.

The PLCO trial also has secondary objectives. It aims to determine the sensitivities and specificities of each of the screening procedures along with the incidence, stage, biological and prognostic characteristics of tumour tissues and biochemical products. Participants were screened at entry. The serum markers have been applied yearly for 5 years, the physical examinations yearly for 3 years and sigmoidoscopy at entry and at 5 years. Control arm participants are followed according to usual healthcare practices. Recruitment began in 1993 and was completed in June, 2001. The trial was designed to last 13 years. A review of the sample size calculations and analysis plan can be found in [18].

Two progress reports on the prostate arm of the PLCO trial have recently been published. These include the findings from the initial screening round [19], the compliance with biopsy indications and the biopsy results obtained at entry and during a 3-year follow-up period [20]. Unlike the ERSPC trial where follow-up examinations after a positive screen include a prostate biopsy, follow up in the PLCO trial relies on the practice standards of a participant’s personal physician and is outside the control of the trial investigators. Follow up information comes from chart reviews.

As of June 2001 38,350 men have been randomly assigned to the screening arm. Based on data obtained from a review of primary health care records compiled by the staff of the PLCO trial, compliance with rectal examination and PSA testing has been more than 89%. Among men in the screening arm, 7.5% have had a positive DRE and 7.9% have had PSA levels higher than 4 ng/mL, values considered abnormal in the PLCO trial. While 74.2% of these men underwent additional diagnostic testing, only 31.5% underwent a prostate biopsy within one year of testing resulting in a detection rate of only 1.4%. Andriole and co-workers [19] report that of 2,717 men with a PSA ≥ 4.0 ng/mL at baseline, 41% and 64% underwent biopsy within 1 and 3 years respectively. PSA values higher than 4 ng/mL lead to significantly higher biopsy rates. Of the 1,793 men who had a positive PSA test after the baseline examination, 50% had a biopsy within 3 years. Of the 4,449 men who had a positive DRE but a negative PSA test, only 27% had a biopsy within 3 years. The biopsy rate rose to 90% when the DRE was positive at a follow-up visit.

The primary goal of the PLCO trial is not to maxmise prostate cancer detection, but rather to study the effect of the application of screening technology as it would be practiced in the US at this time [21]. In contrast, ERSPC is designed to also test the efficacy of prostate cancer screening and therefore the biopsy is part of the screening protocol.

2. **The European Randomized Study of Screening for Prostate Cancer (ERSPC)**

The ERSPC trial was initiated in 1993 in Belgium and in The Netherlands. Since then, six other European countries including Finland, Italy, Sweden, Switzerland, Spain and France have joined. The study is closed to screening in all countries except Switzerland and France. Study power calculations were initially based on the expected number of prostate cancer deaths in each country and have been...
updated using contemporary prostate cancer mortality data. Currently, ERSPC power calculations assume a 25% intervention effect in men who are actually screened and allow a 20% contamination by screening in the control arm. By 2008 the trial will have a power of 0.86 to detect a 25% difference in mortality between the screened arm and the control arm. Lower intervention effects might necessitate longer follow-up periods [22]. Figure 2 shows the power curves over time based on differing effect assumptions and a constant 20% contamination rate in the control arm.

a) Structure of ERSPC

Data within ERSPC is collected in a decentralized fashion according to slightly differing protocols in each participating country. Data are subsequently submitted and evaluated by a centralized data centre semi-annually according to a standardized protocol. The decentralized organisational structure of ERSPC requires quality control and supervision by a complicated committee structure.

The Scientific Committee of the ERSPC has overall responsibility for the study and consists of two voting members per country. The Scientific Committee has appointed an independent Data Monitoring Committee which functions according to mutually agreed flagging and stopping rules. Other committees include the Quality Control Committee, the Pathology Committee, the PSA Committee and the Causes of Death Committee. Causes of deaths are determined according to a mutually agreed algorithm which is available as software to all centres [23].

Differing European legal regulations necessitate country specific randomisation schemes (Figure 3). In Belgium, The Netherlands, Spain, and Switzerland all participants must sign informed consent documents. In Italy, France, Finland, and Sweden randomization occurs before patient contact and only those men invited to undergo testing are required to sign informed consent documents. Control subjects are identified administratively and are followed through national cancer registries.

b) Recruitment, biopsies, and cancer detection

The status of recruitment, biopsy compliance, and cancer detection in each of the 8 ERSPC centres as of March 2005 is summarised in Table 1. A total of 251,133 men have been randomised. Because Finland does not randomise according to a 1:1 ratio, numbers are unequal between the screening and control groups. Compliance with screening varies among men who are randomized to the screening arm according to country. Participation rates are high in those countries that require up front written informed consent and are lower in those countries that require consent after randomisation.

Detection rates in the screening arm remain remarkably stable during the first, second, and third screening rounds. A total of 3,928 cancers have been detected in the screening arm and 2,291 in the control arm. France only recently joined the study (2003). When this site is excluded, the median follow-up is currently 4.4 years.

c) Test procedures

Participating ERSPC centres utilize a PSA cut-off value of 3.0 ng/mL, as an indication for biopsy. Previously several centres also utilised an abnormal rectal examination but switched to PSA testing alone in 1997 after modelling studies showed that substantial numbers of cancers were missed by rectal examination and that the majority of cancers detected by rectal examination in the low PSA ranges had favourable histological characteristics. Four and eight year follow up data revealed that most of these men had a benign course [24-27]. Deleting the digital rectal examination in the Rotterdam section of ERSPC lowered the detection rate from 5.1% to 4.9% in first round screening [27].

Lateralis sextant biopsies are standard within ERSPC. A change in protocol to increase the number of cores sampled has not been accepted by most centres. Throughout the study a Beckman-Coulter Hybritech Tandem E PSA test has been utilized.

d. The screening interval

The screening interval in most ERSPC centres is 4 years. Sweden uses a two-year re-screening interval. The 4-year screening interval was based on the limited number of estimates of lead time that were available in 1994 when the ERSPC study was initiated [28,29]. Contemporary publications that include models based on ERSPC data suggest lead times are in excess of five years and therefore support the 4-year screening interval. Auvinen et al estimated the pre-clinical detectable phase to be 10-14 years depending on patient age and screening procedures. He assumed that lead time might average 50% of preclinical detectable phase [30]. MISCAN modelling using data from the Rotterdam site [31] estimated diagnostic lead time to be 10.3 years (range 9.9-11.2 years) which yielded an over diagnosis rate of 54% (range 51-59%).

222
Figure 2. Effect of different assumptions of intervention effect (20-50%) on the power of the ERSPC trial by end of follow-up year. De Koning et al [22]

Figure 3. Randomization procedures in ERSPC [37]

* Belgium, The Netherlands, Italy, Switzerland
** Finland, Sweden
Prognostic factors of cancers detected at re-screening reveal a stage and grade reduction with respect to the initial screen [32]. This has been confirmed in other centres. The rate of interval cancers is low for the first 4 years in the Rotterdam section with 23 cancer cases detected among 8,350 men who had completed the 4-year re-screening interval. The majority of these interval cases were classified as T1a, T1c or T2a and were managed either by watchful waiting or potentially curative measures. The prostate cancer detection rate at re-screening was 18.5% of the control group. If the 7 men who refused biopsy at the initial screening are excluded the rate would be 13.3%. The sensitivity of the screening tests was calculated to be 85.5% with use of the proportional incidence method [33].

e. Contamination

Contamination is continuously monitored in all ERSPC centres using differing methodologies [34]. For the Rotterdam centre effective contamination was estimated to be in the range of 3% per year. Ciatto has recently summarized the issue of contamination by opportunistic screening in the control arm [35]. A secondary analysis adjusting for contamination and non-participation is planned according to the procedure suggested by Cuzick [36].

f. End-point

Every two years, beginning in 2002, the Data Monitoring Committee evaluates the data to determine whether a decrease in the prostate cancer mortality rate greater than 25% has been observed in the screening arm of the study. To date the DMC has not made such an observation and recommends continuing the study. The first evaluation of the 267 confirmed deaths in the study was carried out in 2005.

g. Concluding remarks

ERSPC seems poised to resolve many questions sur-
rounding screening for prostate cancer and its effect on prostate cancer mortality. In addition the study should provide insights concerning the optimal testing interval and how to avoid over diagnosis and over treatment. In December 2003 the ERSPC published a review of the study since its inception over a decade ago [37]. A complete list of the 248 publications produced by ERSPC centres on details of the study is available through the website of ERSPC www.erspc.org

Should ongoing randomized trials prove the efficacy of PSA based prostate cancer screening, careful evaluation of cost effective screening strategies will be a high priority. The age at which screening should begin, rescreening intervals, and the age at which screening should be discontinued are important public health questions.

1. **AGE TO BEGIN SCREENING**

The optimum age at which to begin prostate cancer screening has not been determined. The American Cancer Society [38] and the American Urological Association [39] recommend that prostate cancer screening be offered annually beginning at age 50 years, and perhaps earlier for those at higher risk (family history of disease, African American). While only 8 percent of black men and men with a family history of prostate cancer who are age 40-50 years will have positive screening tests, 55 percent of those with positive tests have prostate cancer [40]—a finding that supports the recommendation for early screening of high risk individuals. The National Comprehensive Cancer Network (NCCN) has recommended that all men be offered baseline PSA screening at age 40 years, and that the frequency of follow up testing should depend on PSA test results [41].

The incidence and mortality of prostate cancer increase directly with age. For men age 40-49 years the incidence and mortality are 25 and 0.6 per 100,000 males, respectively; compared to 237 and 6.1 for males age 50-59 years [42]. Thus, early detection efforts in men under age 50 years could potentially require more testing per cancer detected compared to men over age 50 years. However, there are reasons to believe that less frequent testing that begins at an early age (under age 50 years) could be a rational and cost effective approach to screening.

First, a substantial number of men whose prostate cancers go undetected prior to age 50 years die of prostate cancer in the next 1-2 decades. Approximately 54 per 100,000 males age 50-64 years die of prostate cancer yearly [42]. Most men with an early prostate cancer that progresses to death, die of the disease 15-20 years after diagnosis [43,44], and thus it is very likely that most of the prostate cancer deaths occurring in men age 50-64 years could have been prevented by detection and treatment when these men were age 40-50 years. Second, younger men are more likely to have curable disease compared to older men and may have improved disease free outcomes [45,46,47]. Third, PSA is a more specific test in younger men who are less likely to have prostate enlargement as a cause of false positive elevations compared to older men [48]. Fourth, since prostate cancers progress slowly [44], it may not be necessary to screen younger men frequently. The screening frequency among younger men could be based on a baseline PSA—the results of which have been shown to predict the risk of a prostate cancer diagnosis over the next 25 years [49].

In the absence of long-term screening data, computer simulations have been used to explore the effectiveness of different screening strategies [50,51], and these suggest that the current standard of yearly screening starting at age 50 years may not be a cost-effective approach to early detection of prostate cancer.

Using a Markov model of the natural history of prostate cancer, Ross and colleagues [51] evaluated the numbers of biopsies and PSA tests per life saved with different screening strategies. They found that a strategy of PSA testing at age 40 years, age 45 years, and biennial (every other year) after age 50 years with a PSA threshold of 4 ng/mL used fewer resources and saved more lives than a strategy that tested annually starting at age 50 years. Since the PLCO and ERSPC randomized screening studies are evaluating the effectiveness of screening in men age 50 years and above, evidence based recommendations for screening men below age 50 years will not be available from randomized trials.

2. **RESCREENING INTERVALS**

Rescreening intervals can influence the effectiveness of a screening program; long rescreening intervals
could miss detecting curable disease for those with fast growing cancers, and short intervals could lead to unnecessary testing, overdiagnosis and overtreatment with no impact on disease mortality for those with slowly growing cancers. Annual screening is recommended for all men over age 50 years -regardless of risk- by the American Cancer Society [38], the American Urological Association [39] and the NCCN [41].

Carter et al [52] suggested that a screening interval of 2 years for men with PSA levels of 2ng/ml or less was not likely to miss a curable cancer. Based on longitudinal data, the authors observed that among cancer cases, conversion to a PSA of 4.1-5.0 ng/ml was rare 2 years after a baseline PSA level that was below 2 ng/ml, but common 2 years after a baseline PSA level of 2-3 ng/ml or 3-4 ng/ml. The authors recommended biennial screening for those men with PSA levels below 2 ng/ml and annual screening for those with PSA levels of 2 ng/ml or above. This concept of using a baseline PSA to determine the rescreening interval is supported by longitudinal studies using frozen plasma samples that show that future prostate cancer risk can be stratified by a baseline PSA measurement [49,53,54]. Furthermore, recent analyses from sections of the European Randomized Study of Prostate Cancer Screening (ERSPC) suggest that annual screening is not necessary to maintain the detection of curable disease in most men.

Hugosson et al [55] reported on the results of biennial screening from the Swedish section of the ERSPC and found that re-screening 2 years after a baseline screen was sufficient to detect prostate cancers at a curable stage for men with PSA levels below 2ng/ml at the initial screen. The authors recommended more frequent screening for those with baseline PSA levels above 2ng/ml. Investigators from the Rotterdam section of the ERSPC [56] have reported on interval cancers detected during 4 years after randomization in the screened arm outside the screening protocol –an indicator of the sensitivity of screening and safety of a re-screening interval of 4 years. Among men who complied with biopsy recommendations at the initial screen, only 18 interval cancers were detected and all were considered low risk disease with a favorable prognosis as defined by D’Amico et al [57]. The rate of interval cancers compared to the number in the control arm was 13 percent and the sensitivity of the screening protocol was 86 percent, suggesting that a re-screening interval of 4 years may even be reasonable.

Evaluation of intermediate pathological end points in the ERSPC suggest that most cancers detected at 2-4 years after the prevalent screen (1st round) will be curable [55,58-61]. Because of the long natural history of prostate cancer, and the ability of PSA screening to uncover advanced life-threatening cancer at the prevalent screen, frequent screening may be unnecessary for most men. Data on interval cancers and intermediate end points among screened and control arms of the PLCO trial (annual re-screening interval) and sections of the ERSPC trial (2 and 4 year re-screening intervals) should provide guidance regarding appropriate re-screening intervals in the near future.

3. AGE TO DISCONTINUE SCREENING

The upper age limit for enrolment in current randomized screening trials of prostate cancer is 74 years. Organizations that have endorsed screening have generally recommended screening for men with a life expectancy of 10 years or more. But the benefits of screening decline rapidly with age. Using a Markov model, Ross et al [62] found that when compared to screening to age 65 years, screening to age 75 years and 80 years required twice and three times, respectively the number of treatments per person-year of life saved as illustrated in Figure 4.

There is reason to believe that a substantial proportion of men with a life expectancy shorter than 10 years may not benefit from screening. Prevalent screening detects most advanced cases [58] and rescreening detects disease at an early curable stage in most men [58,59] with a lead time of 10 years or more [60,61]. Since those men who have non screen detected cancers rarely die of disease before 15 years without treatment [44], it may be that screening
could be discontinued earlier in life (before age 70 years) for most men who have taken part in a screening program and have maintained PSA levels consistent with a low risk of later prostate cancer development [62]. For example, in a prospective cohort study, Carter et al [63] showed that if PSA testing were discontinued in men at age 65 years with PSA levels below 0.5-1.0ng/mL, it would be unlikely that a prostate cancer would be missed later on in life.

**INTRODUCTION**

As the surgical removal of the entire prostate and a step-section histological analysis would be necessary in order to diagnose prostate cancer with a 100% specificity and sensitivity and this strategy is clinically not applicable, the diagnosis of prostate cancer requires obtaining cancerous tissue from the gland during biopsy.

The introduction of transrectal ultrasound has revolutionized prostate biopsy techniques and has greatly increased the diagnostic accuracy [64].

After the introduction of prostate specific antigen (PSA) into screening for prostate cancer, biopsy strategy presents a challenge for the practicing urologist. An optimized early cancer detection requires obtaining cancerous tissue from the gland during biopsy.

Unfortunately DRE is subjective as it is based on the level of experience of the examiner and has a poor predictive value in the detection of prostate cancer if PSA is < 4.0 ng/mL [67].

In addition the contribution of DRE to a screening effort is determined by the the PSA cutoff level employed. In a multicenter trial (>6,000 men) the positive predictive value (PPV) for patients with an abnormal DRE was reported with 10, 40, 8 and 69.1% for PSA values <4ng/ml, 4-10ng/ml and >10ng/ml respectively. The PPV in patients with a PSA>4ng/ml with a normal DRE was 24.4%. More than 50% of patients with diagnosed prostate cancer demonstrated a normal DRE [68].

**V. PROSTATE BIOPSY – WHAT IS CONTEMPORARY PRACTICE?**

**b) PSA greater than 4.0ng/ml**

The use of serum PSA has greatly improved our ability to predict prostate cancer risk in a reproducible way. Since the introduction of PSA into early prostate cancer detection it has been debated at which serum cutoff level a prostate biopsy appears indicated.

While early reports on cancer detection rates in patients with a PSA level of 4-10ng/ml of 5.5%, recent data suggest a current rate between 20-30% [69-73].

Therefore a PSA greater than 4ng/ml is considered an indication for biopsy, even as a rather poor negative predictive value is observed with approximately 20% cancer detection rate in those patients undergoing repeat biopsy [74].

**2. RELATIVE INDICATIONS FOR BIOPSY**

**a) PSA between 2.5-4ng/ml**

The low prostate cancer detection rate (2-4%) described historically in patients with a PSA value of <4.0ng/ml has resulted in the designation of this cutoff as distinction between “normal” and “abnormal”. These findings were very much influenced by factors like adequate sampling and biopsy technique.

Recently several investigators have reported detection rates from 22-27% with extended biopsy strategies among the group of patients with an initial PSA between 2.6-4.0ng/ml [75-77].

The detected cancers are clinically significant in >80% of patients according to current arbitrary definitions [78,79].

In this context it is important to realise that the term “clinical significant” has been defined with great
variability. Prostate cancers with a volume of >0.2ml have been defined as “clinically significant” and so have cancers with a volume of >0.5ml [80-82].

Also an age-adjusted definition of life threatening tumor volumes has been proposed, leading to an age and prostate volume adapted biopsy strategy, which calculated the numbers of cores needed to be taken to ensure a 90% certainty of cancer detection [83]. See tables 2 and 3.

Table 2. Life threatening volume (cc) at diagnosis for various cancer doubling times [83]

<table>
<thead>
<tr>
<th>PSA doubling time (Years)</th>
<th>50</th>
<th>55</th>
<th>60</th>
<th>65</th>
<th>70</th>
<th>75</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.05</td>
<td>0.1</td>
<td>0.3</td>
<td>0.6</td>
<td>1.3</td>
<td>2.3</td>
</tr>
<tr>
<td>4</td>
<td>0.2</td>
<td>0.4</td>
<td>0.8</td>
<td>1.5</td>
<td>2.5</td>
<td>3.9</td>
</tr>
<tr>
<td>6</td>
<td>1.0</td>
<td>1.5</td>
<td>2.4</td>
<td>3.5</td>
<td>5.0</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Table 3. Number of cores per biopsy to ensure certainty of cancer detection as a function of prostate gland size and life threatening tumor volume [83]

<table>
<thead>
<tr>
<th>Prostate Size (cc)</th>
<th>Tumor Volume (cc)</th>
<th>0.1</th>
<th>0.3</th>
<th>0.5</th>
<th>0.7</th>
<th>1.0</th>
<th>1.5</th>
<th>2.0</th>
<th>3.0</th>
<th>5.0</th>
<th>8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td></td>
<td>10</td>
<td>12</td>
<td>14</td>
<td>16</td>
<td>18</td>
<td>20</td>
<td>22</td>
<td>24</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>20</td>
<td>24</td>
<td>28</td>
<td>32</td>
<td>36</td>
<td>40</td>
<td>44</td>
<td>48</td>
<td>52</td>
<td>56</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>30</td>
<td>36</td>
<td>42</td>
<td>48</td>
<td>54</td>
<td>60</td>
<td>66</td>
<td>72</td>
<td>78</td>
<td>84</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>40</td>
<td>48</td>
<td>56</td>
<td>64</td>
<td>72</td>
<td>80</td>
<td>88</td>
<td>96</td>
<td>104</td>
<td>112</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>50</td>
<td>60</td>
<td>70</td>
<td>80</td>
<td>90</td>
<td>100</td>
<td>110</td>
<td>120</td>
<td>130</td>
<td>140</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td>60</td>
<td>72</td>
<td>84</td>
<td>96</td>
<td>108</td>
<td>120</td>
<td>132</td>
<td>144</td>
<td>156</td>
<td>168</td>
</tr>
<tr>
<td>70</td>
<td></td>
<td>70</td>
<td>84</td>
<td>98</td>
<td>112</td>
<td>126</td>
<td>140</td>
<td>154</td>
<td>168</td>
<td>182</td>
<td>196</td>
</tr>
<tr>
<td>80</td>
<td></td>
<td>80</td>
<td>96</td>
<td>112</td>
<td>128</td>
<td>144</td>
<td>160</td>
<td>176</td>
<td>192</td>
<td>208</td>
<td>224</td>
</tr>
</tbody>
</table>

Other authors suggested to start PSA testing at an earlier age (40-50 years) and to use initial cut-off values for determining follow-up regimens including PSA velocity [84]. As a study of Smith and Catalona found that a PSA velocity of 0.75ng/ml/year in men with a PSA of 4.0ng/ml and less yielded a sensitivity of 79% and a specificity of 66% in comparison to a sensitivity of 63% and a specificity of 62% in men with PSA >4.0ng/ml, the optimal use of PSA velocity seems to be in the earlier patient population [85].

b) Age adjusted PSA increase

One obvious shortcoming of using a strategy involving a lower PSA cutoff value is the necessary increase in the number of negative biopsies. Therefore age adjusted PSA determinations were suggest-
ed to improve cancer detection sensitivity in younger and specificity in older men by lowering the number of biopsy sessions at the same time [86].

However it was shown that increasing the PSA cutoff to 4.5ng/ml in patients aged 60-69 years, 8% of organ confined prostate cancers would be missed by reducing the biopsy sessions by 15% at the same time. Increasing the PSA cutoff to 6.5ng/ml among men aged 70years or older would result in a reduction of biopsy sessions in 44% but would also miss 47% of organ confined cancers [87].

c) Presence of high grade prostatic intraepithelial neoplasia or atypia in prostate biopsy

It is known that between 27-79% of patients found with prostatic intraepithelial neoplasia on initial biopsy will develop adenocarcinomas of the prostate [88-90]. A finding of atypia on initial biopsy relates to a risk of 45-49% to detect prostate cancer on repeat biopsies [91,92].

In contrast an indication for immediate rebiopsy is not present in patients diagnosed with high grade PIN in initial biopsies being performed in a screening setting as the rate of positively diagnosed patients was not statistically higher than in negatively diagnosed patients [93]. Only high grade PIN being diagnosed in screened patients in consecutive sextant biopsies predicted for positive findings in further biopsies [94].

Therefore a repeat prostate biopsy with extensive technique is relatively indicated in a 3 to 12 months interval for non-screened patients diagnosed with high-grade PIN in initial biopsies, but not for high-grade PIN diagnosed in screened patients.

In non-screened detected cases with high-grade PIN a repeat biopsy in a 3-12 months interval after the initial biopsy is particularly recommended if the initial biopsy was performed as sextant biopsy. If a 10-12 core protocol was diagnostic for high-grade PIN a time interval of up to 3 years until repeat biopsy appears reasonable. Lefkowitz et al. performed repeat biopsy within one year after HGPIN was diagnosed in a 12-core biopsy and detected cancer in only 1/43 (2.4%) patients. In a follow-up study they performed a repeat biopsy on 31 men who had been diagnosed with HGPIN on 12-core biopsy 3 years previously and identified cancer in 25.8% [95].

d) TRUS-guided biopsy schemes

Over the last 15 years, the most frequently used biopsy protocol was the sextant biopsy protocol described by Hodge et al [64].
In 1995 it was proposed to shift the sextant biopsies more laterally in order to sample better the peripheral zone where most of the cancers are located, however recent reports demonstrated that a single set of sextant biopsies may miss clinically detectable prostate cancer in 15-34% of men [96].

Since the reports of Chen et al [97] we nowadays know that approximately 70% of prostate cancers arise in the peripheral zone. Especially the anterior part of the prostatic base and the near of the midline area of the apex are affected. They also demonstrated that transition zone cancers are mainly situated in the farthest anterior areas of the prostate near the midline. They demonstrated by the use of computer-modeling, that traditional mid-lobe parasagittal sextant biopsy would particularly fail to sample the anterior transition zone, midline peripheral zone and the inferior portion of the anterior horn of the peripheral zone. Therefore the reliability of systemic biopsy for cancer detection relates to the number of cores and their placement.

Several schemes have been suggested with the number of systematic biopsies ranging from 6-18 [98-104]. Most protocols support the use of 10-12 biopsies in a systematic prostate biopsy approach, which today is most regularly applied. These protocols appear safe and well tolerated particularly when carried out under local anaesthesia, which should be generally recommended. The highest rate of cancer detection can be achieved by directing the biopsies laterally towards the apex and the “anterior horn” of the base combined with standard sextant biopsies. Excluding the two parasagittal mid-zone biopsies from the standard sextant biopsy protocol does not have a significant influence on prostate cancer detection rates when 10 core biopsies are performed [105].

Uzzo et al. and Karakiewicz et al. demonstrated an insufficient cancer detection rate especially in prostates with a volume of >50ml. Karakiewicz therefore suggested a volume-adapted biopsy scheme with one biopsy/5ml volume [106,107]. The Vienna normogramme suggested by Djavan et al. proposes an age- and volume-adjusted biopsy scheme with a 90% positive predictive value for prostate cancer [108]. For details see Table 4.

The Vienna normogramme suggested by Djavan et al. proposes an age- and volume-adjusted biopsy scheme with a 90% positive predictive value for prostate cancer [108]. For details see Table 4.

The yield of lesion-directed biopsy of hypoechoic areas is very much determined by the standard systematic biopsy scheme applied. In case 10-core systematic biopsy schemes have been used weighted more towards the lateral aspects of the peripheral prostate zone, the yield of additional puncture of hypoechoic lesions is described between 4-5% [114,115].

In contrast there are some data available which demonstrate higher cancer yield in case some hypoechoic lesions can be found at TRUS. In fact the detection of suspicious lesions very much depends on the quality of the imaging study used. The detection rate of suspect findings for cancer by TRUS varies between 6-88% [99,116].

Table 4. Age- and volume-adapted number of biopsy cores necessary for a 90% positive predictive value of prostate cancer. The “Vienna Normogramme” Djavan et al [108].

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Prostate Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;50</td>
</tr>
<tr>
<td>20-29</td>
<td>6</td>
</tr>
<tr>
<td>30-39</td>
<td>6</td>
</tr>
<tr>
<td>40-49</td>
<td>6</td>
</tr>
<tr>
<td>50-59</td>
<td>10</td>
</tr>
<tr>
<td>60-69</td>
<td>12</td>
</tr>
<tr>
<td>&gt;70</td>
<td>14</td>
</tr>
</tbody>
</table>
Figure 5. Prostatic biopsy schemes according to different authors. (modified after [118])
These data support the fact that lesion-guided biopsies might achieve a higher positive predictive value in the future by introducing new imaging technologies like magnetic resonance imaging and neuronal network guided ultrasound technologies.

3. SUMMARY

Analysing the available data, a general indication for prostate biopsy is seen in patients with an abnormal digital rectal examination (DRE) and/or a PSA of >4ng/ml if they intend to undergo therapy in case of a positive finding. A relative indication is given in patients with a PSA between 2.5-4 ng/ml without abnormal DRE, an age-adjusted increase in PSA, a finding of high-grade PIN and atypia in a non-screened population.

Prostate biopsy should be performed under local anaesthesia with the use of prophylactic antibiotics.

Nowadays standard sextant biopsy is not regarded as an appropriate biopsy procedure for most patients anymore as the rate of false-negative findings is too high. Standard sextant biopsy in combination with four to six additional biopsies directed to the lateral apex and the anterior horn at the base has to be regarded as new standard.

Power Doppler TRUS with the use of prostate enhancing contrast media, the use of artificial neural networks in combination with TRUS and the use of magnetic resonance imaging together with spectroscopy will probably further improve prostate cancer detection.

VI. QUALITY OF LIFE WITH SCREENING – IMPACT OF TREATMENT

1. INTRODUCTION

Researchers must pay careful attention to the effects of different treatment modalities on patients’ health related Quality of Life (QoL), defined as a patient’s physical, psychological and social functioning and well-being. This is particularly relevant because overdiagnosis occurs with screening and is defined as the detection and subsequent treatment of prostate cancer through PSA-testing that otherwise would not have been diagnosed within the patients’ lifetime, is considered a major potential drawback of PSA-testing [119,120]. Prostatectomy is reported to lead to better disease-specific survival than watchful waiting [121], but data are lacking concerning the relative efficacy of radical prostatectomy versus external beam radiotherapy, which are the most commonly used primary therapies.

Editorial remark. The Committee appreciated the description of the longitudinal data from the Rotterdam quality of life studies conducted within ERSPC. However it was felt that a brief literature overview referring to the results of recent cross-sectional quality of life studies and to the results obtained by high volume centres should be included. Dr. H. Ballentine Carter provided the next section, which includes references [122-129].

a) Quality of Life Assessments after Radiation Therapy and Radical Prostatectomy

The most common therapies for localized prostate cancer are radical prostatectomy, external beam radiotherapy, and interstitial brachytherapy. Because disease free outcomes have been comparable between these therapies short term (5 years), health related quality of life outcomes (HRQOL) have assumed increasing importance as an outcome of interest to patients and physicians. Investigators interested in HRQOL outcomes have focused on describing the urinary, sexual, and bowel experiences of patients after surgery and radiation using validated questionnaires instead of physician reported information.

It would appear that general HRQOL is very similar among prostate cancer patients regardless of management choice (including no treatment) even up to 8 years [122,123,124]. However, domain specific dysfunction (urinary, sexual, and bowel) is more prevalent when comparing treatment to no treatment, differs among treatments, and is temporally related to time since treatment.

Prior HRQOL studies comparing radiation therapy and surgery have demonstrated that in general, urinary incontinence is a greater concern for men undergoing surgery when compared to those undergoing radiation, that irritative urinary symptoms are greater for men undergoing radiation when compared to those undergoing surgery, that bowel dysfunction is significantly more prevalent after radiation therapy compared to surgery, and that sexual dysfunction is similar when evaluated long term (beyond 2 years after treatment) [125,126,127]. A recent long term follow-up study comparing HRQOL after radiation therapy and surgery (median follow-up 6 years) suggests that domain specific HRQOL continues to evolve after radiation therapy;
whereas between 2-6 years is more stable after surgery [124]. The authors found that urinary incontinence was a progressive and increasing concern for radiation therapy patients between 4-8 years after treatment, while irritative urinary symptoms and bowel symptoms continued to improve long term after radiation therapy.

There is growing evidence that for surgical intervention, HRQOL outcomes may be better when surgery is performed by high volume surgeons at high volume centers when compared to lower volume surgeons and lower volume centers [128,129].

In 1996 a longitudinal prospective cohort study on the effects of radical prostatectomy and external beam radiotherapy for localized prostate cancer was started in the Netherlands within the context of the European Randomized study of Screening for Prostate Cancer (ERSPC) [130]. The principal aims were to assess the frequency of side effects associated with treatment and to determine QoL in men with localized prostate cancer up to 5 years after primary treatment with radical prostatectomy or external beam radiotherapy.

2. PATIENTS AND METHODS
The present study was published in more detail in [131].

a) Patients

Newly diagnosed prostate cancer patients (n=314) from four Dutch hospitals were enrolled on average 1 month before the start of non-randomly allocated primary treatment, consisting of radical prostatectomy (n=127) or external beam radiotherapy (n=187). Men referred to watchful waiting (n=25) or advanced disease therapy (n=48) were excluded from analysis. For details of patient recruitment and first-year results see Madalinska et al [132,133]. Written informed consent was obtained from all respondents. The Medical Ethical Committees of all four hospitals approved the study design.

b) Health related quality of life measures

Respondents completed postal self-assessment questionnaires on average 1 month before initiation of treatment, and 6, 12 and 52 months afterwards. Disease-specific and generic QoL measures were used. The Prostate Cancer Index (PCI) measures disease-specific QoL in men treated for early-stage prostate cancer [134]. We used its 4 scales for urinary and bowel function and bother. Higher scale scores (0-100) represent better outcomes.

Erectile dysfunction was defined as regularly or often having problems in achieving or maintaining an erection if wished to, or not being sexually active because of erectile problems. We measured it with 12 single items [135,136]. Generic QoL was measured with the SF-36 (8 scales in the physical, mental and social domain) and with the EQ-5D (5 items and a Scale for the valuation of own health).

c) Statistical analysis

Respondents supplied information on age, marital status, educational level, and profession. Baseline clinical information on tumour stage [131], histopathologic tumour (biopsy) grade and urologic treatment history was obtained from the Regional Cancer Registry. Data on progression were obtained from the treating physicians. Statistics for the QoL scales were calculated using SPSS for Windows. A p-value less than 0.01 was considered significant. The development over time of scale scores was analysed with repeated-measures analysis of variance (ANOVA), using SAS. A non-response analysis was performed. Details are available from the authors.

3. RESULTS

a) Cohort characteristics

Age, co-morbidity and average PSA levels differed significantly between the treatment groups. On average prostatectomy patients were 5.9 years younger (p<0.01, Table 5, had 0.4 fewer co-morbid conditions (p<0.01) and a lower PSA level (p<0.01). TNM stages were more favourable in prostatectomy patients (p=0.06). The overall response rate to all four questionnaires was 76%. Median and mean time to long-term follow-up was 52 months (range: 45-58). Information on recurrence was available for 94% of the 52-month respondents, recurrence occurred in at least 7% of prostatectomy patients and 22% of radiotherapy patients.

b) Quality of life scores

Prostatectomy had a marked effect on urinary function (Figure 6A) and bother and on erectile dysfunction. Erectile dysfunction before treatment was reported by 31% of prostatectomy patients and by 40% of radiotherapy patients; at the 52-month assessment, these percentages were 88% and 64%, respectively. Radiotherapy had a marked effect on bowel function. Prostatectomy patients had better generic scores than men treated by radiotherapy. Mental Health score patterns were identical for surgery and radiotherapy patients, showing no influ-
Table 5. Characteristics of the participants after Korfage et al [131]

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Prostatectomy (n=127)</th>
<th>Radiotherapy (n=187)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at baseline (years)</td>
<td></td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>Average (SD, range)</td>
<td>62.3 (5.2; 49-74)</td>
<td>68.2 (5.8; 49-82)</td>
<td></td>
</tr>
<tr>
<td>Comorbidity</td>
<td></td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>Average no. of conditions</td>
<td>0.6</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>PSA level before treatment</td>
<td></td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>in ng/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average (SD)</td>
<td>9.6 (15.6)</td>
<td>15.4 (24.3)</td>
<td></td>
</tr>
<tr>
<td>Tumor stage before treatment</td>
<td></td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>T1</td>
<td>18%</td>
<td>12%</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>67%</td>
<td>61%</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>15%</td>
<td>26%</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>0%</td>
<td>1%</td>
<td></td>
</tr>
<tr>
<td>Tumor grade before treatment</td>
<td></td>
<td></td>
<td>0.84</td>
</tr>
<tr>
<td>G1</td>
<td>51%</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>38%</td>
<td>37%</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>11%</td>
<td>13%</td>
<td></td>
</tr>
</tbody>
</table>

Figure 6. Model results showing average scale scores and 95% Confidence Intervals per treatment group: the Dutch UCLA PCI scale of Urinary function (A) and the SF-36 scale on Mental Health (B). Higher numbers indicate better functioning [131]
ence of either age or treatment choice (Figure 6B). Both treatment groups scored equal to or higher than the US national age-adjusted norms for males [138], except for pre-treatment Mental Health (prostatectomy and radiotherapy group) and General Health (radiotherapy group).

4. DISCUSSION AND CONCLUSION

Our longitudinal study confirmed earlier findings of cross-sectional studies: prostatectomy mainly affected urinary and sexual functioning, whereas radiotherapy had consequences for bowel and sexual functioning. We found that erectile dysfunction at 1 year post-treatment can be considered permanent.

Studies on QoL have given important insight in the prevalence of side effects of primary treatment for prostate cancer, and in the quality of life as perceived by patients after successful primary treatment. Our studies have shown that men are generally able to live well with the side-effects of primary treatment for localised prostate cancer [139]. Furthermore our results suggest the need to search for newer treatment modalities for early-diagnosed prostate cancer that entail a minimum of side effects.

The unclear overall benefit of PSA screening and the high prevalence of side-effects associated with primary treatment complicate decision-making about PSA testing and prostate cancer treatment. Unbiased information on the development of disease-specific and generic functions over the years is useful for both clinicians and patients in this process of decision-making.

VII. NEW DEVELOPMENTS IN SCREENING AND EARLY DETECTION OF PROSTATE CANCER. MODELLING OF OUTCOMES (LEAD TIME, OVERDIAGNOSIS, PC MORTALITY)

Screening for prostate carcinoma (PC) is aimed at reducing prostate cancer specific mortality through early detection that permits treatment and cure. Proper understanding of the outcomes of the screening process implies knowledge of the natural history of the disease and on the possible impact of screening to alter this history.

1. LEAD TIME

Early detection of PC in the asymptomatic phase through screening results in lead time defined as the time elapsing from the date of early detection to the date when PC would have been “clinically” detected in absence of screening. Lead time depends on the sensitivity of the screening test and on the duration of the preclinical detectable phase, a period of time during which PC is detectable by the screening test and yet not symptomatic. Estimates based on prevalent and incident screening detection rates and underlying (in absence of screening) incidence rates suggest that the average detection lead time due to screening by PSA may be in excess of 10 years [140]

2. OVERDIAGNOSIS

Overdiagnosis occurs when PC is diagnosed which would not have surfaced as a clinical problem during the lifetime of a man in the absence of screening. Overdiagnosis may be considered as an extreme example of lead time and depends on the magnitude of lead time, the aggressiveness of the screen-detected PC, and on the life expectancy of the patient being screened. Long lead time, slow cancer growth, and short life expectancy magnify the risk that PC may be over diagnosed.

The risk of overdiagnosis is particularly high for PC because of the long lead time of associated with PSA detected disease, the high prevalence of low grade tumours demonstrated at autopsy, and the high prevalence of indolent PC which may be detected on a random biopsy [141]. Estimates of overdiagnosis from prostate cancer screening by PSA suggest that it may be 50% (one in two screen detected cancers is over diagnosed) or even higher [140,142]. Because we presently have no reliable method to identify over diagnosed cancers, overdiagnosis translates into overtreatment (Table 6).

3. MORTALITY

The efficacy of screening is best demonstrated by evidence of PC mortality reduction in the setting of a randomized trial. Thus far, no such evidence is available. Evidence from a randomized experience in Canada is flawed by major evaluation biases [143]. Mortality comparisons of areas with different screening prevalence (a study design also suffering from major biases) are conflicting [144,145]. Until evidence from the ongoing randomised trials [146] becomes available, no conclusion on the efficacy of screening may be drawn and, due to the negative aspects of screening (mainly overdiagnosis and overtreatment), population screening may not be safely recommended.
Although level one evidence for the value of screening is unavailable, level two and level three evidence is accumulating. The evidence mainly comes from regional comparisons of expected and observed prostate cancer mortality in areas where screening is prevalent and from a number of positive case control studies. Prostate cancer mortality in the US is decreasing. This most likely is a product of many factors including the impact of prostate cancer screening with PSA. The technical feasibility of screening and the usefulness of PSA was first demonstrated conclusively by Catalona [157].

1. DISCLAIMER
The arguments presented in this section do not reflect the personal opinion of the author. They must be considered an academic review of available evidence. The information presented here must be balanced against the information in section IX where arguments against the use of screening are presented. Also, the reader will realise that some of the arguments presented here could just as well be used as evidence against screening.

2. THE DECREASING PROSTATE CANCER MORTALITY IN THE US
The database of the Centre for Disease Control in the United States has been utilised to construct Figure 7. Prostate cancer mortality in the US increased annually until 1992 and then began to decline at a rate of approximately 4% per year. This trend has continued until the year 2002, the most recent date for which accurate data are available. Many researchers have concluded that the decrease in mortality is unlikely to be due to screening but that a fraction of the decrease may be caused by early detection and early treatment [148-151]. These researchers claim that the time between the onset of screening in 1989 and the subsequent decline in prostate cancer mortality observed in 1993 is too short to be attributed to the sequence of screening and subsequent treatment. In a review of the prevalence of treatment regimens in the US, Stanford demonstrated a substantial increase of the use of radiotherapy and radical prostatectomy during the period between 1985 and 1989 [152]. This increase could have contributed to the observed mortality decrease in 1993. This argument has gained recent importance after the randomised comparison of radical prostatectomy and delayed treatment conducted by the Scandinavian prostate group (SPG-4) study have shown that radical prostatectomy can decrease prostate cancer mortality [153].

3. THE INNSBRUCK STUDY
Researchers from Innsbruck have presented evidence of the effectiveness of screening in Tyrol, an isolated area of Austria where PSA screening was advertised and offered free to men aged 40-75 years as of 1993. Approximately 66% of the 65,000 men in this age group were screened at least once in a 4-year period. Initially PSA and rectal examination were used. Subsequently prostate biopsy was recommended when either the PSA increased to one half of the age-specific reference range or as a result of a risk analysis which was based on a neural network that was submitted to participants in support of their own decision process. The development of prostate cancer mortality since 1993 was compared to the expected mortality based on the cancer registry data col-
lected during the years 1988-1993. The change in mortality rates in the Innsbruck area was also compared to the rest of Austria. Significant differences were found in both comparisons. The regional comparison of expected versus observed is shown in Table 7 [154,155]. As seen in the US, the mortality reduction occurred within a three year period after the initiation of active screening. Also, just as in the US, aggressive potentially curative management had already increased prior to the initiation of the screening program.

4. EVIDENCE FROM CASE CONTROL STUDIES

Evidence from case control studies is contradictory. In the context of this section however only the positive studies will be referenced. All case control studies follow the same pattern: they compare the screening history in men who died from prostate cancer to matched controls who did not. Jacobsen et al [156] studied the prevalence of digital rectal examination (DRE) in 173 prostate cancer deaths and 246 control cases. Seventy-five and 84% of the cases and the controls respectively had previously undergone rectal examination. This resulted in an odd’s ratio of 0.51 (95% CI 0.31-0.84) for the utilisation of DRE in favour of the control group.

A study conducted by Weinmann et al [157] within the Kaiser Permanente Organisation evaluated the use of screening procedures in 171 men who died of prostate cancer and 342 controls between 1992 and 1999. A difference in the utilization of screening tests of 69.0% versus 76.4% was found between screened and control cases respectively (OR 0.70, 95% CI, 0.46-1.1).

More recent case control studies have been conducted by Bergstralh et al [158] en Kopecs et al [159]. Both studies are positive. The paper by Bergstralh et
al includes 56 men who died between 1992 and 2000 with prostate cancer as the cause of death. Three (n=142) community controls were matched to the cases on the year of birth and length of the medical record. All of the controls were alive at the time of death of the cases. Logistic regression analysis was used to estimate the odds ratios and 95% confidence intervals for an effect attributable to screening. The combination of DRE and PSA performed in absence of symptoms was considered as “screening”. Odds ratios differed for the use of PSA or DRE and ranged between 0.39 and 0.49. The authors concluded that their results were suggestive of a potential screening effect to reduce prostate cancer mortality from either DRE, PSA or the combination.

The presentation by Kopecs et al [159] had a similar set-up but used the risk of the occurrence of metastatic disease as the clinical endpoint. Cases included 236 men diagnosed with metastatic prostate cancer and 462 controls diagnosed between August 1, 1999 and May 31, 2002. Screening was defined as a PSA test in a man without a prostate cancer diagnosis and without prostate related symptoms. When the odds ratio of not being PSA screened was set at 1, PSA screening between ages 45 to 59 and 60 to 84 led to a significant reduction in the odds ratios to 0.65 and 0.52 respectively. The authors concluded that the study showed a significant reduction in the risk of metastatic prostate cancer in the screened population.

5. Decreasing rates of metastatic disease

Rietbergen et al [160] found a decrease in the rate of metastatic disease in the screening arm of the European Randomized Study of Screening for Prostate Cancer (ERSPC) and in the Amsterdam cancer registry during the years preceding any type of organized screening in The Netherlands. This finding cannot be attributed to the well-recognised stage shift that is produced by screening but could be related to a decline in absolute numbers in the underlying population. Recently this finding was confirmed in a study conducted in Olmsted county Minnesota [161]. The authors compared the age-adjusted incidence of metastatic disease per 100,000 men during the time periods of 1980 –1986, 1987 – 1993, and 1994 – 2000. The rates were 47.4, 65.8, and 33.3% respectively. These differences were significant if metastases were defined only by radiological evidence of regional or distant metastatic disease. The authors concluded that the recent decrease in metastatic disease is most likely due to the increasing rate of screening in Olmsted county.

Since metastatic disease precedes death from prostate cancer in 60-85% of cases depending on the extent of metastatic disease at the time of its diagnosis, these data suggest future declines in prostate cancer mortality among the same cohorts. This evidence however is observational and subject to controversy as shown in section 10 of this chapter.

6. The Rotterdam pilot studies

Prior to initiating ERSPC Rotterdam, 5 pilot studies were conducted. The 5th one is identical to the definitive protocol and is therefore included into ERSPC. Pilot studies 1 – 4 were run between October 1991 and November 1993 and are analyzed outside of the ERSPC. Results from these pilot studies have been reported previously [162]. In 2004 median and average follow-up were 9.9 and 11.0 years, a period of time that was envisaged for the total duration of follow-up within ERSPC as a whole. The results of these pilot studies are summarized in Table 8 [163].

Most cases in the first round of screening were diagnosed by rectal examination. During the largest pilot (pilot 1) men who presented with a PSA >= 10 ng/mL were excluded from randomisation. PSA was therefore used as a pre-screening test because the GP standard indicated the need for urological study with PSA levels of >= 10 ng/mL. The pilot studies were conducted at a time when PSA screening was virtually unknown in The Netherlands and contamination during follow-up, if present, occurred late. All deaths among men with prostate cancer were reviewed by an independent causes of death committee which was blinded to the participation of the patient in either the screening or control arm. The study lacks

| Table 8. The Rotterdam pilot studies: 1-4, after Schröder et al [163] |
|------------------|------------------|
| • 2,367 men age 55-74 randomized to screening vs control, October 1991 - November 1993. |
| • 153 cancers, 97 in the screening (20 interval), 56 in the control arm. Follow-up median 9.9, average 11.0 years. |
| • Diagnosis by abnormal DRE and/or TRUS, not PSA driven |
| • Metastases: screened 4 (2 interval cases), control 18 |
| • Deaths from PC: screened 3, control 12 |

237
sufficient power to demonstrate an effect of screening in comparison to a control population, but did show a large difference in the number of men with prostate cancer deaths between the screened arm and the control arm.

The negative case control studies and negative regional cohort studies contribute to arguments against screening and are not the subject of this review.

The argument against prostate cancer screening is really an argument for caution in its advocacy. Screening has a number of limitations. Candidates for screening should be advised that two randomized clinical trials are underway to assess the value of prostate cancer screening, and that no trial has been completed and properly analyzed in an “intention to treat” manner. While it is an open question as to whether screening saves lives, it is clear that treatment of screen-detected cancers causes morbidity, such as impotence and urinary incontinence, and carries a small risk of death [164].

While screening clearly identifies some cancers that are not clinically significant to the patient and do not need treatment, surveillance and screening studies show that screening misses more cancers than it finds, including some cancers that are clinically significant.

Evidence of over diagnosis in prostate cancer screening comes from comparisons of regions with high intensity screening to areas with low intensity screening [165,166] Lu-Yao and colleagues [167] found higher prostate cancer screening rates among Medicare beneficiaries in an area in the western U.S. (western Washington state) when compared to an area in the eastern U.S (the state of Connecticut). The increased screening area had more than twice the incidence of cancer and a higher incidence of radical prostatectomy and radiation therapy. The two areas had virtually identical prostate cancer mortality rates over 11 years of follow up. Presumably prostate cancer is not more prevalent in the state of Washington as compared with the state of Connecticut. More likely there is more diagnosis and treatment of prostate cancer in the state Washington. Despite different screening rates, men in both states were found to have the same risk of death from prostate cancer. Similar comparisons have been made between the relatively high screening rates in the U.S. as a whole and the low screening rates observed in the United Kingdom [168].

Observations from the placebo arm of the U.S. National Cancer Institute sponsored Prostate Cancer Prevention Trial demonstrate some of the problems associated with prostate cancer screening, especially overdiagnosis. The PCPT enrolled men with a median age of 62, a normal digital rectal exam, and a serum PSA less than 3 nanograms per milliliter. They were randomized to receive a 5-alpha reductase inhibitor, finasteride, or placebo for seven years [169]. During this period 12.2% (571 of 4692) men completing the trial were diagnosed with prostate cancer due to screening. Of note, about half were diagnosed due to an abnormal serum PSA (a PSA greater than 4 ng/ml or an annual increase of greater than 1 ng/ml) and half due to an abnormal DRE. Of those completing seven years with a normal serum PSA and DRE who consented to prostate biopsy 15% (443 of 2950) were found to have prostate cancer [170].

This study suggests that despite seven years of annual screening that diagnosed more than 12% of participating men with the prostate cancer, an additional 15% of cases were missed. Surveillance studies show that less than 3.5 % of all men enrolling in the trial were destined to die of prostate cancer [171,172]. Clinically significant disease, defined as disease that can threaten a patient’s life, was found in only a small subset of this study’s participants. The PCPT suggests that in a well screened group of men the proportion diagnosed with tumours of no threat to the patient may be as high as seven out of eight. Of note, 6% of men completing the trial on the placebo arm had a Gleason 7 disease or greater [170]. Because fewer than 3.5% of men will die of prostate cancer, the prognostic significance of Gleason grading remains uncertain. Better biomarkers that can distinguish prostate cancers that require treatment from those that do not are an urgent necessity. In addition, because half of the prostate cancers were missed during the seven years of follow up, the usefulness of PSA and DRE for finding prostate cancer is also open to question. Carried to the logical extreme, those who believe in early detection of prostate cancer might reasonably forego PSA testing and simply choose to perform a prostate biopsy at some regular interval.

A randomized screening trial comparing outcomes of a cohort screened versus a cohort not screened is one
method of determining whether screening saves lives. Some might argue that the increased survival seen in a screened-diagnosed population when compared to a population diagnosed due to symptoms is evidence of screening benefit. Unfortunately, the lead time introduced by screening compromises survival comparisons when not performed within the context of a randomized trial. The problem is further confounded by including a large number of men with screened detected tumours of uncertain prognosis.

Some argue that the decreasing number of men diagnosed with metastatic disease in the U.S. is evidence of screening value. This is necessary, but not sufficient evidence of screening efficacy. Stage-shift may simply reflect that cancers have been diagnosed earlier, but there has been no impact on long term survival. Prostate cancer outcomes studies have shown that nearly fifty percent of men treated with prostatectomy for presumably local disease have extraprostatic extension on pathologic review [173]. Studies have also shown that more than a third of men receiving radical prostatectomy have evidence of PSA recurrence [174,175]. These men were classified as having localized disease at diagnosis, but with additional follow up these men have been shown to have low volume metastatic disease.
• Level one evidence for the value of screening in terms of reducing prostate cancer mortality is not available. Therefore no grade a recommendations according to ICUD algorithms can be made.

• Level two and three evidence is available from case control and observational studies. This evidence is contradictory and the inherent biases cannot be unravelled.

• Two ongoing randomised studies are far advanced and are likely to produce level one evidence which will allow grade one recommendations. According to protocol predictions definitive answers can be expected between 2009 and 2013 for ERSPC and PLCO studies respectively. Significant differences however may emerge earlier than that and lead to reporting.

ARE THERE UNRESOLVED ISSUES:

• The value of PSA as a biopsy indicator is up for discussion. Current literature shows that presently used cut-off points may miss more cancers than are diagnosed.

• The best time to initiate screening and the best screening interval remain unknown. Future individualisation is likely.

• The most suitable biopsy technique is uncertain. Should all detectable prostate cancers be detected?

• Recent data question the value of a Gleason score above 7 as a predictor of “significant disease”. The rate of diagnosis of such tumours by screening is several fold higher than the lifetime risk of prostate cancer death.

• Overdiagnosis which occurs in about 50% of cases is a major issue. Available prognostic factors which may identify indolent cases are considered not reliable enough for clinical routine. Selection of cases for non-aggressive treatment (active surveillance) is therefore still subject to study.

• Overdiagnosed cases bear the full impact of all disadvantages of screening on quality of life. This phenomenon will in the future be used to determine the value of screening even if prostate cancer mortality is shown to be reduced.

• In the meantime the application of screening tests cannot be refused to well-informed men. Validated, balanced information material however is unavailable in most countries.
REFERENCES

I. INTRODUCTION


II. RESULTS OF OBSERVATIONAL STUDIES OF EARLY DETECTION


21. Editorial comments to [6], p. 750-751/


IV. PSA AND THE TIMING OF EARLY DETECTION MEASURES


74. Keetch DW, Catalona WJ, Smith DS: Serial prostatic biopsies in men with prostate specific antigen levels of 4ng/ml or less. J Urol 1998; 159:920-924


80. Park S, Shinohara K, Grossfeld GD, Caroll PR: Prostate cancer detection in men with prior high grade prostatic intraepithelial neoplasia or atypical prostate biopsy. J Urol 2001; 165:1409


VI. QUALITY OF LIFE WITH SCREENING – IMPACT OF TREATMENT


131. Bianco FJ Jr, Riedel ER, Begg CB, Kattan MW, Scardino PT.


VII. NEW DEVELOPMENTS IN SCREENING AND EARLY DETECTION OF PROSTATE CANCER. MODELLING OF OUTCOMES (LEAD TIME, OVERDIAGNOSIS, PC MORTALITY)


VIII. POPULATION-BASED SCREENING – ARGUMENTS FOR


245


IX. PROSTATE CANCER SCREENING, AN ARGUMENT FOR CAUTION


Committee 11

Prostate Cancer Prevention

Chairman

P.H. Gann (USA)

Members

H. Akaza (Japan),
F. Habib (U.K),
R. Kirby (U.K),
A. Valdes Mendoza (Mexico),
I. Thompson (USA),
H. Van Poppe1 (Belgium)
I. INTRODUCTION

1. DEFINING TARGET POPULATIONS FOR PROSTATE CANCER PREVENTION

II. ANTI-HORMONE AGENTS

1. 5α-reductase inhibitors
2. Toremifene (ER inhibitors)
3. Androgen receptor inhibition

III. ANTI-OXIDANTS

1. Selenium
2. Vitamin E
3. Lycopene
4. Other antioxidants

IV. ANTI-INFLAMMATORY AGENTS

1. Non selective COX inhibitors
2. Other NSAIDs

V. OTHER PHYTOCHEMICALS

1. Soy (including isoflavones)
2. Green tea
3. Cruciferous vegetables (sulfurphane, indole-3-carbinol)
4. Vitamin D and analogs
5. Mixed nutrient trials

VI. ENERGY BALANCE, PHYSICAL ACTIVITY AND DIETARY FAT

1. Obesity
2. Physical activity
3. Dietary fat
4. Statins
5. Multi-faceted lifestyle intervention

VII. METHODOLOGICAL ISSUES IN PROSTATE CANCER PREVENTION

1. Clinically meaningful versus indolent prostate cancer
2. Effects of PSA testing on prevention trials
3. Pros and cons of various Phase II designs, choosing the right disease stage
4. Development of intermediate endpoint biomarkers
5. Choosing the right intervention: whole food versus isolated compounds
6. Gene-nutrient interactions and pharmacogenomics

RECOMMENDATIONS

REFERENCES
This report critically evaluates our current state of knowledge regarding strategies for the primary prevention of prostate cancer. Prostate cancer is a good candidate for prevention, since it is a relatively common cancer with a generally slow rate of growth and progression. Moreover, the costs of screening and treatment – both in terms of financial costs and morbidity – are extremely high. In this discussion, pharmacological approaches to prevention are emphasized since they predominate in the literature; however, we also discuss non-pharmacological approaches such as alterations in diet or physical activity patterns. Chemoprevention has been defined as “the use of pharmacological agents to impede, arrest, or reverse carcinogenesis at its earliest stages”[1]. Since they normally will be applied in large populations that are cancer-free, preventive interventions are held to more stringent standards regarding cost and safety than therapeutic interventions. In reality, most people who use a preventive agent are not expected to derive any benefit, and are expected to take the agent for a long period or indefinitely.

The number-needed to-treat (NNT) is a useful statistic for gaining perspective on a preventive intervention. NNT, which is here the number of people who need to be treated in order to prevent one case of prostate cancer, is calculated as follows:

\[ NNT = \frac{1}{ARR}; \]

where \( ARR = \) absolute risk reduction = \( PCA \) incidence untreated − \( PCA \) incidence treated

Thus, if the incidence of prostate cancer without treatment is 400 cases per 100,000 men per year (the approximate age-adjusted rate for white men in the U.S. age 54-65 years), and a chemopreventive agent has 50% efficacy for reduction in cancer incidence, a total of 500 men will have to receive the agent for one year in order to prevent one case. Less hypothetically, analysts have applied Phase III tamoxifen trial results to a community cohort in Maryland, and estimated that approximately 400 women would have to be treated for one year to prevent each additional case of breast cancer [2]. This not only makes clear the need for a high margin of safety for preventive agents, it also suggests the value of deploying these agents for individuals who are identified at high risk as the net benefits of the intervention will be greater in these populations (i.e., NNT will be lower); while at the same time a higher risk of adverse effects might be considered tolerable. The relationships between NNT, baseline risk and efficacy of a preventive agent are illustrated in Figure 1.

I. DEFINING TARGET POPULATIONS FOR PROSTATE CANCER PREVENTION

The current state of knowledge regarding risk factors for prostate cancer, and hence our ability to identify high-risk subgroups and target them for cost-effective chemoprevention, is discussed in the Report from Group 3 on Epidemiology and Natural History. Unfortunately, at the present time there are no multivariate models for quantifying prostate cancer risk; analogous for example, to the modified Gail model for estimating a women’s breast cancer risk [3]. This is not a technical problem; the problem lies in the lack of strong risk factors – besides age, family history and African-American ancestry - that have been consistently demonstrated in epidemiological stud-
ies. As the Report from Group 3 shows, there has been considerable progress recently in identifying dietary and other lifestyle risk factors for prostate cancer that will require further study. We already know, based on migrant studies, that there are powerful environmental influences on prostate cancer risk, and that these influences affect transition from latent microscopic disease to clinically significant cancer [4]. We also know that a significant proportion of prostate cancer incidence is attributable to genetic traits. At present, it appears that this phenotype can be caused by not one but at least several high-penetrance genetic mutations, and a potentially much larger set of low-penetrance genetic polymorphisms [5]. Thus, we are still some distance from having genetic testing that is feasible in a clinical setting.

Prostate cancer investigators, however, have one major advantage in identifying high-risk men, namely PSA screening. Since the predictive value of an abnormal PSA on initial PSA testing is in the vicinity of 20-30%, large numbers of men can be classified as high-risk following an initial negative biopsy and are thus appropriate candidates for chemoprevention. A small proportion of these men will have HGPIN as a histological finding, which could be a further indication of elevated risk. Although this illustrates a collaborative relationship between secondary prevention (screening) and primary prevention, these strategies for reducing prostate cancer morbidity and mortality are in some sense competitive. Hypothetically, for example, a safe and perfectly effective chemopreventive agent that could be given to men at low or high risk would eliminate the need for screening. More realistically, once primary prevention tools are available, it will be necessary to design population approaches that effectively integrate these tools with screening efforts.

Another interesting target population for preventive agents and interventions are men with localized and presumably indolent prostate cancer. The research community is intensely focused on developing techniques to discriminate these patients – whose numbers have increased in conjunction with PSA testing - from those with more aggressive tumors, who therefore might benefit from aggressive treatment. These patients and their physicians will readily accept low-risk interventions that are proven to inhibit the growth and progression of early tumors. It is encouraging to note from migrant studies that environmental influences on prostate cancer development can affect the rate of tumor diagnosis even if they are introduced during adulthood. In other words, men who migrate from low to high-risk areas rapidly develop a high prostate cancer risk, independent of screening efforts, even if they migrate after onset of adulthood. Thus there definitely are environmental or lifestyle factors that can accelerate or inhibit the growth and development of latent tumors in adult men. This situation is not as clear for breast

Figure 1. The number of men who need to be treated with a preventive agent for one year in order to prevent one case of prostate cancer: number-needed-to-treat (NNT) as a function of the efficacy of the agent and baseline risk in the (untreated) population.
cancer, for example, in which it appears to take one or two generations for migrants to adopt the risk pattern of the host country. It is important to state that patients with aggressive localized or more advanced prostate cancers are not good target populations for evaluating preventive interventions, because these tumors could have developed resistance due to the progression of clonal selection.

Our discussion will refer to clinical trials using the Phase I-III framework; Table 1 summarizes the key elements of these phases in prostate cancer prevention and illustrates the steep increase in time and resources needed for more advanced studies. Phase I trials refer to those with goals related to safety, tolerability and pharmacokinetics. Phase II trials, which are usually randomized and placebo-controlled, evaluate efficacy using intermediate endpoint biomarkers. Phase III trials evaluate efficacy using actual cancer incidence or mortality as endpoints. We will review all the major areas of research, then discuss methodological issues and conclude with recommendations for public health and future research.

## II. ANTI-HORMONE AGENTS

### 1. 5α-REDUCTASE INHIBITORS

By the early 1990’s, a considerable body of evidence was available to indicate that inhibition of the 5α-reductase enzymes, which convert testosterone to the more potent androgen dihydrotestosterone (DHT) in the prostate and other organs such as liver and skin, was a possible means for chemoprevention of prostate cancer. This evidence included studies of kindreds with a rare, inherited deficiency of Type II 5α-reductase [6], pre-clinical studies [7], and results of clinical trials evaluating the safety and efficacy of the Type II inhibitor finasteride as a treatment for BPH. In 2003, the results of the Prostate Cancer Prevention Trial (PCPT) were reported, marking the completion of the first full-scale Phase III trial for prevention of prostate cancer [8]. The PCPT compared prostate cancer occurrence among 18,882 men randomly assigned to either finasteride (5 mg/day) or placebo for seven years. At baseline, participants were age 55 years or older, and had a normal digital rectal exam (DRE) and PSA ≤ 3 ng/ml. Serum PSA and DRE were performed annually and after seven years of follow-up, remaining participants were asked to undergo an end-of-study (EOS) biopsy. The primary endpoint of the trial was the period prevalence of prostate cancer, combining cancers diagnosed while on study and those discovered at the EOS biopsy. The overall prevalence of prostate cancer was 24.8% lower in the finasteride group compared to placebo (95% CI: 18.6-30.4%). However, the prevalence of high-grade cancer was 25.5% higher in the finasteride group: 6.4% vs. 5.1% (P = 0.005). The risk reduction for total prostate cancer did not vary significantly by age, race, family history or baseline PSA. Other secondary findings from the PCPT

<table>
<thead>
<tr>
<th>Study population</th>
<th>Outcome</th>
<th>Duration (approx.)</th>
<th>Subjects needed (approx.)</th>
</tr>
</thead>
</table>
| Phase I          | healthy volunteers | • agent levels  
• toxicity, tolerance | weeks | 20 |
| Phase II         | pre-prostatectomy repeat biopsy | • IEBs  
• IEBs | one month  
3-12 months | 30  
60-80 |
| Phase ?          | PCa, no treatment localized PCa, treated | • clinical progression  
• PSA velocity  
• clinical recurrence  
• PSA “failure” | 1-3 years  
3-5 years | 100-200  
200-500 |
| Phase III        | High-risk men  
elevated PSA (+) family history HGPIN Average-low risk men | o PCa incidence  
o PCa incidence  
o PCa incidence | 5 years  
5 years  
3 years  
7-10 years | 500-1,000  
1,000-2,000  
300-500  
15-30,000 |
included an increase in sexual side effects in the finasteride-treated men and a decrease in symptoms related to urinary function.

The excess of high-grade cancer detected in the finasteride arm of the PCPT has generated considerable debate. The hypothesis that finasteride selectively promotes the growth of aggressive cancers has some plausibility; intraprostatic androgen suppression could provide a competitive advantage to clones that have acquired androgen-independent growth mechanisms. Some investigators have postulated that serum androgen deficiency increases risk of developing aggressive prostate cancer [9], and the pro-differentiating effect of androgens in the prostate under certain conditions is well established [10]. On the other hand, there are at least three possible explanations for the observation of excess high-grade cancer in PCPT that do not involve a pejorative effect of finasteride. First, it is possible that finasteride has effects on the cellular features and architecture of prostate cancer that mimic or exaggerate the appearance of higher grade disease [11]. Second, finasteride reduced overall prostate gland volume by about 25%, based on ultrasound measurements obtained during the end-of-study biopsies. This means that finasteride-treated glands were more intensively sampled during blind biopsy compared to placebo, and that any given tumor had a higher probability of being detected. Since tumors received the highest Gleason score observed by the pathologist regardless of its prevalence in the biopsy sample, increased detection of high-grade tumors in the finasteride group should be expected. The apparent difference in the drug’s effect on high- vs. low-grade tumors would be exacerbated if finasteride shrinks the volume of low-grade cancers more than high-grade ones. Third, the excess of high-grade cancer in the finasteride group was strongest in the first two years of follow-up. If finasteride promoted growth of aggressive cancers, we would expect a gradual increase in the number of excess high-grade cancers as follow-up continued. In fact, the notable excess of high-grade disease early in follow-up suggests that in some men who had aggressive tumors that were present at baseline, finasteride decreased their serum PSA by substantially less than 50%, which in turn made them cross the PSA threshold of 4.0 as soon as their PSA was adjusted upward according to study protocol. In effect, these high-grade cancers could have been unmasked by “finasteride challenge” [12].

A second controversy stemming from the PCPT concerns the clinical significance of the cancers prevented by finasteride in the trial. There were 387 fewer Gleason 2-6 tumors in the finasteride group, and 43 more Gleason 7-10 tumors. However, only 3.8% of all cancers detected in PCPT were Gleason 2-4; Gleason 6 cancers were the majority of those detected in both finasteride and placebo groups. The finasteride group had fewer Gleason 6 tumors both during follow-up and at the end-of-study. Since most men with Gleason 6 cancers opt to undergo curative treatment, it can be argued that finasteride spares these men the cost and morbidity of such treatment. Moreover, although the prognosis for treated Gleason 6 cancers is generally quite good, an important subset of these patients will later develop recurrence and metastasis. In sum, there is no evidence in PCPT that the tumors prevented by finasteride were clinically irrelevant.

A second Phase III trial, REDUCE, is currently underway to determine if dutasteride, another 5α-reductase inhibitor, can prevent a primary diagnosis of prostate cancer. Dutasteride, which is currently approved for treatment of BPH, inhibits both Type I and Type II 5α-reductase. Although Type II enzyme is predominant in benign prostate tissue, Type I is also present and is highly expressed in some cancers [13]. A pooled analysis of three trials of dutasteride versus placebo for BPH provided preliminary evidence that the drug reduces prostate cancer incidence during early follow-up [14]. Men eligible for the REDUCE trial differ from PCPT participants in that they must have a PSA between 2.5 – 10 ng/ml and an initial negative biopsy. Participants will receive biopsies, regardless of PSA, after two and four years of follow-up. The recruitment goal for this international study is 8,000 men; results are anticipated in approximately 2009. The differences between PCPT and REDUCE are summarized in Table 2.

2. TOREMIFENE (ER INHIBITORS)

The incidence of prostate cancer rises sharply with aging, in a hormonal context characterized by an increasing ratio of estrogens to androgens. Adding estrogen to androgen enhances prostate tumor development in rodents and dogs, and inhibition of ER signaling inhibits the growth of cultured prostate cancer cells. Although ERα expression is largely stromal in benign prostate, it is expressed in the epithelial compartment in both premalignant and cancerous lesions, and is associated with increased cell proliferation and increased expression of oncogenic growth factors such as TGFRα, EGF and IGF-1 [15]. Moreover, activation of ERα appears to
increase the transactivation of AR in prostate cancer cells. On this basis, selective estrogen receptor modulators (SERMs) are being investigated for therapeutic and preventive activity in prostate cancer. The relatively low toxicity of these agents in men makes them especially attractive as chemopreventive agents. A Phase II trial of high-dose tamoxifen in men with hormone-refractory prostate cancer did not find evidence for a strong objective response [16]. However, toremifene was found to inhibit early tumor development in the TRAMP mouse model [17]. A recent Phase IIb/III chemoprevention trial compared toremifene (at 20, 40 or 60 mg/day) to placebo among men with isolated HGPIN [18]. Biopsies were repeated at 6 and 12 months. At 12 months, the prevalence of cancer was reduced from 31.2% to 24.4% (22% reduction) in the placebo versus 20 mg toremifene group \((P=0.045)\). Interpretation of this trial is complicated because there were smaller, non-significant reductions in cancer in the 40 and 60mg toremifene groups. A larger Phase III study of toremifene, with a goal of over 1,200 participants with HGPIN, is currently underway, as is a Phase II pre-prostatectomy trial.

### 3. ANDROGEN RECEPTOR INHIBITION

Nonsteroidal antiandrogens, such as bicalutamide, flutamide and nilutamide, interfere with androgen receptor signaling and have been used successfully in treatment of advanced or recurrent prostate cancer [19]. Safety and tolerance of these agents in patients with prostate cancer is quite good: about 30% develop gynecomastia and breast tenderness as the predominant side effect, but only 1-2% withdraw from treatment as a result. Lower doses could conceivably suppress tumor growth in high-risk patients with fewer side effects. In a rat carcinogenesis model, bicalutamide inhibited prostate tumor development in a dose-dependent manner [7]. However, extrapolation of these bicalutamide doses to humans is difficult because of the usual inter-species and model differences as well as the need to use supra-physiologic doses of testosterone to drive tumor growth in the rats. Prevention trials using these agents have not been undertaken due to the difficulty in defining an appropriate balance between cancer risk and toxicity. However, resolution of the crystal structure of the androgen receptor and improved understanding of its signaling mechanisms will help to identify new selective AR modulators with potentially lower toxicity that could hold promise as chemopreventive agents [20].

### III. ANTI-OXIDANTS

The balance between oxidative forces and anti-oxidative mechanisms may play an important role in prostate carcinogenesis. The principal anti-oxidant mechanisms involve exogenous, dietary compounds such as selenium, carotenoids and tocopherols and enzymes, such as the glutathione transferases, which can de-toxify reactive oxygen species in tissue. Loss of expression of GSTP1 has been shown to be very common in prostate cancer compared to adjacent normal tissue [21]. Development of a pro-oxidant state in the prostate can lead to damage to critical proteins and lipids, as well as to DNA [22]. In the following sections we discuss several promising chemopreventive agents in the antioxidant category. It is important to note that although these compounds...
are best known as antioxidants, they all have potential anti-cancer effects through other mechanisms.

1. Selenium

Selenium is a trace element found in bread, cereals, fish and meat. It is an essential element in the functioning of glutathione peroxidase, which protects cellular molecules and DNA against oxidative stress [23]. Laboratory evidence indicates that selenium could suppress carcinogenesis through effects on apoptosis, cell cycling and angiogenesis [24]. Selenium concentration in soils tends to be lower in geographical areas with a high cancer rate [25]. The Health Professionals Follow Up Study collected over 30,000 samples of toenails for the measurement of long-term selenium concentration. There was a strong inverse association between the prediagnostic selenium level assessed in toenails and the risk of advanced prostate cancer (OR = 0.35 for highest vs lowest quintile) [26]. Although toenails are believed to provide a better indication of long-term exposure, recent analyses in other cohorts have also found inverse associations between plasma selenium levels and advanced prostate cancer risk [27]. In a double-blind randomized trial designed to evaluate prevention of non-melanoma skin cancer, the incidence of prostate cancer was reduced by 63% among men assigned to take a daily supplement of 200µg of selenium over a 4.5 year period [28]. Later analyses from this trial showed that risk reduction in the selenium group was greatest among men with the lowest baseline plasma selenium levels, and that prostate cancer risk remained reduced by 50% through an additional two years of follow-up [29]. The effects of selenium according to baseline plasma selenium levels are shown in Figure 2.

This finding has been interpreted with caution since prostate cancer reduction was not a primary or pre-specified hypothesis in the trial. Before routinely administrating exogenous selenium to prevent prostate cancer, data from the SELECT (Selenium and Vitamin E Cancer Prevention Trial) study should be awaited. This Phase III, randomised, double-blind, placebo-controlled trial is designed to test the efficacy of selenium (200 µg) and vitamin E (400 mg) alone and in combination for preventing prostate cancer. This trial recently completed its goal of enrolling 32,400 men who are over age 55 (> age 50 for African-Americans), and have a normal DRE and PSA (≤ 4 ng/ml) [30]. The trial is powered to detect a reduction of 25% in prostate cancer incidence and results should become available by the year 2013. Additional Phase III trials of selenium supplements for primary prevention of prostate cancer are currently underway [31].

2. Vitamin E

Vitamin E, and in particular α-tocopherol, its most active form, is a major antioxidant acting on the level of membrane phospholipids. It has a demonstrable

![Figure 2. The effect of selenium supplements on prostate cancer incidence according to baseline plasma selenium level: complete results from the Nutritional Prevention of Cancer Trial (Duffield-Lillico AJ BJU Int, 2003)](image-url)
ability to protect lipids, proteins and nucleic acids from oxidative damage, and is attributed with a wide range of potential anti-tumor properties. Vitamin E has caused cell cycle arrest and apoptosis in cultured human prostate cancer cells [32]. Interestingly, the chromanyl moiety of vitamin E inhibits androgen receptor signalling in LNCaP cells, with a potency that is similar to bicalutamide [33]. Vitamin E was shown to inhibit growth of androgen-sensitive LNCaP xenografts in nude mice whose tumors were promoted by a high-fat diet [34]. Observational epidemiological studies on vitamin E intake and prostate cancer risk have produced mixed results. Several questionnaire and serum-based studies found no association between tocopherols and risk [35]. The Health Professionals Follow Up Study, which found no overall association between vitamin E supplement use and prostate cancer risk, reported a 44% lower risk of metastatic or fatal prostate cancer in vitamin E users who were current smokers [36]. Three studies that evaluated serum samples also reported a reduced risk of advanced prostate cancer in smokers with the highest \( \alpha \)-tocopherol levels [37-39]. The most important evidence linking vitamin E to prostate cancer prevention comes from the Alpha-Tocopherol Beta Carotene (ATBC) study, a randomized trial in Finland that was performed to assess the effect of vitamin E and beta-carotene supplements on the incidence of lung cancer in over 29,000 adult male smokers [40]. Although neither substance caused a reduction in lung cancer after 6 years of follow-up, the intervention group receiving 50 mg vitamin E daily had statistically significant reductions in prostate cancer incidence and mortality of 32 and 41%, respectively, as shown in Figure 3.

Additional data to clarify the role of vitamin E in the prevention of prostate cancer will be provided by the SELECT study. Although some in vitro studies suggest that both vitamin E and selenium can reduce PSA secretion in LNCaP cells, the SELECT protocol does not include adjustments for serum PSA changes that could complicate comparison of prostate cancer detection across treatment arms. The design schema for SELECT is shown in Figure 4.

The ATBC study and a recent smaller trial indicate that vitamin E supplements have no effect on serum PSA levels [40 41]. Similar data regarding the effect of selenium on serum PSA levels in humans is lacking; however, selenium-induced reductions in PSA secretion by LNCaP cells are due to inhibition of cell growth rather than reduced production of PSA on a per-cell basis [42]. This suggests that selenium, unlike finasteride, should not reduce PSA levels in chemoprevention trial participants in the absence of a growth-inhibitory effect on nascent tumors. Recent evidence also suggests that selenium and vitamin E could act synergistically to promote apoptosis and inhibit growth in prostate cancer cell lines [43]. Although most attention has focused on \( \gamma \)-tocopherol, which is the form of vitamin usually used in supplements, some data indicate that \( \alpha \)-tocopherol might also have anti-carcinogenic properties, and further studies are clearly warranted [44].

In a recent study, no significant reduction in the detection of cancer in the subsequent biopsy was shown in the group of patients with high grade or low grade PIN who were taking selenium-vitamin E supplements, compared to a group that was not. However, it needs to be stressed that this was not a randomized study and that supplement was given for only 6 months [45]. The Women’s Health Study (WHS), a randomized trial of aspirin and vitamin E in 40,000 healthy women, was recently ended after 10 years [46]. There were no reductions in cancer incidence or mortality among women assigned to vitamin E (600 IU alternate days), and no differences in the occurrence of major cardiovascular events. Although the WHS results have only limited relevance for male populations, the lack of any significant adverse effects due to vitamin E supplementation is encouraging for ongoing trials such as SELECT. However, the absence of cardioprotective effects, which had been hypothesized based on preclinical studies, could alter the risk-benefit equation against using vitamin E supplements in healthy individuals.

3. Lycopene

Lycopene is a straight chain carotenoid that cannot be converted to Vitamin A and is highly efficient as an antioxidant in vivo. It is the predominant carotenoid in the circulation in most Western countries, due to the popular consumption of tomatoes and tomato-based foods. Other foods that contain lycopene, such as guava and watermelon, are consumed far less often. The mean daily intake of lycopene among adult men in the U.S. is approximately 2-3 mg and the EPIC study has shown that tomato intake and plasma lycopene levels vary considerably across Europe [47]. There is ample preclinical and epidemiological evidence that lycopene or tomato foods could prevent prostate cancer. Studies in cell cultures have revealed can effect cell proliferation, cell gap junction signaling, and the IGF-1
Figure 3. Vitamin E (50 mg/day) has no effect on lung cancer risk but decreases prostate cancer risk: results from the ATBC Trial in Finland, with 29,133 participants and mean follow-up of 6.1 years (ATBC Study Group, 1994)

Figure 4. Design schema for the SELECT Trial of vitamin E and selenium for primary prevention of prostate cancer
system. In an important study of NMU-testosterone induced prostate cancer in rats, Boileau et al reported that tomato powder in the diet reduced mortality from these cancers by 26% compared to pure lycopene, which was indistinguishable from the control diet in its effect on tumor growth or mortality (see Figure 5) [48].

These results call attention to the potential importance of other substances besides lycopene in tomato foods, such as the less common carotenoids phytoene and phytofluene and small amounts of α-tocopherol. It is possible that these compounds interact to produce biologically important effects.

In the Health Professionals Follow-up Study, men in the highest quintile for lycopene consumption had a 21% lower risk of prostate cancer than men in the lowest quintile [49]. Risk was notably reduced among those with higher intake of cooked tomato products, a finding consistent with pharmacokinetic studies indicating that heating tomatoes and eating them with oil increases the bioavailability of lycopene and other tomato-related compounds. Some other cohort and case-control studies of prostate cancer also observed an inverse association with tomato intake; others did not [50]. Similarly, plasma or serum lycopene levels were inversely associated with risk in some cohort studies, but not others.

A limited number of human trials with lycopene or tomato foods have been conducted. In a small pre-prostatectomy Phase II trial, Kucuk, et al reported that patients receiving 30 mg/day of lycopene had lower PSA and reduced stage at prostatectomy than patients assigned to placebo [51]. Another pre-prostatectomy trial found evidence of reduced oxidative damage in prostate tissue among men receiving tomato-rich foods prior to surgery [52]. A third trial among men without prostate cancer reported a reduction in PSA and DNA oxidative damage in circulating WBCs following consumption of a tomato-rich diet [53]. A number of other Phase II trials of lycopene or tomato foods are currently underway.

4. OTHER ANTIOXIDANTS

In Phase III trials, β-carotene supplements were not associated with a decreased occurrence of prostate cancer, although in the Physicians’ Health Study, there was some evidence for lower risk among men with the lowest levels of serum β-carotene at baseline [54]. In the analysis of serum carotenoids and prostate cancer risk in this trial, assignment to β-carotene significantly lowered risk compared to placebo among men within the lowest quintile for serum lycopene at baseline [37]. These findings are consistent with the hypothesis that the relationship of antioxidant status to risk is non-linear, and that antioxidants such as β-carotene and lycopene can complement each other to some extent. Findings regarding other carotenoid antioxidants, such as lutein, α-carotene and β-cryptoxanthin, have been essentially null. Several cohort studies and at least a dozen case-control studies have evaluated the risk associations for vitamin C and the results have been almost uniformly null, considering serum-based studies as well as questionnaire studies evaluating food or supplement intake [55].

IV. ANTI-INFLAMMATORY AGENTS

A growing body of evidence suggests that chronic inflammation has an active role in the process of carcinogenesis in a number of human malignancies including liver, colon, bladder and lung cancer [56]. Though no such link has been established so far with regard to prostate cancer, histological studies of prostate tumors demonstrated inflammatory cell infiltrates of varying densities in 57.5% of prostate tumors [57]. Inflammatory cells such as lymphocytes and macrophages release inflammatory mediators and growth factors and these, in turn, may contribute to the accumulation of damage due to free radicals and the development of prostate cancer.
Eicosanoids such as prostaglandins and other related compounds have been implicated in the inflammation process. The synthesis of prostaglandins and their metabolism in the prostate by a series of enzymatic reactions involving the cyclooxygenases (COXs) is well recognized [58]. Three COX isoforms have been identified: the constitutively expressed COX-1, a housekeeping gene that has an important role in protecting the gastroduodenal mucosa; the inducible COX-2 gene, an immediate early response gene that is rapidly induced in response to tumor promoters, cytokines and growth factors [59]; a functional role for COX-3 remains to be determined.

The role of prostaglandins in the development of prostate cancer has been substantiated from several experimental studies in both human and animal models. The prostate has the highest level of COX-2 mRNA among human tissues [60]. Additionally it was suggested recently that prostaglandins play a major role in the growth of prostate cancer cells through the activation of COX-2 expression [61]. Furthermore, increased levels of prostaglandins have been widely reported in malignant human prostate tumors. There is also evidence showing that COX-2 is over-expressed in prostate cancer and that tumor grade is positively correlated to COX-2 levels [62]. Cumulatively these findings suggest that inhibition of COX-2 may lead not only to inhibition of metastasis but also to inhibition of prostate carcinogenesis.

1. **Non selective COX inhibitors**

There is an expanding body of information suggesting the potential application of non-steroidal anti-inflammatory drugs (NSAIDs) in cancer chemoprevention. Furthermore, recent population-based case-control and cohort studies have reported that men who regularly took NSAIDs for prolonged periods have a reduced relative risk of developing prostate cancer [63, 64]. However, such observations are not universal; recent epidemiological studies in France and the US were unable to demonstrate an association between NSAID use and prostate cancer risk [65, 66].

One factor which might account for these conflicting patterns stems from varying ability of specific NSAIDs to inhibit COX-1 and COX-2. For example, aspirin is a relatively selective inhibitor of COX-1 whereas conventional NSAIDs such ibuprofen, sulindac and indomethacin inhibit COX-1 and COX-2 to the same extent.

The mechanism of action that defines the role of NSAID as potent agents for the chemoprevention of prostate cancer is not clear. However, there is evidence that the inhibition of the biosynthesis of prostaglandins increases the susceptibility of cancer cells to apoptosis by down regulating the anti-apoptotic protein Bcl-2. There is also evidence that the induction of apoptosis may be independent of Bcl-2 and the process could be mediated through the activation of the key anti-apoptotic kinase Akt [67].

Recently, selective inhibitors of COX-2 isoform have attracted considerable attention because of their ability to selectively inhibit the inducible COX-2 isoform while allowing COX-1 to perform its “housekeeping” functions. By significantly reducing gastrointestinal side effects, these NSAIDs have additional promise as chemopreventive agents [68].

Currently available drugs with these properties are rofecoxib and celecoxib; the latter has been demonstrated to be effective in reducing colorectal cancer in patients with familial adenomatosis syndromes [69]. Other highly selective COX-2 inhibitors such as valdecoxib and etoricoxib are now completing Phase III trials. Development of selective COX-2 inhibitors as chemopreventive agents was effectively halted recently when the APPROVe trial, designed to test the efficacy of rofecoxib (Vioxx) for prevention of recurrent colorectal polyps, revealed that the rofecoxib group had a significant two-fold increase in serious cardiovascular events, an effect that emerged after 18 months of follow-up [70].

As a result, the ViP trial, which was building towards enrolment of 15,000 men with borderline PSA elevations to test Vioxx for prevention of prostate cancer, was abruptly canceled in September, 2004. The future of selective NSAIDs for cancer chemoprevention remains uncertain at this point considering the mandate for high safety margins with this intended use.

2. **Other NSAIDs**

In view of the inconsistencies in the literature concerning conventional NSAIDs and prostate cancer, attention has focused recently on the newly developed nitric oxide (NO) donating NSAIDs. The major advantage of this new generation of NSAIDs is that NO, when released in the gastric mucosa, exerts protective effects by increasing mucosal blood flow, stimulating mucus secretion and so preventing one of the most serious side effects of NSAIDs namely gastric erosion. Recent studies using NO NSAIDs in
colon cancer models have proved successful in vitro and in vivo [71, 72]. In addition, NO NSAIDs have proven to be potent anti-proliferative pro-apoptotic compounds in prostate cell systems [73]. This pro-apoptotic effect is mediated via caspase-3 and it is independent of the type of prostate cell used. These findings have ramifications for the use of these new drugs in prostate cancer chemoprevention and treatment.

1. **SOY (INCLUDING ISOFLAVONES)**

A considerable difference in dietary composition between Western and Asian societies is the consumption of soy. The average consumption of soy food among adult men in Japan has been estimated as 63.6 gms per day [76]. Genistein and daidzein, the isoflavones to which the beneficial effects of soy are attributed, are present in concentrations up to 3 mg/g in soy beans [77]. This results in an average intake of isoflavones of 100 mg/day in Taiwanese adults, far greater than the estimated mean daily intake of 1-2 mg among US males [78]. Isoflavone levels in plasma and prostatic fluid are also orders of magnitude higher in Asian versus European men [79, 80]. Isoflavones and lignans, which are found in certain edible legumes, are considered phytoestrogens in view of their capacity for estrogen agonist or antagonist activity, depending upon dosage.

In a 17-year cohort study performed by Hirayama on 265,000 adult Japanese men, daily soybean consumption had the strongest protective association with prostate cancer risk among dietary factors, with a 40% lower incidence in the high versus low soybean consumption group [81]. The epidemiological evidence on the whole, however, is sparse and interpretation across studies is complicated by differences in type and amount of soy food consumption in various populations. There have been few epidemiological studies in Western populations, where soy intake is minimal. Consumption of tofu was associated with reduced prostate cancer risk among Japanese men living in Hawaii, and similar results were observed for high levels of soy milk intake among vegetarian Adventist men in the U.S. [82, 83]. Recent case-control studies in native Asian populations have observed significant risk reductions associated with tofu, total soy and genistein intake in China, [84] and with higher levels of intake of natto (fermented soybeans) in Japan [85]. Dietary intake of lignan-rich foods such as peas, beans and lentils in European men has been rather consistently associated with reduced prostate cancer risk [86].

Traditional Asian cuisine uses more vegetables, cereals, soy, fruit, nuts and fish; there is a negative relationship to mortality from carcinoma of the prostate [74]. Total intake of fruit and vegetables does not appear to be related to prostate cancer risk [75].

However, several plant-related elements of the traditional Asian diet, including soy, green tea and cruciferous vegetables, have proven to be intriguing. Research currently focuses on both whole foods and specific phytochemicals in these categories (see below for further discussion of this dual approach).

### V. OTHER PHYTOCHEMICALS

The diet consumed in Western societies may be one of the main environmental factors affecting progression from microscopic to clinically significant prostate cancer. The Western diet is characterised by a high intake of energy, total fat and animal products (specifically milk, meat and poultry) and is positively linked to prostate cancer mortality (Table 3).

<table>
<thead>
<tr>
<th>WEST</th>
<th>EAST</th>
</tr>
</thead>
<tbody>
<tr>
<td>o sedentary</td>
<td>o less sedentary</td>
</tr>
<tr>
<td>o high energy intake</td>
<td>o better energy balance</td>
</tr>
<tr>
<td>o high animal fat (meat and dairy)</td>
<td>o low animal fat</td>
</tr>
<tr>
<td>o high red meat</td>
<td>o low red meat</td>
</tr>
<tr>
<td>o low vegetable</td>
<td>o high vegetable</td>
</tr>
<tr>
<td>o low fish</td>
<td>o high fish</td>
</tr>
<tr>
<td></td>
<td>o soy foods</td>
</tr>
<tr>
<td></td>
<td>o green tea</td>
</tr>
</tbody>
</table>

Table 3. Major aspects of the traditional Eastern diet and typical Western diet that are potentially relevant to prostate cancer etiology.

In *in vitro* and *in vivo* studies with isoflavones have demonstrated marked growth inhibition in prostate cancer cell lines and a marked difference in tumor growth in established prostatic tumors [87]. These effects can be estrogen and androgen-independent, and it is now further established that isoflavones can exert hormone-independent effects on tumor cell invasiveness, adhesiveness and angiogenesis [88]. One important soy isoflavone, equol, is not present.
in soy food itself, but must be produced by metabolism of daidzein by gut microflora. A recent study reported that 46% and 59% of Japanese and Korean men were equol producers, respectively, versus only 14% among US men [89]. Furthermore, in a case-control analysis, the ability to produce equol was significantly more prevalent among controls than prostate cancer cases (46% versus 29%, p = 0.004). In rat experiments, equol bound to and sequestered DHT, preventing it from binding to the androgen receptor [90]. These interesting results suggest that a novel approach to prostate cancer prevention might be through manipulation of gut microflora or intestinal metabolism to trigger synthesis of equol in non-producers.

A number of experimental studies involving soy or its constituents and prostate cancer have been conducted. In a randomized trial, Urban, et al found that soy protein supplements had no effect on PSA levels in men with minor PSA elevations; soy exposure was limited to only six weeks [91]. Two other trials in healthy men saw no effect of short-term isoflavone use on PSA [92, 93]. In contrast, Hussain, et al reported that 200 mg per day of soy isoflavone stabilized PSA levels in the majority of patients with rising PSA due to hormone-refractory or hormone-sensitive cancer [94]. However, this was a small pilot study with no control group. In another single-arm trial that included both watchful waiting patients and post-treatment patients, a genistein-rich extract taken over six months stabilized or reduced PSA levels in 8 of 13 patients in the watchful waiting group [95]. Finally, a non-randomized pilot study found that a low-fat diet plus flaxseed (rich in lignans and omega-3 fatty acids) reduced serum PSA and proliferation rates in benign epithelium in 15 men undergoing repeat biopsy due to elevated PSA [96]. These effects, if confirmed, do not appear to be mediated through changes in circulating androgen, since several short-term trials observed no effect of isoflavones or soyfoods on serum androgen levels [97]. Jarred, et al provided isoflavones (160 mg/day) from red clover to 18 patients before radical prostatectomy, and observed greater apoptotic activity in these patients compared to 18 historical controls (p = 0.002); the mechanisms, however, are unknown [98]. Additional early phase trials of soy and its components are currently underway.

2. **GREEN TEA**

Pre-clinical evidence suggests that green tea, or its polyphenol constituents, could inhibit prostate cancer development. Epigallocatechin-3-gallate (EGCG), which accounts for about 50% of total polyphenols in green tea, inhibits proliferation and induces apoptosis in tumor cell lines, and also reduces tumor growth in nude mouse xenografts [99, 100]. Remarkably, oral infusion of green tea polyphenols markedly suppresses the growth and metastasis of prostate cancers in transgenic TRAMP mice, and increases overall survival [101]. In the TRAMP model, these chemopreventive effects – at doses equivalent to approximately six cups of green tea per day in humans - were associated with a decrease in IGF-1 and an increase in IGFBP-3 within the prostate, and with apoptosis mediated through the phosphorylated Akt pathway [102]. These and other studies have reported effects of EGCG or green tea polyphenol mixtures on angiogenic and metastatic pathways via VEGF and matrix metalloproteinases [103]. EGCG also demonstrates potent inhibition of fatty acid synthase (FAS), a molecule whose increased expression is associated with early prostate carcinogenesis, selective inhibition of COX-2 in various tumor cell cultures, and down-regulation of androgen receptor in LNCap cells [104-106]. Interestingly, further studies revealed that other polyphenolic flavonoids found in fruits and vegetables besides EGCG – including quercetin, luteolin and kaempferol – are even more potent than EGCG in suppressing FAS activity and growth in LNCap cells [107]. Dietary sources of these compounds include olives, onions and apples, which emphasizes the potential importance of consuming a diet containing a wide variety of plant-based foods.

Epidemiologic data on green tea and prostate cancer risk is quite sparse, especially from Western countries where tea consumption is low. Jian et al recently reported on a case-control study conducted in China; the odds ratio (OR) for prostate cancer was 0.28 (95% CI: 0.17, 0.47) for tea-drinkers versus non-drinkers, with evidence for even lower risk with higher daily intake or longer duration of use [108]. Although diet interviews were conducted after diagnosis and hospital controls were used, this study had some notable strengths, including a population with a wide range of tea-drinking habits and a validated questionnaire designed for Chinese populations. Another recent case-control study in Japan found a smaller, non-significant (OR = 0.67) inverse association for prostate cancer among heavy green tea consumers (> 10 cups per day) compared to non-consumers [85]. Tea intake was inversely associated with prostate cancer risk in a Canadian case-control...
study; however, most of the tea consumed is presumed to be black tea, which has generally lower concentrations of polyphenols, and has not been associated with cancer risk in most other studies [109, 110].

Experimental studies with green tea are still few. Jatoi, et al conducted a trial, with no control arm, in which 42 men with rising PSA on hormone therapy were given 6 grams per day of green tea powder for a median of four weeks duration. Only one patient had a decline in PSA, and none had clinical or radiographic responses [111]. A similar trial conducted in Canada with 19 hormone-refractory cases found no evidence of response to green tea extract in capsules [112]. In a placebo-controlled trial among men with HGPIN, Bettuzzi et al reported that 600 mg/day of green tea catechins (predominantly EGCG) reduced the prevalence of cancer detected at one year repeat biopsy [113]. Nine of 30 men in the placebo group had cancer detected, versus one of 32 in the intervention group. Patients receiving green tea catechins also had a 17% decline in PSA after 9 months. Additional trials will certainly follow.

3. Cruciferous vegetables (sulforaphane, indole-3-carbinol)

Cruciferous vegetables, which include broccoli, cabbage and bok choy, have been a focus of interest in cancer prevention research for some time. Several epidemiologic studies – both case-control and to a lesser extent cohort studies - have found that consumption of cruciferous vegetables was inversely associated with prostate cancer risk [35]. No association was observed in the first analysis from the large European Prospective Investigation into Cancer (EPIC) study, however [75]. The phytochemicals of interest are sulforaphane (an isothiocyanate) and indole-3-carbinol. Since both of these compounds induce Phase II enzymes that inactivate carcinogens, exposure earlier in life, while prostate cancers are just beginning to develop, could be more important than recent exposure. This complicates most epidemiologic investigations, which have difficulty characterizing long-distant dietary patterns. Interpretation of reported dietary intake is further complicated because cooking can inactivate isothiocyanates in plant foods, and thus reduce their bioavailability [114].

In the laboratory, sulforaphane has been shown to inhibit growth of cultured prostate cancer cells through apoptosis and cell cycle arrest, and it was recently reported that it also inhibits growth of PC-3 cell xenografts in nude mice [115, 116]. Indole-3-carbinol also induces cycle arrest in androgen-responsive LNCaP cells, and there is new evidence that it is a potent repressor of androgen receptor gene transcription [117]. Further pre-clinical and early-phase human trials of these compounds are clearly warranted. Gene-nutrient interactions could prove to be important. A recent case-control analysis found a reduced prostate cancer risk for men in the highest category of cruciferous vegetables (OR=0.58, 95% CI: 0.38,0.89), but also observed that the greatest reduction in risk occurred among men with high broccoli intake and absence of deleting mutations for glutathione S-transferase M1, a potentially important carcinogen detoxification enzyme that is induced by sulforaphane [118].

4. Vitamin D and analogs

Active forms of Vitamin D, or analogs less likely to induce dangerous hypercalcemia, are generating considerable interest as preventive agents for prostate cancer. Epidemiologic data on prostate cancer risk in relation to dietary intake or serum levels of Vitamin D are quite mixed [35], although evidence is accumulating that active forms such as 1,25 (OH)2D3 are highly active in vitro and might operate through non-calcium pathways such as IGF signaling [119]. Nonetheless, epidemiologic evidence of increased risk among men with higher calcium intake, in whom 1,25 Vitamin D is suppressed homeostatically, confer additional plausibility to the Vitamin D hypothesis [120]. Early phase prevention trials with low-toxicity analogs are currently underway, as are studies evaluating the synergy between Vitamin D agents and chemotherapy in patients with advanced prostate cancer [121].

5. Mixed nutrient trials

Some investigators have begun to conduct trials using a mixture of nutritional agents. Although it is usually not possible to determine which ingredient may have been effective in such trials, these studies are important for proof-of-principle, and furthermore recognize the potential significance of interactions among various phytochemicals and micronutrients in the diet. Kranse et al have reported a double-blind crossover trial of a complex dietary supplement versus placebo in 37 men with untreated prostate cancer and rising PSA [122]. The supplement contained vitamin E, green tea extract, soy isoflavones, selenium and carotenoids (including lycopene). Overall,
PSA doubling time during the 6-week treatment phase was unaffected; however, the majority of subjects experienced a decrease in free androgen index while on the supplement and, among these men, PSA doubling time was significantly prolonged.

In a pilot trial without concurrent controls, Joniau et al provided a supplement containing vitamin E, selenium and soy isoflavones to 76 men with HGPIN who were re-biopsied at 3 and 6 months (n=62). Although the prevalence of cancer on re-biopsy, 35.5%, was not different from historical experience, 64% had a decrease in PSA and the cancer prevalence in this subgroup was only 10.2% [123]. Finally, the SU.VI.MAX trial randomly assigned cancer-free men to either a placebo or a low-dose supplement containing vitamin C, vitamin E, ß-carotene, selenium and zinc [124]. After a median follow-up time of nearly 9 years, there was a small reduction in the hazard ratio for prostate cancer (HR=0.88) for the 3,616 men in the analysis. However, among the men with PSA < 3 ng/ml at baseline, the prostate cancer risk was significantly reduced almost by half (HR=0.52, 95% CI: 0.29-0.92). The study also found an elevated but not statistically significant HR for prostate cancer among men receiving supplement whose baseline PSA was above 3.0, raising some concerns about the effect of antioxidant supplementation on growth of pre-existing tumors that are far enough along to elevate PSA. Additional trials of mixed supplementation seem warranted, particularly if the selection of compounds and dose levels is supported by reliable evidence.

VI. ENERGY BALANCE, PHYSICAL ACTIVITY AND DIETARY FAT

In this section, we discuss approaches to prevention of prostate cancer that involve manipulation of diet composition, including dietary fats, and alteration of energy balance through dietary change, physical activity, or both. We also include discussion of statin drugs as potential preventive agents, given their effect on lipid levels in serum and tissues.

1. OBESITY

Numerous epidemiological analyses have evaluated the relationship between obesity, usually measured as body mass index, and risk of developing prostate cancer. The results are complex; in aggregate, they suggest that obesity itself does not increase the incidence of prostate cancer [125]. In fact, several studies report lower risks among obese men, particularly those who are obese at a young age [126]. It is somewhat surprising, therefore, that several recent studies have shown a positive relationship between obesity and mortality from the disease, as well as evidence for increased risk of adverse pathological features and a poor outcome (risk of recurrence or death) among men already diagnosed with prostate cancer [127-130]. The association between obesity and poor prognosis is independent of, and in addition to, the link between obesity and advanced tumor grade or stage. This association does not appear to be due to the greater risk of positive surgical margins in obese men; in one study extremely obese men (BMI ≥ 35) with negative surgical margins had a four-fold increased risk of biochemical failure following radical prostatectomy (see Figure 6) [131].

Several endocrine and cytokine mechanisms have been postulated to explain the possible effect of obesity on prostate cancer development and progression. These include alterations in steroid hormone levels, changes in IGF-1 and its binding proteins, hyperinsulinemia, chronic inflammation associated with obesity, and increases in circulating leptin and interleukin-6 [132]. None of these mechanisms have been conclusively linked as yet to prostate cancer risk, as discussed elsewhere in this volume. Some investigators have focused on abdominal obesity, which is

Moderate-severe obesity had a 4-fold increased risk of failure compared to normal weight in a multivariable model.

Figure 6. Relationship of obesity to biochemical (PSA) failure among 1,250 surgically-treated prostate cancer patients with negative surgical margins (Freedland SJ, J Urol, 2004).
linked to the metabolic syndrome, but few studies have evaluated whether fat distribution is more pertinent to prostate cancer risk than total body fat. Energy (calorie) restriction, leading to negative energy balance and weight loss, is a powerful inhibitor of prostate cancer growth in animal models [48, 133]. It is not known whether milder degrees of weight loss in humans could also be protective. A recent longitudinal analysis of body size and hormones in young-to-middle age men found that, among men who had a decline in BMI over an 8-year period, SHBG levels strongly increased over the same period, whereas among men whose BMI increased the most, SHBG declined significantly [134]. Although changes in bioavailable testosterone did not appear to differ between these groups, the noteworthy increase in SHBG in the men who lost body fat could affect prostate cancer risk through non-androgen dependent effects of SHBG on prostate cells, or indirectly through indication of an improvement in glucose tolerance and insulin load [135, 136].

2. Physical Activity

The hypothesis that increased physical activity can reduce prostate cancer risk, either through its effect on energy balance and obesity or through obesity-independent pathways, has been under investigation for decades. Plausible biological mechanisms include a reduction in androgen signaling, improved glucose uptake and reduced insulin secretion, activated immune function, and upregulation of antioxidant defenses [137]. According to the authoritative review published in 1997 by the World Cancer Research Fund, which categorizes the strength of evidence for potential risk factors as convincing, probably, possible, or insufficient, “no judgment was possible” regarding the role of physical activity in prostate cancer [138]. More recent reviews have reached a similar conclusion [139], and have noted how population studies in this area must overcome difficulties due to inaccuracy in measurement of physical activity, uncertainty regarding the impact of activity during various stages of life, differences in the handling of screen-detected or latent cancer, and varying efforts at controlling for the effects of obesity and diet. It should be noted that the evidence linking physical activity to reduction in risk for colon and breast cancer is considerably stronger.

For prostate cancer, high levels of activity may be needed to influence hormone levels that are potentially implicated in the etiology of this cancer. The majority of studies to date did not have a sufficient number of study subjects who attained very high levels of activity. The existence of a threshold effect was suggested by results from the Health Professionals Follow-up Study, which found that men who engaged in at least 4 hours of vigorous exercise per week had a 54% reduction in the incidence of metastatic prostate cancer compared to men who were the most inactive [140]. Similar findings were reported from the American Cancer Society CPS-II cohort; men with recreational activity at the highest level had a relative risk of 0.69 (95% CI: 0.52,0.92) for aggressive prostate cancer (extraprostatic or Gleason >7) compared to the least active men [141]. A recent case-control analysis from Canada also found evidence for reduced risk only among men with the most vigorous or intense level of activity [142]. In all of these studies, the association for intense physical activity was independent of obesity and history of PSA testing. In addition to problems ascertaining the intensity of physical activity, most earlier studies examined activity later in life, closer to the time of the cancer diagnosis, whereas physical activity performed early in life may be the most etiologically relevant for prostate carcinogenesis. The natural history of prostate cancer is still poorly understood, and the biological mechanisms and etiologically relevant time periods in prostate carcinogenesis when physical activity may be operative are unknown. Despite this, physical activity merits ongoing consideration in prostate cancer prevention research for several alternative reasons: first, physical activity plays an obvious role in controlling obesity; second, there are sound public health reasons to recommend increased activity for the prevention of other cancers, diabetes and cardiovascular disease; and third, evidence is increasing that exercise positively influences other aspects of the cancer experience, including cancer detection, coping, rehabilitation and survival after diagnosis [143].

3. Dietary Fat

The amount of fat in the diet – particularly saturated fat or fat from animal sources – has been suspected as a risk factor for prostate cancer since at least the 1970’s when ecological studies showed strong correlations between per capita fat consumption and prostate cancer incidence rates among countries [144]. The weight of evidence from numerous case-control and cohort studies does support an etiologic role for total, animal or saturated fat intake [145]. Supportive data from pre-clinical studies is lacking, however. Considerable interest has focused on spe-
cific fatty acid types rather than total intake. There is some epidemiological evidence that intake of \(\alpha\)-linolenic acid, an essential polyunsaturated fatty acid, is associated with increased risk, and that linoleic acid – another essential fatty acid and a major component of total polyunsaturated intake - is protective [146, 147]. Animal model data on the effects of linoleic and \(\alpha\)-linolenic acid are sparse, however. In one report, \(\alpha\)-linolenic was observed to be a potent stimulator of LNCaP growth in vitro. [148].

A possible protective role for long-chain omega-3 fatty acids, such as those found in marine oils, has been postulated. An intriguing study involving the Greenland Inuits found that among 61 men whose prostates were fully examined at autopsy, only one invasive cancer was found, which is far below the number expected [149]. The epidemiologic evidence on fish or long chain omega-3 intake is limited and mixed, with some studies showing inverse associations and others the opposite [146]. Two recent cohort studies, however, conducted in Sweden and the U.S., reported lower risk of metastatic prostate cancer and death among men with the highest fish intake [150, 151]. Experimental evidence with cell lines and with transplanted human tumors supports an inhibitory effect of marine oil fatty acids on prostate tumor growth [152].

Long-chain omega-3 fatty acids such as eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) have several potentially interesting effects on mechanisms associated with prostate cancer. Both EPA and DHA interfere with androgen-receptor mediated signaling and may inhibit 5 \(\alpha\)-reductase activity in vitro.[153, 154]. These compounds also inhibit expression of COX-2 and 5-lipoxygenase, which appear to facilitate prostate tumor growth through effects on arachidonic acid metabolism and production of prostaglandin E2 (PGE2)[146]. In a small trial among men with untreated prostate cancer, Aronson et al showed that a low-fat diet plus fish oil supplements produced a significant increase in the omega-3:omega-6 ratio on plasma and adipose tissue, and reduced COX-2 mRNA expression in prostatic tissue in 4 of 7 patients[155]. Further trials of fish oil supplements are underway, and will determine effects on PGE2 expression and other intermediate markers. EPA and DHA are also ligands for PPAR \(\gamma\) and RXR, which have been implicated in early prostate carcinogenesis, and epidemiologic studies in the U.S. and Singapore have reported positive associations between fish intake and levels of IGF binding protein-3 [146].

Dietary fat intake is unavoidably related to energy intake. Many of the epidemiological studies that have evaluated fat as a risk factor have adjusted the estimated association for energy, which means that risk estimates refer to excess risk due to additional fat intake at any given level of caloric intake.

This is parallel to experimental studies of isocaloric fat intake, in which a fixed number of calories are redistributed from fat to either protein or carbohydrate. Ngo et al have reported that an isocaloric low-fat diet inhibited tumor growth in an LAPC-4 mouse xenografts model, and delayed the transition from androgen-sensitivity to androgen independence. [156]. This has important practical implications for prostate cancer prevention, because it suggests that men might be able to reduce their risk by consuming a low-fat diet without calorie restriction. It is important to note that the fat used in these studies was entirely corn oil, which is predominantly linoleic acid, and further study will be needed to test other types of fat intake.

Interest in low-fat interventions as preventive or adjuvant therapy for prostate cancer was heightened when results from the Women’s Intervention Nutrition Study (WINS) were presented at the American Society for Clinical Oncology in 2005 [157]. WINS randomly assigned over 2,400 women with early-stage breast cancer to either standard diet or a diet containing only 15% calories as fat. After a median follow-up of 5 years, WINS investigators reported a 24% improvement in relapse-free survival in the low-fat group. It is not clear how much of this benefit was attributable to significant, unintended weight loss among women on the WINS diet, as opposed to fat intake itself.

4. Statins

Statins, drugs, which inhibit the synthesis of cholesterol, are now widely used for reducing hypercholesterolemia and thereby lowering the risk of cardiovascular disease. Recently, some interesting potential anti-cancer properties of these drugs have come to light. Although cholesterol is a precursor for synthesis of androgens and other steroid hormones, ordinary statin dose levels do not appear to deplete cholesterol enough to reduce androgen levels. On the other hand, cholesterol is an essential component of cell membranes and an emerging body of evidence indicates that cholesterol concentrations influence important signal processes mediated through lipid rafts on the cell surface.

Statins drugs lower the cholesterol content of lipid
rafts in LNCaP cells, and this leads to increased apoptosis and decreased signaling through the stimulatory Akt phosphorylation pathway, which is reversible when cholesterol is added back to the medium[158].

Rodents do not respond to statins, but in a nude mouse LNCaP xenograft model, induction of hypercholesterolemia through diet increases the cholesterol content of lipid rafts and at the same time accelerates tumor growth with increased Akt-p expression [158].

Other possible anti-cancer mechanisms for statins have also been suggested, including inhibition of Ras and Rho signaling due to decreased isoprenoid synthesis, inhibition of signaling through sonic hedgehog-related proteins, and suppression of inflammatory processes [159]. Potential statin mechanisms in prostate cancer are shown in Figure 7.

Epidemiological evidence from pharmacy databases, case-control and cohort studies recently has indicated that long-term statin-users have a lower risk of prostate cancer [121, 160]. For example, in the Health Professionals Follow-up Study, men who used lipid-lowering drugs (primarily statins) for five or more years had a 69% lower risk of advanced prostate cancer compared to men who never used the drugs [161].

5. Multi-faceted lifestyle intervention

Because dietary fat, energy balance and physical activity are almost inextricably linked in everyday life, some investigators have devoted their attention to multi-faceted interventions that alter several aspects at once. Ngo and co-workers showed in a pilot study that a combination of a low-fat high-fiber diet and daily aerobic exercise reduced circulating levels of insulin and IGF-1 in overweight men without prostate cancer, and significantly reduced the ability of their serum to stimulate LNCaP growth in vitro. [162].

More recently, Ornish et al completed a randomized trial of usual care versus intensive lifestyle intervention in 93 men with early, untreated prostate cancer [163]. The intervention included a very low-fat vegetable and soy-based diet (10% fat calories) and moderate daily exercise (walking), plus stress management through meditation. Men in the intervention group had a 4% decrease in PSA after one year, versus a 6% increase in the controls, and their post-intervention serum inhibited LNCaP growth 8-times more strongly than did serum from controls. Neither testosterone nor C-reactive protein was affected; however, intervention group men experienced an average weight loss of 4 kg. The implications of this trial are still under discussion.

![Figure 7. Potential pathways for inhibition of prostate cancer growth by statin drugs](image-url)
VII. METHODOLOGICAL ISSUES IN PROSTATE CANCER PREVENTION

1. CLINICALLY MEANINGFUL VERSUS INDOLENT PROSTATE CANCER

The success of PSA and promise of other markers for early detection of prostate cancer has exacerbated concerns about the detection and treatment of indolent cancer that would not ordinarily have clinical consequences. The prevalence of latent, small foci of prostate cancer is very high in autopsy studies, and the results of the PCPT – the first study to biopsy men regardless of PSA level - indicate that biopsy-detectable tumors are not uncommon even in men who have had serial negative PSA tests. This situation in itself strengthens the argument for the development of safe primary preventive strategies. However, on a more practical level, our difficulty in distinguishing threatening from non-threatening prostate cancer creates a challenge in prevention research as well. Our current model for carcinogenesis assumes that tumors accumulate critical mutations and epigenetic traits as they progress, and that these characteristics render the tumor less vulnerable to both endogenous and exogenous defenses. Therefore, it is reasonable to assume that the effectiveness of preventive agents will generally decrease as tumors progress, and that some agents might be effective in suppressing only the most indolent types. Until we have the ability to accurately distinguish prostate cancers by their level of threat, it will be difficult to interpret the clinical significance of many preventive trials, and to know how many tumors that would have caused substantial morbidity or death have been prevented. On the other hand, particularly in the context of screened populations, it is clear that suppressing the growth of indolent tumors with non-toxic agents will have a beneficial effect on treatment-related morbidity [164].

2. EFFECTS OF PSA TESTING ON PREVENTION TRIALS

When prevention trials are conducted in populations that have a high penetration of PSA testing, there is no way to avoid an effect of PSA on trial design, even if the designers choose not to offer PSA testing as part of the protocol, as in SELECT. Exposure to PSA testing is such a strong determinant of the likelihood of diagnosis, that it is naturally a very important potential confounder in any prevention study. If PSA testing is not offered in a randomized trial protocol, there is a chance that active treatment could be associated with a different exposure to PSA testing, especially in studies in which participants can become unblinded or in those without blinding or placebo control such as dietary intervention studies. This bias would be very difficult to remove from intention-to-treat analyses. On the other hand, if PSA testing is offered in the protocol, the heavy exposure to repeated PSA testing in the trial arms will tend to exacerbate the influence of relatively indolent cancers on the trial results. On the whole this seems preferable to introducing confounding by PSA testing.

A special situation arises when the preventive agent is capable of altering PSA, independent of its possible effect in suppressing tumors. We know that PSA is not cancer-specific, and that elevations are commonly associated with BPH. Finasteride is an obvious example of an agent that alters PSA independent of any cancer preventive effect. This raised complex design challenges in the PCPT and the ongoing REDUCE trial of dutasteride. The main options for dealing with this problem are blind adjustment of PSA values and mandatory biopsy of all participants. Both approaches are difficult, the former because improper adjustment of PSA can bias study results, and the latter because mandatory biopsy increases the potential influence of indolent cancers. Unfortunately, the problem is not limited to drugs with obvious effects on PSA, such as the 5α-reductase inhibitors. PSA is a well-known androgen response gene. Several dietary compounds, such as vitamin E (and especially the pmcol moiety of α-tocopherol) and lycopene have been shown to suppress androgen signaling in vitro, which could in theory affect PSA levels in men on trial and thus alter their probability of diagnosis [33]. Even dietary trials that involve potential weight loss face this problem, because weight loss is expected to alter hormone profiles and potentially could have effects on PSA values. Recent studies have reported an inverse association between obesity and PSA levels among men who are not believed to have prostate cancer [165]. It appears essential to conduct careful preliminary studies to detect an effect of a preventive intervention on PSA in cancer-free men.

3. PROS AND CONS OF VARIOUS PHASE II DESIGNS, CHOOSING THE RIGHT DISEASE STAGE

Since there are far too many interesting agents and
strategies for prevention than we can test in Phase III trials, we must emphasize improvement in our ability to screen agents in Phase II. Four major designs for Phase II prostate prevention trials and their strengths and weaknesses are shown in Table 4 below. These designs have tissue endpoints; some Phase II trials can use PSA or other circulating biomarkers as endpoints, however, we these will often be less informative than trials evaluating tissue changes.

Using the participant as his own control (i.e., comparing pre-treatment and post-treatment endpoints) has a powerful effect on the required study size, by reducing the variance of key endpoints. Phase II prevention trials in accessible organs can use a reduction in prevalence or volume of premalignant tissue as an endpoint; for example, this has been done in studies on Barrett’s esophagus. HGPIN, on the other hand, cannot be visualized for targeted repeat biopsy. Thus, failure to find HGPIN after treatment using random needle biopsy frequently will occur due to sampling error. In fact the probability of finding HGPIN again on repeat biopsy is estimated to be in the vicinity of 30%. Trials which utilize changes in benign tissue as endpoints, such as the biopsy/re-biopsy designs, will be feasible with 50-100 subjects as opposed to 500-1000.

Among men with isolated HGPIN, the probability of finding prostate cancer on repeat biopsy is similar to the probability of finding HGPIN again (approximately 30%). Therefore, prevention trials among men with isolated HGPIN, with prostate cancer on subsequent biopsy as the major endpoint, are not impractical, and will have study size requirements of about 500-1000 patients. These are technically Phase III because of the cancer endpoint, but in fact the exposure duration is likely to be relatively short (1-3 years) and most certainly any reductions in tumor incidence observed are due to effects of the agent on tumors that are already biopsy-detectable. Caution is advisable when designing or interpreting prevention trials that involve patients with clinically significant, and especially advanced or recurrent disease. As stated earlier, these cancers might not respond to preventive agents that could be highly effective at earlier stages of tumorigenesis.

4. DEVELOPMENT OF INTERMEDIATE ENDPOINT BIOMARKERS

The success of Phase II prevention studies in making correct decisions about which agents should be promoted to Phase III depends upon development of reliable and valid intermediate endpoints. Circulating markers that reflect tumor size and differentiation to some extent, such as serum PSA, are of limited value in this context. PSA, for example, is only weakly correlated with tumor characteristics such as volume and therefore, small increases or decreases in PSA during a trial cannot be expected to be reliable indicators of meaningful changes in tumors. For a change in PSA due to a treatment to be both statistically and biologically meaningful, a preventive agent would have to be capable of having a large effect on a tumor that has already reached a relatively advanced stage of development. As argued above, we can conceivably reject many useful preventive agents if we require them to have substantial effects on clinically significant tumors. Circulating markers

---

**Table 4. Pros and cons of Phase II design options with tissue endpoints**

<table>
<thead>
<tr>
<th>Design</th>
<th>Pro</th>
<th>Con</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-prostatectomy</td>
<td>o  abundant tissue</td>
<td>o  short exposure</td>
</tr>
<tr>
<td></td>
<td>o  fresh tissue (mRNA)</td>
<td>o  difficulty comparing biopsy to surgical sample</td>
</tr>
<tr>
<td>Pre-biopsy</td>
<td>o  many patients</td>
<td>o  shorter exposure</td>
</tr>
<tr>
<td>Biopsy/re-biopsy (with or without HGPIN)</td>
<td>o  3-12 month exposure</td>
<td>o  fewer patients</td>
</tr>
<tr>
<td></td>
<td>o  low endpoint variance</td>
<td>o  changing criteria for repeat biopsy</td>
</tr>
<tr>
<td>Watchful waiting with biopsy</td>
<td>o  may detect effects on tumor if re-sampled</td>
<td>o  biopsy not clinically indicated</td>
</tr>
</tbody>
</table>
that involve surrogate targets, such as changes in WBC DNA damage, are interesting but provide only the most circumstantial type of evidence. The most relevant intermediate markers will therefore involve analysis of changes in prostate tissue. Markers related to important generic processes in early cancer development - such as proliferation, apoptosis and differentiation – are important, as are markers whose functions are less well-characterized, but which are strongly associated with the earliest steps in prostate tumor progression. Epidemiological validation of these intermediate markers – that is, studies quantifying the degree of association between the potential intermediate markers and actual cancer risk – are very valuable; however, they are difficult to conduct and judgments about the significance of certain markers will sometimes have to be made on prima facie evidence. Phase III trials themselves offer perhaps the best opportunity for epidemiological validation of intermediate biomarkers.

5. CHOOSING THE RIGHT INTERVENTION: WHOLE FOOD VERSUS ISOLATED COMPOUNDS

Where dietary elements are involved, there is an obvious choice between testing specific compounds believed to carry important biological activity and testing whole foods or dietary patterns. Evidence is accumulating to support the contention that the effects of dietary factors or foods on cancer risk involve interactions among perhaps many specific elements in the food. A compelling example is provided by the unanticipated results of two Phase III trials of supplemental β-carotene, in which supplements appeared to cause an actual increase in lung cancer risk among the participants, who were male smokers [166].

Numerous diet-history and serum-based observational studies had indicated that men with higher intake or higher blood levels of β-carotene, due to consumption of certain fruits and vegetables, had reduced lung cancer risk. The aforementioned study in an animal model comparing the effects of tomato powder versus pure lycopene on inhibition of prostate tumor growth provides a similar note of caution. It is now recognized as a challenge for prevention researchers to use the power of focusing on single compounds for understanding mechanisms while also being aware of the potential importance of interactions when considering translational studies.

Whole food or dietary intervention studies involve some tradeoff, as they often must give up the benefits of participant blinding and placebo control. Intermediate approaches, such as the use of capsules containing complex mixtures or extracts of foods, can offer an attractive alternative that retains blinding and placebo control. Research involving single compounds and whole foods are both necessary and should be viewed as complementary rather than competing strategies.

6. GENE-NUTRIENT INTERACTIONS AND PHARMACOGENOMICS

It is intuitively correct that response to any pharmacologic or dietary intervention aimed at preventing prostate cancer could be significantly modified by an individual’s genetic background. We are at only the earliest beginning of efforts to identify and understand the important drug-gene and diet-gene interactions. Important early examples of this work include efforts to characterize genetic polymorphisms in SRD5A2 that modify the response to 5α-reductase inhibitors such as finasteride [167].

Recently, Li et al reported that an amino acid-substituting polymorphism in the gene for manganese superoxide dismutase (MnSOD) – a key antioxidant enzyme in mitochondria – had no overall association with prostate cancer risk in the Physicians’ Health Study, but significantly modified risk when pre-diagnostic plasma levels of various antioxidants was taken into account [168]. For example, among men with the activating alanine/alanine (AA) genotype, high levels of plasma selenium were associated with a 70% reduction in prostate cancer risk, with similar patterns observed for plasma vitamin E and lycopene.

Moreover, the Physicians’ Health Study involved randomized assignment to a β-carotene supplement, and while there was no main effect of β-carotene on prostate cancer occurrence, for men with the AA genotype for MnSOD, the RR=0.37 (95% CI: 0.15,0.94) for β-carotene versus placebo. This is one of the first important observations regarding gene-nutrient interaction in prostate cancer, but certainly not the last.
1. **5α-Reductase inhibitors**

The results of the PCPT – the first Phase III primary prevention trial in prostate cancer to be completed – have established that finasteride, and 5α-reductase inhibitors in general, offer a highly promising approach to chemoprevention. However, the excess rate of detection of high-grade cancer in participants assigned to finasteride is a matter of serious concern, and resolution of this concern deserves high priority. A recommendation for the widespread use of finasteride as a chemopreventive cannot be made until additional evidence is examined to determine whether the excess of high-grade cancer was due to a true biological effect or an artifact related to study design.

2. **Anti-oxidants**

Selenium and vitamin E have shown some promise in both observational and experimental studies, and results from important Phase III prevention trials are pending. Recent trial evidence suggesting no reduction in cardiovascular disease with vitamin E supplements and possible risks associated with high doses will undoubtedly have an effect on the cost-benefit evaluation of this strategy for prostate cancer prevention. Tomato products including lycopene have also shown notable promise, but have not yet been tested in rigorously controlled trials.

3. **Asian diet**

Certain aspects of the traditional Asian diet, including soy foods, green tea and fish, deserve some priority in future research. The epidemiological evidence for a suppressive effect of this diet pattern on prostate tumor promotion, compared to the typical Western diet, is quite extensive. However, translational research evaluating this diet or its various components is at too early a stage to have yielded significant results.

4. **Weight control, energy balance**

Weight control and possibly cholesterol-lowering (through a combination of diet and physical activity modification) can be encouraged prudently and should be studied further as a means for prostate cancer prevention. Changes in the social and physical environment will be crucial for initiating and maintaining any public health benefits, long-term.

5. **Methodological recommendations**

5a. **Risk stratification**

Better risk stratification methods are greatly needed to identify target populations for preventive interventions. Improvements are likely to come through development of genetic testing, refinement and validation of risk factors in epidemiological research and appropriate risk modeling, and through development of new techniques for early detection. More accurate characterization of individual risk – especially risk for more aggressive forms of prostate cancer – will allow better decisions about balancing risk and benefit in preventive interventions.

5b. **Selecting agents for Phase III**

International consensus on the *optimal process* for screening and selecting preventive interventions for Phase III trials would be extremely useful. This would include establishing some common ground regarding advantages and disadvantages of various pre-clinical (animal) models, and various Phase I and Phase II studies.

5c. **Isolated compounds and whole foods**

We recommend active engagement of investigators in the field to increase the complementary relationship of research focusing on single compounds and that which is focusing on whole foods or lifestyle/dietary patterns. Greater consideration should be given to exploring the interactions between phytochemicals in food and other naturally occurring compounds in the diet.

5d. **Intermediate biomarkers in Phase II**

Given the number of agents and strategies that require testing, the Committee believes it is imperative to strengthen the array of tools available as intermediate biomarkers in Phase II trials. Incorporation of biomarker validation substudies into Phase III trials is an important way to accomplish this. Emerging technologies offer many new opportunities to measure potentially relevant effects of preventive agents on tissue.

5e. **Integrating primary prevention and screening**

Effective primary prevention approaches will affect secondary prevention (screening) efforts, and vice versa. The two approaches to reducing suffering and mortality due to prostate cancer do not necessarily have to conflict, however. The Committee believes it is not too early to begin discussion of ways in which the two approaches can be integrated to maximize the benefit in populations of men at risk.

5f. **Gene-nutrient interactions**

The effects of diet on prostate cancer risk are complex and are not likely to be uniform within broad populations. Identification of subgroups with differential responses to diet is important, and one of the most promising approaches lies in the evaluation of gene-nutrient interactions. Opportunities for studies in this area will expand rapidly once candidate genetic polymorphisms that are functionally significant are identified in larger numbers.
REFERENCES


22. Malins DC, Polissar NL, Gundersen SJ. Models of DNA structure achieve almost perfect discrimination between normal prostate, benign prostatic hyperplasia (BPH), and adenocarcinoma and have a high potential for predicting BPH and prostate cancer. Proc Natl Acad Sci U S A. 1997;94(1):259-264.


37. Gann PH, Ma J, Giovannucci E, et al. Lower prostate cancer risk


Gapstur S. Longitudinal analysis of changes in BMI associated with changes in steroid hormones: CMHS. 2005.


Committee 12

New Developments in the Treatment of Localized Prostate Cancer

Chairman

G. BARTSCH (AUSTRIA)

Vice-Chairs

W. CATALONA (USA),
M. GOSPODAROWICZ (CANADA)

Members

F. ABBAS (PAKISTAN),
C. ABBOU (FRANCE),
Y. Arai (JAPAN),
M. BOLLA (FRANCE),
P. CONORT (FRANCE),
D. DEARNALEY (GREAT BRITAIN),
A. GELET (FRANCE),
R. LEE (USA),
M. MENON (USA),
T. PIECHAUD (FRANCE),
J. TRACHTENBERG (CANADA),
G. VALLANCIEN (FRANCE)
# CONTENTS

<table>
<thead>
<tr>
<th>Introduction</th>
<th>III. Adjuvant Therapy for High Risk Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Staging of Localized Prostate Cancer</td>
<td>IV. External Beam Radiotherapy</td>
</tr>
<tr>
<td>I. Characterization of the Local Tumor (see committee 4)</td>
<td>V. Brachytherapy</td>
</tr>
<tr>
<td>II. Informatics Tools for Prognosis and Staging: Nomograms</td>
<td>VI. Hormonal Therapy as a Primary Treatment Option for Localized or Locally Advanced Prostate Cancer</td>
</tr>
<tr>
<td>III. Management Consideration</td>
<td>VII. Other Therapies with Lower Level of Evidence</td>
</tr>
<tr>
<td>B. Management Options</td>
<td></td>
</tr>
<tr>
<td>I. Active Monitoring (Watchful Waiting)</td>
<td>RECOMMENDATIONS</td>
</tr>
<tr>
<td>II. Radical Prostatectomy</td>
<td>Algorithm for Management of Localised Prostate Cancer (by order of Preference)</td>
</tr>
<tr>
<td>REFERENCES</td>
<td></td>
</tr>
</tbody>
</table>

276
New Developments in the Treatment of Localized Prostate Cancer

G. Bartsch

W. Catalona, M. Gospodarowicz

F. Abbas, C. Abbou, Y. Arai, M. Bolla, P. Conort, D. Dearnaley, A. Gelet, R. Lee, M. Menon, T. Piechaud, J. Trachtenberg, G. Vallancien

INTRODUCTION

Prostate cancer, the most common cancer in American and European men, is increasingly being diagnosed in men from other parts of the world. Management decisions are influenced by the variable natural history of the disease: while prostate cancer is unequivocally lethal in some patients, others do indeed die with rather than of their cancer. Further highlighting the remarkable variation in behavior of this disease, histologically apparent cancer can be found incidentally in the prostates of 42% of 75-year-old men who die of other causes. While the lifetime risk that an American man will be diagnosed with prostate cancer is estimated to be about 16%, his risk of dying from this disease is only 3.6% [1,2]. Consequently, appropriate management of this disease requires assessment of risk: How likely is a given man’s cancer to progress or metastasize over his remaining lifetime? What is the probability of success with treatment? What are the risks of side effects and complications with each type of treatment?

To quantify the risk posed by a particular cancer, modern medical informatics makes it possible to use nomograms, or algorithms, that can account for the interactive effects of multiple factors, such as stage, grade, and serum prostate-specific antigen (PSA) level. Decision-analysis models can help us estimate the probable gain (or loss) in quality-adjusted life years from active versus expectant management for the “average” [3].

Decision-making can be further refined to reflect the particular values (“utilities”) of an individual patient through utility assessment. For example, how concerned would an individual be if he were incontinent, had radiation proctitis, or were living with an untreated cancer? The ultimate goal is to select and apply the right treatment at the right time, but only to those patients who need it. In this section, we will focus on clinically localized prostate cancer.

A. STAGING OF LOCALIZED PROSTATE CANCER

In the treatment of localized prostate carcinoma, the accurate determination of tumor stage is necessary to inform patients, to direct therapeutic options, to interpret outcome data, and to stratify different treatment arms in clinical studies.

Several staging systems for prostate cancer have been described. The most often used are the Jewett-Whitmore (A, B, C, D) and the TNM systems (Table 1). It is the consensus of this committee that the modified TNM system of 2002 provides us with the best available clinical classification [4,5].

Note that non palpable cancer detected by PSA is categorized as stage T1c, and that T2 lesions (palpable but confined tumors) have been divided to include T2a, T2b, and T2c once again, as in the 1992 system. The committee recommends that the definition of T1a also include low (Gleason 2 to 5) tumor (Table 2).
For patients with localized disease, a critical assessment of the location, size, extent, and histologic features (zone, grade) of the primary tumor provides prognostic and staging information and is essential for treatment planning. These cancers are best characterized by the clinical stage (determined by DRE), Gleason grade, and serum PSA level. These are the only features independently predictive of pathologic stage and prognosis [6]. Imaging studies (TRUS, MRI) have been intensively investigated but have yet to be accepted as essential adjuncts to staging.


### I. CHARACTERIZATION OF THE LOCAL TUMOR (see committee 4)

### Table 1. Comparison of the ASCC/UICC 1992 TNM staging system and Whitmore-Jewett Staging System

<table>
<thead>
<tr>
<th>TNM 1992</th>
<th>TNM 2002</th>
<th>Whitmore-Jewett</th>
<th>Definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>T1</td>
<td>A</td>
<td>Clinically inapparent tumor not palpable, nor visible by imaging</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tumor an incidental histologic finding</td>
</tr>
<tr>
<td>T1a</td>
<td>T1a</td>
<td>A1</td>
<td>&lt; 5 % of tissue resected</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt; 3 chips</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tumor an incidental histologic finding</td>
</tr>
<tr>
<td>T1b</td>
<td>T1b</td>
<td>A2</td>
<td>&gt;5 % of tissue resected</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;3 chips</td>
</tr>
<tr>
<td>T1c</td>
<td>T1c</td>
<td></td>
<td>Tumor identified by needle biopsy (e.g., for elevated serum PSA)</td>
</tr>
<tr>
<td>T2</td>
<td>T2</td>
<td>B</td>
<td>Tumor confined within the prostate</td>
</tr>
<tr>
<td>T2a</td>
<td>T2a</td>
<td>B1N</td>
<td>Tumor involves half of a lobe or less</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B1</td>
<td>Palpable nodule &lt; 2 cm and confined to one lobe</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Palpable nodule &lt; 2 cm and confined to one lobe</td>
</tr>
<tr>
<td>T2b</td>
<td>T2b</td>
<td>B2</td>
<td>Tumor involves more than half of a lobe but not both lobes</td>
</tr>
<tr>
<td>T2c</td>
<td>T2c</td>
<td>B3</td>
<td>Tumor involves both lobes</td>
</tr>
<tr>
<td>T3</td>
<td>T3</td>
<td>C</td>
<td>Tumor extends through and beyond the prostate capsule</td>
</tr>
<tr>
<td>T3a</td>
<td>T3a</td>
<td></td>
<td>Unilateral extracapsular extension</td>
</tr>
<tr>
<td>T3b</td>
<td>T3b</td>
<td></td>
<td>Bilateral extracapsular extension</td>
</tr>
<tr>
<td>T3c</td>
<td>T3c</td>
<td>C1</td>
<td>Tumor invades seminal vesicle(s)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C2</td>
<td>&lt; 6 cm tumor beyond prostatic capsule</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt; 6 cm tumor beyond prostatic capsule</td>
</tr>
<tr>
<td>T4</td>
<td>T4</td>
<td></td>
<td>Tumor is fixed or invades adjacent structures other than seminal vesicles</td>
</tr>
<tr>
<td>T4a</td>
<td></td>
<td></td>
<td>Tumor invades bladder neck and/or external sphincter and/or rectum</td>
</tr>
<tr>
<td>T4b</td>
<td></td>
<td></td>
<td>Tumor invades levator muscles and/or is fixed to pelvic wall</td>
</tr>
</tbody>
</table>

### II. INFORMATICS TOOLS FOR PROGNOSIS AND STAGING: NOMOGRAMS

#### 1. Predicting Pathologic Stage

Pathologic stage is determined by histologic examination of radical prostatectomy specimens, including the seminal vesicles and the pelvic lymph nodes. Nomograms are mathematical algorithms derived from statistical models that are used to predict outcomes for an individual patient or for groups of patients. Partin et al developed an algorithm that combined clinical T stage, Gleason grade in the biopsy specimens, and preoperative PSA levels to predict
pathologic stage, which is then assigned as one of four mutually exclusive groups: organ-confined, established capsular penetration, seminal vesicle invasion, or lymph node metastasis [7]. These “staging tables,” which offer categorical (rather than continuous) probabilities, are widely used in clinical practice and have been prevalent at several institutions. However, their accuracy may vary depending on the extent of cancer in any given community, which is, itself, highly dependent on the frequency of screening in the population.

2. Predicting Prognosis

Predicting pathologic stage is important in clinical decision making, and may help to determine the need for more intensive therapy, i.e., high-dose, 3-D conformal external beam irradiation therapy or for modifying surgical technique to resect a neurovascular bundle. But pathologic stage is only a proxy for prognosis. Patients and their physicians are interested not only in the chance of the success of a given treatment, but in the microscopic extent of the cancer as well. Many risk stratification schemes have been published to predict outcomes after therapy [8,9], but none, thus far, predicts the chances of success or failure as well as a nomogram does [10]. Kattan et al developed the first continuous, rather than categorical, scale to predict the probability (with 95% confidence intervals, CI) that a given patient will remain free of recurrence for > 5 years [11]. Prognostic nomograms are available for patients treated with external radiation therapy as well as with brachytherapy [12].

III MANAGEMENT CONSIDERATION

1. Medical Decision-Making

The end point of cancer treatment should be quality-adjusted survival rather than survival at all costs to the patient [13]. The selection of treatment should be based on the life expectancy of the patient (age, comorbidity), the nature of the cancer (stage, grade, PSA, biopsy results), the effectiveness and side effects of a given treatment in the hands of the treating physician, and the patient’s own preferences. Three key considerations in defining optimal therapy for a given patient include the characteristics of the cancer, the characteristics of the patient, and environmental factors, such as availability and quality of each type of therapy in a given community.

2. Predicting Outcomes

A randomized prospective trial has shown that, compared to watchful waiting, radical prostatectomy reduces the chances of metastases and of death from prostate cancer [14]. Nevertheless, such trials can not establish whether some patients benefit greatly from aggressive treatment and others suffer harm, depending on their age, health, and the characteristics of their cancer. It would be valuable to have more information about the chances that a cancer with particular characteristics will metastasize or cause death [15].

Nomograms allow us to incorporate all established prognostic factors for quantifying the risk posed by a particular cancer. While nomograms have been published predicting biochemical progression after surgery, [11] external radiotherapy, [10] and brachytherapy, [12] there are none yet that predict long term cancer-specific survival or the development of metastases after the various treatment options, nor do we have nomograms for patients managed expectantly (watchful waiting).

3. Decision Analysis

The decision to observe or treat a patient involves balancing risks, benefits, and uncertainties. A framework known as decision analysis can be used to develop an explicit approach to the tradeoffs involved in such decisions. An analytical model for decisions about clinically localized prostate cancer requires quantitative information about the life expectancy (age, comorbidity) of the patient; the probability of metastases and of death from prostate cancer over time in the untreated (or expectantly managed) patient, the particular characteristics of the primary tumor (prognostic features), the effectiveness of the treatment being considered, the complication rate and side effects from the treatment, and patient utilities (values) for each health state affected by the cancer and its treatment (e.g., having rectal bleeding, living with an untreated cancer, being incontinent).

The decision about treatment of a localized prostate cancer compares the immediate risk of perioperative mortality and morbidity to the ongoing risk of metastatic spread. For such problems, the Markov process is a particularly effective model [16,17]. Decisionmaking can be further tailored to the particular values (“utilities”) of an individual patient through utility assessment. That is, compared to the value of a year lived as a healthy man, how would a man with prostate cancer value a year lived with an
untreated cancer, or in a state of incontinence, or with loss of libido from androgen ablation? [13]. Application of decision theory to the problem of clinically localized prostate cancer is particularly appropriate. This disease requires a physician’s careful deliberation regarding the alternatives, a prerequisite for benefit from a decision aid [18]. Of course, such models require proof in clinical trials. The ultimate goal is for the patient, in consultation with his physician, to be able to select and receive appropriate treatment at the right time.

4. RISK STRATIFICATION

Selection of therapy for an individual patient requires assessment of risk: How likely is a given man’s cancer to progress or metastasize over his remaining lifetime? What is the probability of success with treatment? What are the risks of side effects and complications with each treatment? While some small, well-to-moderately differentiated prostate cancers may progress very slowly over time, many cancers detected clinically will grow locally, metastasize, and eventually result in death [15].

Taking into consideration the clinical stage, serum PSA level, and biopsy Gleason score, patients can be stratified into “risk groups.” (Table 2) While the extremes are accurate (low risk and very high risk), the two intermediate groups are remarkably heterogeneous and contain patients across the risk strata. More accurate assignment of risk can be computed with one of the prognostic nomograms. Using a nomogram, we could define “low risk” as > 80% chance of freedom from recurrence after surgery, “intermediate risk” as 50–80% chance, “high risk” as 15–50%, and “very high risk” as < 15% chance.

The optimal treatment strategy for a patient with prostate cancer should provide long term, disease-free survival with minimum treatment-related morbidity and maximum preservation of quality of life. When expectant management (active monitoring, watchful waiting) is not appropriate, active treatment options include radical prostatectomy, external beam radiotherapy, or brachytherapy, each used with or without adjuvant androgen deprivation. The selection of treatment should be tailored to each individual and the particular characteristics of his cancer.

The selection of optimal therapy for an individual patient should depend as much on the availability of high quality delivery of that therapy as on absolute differences in outcomes between treatments.

I. ACTIVE MONITORING (WATCHFUL WAITING)

The management option of active monitoring is also called observation, surveillance, watchful waiting, and, more recently, active observation or active monitoring. These terms are all associated with a management policy in which there is an active decision not to eradicate the primary tumor and to defer active treatment until the occurrence of symptoms. In many series of patients managed with noncurative intent, some patients receive no treatment, while others receive immediate hormonal therapy. For series with such mixed noncurative approaches, the term con-
servation management may be the most appropriate description.

Prostate cancer used to be diagnosed in men aged = 70 years; with the advent of PSA-based early diagnostic regimens the lead-time (the period between screen detection and presumed clinical diagnosis) is reduced and the age at diagnosis declines to = 60 years.

In all geographical areas referred to, the ratio between incidence and mortality has increased over time. However, this change is much more drastic in North America, where screening for prostate cancer is highly prevalent. Here, based on statistics for 2000, one in almost six men diagnosed with prostate cancer was likely to die from the disease.

How do these epidemiological data translate to the clinical situation? Can those patients who are unlikely to die from their disease be identified with reasonable certainty? Is it possible to avoid unnecessary treatment safely and how can that be achieved? [21]

WW should be clearly differentiated from “observational” treatment as used in the natural history studies and in the control arm of the Scandinavian Prostate Cancer Group study 4 [14]. Treatment by observation does not aim at the eventual cure of a given patient but a determining the proper timing for instituting endocrine (palliative) treatment. Such policies were previously based on doubts about the effectiveness of curative forms of management of localized disease, e.g. radical prostatectomy and radiotherapy. Observational treatment may be indicated in men with prostate cancer whose life-expectancy is limited by age or concomitant disease. This option entails that these two factors must be considered in each treatment decision. Estimates of comorbidity are possible according to well established scoring systems. WW entails the observation of selected patients to determine which cancers should be treated by potentially curative measures and when. The expected result is that some men, because they do not have aggressive disease, may not require any treatment because their cancer does not progress to clinical disease during their lifetime.

The advantages of watchful waiting are that it reduces the risk of overtreatment, and it limits treatment side effects, either by eliminating or by postponing them. The disadvantages are the risk that the tumor will progress beyond the possibility of cure, eventually causing severe disability and death. Delay may also make later definitive treatment more difficult, with increased side effects for the patient. Many patients also suffer from the anxiety of living with an untreated tumor.

There is now Class I evidence from a prospective randomized clinical trial that watchful waiting leads to significantly greater risk of metastases (27 % compared to 13 % after radical prostatectomy), and cancer related death (13 % compared to 7 %) 8 years later, compared to radical prostatectomy for patients with low and intermediate risk cancer [14].

More recently, in some series and clinical trials, the initial treatment is deferred, but with the stated goal of giving curative treatment when needed (i.e., when there is clear evidence that cancer is progressing). In such a “deferred definitive therapy” approach, the patient is closely followed with regular, periodic DRE, PSA, and repeat needle biopsy. The cancer is “actively monitored,” and definitive treatment – radical prostatectomy, external radiation, or brachytherapy – is offered with curative intent upon progression. The major limitation of this approach is the absence of established criteria for progression [1,22-24].

Nevertheless, the “trigger” point, or constellation of signs and symptoms, that appropriately signals the optimal time for treatment remains elusive. Some studies report a much greater risk of eventual progression if a repeat needle biopsy finds cancer [22,24]. Others use the rate of rise of PSA (PSA doubling time) (1). Various trigger points based on PSA have been used [1-3,22]. Also, in serial biopsies in ploidy and grade have been seen before clinical progression [25]. In contemporary series on active monitoring, change in biopsy grade has been seen before clinical progression [25]. In one series, 12 of 13 men undergoing deferred radical prostatectomy had curable cancers [22]. Whether these trigger points are valid or not, however, still needs to be proven.

One strategy is to follow patients with DRE and PSA every 3 months during the first year, and every 6 months during the second year, and every 6 months, thereafter. However, the follow-up must be individualized. For instance, if the aim is to provide deferred active treatment with curative intent, then periodic needle biopsies should be included in the follow-up strategy, and the intervals between the visits could be shorter. On the other hand, in a patient with high age and a short life expectancy and with an early prostate cancer, monitoring for clinical progression with an occasional PSA might be sufficient.

The proportion of patients “failing” active monitor-
ing and then receiving active treatment varies with the trigger points used in different series. In one study using clinical signs as a trigger, the probability of remaining untreated after diagnosis was 71% at 5 years and 43% at 10 years. [26]. In another study with a PSA-based trigger point, the probability of remaining on active monitoring was 67% at 2 years and 48% at 4 years after diagnosis [1].

In both series, some patients demanded treatment in the absence of objective evidence of progression. Neither of these series makes it clear what proportion of patients treated late was successfully cured.

Future research must concentrate on evaluating entry criteria and follow-up procedures to establish the appropriate trigger points for treatment. In this respect observational and WW studies need to be separated.

a) Cancer control

The survival outcome for watchful waiting is described in several reports. Before 1995, the 10-year cancer-specific survival in patients with low- or intermediate-grade clinically localized prostate cancer was around 85% [20,27,28]. This figure has been confirmed in more recent reports in population-based series [15,29,30] and in large cohort series [31-33]. The outcome of deferred treatment is much worse in patients with high grade tumors, as the 10-year disease-specific survival rate for poorly differentiated tumors ranges from 34% to 59% [15,30-32]. In the watchful waiting arm of the recent Swedish randomized trial, 27% of the patients developed metastases at 8 years, compared to 13% of those treated with radical prostatectomy [14]. Beyond 10 years after diagnosis, however, survival data is sparse. Most series with 10 years or longer of follow-up were accrued before the PSA-era, and at that time most patients had palpable lesions or lesions that were found at transurethral resection of the prostate because of a presumed benign hyperplasia. With active PSA testing, cancers detected today are found 5 to 8 years earlier than before the PSA-era [34-36]. Thus, it is problematic to extrapolate the cancer metastatic and mortality rates over time from the older series to contemporary PSA-detected cancer.

b) Complications and quality of life

The worst consequence of deferring active treatment is that the tumor might progress beyond curability and eventually kill the patient. This possibility cannot be excluded in an individual patient but, to date, there is little data documenting this risk. With regard to quality of life, men with prostate cancer on active monitoring seem to have the same degree of sexual dysfunction and of urinary and bowel symptoms as age-matched controls [37-40]. The overall quality of life was similar in patients on observation, as in age matched controls in one study, [37-40] and did not change during the first year of follow-up in another [41]. Patients on watchful waiting seem to have similar psychological morbidity to patients subjected to radical prostatectomy, three and ten years after treatment [42].

Watchful waiting, in the traditional sense, is rarely an optimal strategy for men with prostate cancer. Active monitoring with periodic medical evaluation can be an appropriate option for well informed patients who wish to minimize the short-term risks of immediate therapy and who accept the risks of deferred treatment. For men with a short life expectancy, active monitoring may be appropriate for any stage of cancer in the absence of symptoms or signs of impending morbidity from the disease.

If this management is chosen, the patient should be thoroughly informed about the expected course of the disease, and then closely followed. Possible trigger points for reevaluation of the management plan have to be discussed with the patient. Most importantly, the patient’s preferences must be considered, and patient anxiety accepted as an appropriate indication for reconsidering active treatment.

RECOMMENDATION

In the era of PSA testing and early detection about 85 to 90% of patients with clinically significant localized disease are considered good candidates for definitive surgical treatment, external-beam radiotherapy or interstitial radiation therapy. The watchful waiting strategy may be a reasonable alternative in carefully selected elderly men, patients who are too ill to survive longer than 10 years, and those who are assumed to have small-volume cancers. However, patients with small-volume and low-grade disease subjected to the watchful waiting strategy have to be informed that it is not possible to accurately diagnose small-volume disease.

The patients have also to be informed that for the time being there is no specific test to assess progression of disease. We do not know how many of the patients in the follow up will still have curable disease at the time of progression. Therefore, patients in the watchful waiting group have to be informed that without treatment of curative intent about the risk of
missing the therapy window of curative strategy as external beam radiotherapy, interstitial radiation therapy or surgery. Finally the major concern with any observation protocol would be the deprivation of effective therapy in individuals who need it.  

Table 3 shows the indications, contraindications, advantages, and disadvantages of active monitoring.

**Table 3. Indications, Contraindications, Advantages, and Disadvantages of Active Monitoring**

<table>
<thead>
<tr>
<th>Indications</th>
<th>Low grade, low volume disease</th>
<th>Expected survival less than 10 years</th>
<th>Patient’s preference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contraindications</td>
<td>High grade disease</td>
<td>Locally advanced disease</td>
<td>Expected survival more than 10 to 15 years</td>
</tr>
<tr>
<td>Advantages</td>
<td>Reduces over treatment</td>
<td>Avoids or postpones treatment-associated complications</td>
<td>No effect on work or social activities</td>
</tr>
<tr>
<td>Disadvantages</td>
<td>Disease progression beyond possibility for cure</td>
<td>Under staging</td>
<td>Treatment with curative intent in a later stage may result in more severe side effects</td>
</tr>
</tbody>
</table>

### II. RADICAL PROSTATECTOMY

Radical prostatectomy was the first treatment for prostate cancer. It was first performed in Europe in 1866 [43] and later was modified and popularized by Young in 1905 in the United States (US) [44]. Because of the anatomic inaccessibility of the prostate gland, radical prostatectomy is a technically formidable operation. Therefore, simpler methods are always being sought to treat patients with clinically localized prostate cancer. Although some of these have provided alternative treatment options, none has supplanted radical prostatectomy. This is because hormonal therapy is never curative and, in many patients, not all prostate cancer cells can be eradicated by radiotherapy, freezing or thermal energy. Radical prostatectomy remains one of the most commonly performed treatments for clinically localized prostate cancer [45].

Walsh’s modification of radical retropubic prostatectomy improves hemostasis and thus provides better visualization during the dissection and allows preservation of the neurovascular bundles that innervate the corpora cavernosa and are responsible for producing erections [46]. As a result, radical prostatectomy is now performed with a high cure rate while preserving urinary continence and erections in most patients [47,48]. Other innovations that have enhanced the use of radical prostatectomy are the development of outpatient transrectal ultrasound-guided biopsy techniques under local anesthesia and the widespread use of the prostate-specific antigen (PSA) blood test as a first-line screening test for prostate cancer [49]. Beginning in the 1990s, the laparoscopic approach to radical prostatectomy, including robotic-assisted laparoscopic prostatectomy, was added to the treatment options.

The main advantage of radical prostatectomy is that it offers the possibility of cure with minimal collateral damage to surrounding tissues while providing more accurate tumor staging. Furthermore, treatment failure is more readily identified following radical prostatectomy, and patients with recurrence often can be salvaged with postoperative radiotherapy. Erectile dysfunction and rectal complications are less likely with nerve-sparing radical prostatectomy than with radiotherapy, and both urinary incontinence and erectile dysfunction can be corrected. The postoperative course following radical prostatectomy now is more uneventful than in the past: few patients require non-autologous blood transfusions, hospital stays usually are two to three days, and operative mortality is rare [50] (see Table 4).

With the recent proof from a prospective, randomized clinical trial that radical prostatectomy reduces the rate of metastases and death from prostate cancer and improves overall survival, as compared with watchful waiting, the rationale for radical prostatectomy for clinically localized disease is more compelling than ever [51].

Of the commonly used operations, most urologists currently prefer the open retropubic approach; however, each operative approach has its advantages and disadvantages as well as its advocates and detractors. With the open retropubic approach, the surgical anatomy is more familiar, there is less risk for rectal injury, pelvic lymphadenectomy can be readily per-
formed, the wide exposure provided is adaptable to individual anatomic variations, and there is better preservation of the neurovascular bundles and less risk for positive surgical margins. The perineal approach is preferred by some urologists because it is often quicker and less bloody and is effective when performed by those who are skilled in its use. It has the disadvantage of not allowing a lymph node dissection through the same incision.

The laparoscopic approach is by far the most difficult, and there are limited data on long-term outcomes with its use. The theoretic advantages are less bleeding, better visualization with magnification, less postoperative pain, and a shorter convalescence time. However, because of the steep learning curve, the risks for complications are greater, and during the learning phase, an expert laparoscopic surgeon should be present in the operating room. Laparoscopic prostatectomy can be performed via a transperitoneal or extraperitoneal approach. The transperitoneal approach may facilitate the lymphadenectomy, but it carries a higher risk of intestinal, vascular, and ureteral injury, as well as postoperative intestinal obstruction. Laparoscopic surgery carries a higher risk for severe complications. Hemostasis is difficult to obtain in the neurovascular bundles because of the intrinsic difficulty of intracorporeal suturing and inconvenience of applying hemostatic clips. Heat from a harmonic scalpel or electrocautery can irreversibly damage the cavernosal nerves [52]. Although, intraoperative blood loss may be less, postoperative bleeding is more frequent after insufflation of the operative field has been discontinued. Anastomotic leaks are also more common. In expert hands, reported incontinence and anastomotic stricture rates are comparable to those achieved with open surgery, and it has been claimed that nerve sparing is equivalent or even better. However, direct comparisons and validated results are lacking, and the adequacy of long-term cancer control is as yet uncertain.

Remotely controlled laparoscopic surgery — so-called robotic prostatectomy — recently has been popularized and aggressively marketed because of its greater technical ease for the surgeon, especially for facilitating intracorporeal suturing. It has appeal for patients as a less invasive “high-tech” advance in surgery. The 3-dimensional visualization provided by the robot is also an advantage over standard laparoscopic techniques. Early reported results, though favorable, also require validation.

Salvage radical prostatectomy can be performed in patients who have failed radiation therapy; however, there are more complications and they are much more difficult to manage. Moreover, the prospects for cure are far less that for primary prostatectomy.

### 1. Patient Selection and Preoperative Assessment

An ideal candidate for radical prostatectomy should be healthy and free of co-morbid conditions that might make the operation risky. He should have a life expectancy of at least 10 years, and his tumor should be biologically significant and completely resectable. The generally accepted upper age limit for radical prostatectomy is about 75 years old.

Digital rectal examination and prostate ultrasound findings provide limited information about the extent of the primary tumor. The serum PSA data, including, rate of change of PSA (PSA velocity or PSA doubling time), PSA density (serum PSA divided by prostate volume), percentage of free or complexed PSA are all related to prostate cancer aggressiveness. The biopsy results, including the Gleason grade, number of cores containing cancer, distribution and volume of cancer in the cores, presence of perineural or lymphovascular invasion also correlate with cancer aggressiveness and the likelihood of organ-confined disease.

There is no general agreement about how extensive the staging evaluation should be. In patients in whom surgery is contemplated, a more extensive evaluation...
is indicated. Some physicians believe that a radionuclide bone scan and abdominal and pelvic computed tomography (CT) or magnetic resonance imaging (MRI) scan are not indicated if the tumor has a Gleason sum of less than 7 and the serum PSA level is less than 10 ng/ml because of the low likelihood of metastases. However, others prefer a more complete workup, including a complete blood count, coagulation studies, a metabolic profile, base-line bone scan with confirmatory imaging studies, if necessary, and a CT or MRI scan of the abdomen and pelvis to evaluate the primary tumor and regional lymph nodes and to rule out other pathologic conditions. A chest x-ray is usually obtained to evaluate the lungs and mediastinum, and cardiac clearance with an electrocardiogram, stress echogram and coronary angiography, if necessary, should be considered if surgery is anticipated. Tumor-selective imaging, such as monoclonal antibody scans, positron emission tomographic (PET) scans, and magnetic resonance spectroscopy (MSR) are not widely used.

The lack of accuracy of imaging studies in staging prostate cancer has lead to the development tables, algorithms and nomograms, based on preoperative clinical and pathologic parameters that predict the pathologic stage and thus identify patients most likely to benefit from radical prostatectomy [7,53]. Additionally, nomograms predicting the probability of not having tumor recurrence after radical prostatectomy or radiotherapy also have been developed [10,11,54]. However, statistics are more useful in predicting results in groups of patients than in individuals, and wide confidence intervals limit the usefulness of risk assessment.

For patients with a low probability of curable disease or a short life expectancy, an alternative treatment should be recommended. In this regard, the use of neoadjuvant hormonal therapy with the hope of increasing the resectability has proved futile.

The urologist should realistically counsel the patient on the nerve-sparing aspects of the operation. Nerve sparing does not materially compromise cancer control in appropriately selected patients. However, nerve sparing is inappropriate in men with locally advanced disease. The feasibility of performing nerve-sparing surgery is also questionable when there is extensive cancer in the prostate biopsies, palpable evidence of possible extraprostatic extension, a serum PSA greater than 10 ng/ml, a biopsy Gleason score higher than 7, poor-quality erections preoperatively, lack of interest and/or willingness of a partner in sexual relations, or other medical conditions that may adversely affect erections, such as atherosclerotic cardiovascular disease, diabetes, hypertension, psychiatric or neurological diseases, or medications that produce erectile dysfunction.

The urologist should discuss postoperative treatment of erectile dysfunction that might result from the operation, including information on phosphodiesterase (PDE)-5 inhibitors, intraurethral and intracorporal vasodilators, the vacuum erection device, venous flow constrictors, and the implantable penile prosthesis. The discussion also should include the timing of the return of erections that usually begins 3 to 6 months postoperatively and continues to improve for up to 36 months. The urologist also should discuss the risk for developing Peyronie’s disease that can occur from injury to the penis during sexual activity without a rigid erection. If erectile function is of paramount importance, the patient should be reassured that erections can be almost always be restored, regardless of whether or not nerve-sparing surgery can be successfully performed.

From the preoperative evaluation, the urologist and patient should have insights into the likelihood of success in achieving all goals of surgery and in determining whether nerves safely can be spared. The urologist also should discuss the possibility of cutaneous nerve graft interposition, if one or both cavernosal nerves must be resected. There are limited data concerning the effectiveness of such grafts, and most patients whose tumor appears to be so advanced that one or both nerves must be resected will require nerve-sparing surgery can be successfully performed.

From the preoperative evaluation, the urologist and patient should have insights into the likelihood of success in achieving all goals of surgery and in determining whether nerves safely can be spared. The urologist also should discuss the possibility of cutaneous nerve graft interposition, if one or both cavernosal nerves must be resected. There are limited data concerning the effectiveness of such grafts, and most patients whose tumor appears to be so advanced that one or both nerves must be resected will require nerve-sparing surgery can be successfully performed.

2. SURGICAL TECHNIQUE

Radical prostatectomy involves complete resection of the prostate gland and seminal vesicles and usually includes a modified pelvic lymph node dissection as well. This is accomplished most readily through the open retropubic approach. With the perineal approach, the dorsal venous complex and the anterior fibromuscular tissue are usually left behind, risking positive anterior margins. The apex of the prostate, where the capsule becomes discontinuous, is another common site of positive margins.
The main steps in performing anatomic nerve-sparing radical prostatectomy are: 1. a pelvic lymphadenectomy; 2. opening the endopelvic fascia with limited incision of the puboprostatic ligaments; 3. ligating and transecting the dorsal venous complex of Santorini; 4. dissecting the prostate from the neurovascular bundles from the apex to the base of the prostate; 5. securing and transecting the prostatic pedicles; 6. transecting and reconstructing the bladder neck; 7. dissecting the seminal vesicles and ampullary portions of the vasa deferentia; 8. reconstructing the bladder neck, and 9. performing the vesico-urethral anastomosis.

Pelvic lymphadenectomy is optional in patients at low risk for metastases by virtue of a low Gleason grade, low PSA, and low biopsy tumor volume. Patients who elect to undergo lymphadenectomy should decide in advance whether they wish to have their prostate gland removed if there are nodal metastases. If they do not, the excised lymph nodes should be sent for frozen-section examination during the operation. Otherwise, intraoperative frozen section analysis is not necessary. It is disputed whether a limited or extensive pelvic lymphadenectomy should be performed. A limited dissection is quicker, has less risk for complications and lymphedema, and is appropriate for most patients who have a low risk for pelvic lymph node metastases. However, some urologists prefer a more extensive lymph node dissection, and believe that it might achieve better cancer control.

The key to preserving urinary continence is performing a meticulous dissection, avoiding injury to the external urinary sphincter. It is not necessary to preserve the bladder neck to achieve complete urinary continence, and in patients with high-volume or high-grade tumors involving the base of the prostate, preserving the bladder neck risks positive surgical margins.

Meticulous dissection is required to maximally preserve the neurovascular bundles. In performing the nerve-sparing surgery, the neurovascular bundles are identified at the apex of the prostate (this can also be done in an antegrade fashion beginning at the base), and the bundles are dissected free of the posterolateral surface of the prostate gland. Hemostatic sutures or clips may be used to control bleeding from bridging vessels in the neurovascular bundles. Neither electrocautery nor a harmonic scalpel should be used on the neurovascular bundles.

If the neurovascular bundles must be resected, a cutaneous nerve graft can be harvested from the leg or arm and interposed [55-57]. A surgeon who has training and expertise in microsurgical nerve grafting techniques should perform this part of the operation.

The vascular pedicles of the prostate are ligated or clipped and divided close to the gland, taking care to avoid incising into the prostatic capsule. In performing the seminal vesicle dissection, care must be taken to avoid injury to the neurovascular bundles situated immediately lateral and posterior to them.

Patients should begin ambulating on the afternoon or evening of the day of the operation. The catheter may be removed 1 to 2 weeks after surgery, depending upon how completely the bladder neck and urethra are apposed and the amount of tension on vesicourethral anastomosis. The surgeon should strive for complete apposition and a watertight, tension-free anastomosis, but this cannot always be easily accomplished. Adjusting the operating table to flex the abdomen facilitates apposition of the urethra and bladder neck.

After the catheter has been removed, Kegel exercises should be initiated. A protective pad is used until a complete urinary control is achieved. The first postoperative serum PSA level should be measured one month after the operation.

### 3. CANCER CONTROL

The prime objective of radical prostatectomy is to cure the cancer. Important cancer control endpoints are: pathologically organ-confined disease with clear surgical margins, biochemical recurrence (detectable serum PSA), local progression, metastases, cancer-specific-survival, and overall survival. Depending upon the Gleason grade and the PSA doubling time, biochemical (PSA) evidence of recurrence precedes clinical metastases by a mean of about 8 years and cancer-specific mortality by about 13 years.

Non-progression rates vary with clinical and pathologic risk factors. Independent preoperative prognostic factors are tumor stage, Gleason grade, and PSA level. Additional adverse prognostic features include: non-organ-confined disease, perineural and/or lymphovascular space invasion, extracapsular tumor extension, positive surgical margins, seminal vesicle invasion and lymph node metastases (Table 5).

A rising serum PSA is usually the earliest evidence of tumor recurrence following prostatectomy (58). Biochemical recurrence is frequently used as an
intermediate endpoint for treatment outcomes; however, not all patients with biochemical recurrence ultimately develop metastases or die of prostate cancer [6,59,60]. In rare instances with very undifferentiated tumors or neuroendocrine tumors that do not produce much PSA, there can be palpable evidence of recurrence despite an undetectable PSA level. Accordingly, it is reasonable to perform postoperative digital rectal examinations.

The Catalona series, now including more than 4,200 open anatomic radical retropubic prostatectomies, is fairly representative of other similar large series reported from high-volume institutions and includes all men who underwent surgery in the analysis, even those with adverse preoperative prognostic features. It is important to emphasize that patient selection and the duration and frequency of follow-up monitoring are critical in determining outcomes(61). In this series of men who underwent anatomic radical retropubic prostatectomy, the actuarial 10-year cancer progression-free survival probability was approximately 79% for patients with organ-confined disease, 62% for men with extracapsular tumor extension without cancerous surgical margins, 53% for men with extracapsular tumor extension and cancerous surgical margins, 26% for patients with seminal vesicle invasion, and 12% for patients with lymph node metastases (Table 5).

Within these pathologic tumor stages, cancer progression rates also were strongly associated with other parameters, as well as with the era of treatment and patient age. For example, the preoperative serum PSA level was inversely related to both the percentage of patients with organ-confined disease and the 10-year progression-free survival rate.

a) Management of Biochemical Recurrence

Patients with a detectable PSA level (>0.1 ng/ml) following radical prostatectomy usually have persistent prostate cancer, although some might have retained benign prostate tissue causing the PSA elevation. In the latter case, the PSA usually increases very slowly. The PSA velocity or doubling time and the Gleason grade usually reflect how rapidly the tumor will progress, and there is a wide spectrum of cancer progression rates.

Patients with biochemical recurrence after radical prostatectomy have the option of following their PSA levels and receiving additional therapy only if there is rapid disease progression or, alternatively, receiving salvage radiotherapy or hormonal therapy. Salvage radiotherapy is widely used despite the fact that no reduction of the rate of distant metastases or improvement of long-term survival has been formally demonstrated; however, anecdotal evidence suggests that salvage radiotherapy might reduce the clinical tumor recurrence rate. Salvage radiotherapy is most beneficial in patients with positive surgical margins or extracapsular tumor extension without seminal vesicle invasion or lymph node involvement. Nevertheless, it is possible that some patients with seminal vesicle invasion or lymph node metastases also might benefit.

If salvage radiotherapy is anticipated, it should be initiated before the PSA level rises much above 0.5ng/ml. Patients most likely to have favorable responses to salvage radiotherapy are those whose PSA recurrence occurs long after surgery, those with a slow rate of PSA increase, those without seminal vesicle invasion or lymph node metastases and those

---

**Table 5. Actuarial (PSA-based) Non-Progression Rate in Patients Undergoing Radical Retropubic Prostatectomy for Clinical Stages T1-T3 Prostate Cancer**

<table>
<thead>
<tr>
<th>Series</th>
<th>Patients, N (Year)</th>
<th>Mean Age</th>
<th>Months, F/U Mean (range)</th>
<th>Clinical Stage</th>
<th>5-Year Progression-Free Rate (%)</th>
<th>10-Year Progression-Free Rate (%)</th>
<th>15-Year Progression-Free Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walsh</td>
<td>2404</td>
<td>58±6</td>
<td>76 (12-204)</td>
<td>T1-T3a</td>
<td>84</td>
<td>74</td>
<td>66</td>
</tr>
<tr>
<td>*</td>
<td>(1982-1999)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catalona</td>
<td>3478</td>
<td>61±7</td>
<td>65 (0-233)</td>
<td>T1-T3</td>
<td>80</td>
<td>68</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations/Symbol: PSA, prostate-specific antigen; N, number of patients in study; -, data unavailable.

*Patients with adjuvant radiation therapy before PSA recurrence were excluded. [20]

**Patients with adjuvant radiation therapy before PSA recurrence were included (61)**
with lower Gleason sums. There is conflicting evidence about whether positive surgical margins is a favorable or unfavorable parameter for predicting response to postoperative radiation therapy, although several reports suggest that patients with positive margins are more likely to respond, the notion being that patients with positive margins are more likely to have local recurrence only as opposed to systemic disease. The beneficial effects of salvage radiotherapy are controversial. Some patients fare well without salvage radiotherapy and others fail with distant metastases despite salvage therapy.

Salvage radiotherapy is usually administered to the bed of the prostate gland in a dose of approximately 64 Gy. It is advisable to wait at least 3 to 4 months after surgery to allow the surgical wound to heal completely and urinary continence to return. Radiation to the whole pelvis is usually discouraged because of the higher risk for bowel complications. The side effects of postoperative radiotherapy also include a 5% to 10% risk of radiation proctitis and a 50% probability that return of erectile function will be materially compromised. Radiotherapy also can compromise borderline postoperative urinary continence in some patients.

Patients with very unfavorable prognostic parameters who are more likely to fail with distant metastases are more likely to benefit from androgen-deprivation therapy. In high-risk patients with high-grade tumors who opt for postoperative radiotherapy, it is uncertain whether they should receive adjuvant hormonal therapy as well. Clinical trials are underway to answer this question; however, the only disadvantage of adding hormonal therapy in this setting is the expense and associated side effects.

In patients with lymph node metastases, a prospective, randomized trial has demonstrated significantly improved survival in patients treated with early androgen-deprivation therapy.

4. COMPLICATIONS AND QUALITY OF LIFE

Anatomic nerve-sparing radical retropubic prostatectomy provides excellent cancer control with an acceptable complication rate in appropriately selected patients.

The overall complication rate following radical prostatectomy can be less than 10% in experienced hands [8]. With a careful selection of patients and performance of necessary preoperative cardiovascular evaluation, perioperative mortality can be largely avoided. The perioperative mortality rate following radical prostatectomy is less than 0.5% [62].

\[\text{a) Early Complications}\]

Comparable results can be obtained with either regional or general anesthesia. Blood loss is usually less than 1 liter, and non-autologous blood transfusions are seldom required.

Early complications include hemorrhage, rectal, vascular, ureteral, or neurologic injury, urinary leak or fistula, thromboembolic and cardiovascular events, urinary tract infection, lymphocele, and wound problems (Table 6).

| Table 6. Perioperative Complications of Radical Prostatectomy in Catalona Series (%) (40) |
|-----------------------------------|-----|
| Anastomotic stricture             | 2.7 |
| Inguinal hernia                   | 2.5 |
| Thromboembolic                    | 1.3 |
| Infectious                        | 0.7 |
| Wound                             | 0.2 |
| Lymphatic                         | 0.2 |
| Neurological                      | 0.1 |
| Myocardial infarction             | 0.1 |

It is advisable to routinely use support stockings and early ambulation. Prophylactic anticoagulation and sequential compression stockings should be considered in high-risk patients.

Inadvertent injury to the obturator nerve can occur during the pelvic lymphadenectomy. When a tension-free primary nerve repair is not feasible, nerve grafting can be performed utilizing a cutaneous nerve [63]. However, even without repairing the nerve, conservative management with physical therapy can compensate for the deficit, and many patients do not exhibit significant thigh adductor deficit following the injury [64].

Ureteral injury is an infrequent complication of radical prostatectomy. A minor injury or ligation can be managed with de-ligation and stenting. Mobilization of the distal ureter and ureteroneocystostomy should be performed for more severe injuries.

Usually, a rectal injury can be repaired primarily using a multiple layer closure [65]. However, a diverting colostomy should be considered in men with a large rectal defect, a history of pelvic radiotherapy, or men taking long-term glucocorticoid therapy. Vesicorectal fistula can be repaired through
an abdominal, transrectal, or laparoscopic approach. Lymphoceles are usually drained externally through a percutaneous approach or internally through and open or laparoscopic approach.

b) Late Complications:
The most common late complications of radical prostatectomy are erectile dysfunction, urinary incontinence, inguinal hernia, and anastomotic or urethral strictures. Early rehabilitation measures, including Kegel exercises and the use of PDE-5 inhibitors, a vacuum erection device, intraurethral or intracavernosal administration of vasodilators appear to be helpful in minimizing the likelihood of these complications.

Urinary continence recovers more quickly that erectile function, and up to half of men are continent almost immediately after removal of their catheter. Continence may continue to improve for up to 18 months after surgery. The overall urinary continence outcome following radical retropubic prostatectomy is generally very favorable, depending upon the experience and skill of the surgeon. In experienced hands, more than 90% of men recover complete urinary continence. The return of urinary continence is strongly associated with patient age. For example, in most large series, more than 95% of men younger than age 50 were continent following surgery, and 85% of men older than 70 years of age regained continence. Very few require implantation of an artificial urinary sphincter or a sling procedure for stress incontinence.

Patients with intact libido and erections usually wish to maintain these functions or, at worse, have erections sufficient for penetration with the help of PDE-5 inhibitors. Others with poor quality erections preoperatively would find it acceptable if their postoperative erections would at least afford some rigidity to provide sensory satisfaction for both sexual partners. However, erectile potency following radical prostatectomy usually is defined as the ability to maintain erections sufficiently rigid for penetration with or without the help of oral PDE-5 inhibitor. The return of erectile function following radical prostatectomy is strongly associated with the age of the patient, preoperative potency status, extent of nerve-sparing surgery (bilateral versus partial nerve sparing), and the era of surgery. With few exceptions, erectile function usually begins to return as partial erections 3 to 6 months after surgery and may continue to improve for up to three years. In the most favorable candidates in whom preoperative potency is normal and bilateral nerve-sparing surgery can be performed, up to 95% in their 40s, 85% in their 50s, 75% in their 60s and 50% in their 70s will recover erections sufficient for penetration and intercourse with or without the aid of PDE-5 inhibitors.

Anastomotic or other urethral strictures should be managed initially with a gentle, serial dilation, but often internal incision and injection of corticosteroids may be required. For a long or persistent anastomotic stricture, a transurethral resection of the scar tissue cephalad to the external sphincter may be necessary. Usually, an interval of self-catheter dilation of the anastomosis is required. Continued self-dilation or intermittent dilation by an urologist is needed in difficult, persistent cases. Urethroplasty is rarely necessary.

5. ROBOTIC RADICAL PROSTATECTOMY

Standard laparoscopy has several inherent limitations including a two dimensional view, rigid instruments, and counterintuitive movement. As a result, LRP has a steep learning curve and many experts in “open” surgery find the technique difficult to master leaving the procedure to those with advanced laparoscopic skills [66-68].

The daVinci system offers several advantages over standard laparoscopy. There is wristed movement of the robotic arms allowing six degrees of freedom (two more than laparoscopy), three dimensional visualization, elimination of physiological hand tremor with a sophisticated filtering system, and detailed magnification for superior visualization of tissues.

Robotic radical prostatectomy has overcome the limitations associated with traditional laparoscopy. Performance of the vesicourethral anastomosis is likely the most difficult step of LRP, and case prohibitive for many urologists. The daVinci system allows for six degrees of freedom of its instruments, allowing for easy and efficient performance of suturing resulting in a more precise anastomosis. The three dimensional visualization and magnification allows for superior visualization of tissues, and has permitted like in open surgery to develop the nerve sparing technique [69-71]. The improved ergonomics of the robot systems deters from surgeon fatigue that is readily seen in complex laparoscopic procedures. Several authors have found a smooth transition from open prostatectomy to robotic prostatectomy with comparable experiences to the most skilled laparoscopic surgeons after more than 100 LRPCs [72,73].
Given these advantages, the Vattikuti Institute Prostatectomy (VIP) study has been shown that the steep learning curve of LRP can be overcome by those with minimal laparoscopic skills, while simultaneously providing superior outcomes in both continence and potency, rivaling the best open and LRP series [74].

In this series over 1500 cases of Robotic Radical Prostatectomy the operating time ranged from 70 to 160 minutes. Port placement and specimen retrieval took approximately 20 to 40 minutes leaving an actual robotic console (dissection) time of 90 to 100 minutes. Pelvic lymphadenectomy took 18 minutes on average. Estimated blood loss ranged from 50 to 250 mL. No patient required an intraoperative transfusion, and none provided autologous blood preoperatively. Over 95% of patients were discharged within 24 hours of hospital stay.

Total continence, defined as using no pad at all, was achieved in 96% of patients at a follow-up of 6 months. At the time of catheter removal, 50% of patients were found to be continent (requiring no pad at all, or just one liner for security). Regarding potency at a follow up of six months, 82% of preoperatively potent patients younger than 60 years old had a return of some sexual function, and 64% were able to achieve erections sufficient for intercourse [74]. Using the prostatic fascia preserving technique, at 12 months follow up 97% were able to achieve erections sufficient for intercourse with or without phosphodiesterase 5 inhibitors [70].

### III. ADJUVANT THERAPY FOR HIGH RISK CANCER

Some studies support immediate postoperative androgen ablation in patients with lymph node metastases [75] or seminal vesicle invasion (76). Large studies are underway to define the role of adjuvant hormonal therapy in patients with localized prostate cancer [77]. At present, there is no established survival benefit for long term postoperative androgen ablation or antiandrogen therapy (Tables 7 and 8).

Until some benefit is demonstrated, neoadjuvant hormonal therapy should not be used as a substitute for optimal surgical technique and is not recommended for a presumed survival advantage.

The benefit of immediate adjuvant radiation therapy for men at increased risk of local recurrence (extra-capsular extension, positive margins, seminal vesicle invasion) has been debated for years [78-80]. In some studies, the risk of subsequent local recurrence was reduced substantially, but there was no effect on distant metastases or cancer-specific survival [81]. Pending the outcome of appropriate clinical trials, there is little rationale for treating microscopic extra-capsular extension with adjuvant radiotherapy routinely, as 71% remain free of progression with surgery alone. Those patients with seminal vesicle invasion typically develop distant metastases rather than local recurrence [82].

Adjuvant radiotherapy (in those with an undetectable PSA level) seems most appropriate for patients with positive margins in the absence of seminal vesicle invasion or lymph node metastases, as cancer cells may have been left within the field of irradiation. However, only 33% to 50% of patients with a positive margin who do not receive immediate radiation therapy develop an elevated PSA level within 10 years [83-85].

While a significant risk factor for recurrence, positive margins do not prove unequivocally that the cancer is destined to recur or that local tumor has been left behind.

### Table 7. Indications of Hormonal Monotherapy for Men with Localized or Locally Advanced Prostate Cancer

<table>
<thead>
<tr>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced age</td>
</tr>
<tr>
<td>Patients refusing curative treatment</td>
</tr>
<tr>
<td>Patients unsuitable for curative treatment</td>
</tr>
<tr>
<td>treatment because of co-morbidity</td>
</tr>
</tbody>
</table>

### Table 8. Advantages and Disadvantages of Hormonal Monotherapy

<table>
<thead>
<tr>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced risk of disease progression</td>
<td>No evidence of survival benefit</td>
</tr>
<tr>
<td>Patient’s reassurance of receiving treatment for prostate cancer</td>
<td>Cost</td>
</tr>
<tr>
<td>Relief of fear against disease progression</td>
<td>Adverse effects such as gynecomastia and/or breast pain</td>
</tr>
</tbody>
</table>
External beam radiotherapy is a proven modality in the radical treatment of localized prostate cancer [86]. Full clinical assessment and staging is essential to select the appropriate treatment for men presenting with prostate cancer. The factors to consider in the selection of treatment include tumour factors including stage, Gleason score, and PSA; patient factors - age, symptoms, sexual function, co-morbid conditions such as inflammatory bowel disease, potential life expectancy and individual preference; and external factors such as treatment prescription, technique, and quality. Curative approach is offered based on the balance of probabilities that the cancer is likely to progress during this time span in that individual. There are no randomized trials comparing radical radiotherapy with radical prostatectomy. Recent comparisons of non-randomized patient series have not showed any consistent differences between radical radiotherapy and prostatectomy approaches. The largest comparison collated pooled data from six large USA centers with 6877 men treated over a ten-year period between 1989 and 1998 [87]. The overall 5-year PSA failure free results were similar for patients in the same prognostic risk groups regardless of the form of therapy. These data suggest that there is little difference between radical radiotherapy and radical prostatectomy for early stage localized disease. In a multi-institutional series of 1765 men with T1-T2 cancers treated to a median dose of 69.4 Gy between 1988-1995, 5 year PSA control rates were 81%, 68%, 51% and 31% for men with initial presenting PSA levels of <10, 10 to <20, 20 to <30 and ≥ 30 ng/ml respectively [88]. The largest comparison collated pooled data from six large USA centers with 6877 men treated over a ten-year period between 1989 and 1998 [87]. The overall 5-year PSA failure free results were similar for patients in the same prognostic risk groups regardless of the form of therapy. These data suggest that there is little difference between radical radiotherapy and radical prostatectomy for early stage localized disease. In a multi-institutional series of 1765 men with T1-T2 cancers treated to a median dose of 69.4 Gy between 1988-1995, 5 year PSA control rates were 81%, 68%, 51% and 31% for men with initial presenting PSA levels of <10, 10 to <20, 20 to <30 and ≥ 30 ng/ml respectively [88].

The likelihood of radiation related complications is dependent on the dose delivered, irradiation technique used, volume of normal tissues or organs-at-risk irradiated and tolerance / radiosensitivity of the respective normal tissues. Radiotherapy related complications are reported as acute side-effects, those occurring during radiotherapy and/or within 3 months of radiotherapy, and late side-effects, those occurring usually months to years post-irradiation. Acute side-effects can include rectal symptoms of proctitis, diarrhoea, bleeding, urinary symptoms of frequency, nocturia, dysuria, or bleeding. The majority of acute complications resolve completely within 2-6 weeks following completion of external beam radiotherapy. For external beam radiotherapy in prostate cancer, late rectal side-effects are the major dose limiting complications and include persistent rectal discharge, tenesmus and rectal urgency, rectal bleeding, ulcer or stricture. Important late genitourinary complications include chronic cystitis, urinary incontinence, bladder ulceration, haematuria, urethral stricture and impotence. An important aspect of prostate radiotherapy side-effects is erectile dysfunction. Etiology of this complication is multifactorial and is influenced by factors such as pretreatment function, age, small vessel disease and previous urological surgery. Its incidence is estimated between 30-40% of treated men. Recent studies suggested that the radiation dose to the penile bulb is related to post radiation impotence. An important determinant of radiotherapy treatment outcome is local control [89-91].

Strategies to improve local control include either an increase in the prescribed dose or to employ the use of neo-adjuvant and/or adjuvant androgen deprivation. Retrospective studies have reported lower local control rates of 62-63% when prescribed doses were < 60 Gy increasing to 74-80% for doses between 60-70 Gy and 81-88% when doses were > 70 Gy. There is potential for an increase in radiation related complication rates when dose is increased using conventional external beam radiation techniques. Modern radiotherapy planning techniques such as 3D CRT and IMRT are used to confine high dose radiation to the target and protect normal tissues. With 3D CRT, the incidence of late radiation toxicity such as rectal proctitis is significantly reduced (5% vs. 15%) compared to conventional techniques. 3D CRT is currently the minimum standard for prostate radiotherapy. The use of 3D CRT provides the opportunity for safe dose escalation. In randomized control trials of dose escalation in prostate radiotherapy showed benefits of dose escalation. The M.D. Anderson Cancer Center trial randomized 305 men with T1-T3 localized prostate cancer to either 70 or 78 Gy [92]. With a median follow-up of 60 months, the 6 year freedom from failure including biochemical PSA failure rates were 64% for 70 Gy and 70% for 78 Gy (p = 0.03). The Institute of Cancer Research/Royal Marsden Hospital randomized pilot study of 125 men with T1-T3 cancers treated with 3-6 months of neoadjuvant androgen suppression followed by RT showed a higher 5 year PSA control 74 Gy (71%) than the 64 Gy (59%) group (p = 0.10). The other trials shortly
to be reported include MRC RT-01 trial with over 850 men with localized prostate cancer, the Netherlands CKVO 96-10 trial with 670 men, the French Collaborative trial recruited 306 patients. Outcomes for biochemical PSA control rates and incidence of late complications from these trials will provide further clarification for optimization of patient management.

IMRT techniques can permit better dose distributions for irregularly shaped volumes and improved avoidance or sparing of adjacent dose limiting normal structures [93]. The delivery of appropriately designed non-uniform dose intensities with IMRT can produce better sculpturing of the high dose region to complex irregular shapes particularly concave shaped target volumes such as the coverage of seminal vesicles or pelvic nodal volumes. IMRT plans provide a very steep high to low dose gradient at the edge of the target volume for improved avoidance of adjacent normal structures such as the rectum, bowel and bladder. With IMRT, the complications may be further reduced and higher dose escalation may be safely achieved. Using this technique, clinicians at the Memorial Sloan-Kettering Cancer Center treated 772 men to doses ≥ 81 Gy and reported low incidence of late rectal complications [93]. With a median follow-up time of 24 months, the 3-year actuarial likelihood of grade ≥ 2 rectal toxicity was 4%.

Patient positioning and immobilization is important to ensure reproducibility for each treatment. Rectal filling and emptying can influence prostate position during radiotherapy. Strategies to achieve a more reliable rectal ‘state’ include the use of daily laxatives or enemas prior to each radiotherapy fraction, endorectal balloon [94,95]. The position of the prostate may be visualized via the use of implanted intra-prostatic markers. These radio-opaque markers allow daily verification of the prostate location using electronic portal imaging devices [96].

External beam prostate radiotherapy is usually given by conventional fractionation (i.e. 1.8 to 2.0 Gy per fraction). It has been suggested that prostate cancers possess higher fractionation sensitivity similar to that for late responding normal tissues. This means that the use of hypofractionation (i.e. larger dose per fraction and less number of fractions) may provide an improved therapeutic ratio by reducing the probability of late radiation morbidity for the same level of local control. A recent Australian trial reported on a randomized comparison of 64 Gy in 32 fractions with 55 Gy in 20 fractions in men with early stage prostate cancer [97]. The interim analysis revealed similar PSA relapse-free survival rates and toxicities between the two radiation schedules. One large UK series has recently reported on 703 clinical staged T1-4N0M0 prostate cancer patients treated to 50 Gy in 16 daily fractions with overall 5 year PSA relapse-free survival and radiation morbidity rates are similar to that obtained with 65-70 Gy delivered using 1.8-2 Gy fractions. There are a variety of phase I/II studies currently being undertaken to evaluate the use of hypofractionation regimes. At the Christie Hospital in Manchester, Princess Margaret Hospital in Toronto and Notre-Dame Hospital in Montreal, 3 Gy per fraction to escalated doses of 60 – 66 Gy are being studied for different prognostic groups. A randomized phase III study comparing 74 Gy in 2 Gy per fraction with 57 Gy and 60 Gy using 3 Gy per fraction has been started at the Institute of Cancer Research/Royal Marsden Hospital.

The new technologies that allow shaping treatment beam and radiation dose distributions require accuracy in daily dose placement to internal targets. The accurate daily patient positioning is assisted by laser guidance systems and patient immobilization devices. New approaches involve the systems that use patient surface or multi-region sensing with automated linkage to pre-defined topological maps or treatment portals and delivery simultaneous correlated to automated indexed treatment couches based on the real time information. A variety of methods have been investigated to address the issue of prostate organ motion. Some investigators have used rectal obturators or balloons whilst others have utilized radiopaque markers to help reproduce the treatment position [94,96]. Daily localization of the prostate gland can be performed using a portable ultrasound machines (BAT or B-mode acquisition targeting ultrasound system, Nomos Corporation, USA) which provides spatial localization of the prostate correlated to the position of the patient on the therapy couch [98]. More recent approaches to this consider a 4D strategy or the real-time element of the prostate position during radiation delivery. New technologies allow on line volumetric imaging of the target. One such system is Elekta Synergy (Elekta Oncology Systems, UK) where a X-ray volumetric imager device capable of kilovoltage (kV) cone beam imaging forms part of the linear accelerator. This digital kV flat panel device provides the opportunity of 3D cross-sectional and fluoroscopic imaging of the patient on a linear accelerator [99]. This system can also provide 3D information for
treatment verification. Similar systems for 3D and kV imaging on the linear accelerator have been developed by Varian Oncology Systems with their On-Board Imager and by Siemens – Artiste. The Siemens Artiste system differs from both the Varian and Elekta systems by having its kV imager along the same axis as the gantry head instead of perpendicular to the irradiation axis. Another novel system that has been developed and is in current usage incorporates a CT scanner within the linear accelerator delivering treatment in a helical tomotherapy method (TomoTherapy Inc, Wisconsin). Other systems available to undertake tracking of the prostate include the system sponsored by Mitsubishi Electronics at the Hokkaido University School of Medicine and the Cyberknife (Accuray Inc, Sunnyvale, CA). The guiding principle of all these systems is that by accurate localization to the actual position of the target at the time of delivery, geographical miss and set up errors will be avoided, treatment margins can be reduced and dose escalation can be safely delivered. However, irregardless of the system used and its possible complexity, it is imperative that protocols and adequate quality assurance is present to ensure safety of the system for patient use.

The use of MR imaging in radiotherapy planning can result in smaller treatment volumes leading to more appropriate shaping of the treatment fields and thereby reducing the risk of treatment related complications. The addition of multi-modality morphological imaging such as pelvic MRI has enhanced volume definition for treatment planning in prostate cancer [100,101]. In addition, the use of MR spectroscopy in defining volumes of biologically aggressive tumour within the prostate gland has led to new protocols investigating focal dose escalation [102,103].

External beam irradiation is a well established curative treatment modality for men with non-metastatic prostate cancer and additionally has a role in palliation of patients with metastatic disease. Recent technological developments have changed prostate radiotherapy practice. The widespread availability of cross-sectional CT imaging with the development of software to reconstruct anatomy and manipulate 3D images together with hardware such as MLC has enabled the routine use of conformal radiotherapy. Sophisticated computing has allowed the development of IMRT. These developments and techniques have permitted the safe escalation of radiation dose in prostate cancer. The published data suggest benefit from dose escalation but long term results are needed to confirm these outcomes. Further refinements in radiotherapy strategy for prostate cancer will include consideration of the radiobiological rationale for hypofractionation in prostate cancer and the assessment of volumes appropriate to the prognostic group for high dose irradiation. The utilisation of biological images to include functional, biochemical, metabolic and physiological data may aid in defining more appropriate treatment volumes. Technological advances have ushered the capability to consider 4D treatment strategies for online real-time and/or gated treatments to further enhance accuracy and reliability of high dose radiation schemes. Further developments for 4D treatments are expected to continue into the next decade. This will substantially alter the way we perceive the practice of radiotherapy and influence the manner in which patients are treated with radiotherapy.

**ADJUVANT RADIOTherapy After Radical ProstateCTomy**

Radical prostatectomy provides excellent control as long as prostate cancer is organ confined. For patients with cancer extending beyond the capsule (pT3) the risk of local failure varies from 10 to 50%. Initial PSA level, Gleason score, and positive surgical margins were shown to be independent predictors of biochemical relapse. Postoperative radiotherapy was reported to eradicate the microscopic disease left in the surgical bed and reduce significantly the local relapse and PSA failure rates without any impact on disease free survival, but no randomized studies have been published so far to conclude to the efficiency of this concept. Recently completed randomized trials presented here do provide level 1 evidence for benefit of adjuvant radiotherapy after radical prostatectomy in patients at high risk for recurrence.

a) **EORTC 22911 [104]**

1005 patients from 37 institutions were randomly assigned to a wait-and-see policy or immediate postoperative radiotherapy. Eligible patients had a clinical stage T0-3 N0 M0, and pathological stage pT2-3 N0 with at least one of the following risk factors: tumour growth beyond the capsule (capsule perforation), positive surgical margins (including the level of prostate apex where the capsule is not existent) or invasion of seminal vesicles. Biochemical progression was defined as every increase over the lowest postoperative value to a value >0.2 ng/ml confirmed twice, at minimum 2-week intervals. The median age was 65 years. The clinical tumour extension was classified T0-1 (17.6%), T2 (65.1%), T3 (17.2%), Tx
The WHO histological grade, was distributed as follows: G1 (12.5%), G2 (62.7%), G3 (23.6%), Gx (1.2%). The median pre-operative PSA was 12.3 ng/ml (range: 0.3-159.4). PSA was undetectable (<0.2 ng/ml) in 69.5% of the patients within 3 weeks after surgery and in another 19.2% during follow-up, prior to any relapse or further treatment. In the post-operative irradiation group, 457 patients (91.8%) were irradiated and forty-one (8.2%) were not. Five patients in the wait-and-see group (1%) were irradiated. In the 457 irradiated patients, irradiation was initiated a median of 90 days after surgery [14-156] and lasted a median of 44 days [18-106]. The target total was 60 Gy: 415 patients (90.8%) received exactly 60 Gy, 4 patients (0.9%) received a dose <60 Gy and 38 a dose above 60 Gy (8.3%). After a median follow-up of 5 years, biochemical progression free survival was significantly improved in the irradiated group with 5-year rate of 74.0% compared to 52.6% in the control group (P=0.0001). Clinical progression-free survival was also significantly improved (P=0.0009). The cumulative loco-regional failure rate was significantly lower in the irradiated group (P<0.0001): 5.4% versus 15.4% in the control group, at 5 years. Grade 2-3 late effects were significantly more frequent in the post-operative irradiation arm (P=0.0005), but the events of severe toxicity (grade 3 or higher) were rare with a 5-year rate of 2.6% in the wait-and-see arm and 4.2% in the post-operative irradiation arm (P=0.0726).

b) ARO 96-02 [105]

385 patients with pT3 N0 were randomized to either 60 Gy (arm A: 193 patients) or to follow a wait and see policy (arm B: 192 patients) before achieving an undetectable PSA. Patients were stratified for Gleason score, margin status, neoadjuvant hormonal treatment and stage (pT3A+B versus C). When the undetectable PSA-level after radical prostatectomy was not achieved, the patients were stated as progressive disease and were irradiated. PSA progression for patients with undetectable PSA was stated after two consecutive increasing PSA out of the undetectable range. Primary endpoint was biochemical progression free survival. 78 patients (20%) did not achieve an undetectable PSA and were stated as progressive disease (arm A: 45 patients, arm B: 33 patients) and 32 patients (21%) from the radiotherapy arm did not receive radiotherapy. After a median follow-up of 40 months for arm A and 38.5 months for arm B, biochemical progression free survival was significantly improved in the irradiated group with 4-year rate of 81% compared to 60% in the control group (P<0.0001). The rate for grade II side effects for the rectum was 3%.

c) SWOG 8794 Trial [106]

This was a randomized study of 473 patients with pT3 prostate cancer enrolled between 1988-1995 and randomized to receive adjuvant RT - 60-64Gy or observation only. Patients were followed with PSA every 3 months for one year, every 6 months for two years, and annually thereafter. Bone scans were performed if clinically indicated. Subjects were followed until death. The primary endpoint was metastasis-free survival; relapse-free and overall survival were the secondary endpoints. Subjects were followed until death. With a median follow-up of 9.7 years adjuvant RT was not shown to be significantly better for the primary endpoint (metastasis-free survival, potentially due to a lower than expected event rate), the results were concordant with two statistically significant secondary endpoints: PSA progression and relapse-free survival. 32% of subjects on observation ultimately received radiation at a median of 2 years following randomization. The conclusions of the study was that the PSA and relapse free survival were significantly improved with adjuvant RT but the improvement in metastasis-free and overall survival did not reach statistical significance. This trial also compared the impact of adjuvant RT on clinical outcomes and quality of life (QOL): 219 of the 431 patients included, were registered to the QOL study at baseline, 6 weeks, 6 months and annually for five years. Men receiving RP+RT reported significantly more tenderness/urgency with bowel movements, more frequent urination, and unpleasant global QOL early on during the trial; nevertheless, all significant treatment arm differences had disappeared by 5 years.

The results of these three trials show a statistically significant improvement in biochemical progression free survival with immediate post-operative irradiation (P<0.0001) and a statistically significant reduction of the loco-regional failure rate (P<0.0001). A longer follow-up is needed to assess if post-operative irradiation impacts on the occurrence of distant metastases and/or survival, but the target accrual of these trials may be inadequate to determine survival benefit. Limitations of these studies, that were started in the early PSA era, include conventional irradiation, low dose of 60 Gy, a variable postoperative nadir. Small retrospectives series have reported similar results on local control, one of them on the five-year freedom from PSA relapse rate(107); nevertheless, results as shown by these two randomized stud-
ies have never been reported so far. The indications of salvage radiotherapy can be divided in 3 scenarios: clinically palpable and/or biopsy proven isolated local recurrence, persistently detectable PSA, delayed PSA rise without any clinically evident disease after initially undetectable postoperative levels. It is quite obvious that the earlier the initiation of salvage radiotherapy, the better the impact on clinical or biochemical free survival, which means that the two first scenarios have the poorest outcome; the best situation is the third one with a delayed rise of PSA in patients who are likely to have a lower tumoral burden. At the time these trials were running, there were no data based on randomized trials in favour of an immediate salvage radiotherapy versus a deferred one in case of a rising PSA, and the threshold value of PSA relapse to start with radiotherapy was not known. Further trials are needed to determine whether the results of immediate irradiation might be equivalent to withholding irradiation until the PSA rises to 0.5 and 1 ng/ml. Today, the rate of positive surgical margins is far lower as is the median PSA before surgery. For this reason the results of radical prostatectomy and immediate external irradiation for pathological tumour stage T3 might be even further improved by: i) treating pT3 patients with cT1-2 N0, baseline PSA lower than 20 ng, and a negative postoperative PSA, ii) using contemporary conformal radiotherapy and promoting dose escalation up to 64 Gy.

As regard clinical research, two directions are discussed: i) the EORTC is developing a new clinical trial which randomises patients after radical prostatectomy between adjuvant radiotherapy and short term androgen deprivation. ii) the Institute of Cancer Research (UK) is planning a RADICALS Trial, a randomized phase III trial of adjuvant versus selective salvage treatment after radical prostatectomy for localized prostate cancer.

V. BRACHYTHERAPY

Prostate brachytherapy techniques have evolved over the past four decades coincident with advances in technology. The development of transrectal ultrasound (TRUS), perineal template guidance and computer-based treatment planning systems more than 20 years ago has led to the gradual abandonment of the retropubic approach and the wide adoption of the closed transperineal method of prostate brachytherapy [108]. The early practitioners of the transperineal approach relied on TRUS or computed tomography (CT) to image the prostate and used these images to create a "preplan", typically several days prior to the operative procedure. The preplan outlined the three-dimensional placement of the sources and the objective at the time of the procedure was to carry out the "preplan". Further improvements in image-based treatment planning systems over the past decade have led to the introduction of intraoperative treatment planning (ITP); obviating the need for a preplan and allowing for dosimetric optimization as sources are placed in the operating room.

A number of investigators have reported on ITP for prostate brachytherapy [109,110]. The techniques differ slightly but all have several components in common. An ultrasound probe is positioned in the rectum, and the prostate and normal anatomy (rectum, urethra) are identified. These organs are then contoured and transferred to the treatment planning system. Some investigators will insert needles through the perineal template around the periphery of the prostate and identify needle positions on each image. The treatment-planning computer then creates an optimized source placement within the needles based on pre-specified dose volume limits for the prostate, urethra and rectum. Other investigators will begin to place sources manually and attempt to identify the position of the sources as they are placed. Dose-volume histograms can be calculated as sources are placed. Either method allows the operator to avoid overdose to critical structures and identify potential areas of undertreatment. If the undertreated areas are thought to contain cancer then it is possible to place sources in the appropriate position before the procedure is completed. A number of centers have reported that dosimetric outcomes are improved with ITP compared to the preplanned method. One of the early problems with ITP was the inability to accurately localize the source positions in relation to the prostate gland. Sources that moved such they were more than 20 degrees from the long axis of the ultrasound probe were not very echogenic and difficult to identify. There is at least one source that has been designed specifically for intra-operative localization. This source (Echoseed™, Oncura) has a knurled outer surface that has been shown to increase the echogenicity and visibility relative to non-knurled sources.

In the next several years it will be important to better understand the dose-volume relationship for cancer control and morbidity. To date it appears that a higher dose to the prostate gland following prostate brachytherapy is associated with better biochemical outcomes but the data has not been entirely consis-
tent [111,112]. The dose distributions achieved with brachytherapy are heterogeneous and the anatomic distribution of prostate cancer is non-uniform making dose-response relationships difficult to uncover. Normal tissue dose-response relationships on the other hand may be easier to identify. In particular, it will important to quantify any dose-volume relationships that can be incorporated into ITP to further reduce the sexual, urinary, and rectal dysfunction occasionally observed following prostate brachytherapy.

### VI. HORMONAL THERAPY AS A PRIMARY TREATMENT OPTION FOR LOCALIZED OR LOCALLY ADVANCED PROSTATE CANCER

Radical prostatectomy and radiotherapy are the standard treatment options for men with localized prostate cancer. However, in older men and those who are not willing or cannot receive such curative treatments because of their co-morbid conditions, active monitoring, with or without primary hormonal therapy, is a valid option. With active monitoring (watchful waiting), though disease progression is a concern, more than 50% of patients may require initiation of treatment within 5 years of diagnosis [113]. While hormonal therapy is an established treatment option for patients with locally advanced prostate cancer, it is rarely used alone as primary treatment for patients with earlier stage prostate cancers. Hormonal therapy is palliative and is likely to affect a patient’s quality of life, especially sexual function immediately, and body strength, fat distribution, hair loss, hot flashes, and bone density in the long term. Data from recent clinical trials, however, suggest that non-steroidal anti-androgen monotherapy could be a viable treatment option for both localized and locally advanced prostate cancer. Wirth et al [77] reported that immediate treatment with bicalutamide significantly reduced the risk of disease progression when compared with watchful waiting in these patients, although there was no difference in survival.

While more data is awaited, the existing evidence suggests that hormonal monotherapy might be a viable therapeutic option in select men with prostate cancer who either refuse, or cannot undergo, curative treatment because of underlying disease or advanced age. Table 7 shows indications and contraindications of hormonal monotherapy therapy for men with localized or locally advanced prostate cancer. Table 8 lists the advantages and disadvantages of hormonal monotherapy.

### VII. OTHER THERAPIES WITH LOWER LEVEL OF EVIDENCE

A number of innovative local therapeutic approaches have been investigated for men with localized or locally advanced prostate cancer, including cryotherapy, thermotherapy, and high intensity focused ultrasound ablation (HIFU). None of these approaches, however, has sufficiently matured to be considered as a standard therapy for men with prostate cancer. These investigational treatments, though, appear promising, and it is likely that, with con-inuing technological refinements, some of these could become established methods of treatment in future.

#### 1. CRYOTHERAPY

The mechanism of cellular death after freezing has been carefully analyzed in the past [114,115] In situ ablation for malignancy requires more extensive and precise control of energy than that for benign prostatic hyperplasia. The location of prostate cancer is often not clearly definable by any imaging modality. The tumor can be multi-focal and affecting various parts of the gland. Therefore, it generally requires ablation of entire gland. Cryoablation of the prostate is used to cause ablation of the entire gland or areas of local tumor extension.

The proposed indications include primary treatment of clinically localized cancer, salvage therapy for local failures of radical prostatectomy or external beam irradiation, and control of local complications. Initial results of cryotherapy were poor, as it was shown to be incapable of completely eradicating the local tumor. Complications were troublesome, and loss of erection was almost uniform. With improved technology, however, the recent series show better cancer control rates and lower morbidity.

Ennis et al reported an 80% (110 of 137 patients) negative biopsy rate at 1 year, and a 91% (20 of 22 patients) negative biopsy rate at 3 years, following cryotherapy for T1-T4; N0-N1 prostate cancer [116]. Another study reported no fistulas or major complications in 92 patients who were treated with a more advanced technique [117]. A helium/argon cryogen was delivered through a 17-gauge brachytherapy template, thus the technique parallels that of
brachytherapy. Han et al reported a multicenter study using the third generation cryosurgery [118]. A total of 79 (75%) of 106 patients remained PSA nadir of 0.4 ng/ml or less at 12 months. The complication rates were significantly lower than those reported with larger probes. One outcome that has not changed was erectile dysfunction. Longer-term follow-up was provided by Bahn et al evaluating a series of 590 patients with localized or locally advanced prostate cancer [119]. The mean follow-up was 5.4 years. The 7-year biochemical disease-free (PSA of 0.5 ng/ml or less) survivals for low-, medium- and high-risk patients were 61%, 68% and 61%, respectively. Incontinence and impotence (of men who were potent before cryotherapy) rates were 4.3% and 94.9%, respectively.

In a recent study using the Argon based cryosurgery system, 38 men underwent salvage cryosurgery for radiation therapy failure [120]. The PSA based progression-free survival was 86% at 1 year and 74% at 2 years. The reported complications included rectal pain (40%), urinary tract infections (3%), urinary incontinence (8%), hematuria (8%), and scrotal edema (11%). There was no fistula formation, urethral sloughing, or urinary retention.

There are only several papers evaluating QOL outcomes in patients with prostate cancer treated with cryosurgery. Robinson et al assessed 69 patients having cryotherapy for localized prostate cancer [121]. Total Functional Assessment of Cancer Treatment-Prostate (FACT-P) score showed a significant decline from baseline at 6 weeks, with a steady increase over the year of follow-up, to scores not different from baseline. The sexual function scores were significantly below baseline at one year. The same authors reported the 3-year follow-up QOL data: the QOL remained stable over the following 2 years [122]. The only exception to this general trend was persistent impairment in measures of social/family well-being. At 3 years, only 13% of patients regained erectile function. Perrotte et al evaluated QOL in 150 patients undergoing salvage cryotherapy, using the UCLA Prostate Cancer Index and questions specific to cryotherapy-related complications [123]. Most of the patients had side effects resulting in significant morbidity, particularly incontinence, impotence and perineal pain. The mean overall satisfaction rate was 33%, with a trend to higher dissatisfaction rates in patients with perineal pain.

There are some advantages to the patients in choosing cryotherapy for treating clinically localized prostate cancer. It is minimally invasive, does not involve exposure to radiation hazards, and lower surgical risk than radical prostatectomy. However, this must be countered by the high rate of impotence after cryotherapy. New techniques such as “nerve-sparing” cryosurgery may decrease impotence rate [124]. Although complication rate is relatively high, cryotherapy appears to be a good treatment option for patients with local recurrence after radiation therapy, since there has been no effective treatment option for this patients group. To maintain potency and preserve genitourinary function, focal cryosurgery has recently emerged as an alternative option for men with low-risk unifocal disease [125].

There were few long-term follow-up data on modern cryosurgical techniques, because of the rapid and constant advance in surgical technique and cryosurgical technology over the last decade.

2. THERMAL IMPLANTS

Heat applied to prostate tissue results in various changes in cellular composition [115]. Thermal therapy could be considered as hyperthermia, when the tissue temperatures range between 41.5 and 46, and thermal ablation, when tissue temperatures are greater then 46. In treating cancer, hyperthermia is employed in an attempt to kill cancerous cells while sparing normal cells. Thermal ablation does not make this distinction but rather kills all treated cells, both normal as well as cancerous.

The proposed indications include: early stage localized prostate cancer, combination with external beam radiation therapy in patients with intermediate risk localized disease, and salvage therapy for locally recurrent prostate cancer following radiation therapy. Until the late 1980s there were no studies evaluating the effect of heat or heat plus radiation on human prostatic tumor cells. During 1990s, laboratory and animal studies assessed the feasibility of thermal therapy for prostate cancer [126-130]. Deger et al [131,132] reported results in 57 patients with localized prostate cancer who were treated with interstitial hyperthermia using cobalt-palladium thermo seeds and conformal radiation therapy. The intraprostatic temperatures were between 42° and 46°. Median PSA decreased from 12.2 ng/ml to 2.6ng/ml 3 months after treatment, to 1.3 ng/ml 12 months after treatment, and to 0.55 ng/ml 2 years after therapy. Master et al reported PSA results and immediate/intermediate morbidities in 14 men who underwent thermal ablation for locally recurrent prostate cancer.
following radiotherapy. Six months after the procedure 57% had a PSA decrease to less than 0.1 ng/ml. Morbidity was predominantly in the form of incontinence and impotence [133].

While the perceived advantages of the synergistic interaction of heat and radiation seem beneficial, there are only a few studies available to assess long-term safety or efficacy of thermal therapy. The role of focal therapy in the management of prostate cancer also needs to be defined. Thermotherapy needs a relatively short period of time (1 hour), patients can receive repeated treatments as clinically needed, and it does not preclude the use of other forms of therapies. The disadvantages include the risk of damage to the adjacent normal tissue, need for constant temperature monitoring, and the equipment is expensive and requires highly trained personnel. Ablation therapy appears to be a good treatment option for the patients with local recurrence after radiation therapy, since there has been no effective treatment option for locally advanced prostate cancer.

3. TRANSCERAL HIGH-INTENSITY FOCUSED ULTRASOUND (HIFU)

a) Techniques

1. PRINCIPLE OF TREATMENT WITH TRANSCERAL HIGH-INTENSITY FOCUSED ULTRASOUND

The piezoelectric transducer produces bursts of convergent beams of high-intensity ultrasound at the focal point. Tissue destruction in the target zone is due to thermal effect and cavitation leading to coagulation and necrosis of the prostate:

- the thermal effect is achieved at the focal point (between 85 °C and 100 °C). The short duration of the phenomenon limits diffusion of heat around the focal point. Repeated shots after displacement of the focal point allows juxtaposition of elementary lesions and destruction of prostatic volume.

- the cavitation phenomenon [134] corresponds to vibration of microscopic gas bubbles dissolved in the tissues by successive ultrasound impulses.

2. EQUIPMENT

The equipment comprises an operating table, an ultrasound generator with a treatment head placed in a chilled balloon connected to a transrectal ultrasonograph (for identification of the target volume), and to a computer which directs shots to the target volume determined by the urologist and monitors the temperature in the rectum. Safety devices have been gradually reinforced, following the first investigational clinical phase, to ensure continuous control of the position of the transducer in relation to the rectal wall, detection of the patient’s movement and interruption of shots in the case of an abnormality. The rectal mucosa is cooled to a temperature between 12 °C and 14 °C, avoiding any rectal damage.

The procedure is now standardized. Nevertheless Ablatherm® and Sonablate® have not the same parameters and control unit. Ablatherm®’s parameters are now well validated and the apparatus has obtained the CE Mark in 2000. Different durations of each shot have been evaluated for first-line standard treatment, for repeat treatments or for local recurrence after radiotherapy, according to the thermal conductivity of the tissue in these different situations.

3. TECHNIQUE

A transrectal resection of the prostate or a bladder neck incision is often performed under the same anaesthesia at the beginning of the procedure to reduce the risk of prolonged urinary retention (see below). Then the firing head is placed in the rectum, the prostate volume is calculated before to start the shots. A bladder catheter of 18 French is inserted before or after the procedure. Between 350 to more than 1000 shots are delivered with an average of 120 min of treatment.

b) Cancer control

1. CLINICAL RESULTS

• Studies with short term follow-up

Gelet et al. presented the results concerning 242 patients treated between 1993 and 2002 at Edouard Herriot Hospital in Lyon with a minimum follow-up of one year (under publication). This prospective study is the first to present actuarial recurrence-free survival rates for groups stratified according to their prognostic risk (25.6% low-risk, 44.6% intermediate-risk, 29.8% high-risk of recurrence) with a minimum follow-up of one year.

The Ablatherm prototype was used from 1993 to 1999 (104 patients) and the standard apparatus has been used since 2000 (138 patients). The patients included, with a mean age of 71±5.43 years (median: 71 years), essentially presented localized prostate cancer (48.8% T1, 47.5% T2 and 3.7% T3) with PSA < 30 ng/ml (mean PSA: 9.22±5.76 ng/ml). The mean prostate volume was 32.4±16.6 cc. 12.8% of patients
had a poorly differentiated tumour (Gleason score: 2 to 4: 59.5%, score 7: 27.7% and > 7: 12.8%). An average of 1.6±0.8 sessions were performed per patient in the overall series. A mean of 1.9±0.9 sessions per patient were performed for the 104 patients treated with the prototype (corresponding to 2 systematic sessions, i.e. one session per lobe), and 1.3±0.4 sessions per patient for the 138 patients treated with the standard device. All patients were followed for more than one year (mean follow-up: 29±21 months; range: 1 to 9 years; median: 24 months). Criteria of failure for calculation of progression-free survival were a positive biopsy regardless of the PSA value or 3 consecutive PSA elevations with a PSA velocity > 0.75.

The median PSA nadir was 0.16 ng/ml; 72.7% of patients had a PSA nadir < 0.5 ng/ml; 81.8% of biopsies were negative after treatment. The actuarial 5-year negative-biopsy rate was 74% (82%, 71% and 48% for Gleason 2 to 6, 7 and > 7, respectively).

The 5-year recurrence-free survival rate was 63% (low-risk: 78%, intermediate-risk: 61%, and high-risk: 47%). 16.5% of patients had received adjuvant therapy after treatment failure (radiotherapy in one half of cases, endocrine therapy in one half of cases including 2 combined treatments). 7% of patients in failure did not receive any complementary treatment because of a low PSA with low velocity. The actuarial 5-year adjuvant treatment-free survival rate was 72% (low-risk: 94%, intermediate-risk: 62%, and high-risk: 65%, p<0.05).

Chaussy and Thuroff (135,136) reported the results of a series of 184 patients with clinically localized prostate cancer and a life expectancy greater than 5 years, not candidates for total prostatectomy. One half of patients (48%) had previously received androgen suppression. The results showed 57% of negative biopsies after treatment for the entire population and 79% for the 94 patients treated since November 1997 according to the standard procedure (optimized technical parameters and protection and alarm systems). One third of patients required transurethral resection 6 to 8 weeks after HIFU for persistent retention. The median value of the last postoperative PSA was 1.3 ng/ml (range: 0 to 14.3 ng/ml).

2. The European multicenter trial

This prospective trial included 652 patients from November 1995 to October 2000 [137]. These intermediate results concern the 402 patients with localized prostate cancer (T1-2 N0-x M0) out of the 559 patients treated between November 1995 and November 1999. All these patients were treated by HIFU as first-line treatment and did not present indications for total prostatectomy. Patients previously treated by total prostatectomy (8 cases), external beam radiotherapy (35 cases) or androgen suppression (104 patients) and 10 patients with locally advanced or metastatic disease (T3-4 and/or N1 and/or M1) were excluded from the analysis.

Treatment was administered in 2 distinct sessions (one per lobe) until 1998. Another session could be proposed in the case of positive follow-up biopsy or local progression after initial HIFU therapy. Recurrences after HIFU treated by other modalities (radiotherapy, androgen suppression) were considered to be failures of HIFU. Surveillance was ensured by PSA assay and prostatic biopsies (> 6 weeks after HIFU). Analysis of positive biopsies or a high PSA nadir was correlated with patient and tumour characteristics and successive technical protocols. These biopsy results were studied by classifying patients according to 3 prognostic groups (low-risk: T1-T2a and PSA ≤ 10 ng/ml and Gleason score 6, intermediate-risk: T2b or 10 < PSA ≤ 20 ng/ml or Gleason = 7 and high-risk: T2c or PSA > 20 ng/ml or Gleason score ≥ 8).

The analysis was based on 402 T1-2 N0 M0 patients with a mean age of 69.3±7.1 years. The mean prostate volume was 28±13.8 cc; the initial PSA value was 10.9±8.7 ng/ml. 90.7% of patients had a Gleason score ≤7. A total of 602 sessions were performed in these 402 patients (1.47 session/patient). The mean follow-up was 407.3 days (range: 0 to 1,541 days).

After treatment, 87.2% of the 288 patients evaluable for prostatic biopsy had negative biopsies. The biopsy results according to prognostic groups showed that 92.1% of biopsies were negative in patients presenting a low-risk versus 86.4% in the intermediate-risk group and 82.1% in the high-risk group.

Negative biopsy rates were also similar regardless of the prostatic volume (88.4% of negative biopsies for prostatic volume ≤ 40 cc vs 85.0% > 40 cc), the anteroposterior diameter of the prostate (85.4% for AP diameter ≤ 25 mm vs 88.1% for AP diameter > 25 mm), and complete or partial treatment of the prostatic volume (91.7% after complete treatment vs 87.2% after partial treatment). The PSA nadir was usually obtained 3 to 4 months after HIFU (mean interval: 163.5 days, median: 111.5 days). The mean PSA nadir was 1.8 ng/ml (range: 0-27 ng/ml).
Uchida et al. has published the preliminary results with the system of Sonablate® [138] 140 patients (mean age 70 yo) with PSA 14.7 ng/ml (3.39 to 89.6), mean prostate volume 25.2 cc, mean follow-up of 19.4 months had an overall disease free rate of 66%. According to the PSA level, results were respectively 88% (PSA <10), 67% (PSA >10 and <20) and 34% (PSA >20). According to the Gleason score the disease free rate was 75% (2-4), 65% (5-7) and 57% [8-10].

b) Clinical Results: Studies With 5 Years Of Follow-Up

Gelet et al., in 2001 [139], published a series of 102 patients with clinically localized prostate cancer (46% T1b-c and 46% T2) or local recurrence after external beam radiotherapy (8%). 8% of patients had received neoadjuvant endocrine therapy, which was stopped before HIFU. These patients were not candidates for prostatectomy, but had a life expectancy of at least 10 years or refused the other treatment options proposed or simple surveillance. The mean age at treatment was 70.8±6.13 years. The mean follow-up was 19 months (range: 7 to 76 months). Over this period, 75% of patients remained recurrence-free (negative biopsy). The actuarial 5-year progression-free survival rate (negative biopsy, no PSA elevation) was 66%. The progression-free survival rate ranged from 73% for PSA <10 ng/ml (vs 50% for PSA >10, p=0.02), to 81% for a Gleason score <6 (vs 46% for a gleason score >6, p<0.001) and 68% for 1 to 4 positive pretreatment prostatic biopsies (vs 40%, p=0.01). No significant difference was observed according to the prostate volume treated.

Gelet et al., published in 2003 [140], combined the results of 120 patients treated by HIFU since 1993 for localized prostate cancer (T1-2 N0 M0) with preoperative PSA < 10 ng/ml, a mean age of 71 (range: 56 to 86) years, not candidates for radical prostatectomy with a life expectancy greater than 10 years. The 5-year progression-free survival was 76.9%. It increased significantly (p=0.024) to 85.4% in the case of well differentiated tumour (Gleason 2-6) versus 61.3% for poorly differentiated tumours (Gleason 7-10). No significant difference was observed according to the volume of the prostate (71.5% if volume < 40 cc vs 72.3% if volume > 40 cc), number of positive biopsies at diagnosis (78% if one to 2 positive biopsies vs 77.2 if 3 to 6 positive biopsies), or baseline PSA (88% if PSA < 4 ng/ml vs 73.1% if PSA > 4 ng/ml).

The PSA nadir is a major prognostic factor: a PSA nadir < 0.5 ng/ml was associated with a 91% negative biopsy rate and an 86% progression-free survival rate. Progression-free survival was even 93% when follow-up biopsies were negative and the PSA nadir was < 0.5 ng/ml.

In another 5 years follow up study the results of 146 consecutive patients (mean age: 66.9±6.7 years) with localized T1-T2 N0 M0 prostate cancer treated between October 1997 and November 2002 are presented [141]. 43% of patients had already been treated by endocrine therapy. No adjuvant endocrine therapy was administered after HIFU.

These patients presented a contraindication to total prostatectomy or refused this operation. PSA had to be less than 15 ng/ml (mean PSA: 7.6±3.4 ng/ml) and the Gleason score had to be less than 7 (mean Gleason score: 5±1.2). The mean prostatic volume was 23±7.7 cc. All patients were treated under spinal anaesthesia with Cystocath bladder drainage that was removed after an average of 12.7 days (range: 1 to 59 days). A total of 171 sessions were performed in these 146 patients (1.17 session per patient). The mean follow-up was 22.5 months (range: 4 to 62 months). Analysis of the results shows a median PSA nadir of 0.07 ng/ml (range: 0 to 5.67 ng/ml) and 93.4% of patients presented negative follow-up biopsies. The median PSA at 22 months was 0.15 ng/ml (range: 0 to 12.11 ng/ml). 87% of patients had a PSA value less than 1 ng/ml.

c) Results of HIFU in salvage therapy after failure of radiotherapy.

The publication [142] analysed the results of 71 patients treated at Edouard Herriot hospital in Lyon and at the Institut Montsouris in Paris. The tumour stage before radiotherapy was T1, T2 and T3 for 21.1%, 39.5% and 21.1% of patients respectively and was unknown in 18.3% of cases. The mean PSA at diagnosis was 20.4 ng/ml (range: 3.5 to 60) and the mean PSA nadir after radiotherapy was 1.46 ng/ml (range: 0 to 4.3 ng/ml). The initial dose of radiotherapy was 64.6 Gy (range: 56 to 88). Failure of radiotherapy was reflected by elevation of PSA and was confirmed by biopsy in each patient. No lymph node or bone metastasis was detected (N0 M0). The mean interval before relapse was 38.5 months (range: 6 to 120 months). One third of patients had received endocrine therapy before HIFU, either as an adjuvant to radiotherapy or following the diagnosis of failure of radiotherapy. These treatments were stopped before HIFU.
The mean age of the patients at the time of HIFU was 67±5.86 years, the mean prostatic volume was 21.4±11.1 cc, and the mean PSA before HIFU was 7.7±8.10 ng/mL. The Gleason score before HIFU was between 2 and 6 in 33.8% of patients, 7 in 18.3% of patients and between 8 and 10 in 47.9% of patients. The mean follow-up after HIFU was 14.8 months (range: 6 to 86 months).

Follow-up consisted of PSA assay and prostatic biopsies (systematic at 3 months and in the case of PSA elevation), CT and bone scan in the case of PSA elevation. 80% of post-HIFU prostatic biopsies were negative. The mean PSA nadir after HIFU was 1.97±4.58 ng/mL, with a median of 0.20 ng/mL. A PSA nadir < 0.5 ng/mL at 3 months was observed in 61% of patients. 40 patients (56.3%) required adjuvant therapy after HIFU in the form of endocrine therapy alone (49.3%) or a combination of endocrine therapy and chemotherapy (7%), due to isolated PSA elevation (36.6%) or residual localized cancer (19.7%). Metastatic disease was diagnosed during follow-up in 9 patients (12.7% – bone: 8.4%; lymph node: 2.8%; lung: 1.4%). Four patients died from metastatic disease.

d) Complications and Quality of Life

All the series using the Ablatherm® system have shown a very good immediate and late tolerance, probably better than other treatments indicated in localized prostate cancer.

Four studies have reported the complications of HIFU: one study based on 120 patients treated for localized prostate cancer with PSA ≤ 10 ng/mL [140], the European multicentre study published in 2003 [137], Chaussy’s paper comparing HIFU therapy alone versus combined resection followed by HIFU [143] and the Lyon series of 242 patients with a minimum follow-up of 1 year.

1. Early Complications

**Chronic perineal pain,** observed in 3.3% of patients: **Urinary tract infections** were observed in 13% of patients in the European multicentre study and responded to the usual antibiotics. Gelet’s study of 242 patients with a minimum follow-up of 1 year indicated 1.4% of symptomatic urinary tract infections in 138 patients treated according to current standards versus 4.8% for patients treated with the prototype before 1999.

**Urinary retention** is frequent during the immediate postoperative period when resection is not systematically performed prior to HIFU. It is related to postoperative prostatic edema and elimination of necrotic debris (late retention). The prostatic volume increases by 20% to 40% over the days following the HIFU session. The median duration of bladder drainage ranged between 5 days (urethral catheter) and one month (suprapubic catheter). The prolonged retention rate was estimated to be 8.6% with a secondary resection rate of between 5% and 30%. Since January 2000, prostate resection (TURP) is performed quite systematically, which has considerably decreased the frequency of urinary retention (mean duration of post-HIFU catheterization: 11 days without TURP versus 6 days with TURP). The duration of systematic bladder drainage is currently 3 to 4 days. Prolonged retention was observed in 3.6% of the 138 patients treated according to current standards versus 4.8% for patients treated with the prototype in the analysis of 242 patients with a follow-up of at least 1 year. The role and benefits of transurethral resection of the prostate before HIFU must be stressed. Vallancien [144], in a series of 30 patients (22 resections, 8 bladder neck incisions), reported prolonged retention in 6.6% of cases with improvement of the IPSS score after HIFU and only 2 patients with an IPSS score > 12 after treatment. One half of patients experienced urgency, which resolved over 3 weeks. Resection induces moderate haematuria in 75% of cases, but does not accentuate the incontinence rate (3.3% grade 1, persisting at 1 year). Overall, 88% of patients are satisfied with their quality of life for a reason related to their urinary problems after HIFU versus 63% who were satisfied before treatment (IPSS QoL). Chaussy compared the respective urinary complications of his two patient groups treated by HIFU only during a first period (96 patients) and then by a combination of resection and HIFU from 2000 onwards (175 patients). He also reported a benefit of associated resection on the patient’s quality of life and the lower incidence of urinary adverse effects and even a decreased retreatment rate for residual cancer (4% versus 25% without resection). He reported prolonged retention in 6.9% of cases. The drainage time after combined treatment was 7 days versus 40 days after HIFU only, the IPSS score was 3.37 versus 8.91 after HIFU.

The development of urethrectal fistula is the main complication identified after transrectal prostatic HIFU (5 patients in the European multicentre study). This risk has been eliminated for the treatment of localized lesions with integration of safety devices,
essentially permanent control of the transducer-rectum distance between each shot and the rectal wall cooling system by circulation of cooling fluid. The risk is still high after post-radiotherapy salvage therapy. Two of the 5 fistulas reported in the European multicentre study were observed before integration of the rectal cooling system, 2 in patients with a very thick rectal wall (> 6 mm) (which is currently considered to be a contraindication) and one case during early retreatment at 2 months. Treatment consisted of bladder drainage in 3 cases, collagen injection in one case and surgical revision in one patient. In the series of 242 patients with a follow-up of at least 1 year, no urethroeccal fistula was observed when HIFU was performed according to current standards (since 2000) versus 0.9% in patients treated up until 1999 with the prototype.

2. LATE COMPLICATIONS

Strictures of the prostatic urethra or bladder neck have considerably decreased since systematic use of the TURP-HIFU combination (9% after resection versus 26% after HIFU only). These strictures were corrected by bladder neck incision (usually performed with a cold scalpel). They occur at an average of 6 months after the HIFU session. At long-term follow-up, 3.6% of patients presented a urethral stricture, treated simply by urethrotomy. 8.7% of urethral strictures or bladder neck sclerosis were reported in 138 patients treated according to current standards from 2000 to 2002 in the study of 242 patients with a minimum follow-up of 1 year versus 25.9% for patients treated with the prototype.

The risk of incontinence is not increased by associated transurethral resection: no cases of grade 3 incontinence were observed, and the rate of grade 1/2 incontinence has decreased (13% versus 20%) among the 120 patients treated for localized prostate cancer.

Grade I and grade II urinary incontinence were observed in 10.6% and 2.5% of cases, respectively, in the European multicentre study (Ablatherm prototype, without associated resection). These moderate forms of incontinence either resolved spontaneously or after retraining by physiotherapy. Severe incontinence (Grade III) occurred in 6 patients (1.5%) and was treated by retraining (1 case), collagen injection (1 case) or artificial sphincter (4 cases). Gelet, in his series of 242 patients with a minimum follow-up of 1 year, reported an incontinence rate of 7.9% for 138 patients treated according to current standards from 2000 to 2002 versus 22% for patients treated with the prototype. The respective rates were 6.5% versus 13.4% for grade I, 1.4% versus 6.7% for grade II and no cases of grade III versus 3.8%. Urgency was also reported in 8% of patients.

Overall, the risk of incontinence after combined TURP and HIFU treatment of a localized tumor on the standard machine and according to the standard procedure is 4.6% to 6.5% for grade I incontinence, 1.4% to 2.3% for grade II and zero for grade III.

The impotence rate was evaluated by questionnaire in the study of 120 patients (140): 70 patients achieved erections allowing penetration before treatment and 36% of patients retained erections allowing penetration after HIFU treatment. Impotence was not studied in detail in the European multicentre study. 8.7% of patients spontaneously reported erectile dysfunction after treatment, but the previous quality of erection was not established in all centres. Gelet’s study of 242 patients with a minimum follow-up of 1 year showed an impotence rate of 66% in patients with no pre-existing disorders (75 patients evaluated out of 242).

RECOMMANDATION

Long term results with the Ablatherm® system (5 years of follow-up) have shown a statistical difference between low-risk group (PSA<10 ng/ml and Gleason <7) with a disease free survival rate of nearly 80% and higher-risk group with about 50% of success for the intermediate group. The retreatment rate is between 20 to 30%. HIFU is obviously a promising option in localized prostate failure after radiotherapy with about 40% of progression free rate (56% if Gleason score <6). Nevertheless the complication rate is higher in term of incontinence, impotence and rectal injury. TURP is often performed at the beginning of the HIFU session; this procedure has dramatically reduced the urinary retention rate.
## Management of Localised Prostate Cancer

**Risk**

<table>
<thead>
<tr>
<th>Life Expectancy</th>
<th>&lt; 5 Years</th>
<th>5-10 Years</th>
<th>&gt; 10 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low</strong>&lt;br&gt;T Stage: 1a or 1c and&lt;br&gt;Gleason: 2-5 and&lt;br&gt;PSA: &lt;10 and&lt;br&gt;Biopsy Findings: Unilateral &lt;50%</td>
<td>1. Active monitoring&lt;br&gt;2. RT (3D-conformal or Brachytherapy)&lt;br&gt;3. Investigational therapy</td>
<td>1. Active monitoring&lt;br&gt;2. RT (3D-conformal or Brachytherapy)&lt;br&gt;3. Investigational therapy</td>
<td>1. Radical Prostatectomy&lt;br&gt;2. RT (3D-conformal or Brachytherapy)&lt;br&gt;3. Investigational therapy</td>
</tr>
<tr>
<td><strong>Intermediate</strong>&lt;br&gt;T Stage: 1b, 2a or&lt;br&gt;Gleason: 6, or 3+4 = 7 or&lt;br&gt;PSA: &lt; 10 or&lt;br&gt;Biopsy Findings: Bilateral, &lt;50%</td>
<td>1. Active monitoring&lt;br&gt;2. RT (3D-conformal or Brachytherapy)&lt;br&gt;3. Investigational therapy</td>
<td>1. RT (3D-conformal or Brachytherapy)&lt;br&gt;2. Radical Prostatectomy&lt;br&gt;3. Investigational therapy</td>
<td>1. Radical Prostatectomy&lt;br&gt;2. RT (3D-conformal or Brachytherapy)&lt;br&gt;3. Investigational therapy</td>
</tr>
<tr>
<td><strong>High</strong>&lt;br&gt;T Stage: 2b, 3a, 3b or&lt;br&gt;Gleason: ≥ 4+3 = 7 or&lt;br&gt;PSA: 10-20 or&lt;br&gt;Biopsy Findings: or &gt;50 %, perineural, ductal</td>
<td>1. Hormonal Tx&lt;br&gt;2. RT (3D-conformal) + Hormonal Tx&lt;br&gt;3. Investigational therapy</td>
<td>1. RT (3D-conformal)+ Hormonal Tx (2-3y)&lt;br&gt;2. Hormonal Tx&lt;br&gt;3. Radical Prostatectomy + Pelvic lymphnodes dissection (in seleted patients)&lt;br&gt;4. Investigational therapy</td>
<td>1. RT (3D-conformal)+ Hormonal Tx (2-3y)&lt;br&gt;2. Radical Prostatectomy + Pelvic lymphnodes dissection (in selected patients)&lt;br&gt;3. Investigational therapy&lt;br&gt;4. Hormonal Tx</td>
</tr>
</tbody>
</table>

1. Active monitoring contraindicated for symptomatic patients. It is not recommended in intermediate, and high risk patients with a life expectancy > 10 years.

2. + pelvic node dissection unless predicted probability (staging nomograms) is < 3%

3. There is a "move" to recommend radical prostatectomy for high risk and very high-risk patients as a part of a multimodal treatment program including hormonal therapy and postoperative radiotherapy and possibly even chemotherapy.


