From the Department of Oncology-Pathology
Karolinska Institutet, Stockholm, Sweden

MORPHOLOGICAL, IMMUNOHISTOCHEMICAL AND GENETIC ASPECTS OF ACINAR AND DUCTAL ADENOCARCINOMA OF THE PROSTATE

Claes Lindh

Karolinska Institutet

Stockholm 2022

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2022

ISBN 978-91-8016-626-3

MORPHOLOGICAL, IMMUNOHISTOCHEMICAL AND GENETIC ASPECTS OF ACINAR AND DUCTAL ADENOCARCINOMA OF THE PROSTATE

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Claes Lindh

Principal Supervisor:
Professor Lars Egevad
Karolinska Institutet
Department of Oncology-Pathology

Co-supervisor:
Assistant professor Johan Lindberg
Karolinska Institutet
Department of Medical Epidemiology and Biostatistics

Opponent:
Professor Lars Akslen
University of Bergen
Department of Clinical Medicine

Examination Board:
Professor Jaakko Patrakka
Karolinska Institutet
Department of Laboratory Medicine

Associate Professor Michael Häggman
Uppsala University
Department of Surgical Sciences

Associate Professor Sten Stemme
Karolinska Institutet
Department of Oncology and Pathology

For my wife Rika and children, Sakura and Hugo.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Prostatacancer är en av de cancersjukdomar som orsakar flest cancerrelaterade dödsfall i utvecklade länder. De flesta prostatacancrar tillhör cancertypen acinär prostatacancer, vilken utgör över 90% av alla diagnostiserade prostatacancrar. Den näst vanligaste cancertypen i prostata är duktal prostatacancer. Duktal prostatacancer beskrevs första gången 1967 och man trodde först att det inte var en form av prostatacancer, utan en egen tumörtyp som uppkom i vävnaden intill urinröret i prostata. Duktal prostatacancer är en aggressiv typ av prostatacancer med sämre prognos än konventionell prostatacancer.

I vårt första arbete beskriver vi hur ofta man med blotta ögat kan identifiera prostatacancer i ofixerade prostatektomier, bortopererade prostator, och vilka särdrag de identifierade tumörerna uppvisar. I studien visar vi att man i 52% av fallen kunde identifiera förändringar konklusiva för cancer och i 24% förändringar misstänkta för cancer. I dessa identifierade områden bekräftades cancer mikroskopiskt i 94% respektive 69% av fallen. Men även i fall där man visuellt inte kunde identifiera någon cancer på preparatens snittytor, förelåg mikroskopiskt cancer, någonstans på snittytan, i 56% av fallen. Av visuellt konklusivt identifierade cancrar var 30% beigea, 30% vita, 16% gula och 24% orangea. Visuellt uppskattad tumörstorlek var mindre än den verkliga, mikroskopiskt uppmätta, tumörstorleken. Vi visar också att det är svårare att visuellt identifiera tumörer belägna i vävnaden kring urinröret (transitionszonen) än tumörer belägna längs prostatas ytterkanter (perifera zonen). Tumörer med hög tumörgrad (aggressiva tumörer) identifierades oftare än tumörer med låg tumörgrad. Sammanfattningsvis så visade sig förändringar som visuellt bedömdes som konklusiva för cancer oftast vara cancer vid mikroskopisk undersökning. Dock så förelåg cancer oftast även i de fall där man visuellt inte kunde identifiera någon cancer.

Biobankning är en process där man tillvaratar biologisk vävnad för användning i forskningsstudier. För många forskningsstudier önskar man tillgång till färsk vävnad, d.v.s. vävnad som inte är formalinfixerad. Biobankat vävnadsmaterial omhändertas direkt efter operation, fryses ned i flytande kväve och förvaras i -80°C. Detta förfarande gör att vävnadens kvalitet förblir hög och möjliggör avancerade studier av tumörcellers arvsmassa. Att tillvarata ofixerad tumörvävnad från prostatektomier är problematiskt eftersom det ofta är svårt att se var tumörerna är lokaliserade. Därtill så riskerar man att bara identifiera och ta prover från större tumörer vilket kan göra att selektionen av tumörer blir skev. Detta skulle kunna medföra att det tumörmaterial som ligger till grund för forskningsstudier inte blir representativt för prostatacancer i stort. I vår andra studie beskriver vi en ny metod för att biobanka en hel skiva prostatavävnad från operationspreparat. Tekniken möjliggör analyser av förstadier till cancer men även av fall där flera separata tumörer samtidigt föreligger i prostata. En stor fördel med den nya metoden är att man från de tillvaratagna vävnadsbitarna

också kan snitta fryssnitt som sedan kan användas för att rapportera klinisk information som ligger till grund för patienters behandling och prognos. I studien visar vi att biobankat vävnadsmaterial håller hög kvalitet, d.v.s. att tumörcellernas arvsmassa huvudsakligen är intakt, vilket gör att det kan användas för avancerad molekylärbiologisk forskning.

Immunförsvaret patrullerar ständigt vår kropp och letar efter kroppsegna celler som uppvisar tecken på cancerutveckling. Då sådana celler upptäcks blir de eliminerade av immunförsvaret. Denna immunövervakning är central för att förebygga cancerutveckling och detta exemplifieras väl av att individer med nedsatt immunförsvar löper en förhöjd risk att drabbas av vissa cancerformer. Det finns således ett evolutionärt tryck på cancerceller att utveckla metoder för att undvika immunövervakningen. Ett sätt för tumörceller att göra detta är att öka uttrycket av proteinet PD-L1 på cellytan. Det ökade PD-L1-uttrycket bromsar immunförsvaret och tumörcellerna kan därmed undvika eliminering. Under senare tid har den nya läkemedelsklassen ”immune check point inhibitors” introducerats. Dessa läkemedel ”avmaskerar” tumörceller genom att minska PD-L1-molekylens bromsande effekt på immunförsvaret, så att tumörcellerna kan elimineras av immunförsvaret. De har visats förlänga överlevnaden i flera svåra tumörsjukdomar. För att utreda vilka patienter som kan tänkas ha nytta av behandlingen undersöker man immunohistokemiskt uttrycket av PD-L1 på tumörceller och/eller i tumörinfiltrerande immunceller. Om PD-L1 uttrycks i riklig mängd talar detta för att läkemedlet kan ha effekt. När kroppens celler genomgår celldelning sker mängder av små kopieringsfel i arvsmassan. Dessa fel repareras normalt av speciella reparationsprotein (MMR-protein) för att undvika utveckling av skadliga mutationer. I maligna tumörer kan inaktiverande mutationer i dessa reparationsproteiner uppkomma, vilket leder till att tumörerna ackumulerar ett stort antal mutationer i arvsmassan. Detta fenomen kallas mikrosatellitinstabilitet. I vårt tredje arbete visar vi att uttryck av PD-L1 i tumörceller är ovanligt i både duktal och acinär prostatacancer, 3% respektive 5%. Däremot är PD-L1-uttryck i tumörinfiltrerande immunceller betydligt vanligare, 29% och 14% i duktal respektive acinär prostatacancer. Defekt uttryck av MMR-protein, vilket leder till mikrosatellit-instabilitet, noterades endast i 5% av fallen.

Sedan prostatacancervarianten duktal prostatacancer första gången beskrevs har dess ursprung och relation till ”vanlig”, acinär, prostatacancer varit föremål för diskussion. Duktal prostatacancer förekommer vanligen tillsammans med acinär prostatacancer i blandade tumörer och ”ren” duktal prostatacancer är mycket ovanligt. I vår fjärde studie ville vi klarlägga den klonala relationen mellan duktala och acinära prostatacancerkomponenter i blandade prostatacancrar, d.v.s. utreda huruvida komponenterna har uppkommit ur en gemensam ursprungscell eller om de uppkommit oberoende av varandra. Vi identifierade fall av blandade prostatacancrar, dissekerade fram duktala och acinära prostatacancerkomponenter, extraherade DNA och genomförde genomisk sekvensering. Studien visar att det i 12 av 15 undersökta fall förelåg ett gemensamt klonalt ursprung, d.v.s. i

majoriteten av undersökta fall representerade de två tumörkomponenterna delar av samma tumör. I tre av 15 fall påvisades inget gemensamt klonalt ursprung, i dessa fall är det tänkbart att det rör sig om två separata tumörer som vuxit in i varandra. Slutligen visar vi att duktala prostatacancrar uppvisar genetiska förändringar som man ofta ser vid höggradig prostatacancer, såsom aneuploidi (avvikelser av antalet kromosomer i en cell).

ABSTRACT

Prostate cancer is one of the most common causes of cancer-related death in developed countries. Acinar adenocarcinoma is by far the most common subtype of prostate cancer, with ductal adenocarcinoma being the second most common subtype.
Biobanking of prostate cancer tissue is important for basic research, development of new biomarkers and a move towards personalized medicine. Various biobanking techniques have been described but harvesting of tissue is still often based on macroscopic identification of cancer in radical prostatectomy specimens. In the literature, the macroscopic features of prostate cancer in unfixed prostatectomy specimens are incompletely described. In our first study, we investigated the macroscopic features of identifiable tumors and their zonal distribution in 514 radical prostatectomy specimens. Grossly detected findings conclusive for cancer were seen in 52% of cases and suspicious for cancer in 24%. Macroscopic findings conclusive for cancer predicted microscopic identification of prostate cancer on microscopic examination in most cases. Cancers ≥2 mm were present somewhere on the cut surface in the majority of cases even when no suspicious or conclusive cancers had been identified macroscopically. Tumors in the transition zone of the prostate were more difficult to identify macroscopically. In our second study, we report a novel biobanking protocol for harvesting a full horizontal slice of unfixed prostate tissue from 20 radical prostatectomy specimens. In 18 of 20 cases, cancer was found in the biobanked tissue material. The biobanking protocol facilitated harvesting of a large slice of prostatic tissue, allowing studies of multifocal tumors and tumor heterogeneity. Clinical histopathological parameters could be reported from frozen sections of the biobanked material. The morphological quality, using cryogel, and the RNA quality, measured by RNA integrity number (RIN), were excellent.

Ductal adenocarcinoma is a high-grade neoplasm with an adverse prognosis compared to acinar adenocarcinoma. The definition of ductal adenocarcinoma is based on histological features. Ductal adenocarcinoma usually presents in mixed tumors together with acinar adenocarcinoma. For a long time, the histogenesis and definition of ductal adenocarcinoma has been controversial. Some studies have suggested that acinar and ductal adenocarcinoma components may have a common clonal background. Expression of Programmed Death Ligand-1 (PD-L1) is a predictive biomarker for a new group of oncological drugs, immune checkpoint inhibitors. The frequency of PD-L1 expression in ductal adenocarcinoma is not well described. Deficient mismatch repair (dMMR) results in an accumulation of mutations in cancer cells. dMMR has been reported to be uncommon in prostate cancer. In our third study, we investigated the expression of PD-L1, dMMR and tumor infiltrating immune cells in acinar and ductal adenocarcinoma using a tissue microarray (TMA). PD-L1 expression in tumor cells was rare but more common in tumor infiltrating immune cells. PD-L1 expression was identified in tumor infiltrating immune cells in 29% of ductal adenocarcinomas. dMMR was rare, identified in only 5% of cases. There was a statistically significant increase in the number of CD8+ lymphocytes in ductal adenocarcinoma compared to acinar

adenocarcinoma. In our final study, we investigated the clonal relationship between acinar and ductal adenocarcinoma components in mixed prostate cancers. Targeted sequencing was performed in 15 cases, followed by bioinformatic processing and manual curation of data. A common somatic denominator for both tumor components could be identified in 12 out of 15 cases indicating a common clonal origin. Increased ploidy, which is associated with advanced prostate cancer, was seen in more than half (53%) of ductal adenocarcinomas but not in any acinar adenocarcinoma. PTEN and CTNNB1 mutations were common in ductal adenocarcinoma (40%) but not seen in any acinar adenocarcinoma. In both acinar and ductal adenocarcinomas, ERG gene fusions were detected in 47%. No cases showed microsatellite instability or high tumor mutation burden. The genetic signature of ductal adenocarcinoma was consistent with its characterization of ductal adenocarcinoma as an aggressive form of prostate cancer

LIST OF SCIENTIFIC PAPERS

I. Lindh C, Delahunt B, Egevad L. Macroscopic features of prostate cancer. Pathology. 2018 Jun;50(4):382-388.

II. Lindh C, Delahunt B, Samaratunga H, Yaxley J, Gudjónsdóttir J, Clements M, Lindberg J, Egevad L.A novel technique for biobanking of large sections of radical prostatectomy specimens. Histopathology. 2018 Feb;72(3):481-489.

III. Lindh C, Kis L, Delahunt B, Samaratunga H, Yaxley J, Wiklund NP, Clements M, Egevad L. PD-L1 expression and deficient mismatch repair in ductal adenocarcinoma of the prostate. APMIS. 2019 Aug;127(8):554-560.

IV. Lindh C, Samaratunga H, Delahunt B, Bergström R, Chellappa V, Yaxley J, Lindberg J, Egevad L. Ductal and acinar components of mixed prostatic adenocarcinoma frequently have a common clonal origin. Prostate. 2022 Apr;82(5):576-583.

CONTENTS

LIST OF ABBREVIATIONS

dMMR	Deficient mismatch repair
HE	Hematoxylin and eosin
HGPIN	High-grade prostatic intraepithelial neoplasia
IHC	Immunohistochemistry
ISUP	International Society of Urological Pathology
mCRPC	Metastatic castration-resistant prostate cancer
MSI	Microsatellite instability
PARP	Poly (ADP-ribose) polymerase
PD-L1	Programmed Death Ligand-1
PSA	Prostate-specific antigen
PSMA	Prostate-specific membrane antigen
PZ	Peripheral zone of the prostate
TMA	Tissue microarray
TUR-P	Transurethral resection of the prostate
TZ	Transition zone of the prostate

1 INTRODUCTION

"All men by nature desire to know"

—Aristotle

1.1 EPIDEMIOLOGY OF PROSTATE CANCER

Prostate cancer is one of the leading causes of cancer-related death in the developed world (1). Prostate cancer is the second most common cancer among men after lung cancer and globally accounts for 7% of newly diagnosed cancers in men (2). In Sweden, it is the most common form of cancer with approximately 10500 new cases, accounting for approximately 30% of all cancer cases, and 2300 prostate cancer-related deaths per year (3). The median age for diagnosis of prostate cancer in Sweden is 70 years and only 1% of cases are diagnosed in men younger than 50 years. The risk for prostate cancer increases with age and more than 85% of newly diagnosed cancers affect men >60 years of age (3). The incidence of prostate cancer increases with human development index (HDI), GDP and high life expectancy. The disease is most common in North America, Europe, Australia and New Zeeland (4). A few countries with a high HDI, such as Japan and South Korea, have a lower incidence of prostate cancer compared to western countries but the incidence in these countries is rising (4). The incidence of prostate cancer has increased considerably the last decade due to increased serum-prostate-specific antigen (PSA) screening and increased life expectancy (5). Several factors affect the risk of developing prostate cancer such as age, race and family history (6). There is a strong association between prostate cancer risk and a family history of cancer. To determine risk, the number of affected relatives, the degree of relationship and age of disease presentation is considered. A man with two first-degree relatives with prostate cancer has been shown to have a 3.5-fold increased risk of developing the disease (7).

1.2 SYMPTOMS AND CLINICAL DIAGNOSTICS

Prostate cancer usually does not give any symptoms in its early stages and most new prostate cancers are detected through increased serum-PSA and/or palpable tumors on digital rectal examination (8). Serum-PSA is often elevated in prostate cancer, but it can also be elevated in other conditions such as prostatitis or prostate hyperplasia (9). Prostate cancer metastasizes via lymphogenous spread, initially to the pelvic lymph nodes, and via hematogenous spread, primarily to bone (10). Locally advanced or metastatic prostate cancer can present with skeletal pain, lower urinary tract symptoms or fatigue (8).The foundation of prostate cancer diagnostics is ultrasound guided core needle biopsies of the prostate for histopathological examination or more rarely fine needle aspiration cytology. Core needle biopsies are traditionally taken in a systematic manner, meaning that biopsies are taken from the entire prostate in a grid-like fashion guided by transrectal ultrasound. Recently multiparametric magnetic resonance imaging (MRI) has been added to the diagnostic armament to identify

and localize prostate cancer. This enables targeted biopsies of prostatic lesions. Targeted lesions can be obtained through three different approaches: MRI image fusion with transrectal ultrasound using computerized software, percutaneous biopsy during the actual MRI investigation and visual review of the MRI followed by prostate biopsy using transrectal ultrasound (so called “cognitive biopsy”) (11). Prostate cancer is sometimes diagnosed incidentally in transurethral resection of the prostate (TURP) specimens or in patients with disseminated disease where the diagnosis of prostate cancer is made on biopsy specimens of distant metastases (12).

1.3 MACROSCOPIC FEATURES OF PROSTATE CANCER

For cancer of most organs, macroscopic features such as color, texture and demarcation are well-documented and described in the literature (13,14). In contrast, prostate cancer is often anecdotally described as white or tan, vaguely demarcated and difficult to identify macroscopically (13–15). Prostate cancers are known to be difficult to identify on macroscopic examination and are sometimes not visible, even when the tumors are large. The difficulty of identifying tumors on macroscopic examination complicates harvesting of fresh tumor tissue. This has led to the recommendation that radical prostatectomy specimen should be completely embedded for microscopic examination.

Few studies have addressed the prevalence of grossly identifiable prostate cancers in radical prostatectomy specimens. None of these studies contains detailed descriptions of macroscopic findings such as the colors or zonal distribution of cancers in unfixed prostatectomy specimens (15,16). Renshaw *et al.* completely sliced 211 unfixed radical prostatectomies and noted macroscopically visible tumors. Conclusive tumor foci were identified in 41% of cases, while lesions suspicious for cancer were seen in 37%. On microscopic examination, tumors were confirmed in 96% of cases with findings conclusive for cancer macroscopically and in 64% of cases with findings suspicious for cancer (15). In a series of 104 formalin fixed prostatectomy specimens, Hall *et al.* reported that 72% of Stage A and 92% of Stage B tumors were identifiable macroscopically (16).

1.4 HISTOPATHOLOGY

Most of primary prostatic tumors are adenocarcinomas and they most often arise in the peripheral zone of the prostate (17). Acinar adenocarcinoma is the most common subtype of prostate cancer accounting for over 90% of cases (18). Acinar adenocarcinoma is microscopically characterized by infiltrating small or large glands, cribriform structures, glomerulations, solid sheets or strands of tumor or single tumor cells. The classical cytological feature of prostate cancer is prominent nucleoli (19,20). Other nuclear features

include nuclear enlargement and hyperchromasia (19,20). Mitotic figures are more common in high-grade prostate cancer than in benign glands (21). Another feature sometimes seen in prostate cancer is the presence of intraluminal crystalloid structures (19). Most carcinomas exhibit marked desmoplastic stromal response but prostate cancer, in contrast, often shows little or no stromal reaction. When desmoplasia is present, it is usually in high-grade carcinoma (22).

The prostate is anatomically divided into three zones: the peripheral zone (PZ), the transition zone (TZ) and the central zone (CZ), *Figure 1*. Benign prostatic glands are normally lined by a two-layered epithelium with apical luminal secretory cells and underlying basal cells. Prostate cancer is characterized by loss of the basal cells, but this can be difficult to assess by light microscopy of hematoxylin and eosin (HE)-stained sections (23). The presence or absence of basal cells can be highlighted by immunohistochemistry (IHC) for p63 or high molecular weight cytokeratin. A potential pitfall is that IHC for basal cell markers can also be negative or patchy in benign lesions such as adenosis, partial atrophy and HGPIN (24).

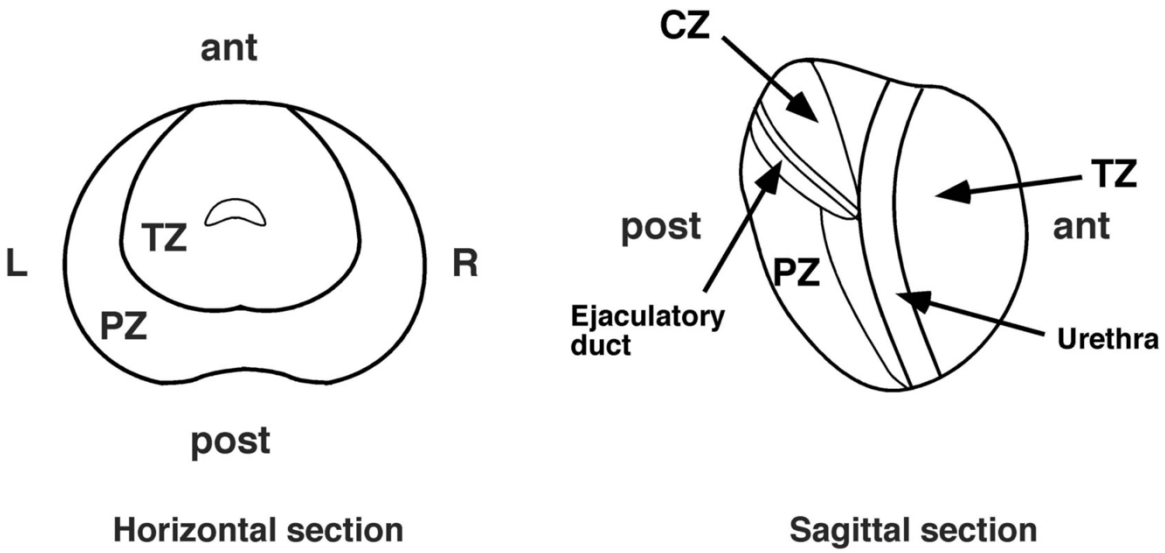


Figure 1. Schematic figure illustrating the anatomic zones of the prostate. Peripheral zone (PZ), Transition zone (TZ), Central zone (CZ).

1.5 PROSTATE CANCER SUBTYPES

Histological variants of acinar adenocarcinoma include pseudohyperplastic, foamy, atrophic, oncocytic, signet-ring, colloid and lymphoepithelioma-like adenocarcinomas. Other non-acinar prostate cancer types include urothelial carcinoma, neuroendocrine tumors, basal cell carcinoma, squamous, adenosquamous carcinoma, sarcomatoid carcinoma, lymphomas and various forms of mesenchymal neoplasms (18). Several of these tumor types are very uncommon and rarely encountered, while ductal adenocarcinoma is more commonly seen. Correct identification of prostate cancer subtypes is of great importance because they can have a different biological behavior and may be

assigned different Gleason scores. This can impact medical patient management and clinical outcome (25).

Ductal adenocarcinoma of the prostate is the second most common histological subtype of prostatic cancer after acinar cancer. It has been reported to account for 3.2% of all prostate cancers although the reported incidence varies widely (13). Most commonly it presents in mixed tumors together with acinar prostate cancer, while a pure form is found in only 0.2%-0.4% of cases (26). Ductal cancer is reported to have an adverse prognosis compared to conventional acinar prostate cancer with shorter time to biochemical recurrence and increased mortality rate (27,28). Also, an increasing percentage of the ductal component has been shown to correlate with an increased risk of extraprostatic extension and seminal vesicle invasion (29). Ductal cancer was first described by Melicow and Patcher in 1967. It was initially called ”endometroid carcinoma” due to its histopathological similarity to endometroid carcinoma of the uterus (30). In the early literature, ductal cancer was thought to arise from remnants of a Mullerian duct structure (31) but it was later shown that it was a variant of prostate cancer (32). The mean age of patients at the time of diagnosis ranges from 60-80 years (27,32–35) i.e., somewhat older than patients with acinar cancer (27). Periurethral tumors most commonly arise around the mons verumontanum and form exophytic tumors in the urethra lumen. Patients sometimes presents with urinary obstructive symptoms or gross/microscopic hematuria (34). Ductal cancer can also arise in the PZ (38). Some authors have described serum-PSA as elevated while others report that patients with ductal cancer present with a lower serum-PSA (34).

The diagnosis of ductal cancer is based solely on morphological findings. The tumors show pseudostratified, columnar tumor cells with abundant cytoplasm. The tumor cells exhibit basally located, elongated nuclei with macronucleoli and a clumped chromatin pattern. Mitotic figures and luminal necrosis can also be identified (14). Ductal cancer grows in several architectural patterns, often varying within the same tumor although one pattern is usually predominant (36). The two most common patterns are papillary and cribriform (37). Papillary architecture is usually not seen in conventional acinar adenocarcinoma (36). Poorly differentiated ductal cancer can grow in solid sheets with central necrosis without glandular formation. In the 2005 upgrade of the Gleason grading system, it was decided that ductal cancer should be considered Gleason pattern 4. In cases with comedo necrosis Gleason pattern 5 is warranted (39).

1.6 STAGING, RISK STRATIFICATION AND PROGNOSIS.

Prostate cancer is risk stratified into prognostic categories according to the Gleason grading system (39). The Gleason score combined with clinical stage gives an estimation of the prognosis (40). Clinical staging is a part of the TNM staging system, which describes the extent and size of the primary tumor (T), involvement of regional lymph nodes (N) and

presence of distant metastases (M). Patients typically presents with an elevated serum-PSA and/or a palpable nodule in the prostate on digital rectal examination (8). Core needle biopsies are performed to establish a morphological diagnosis. When prostate cancer is diagnosed on core needle biopsies, pathological parameters such as Gleason score (tumor differentiation), tumor amount, perineural growth, extraprostatic extension and intraductal carcinoma are reported (41). Mimics of prostate cancer such as adenosis, sclerosing adenosis, basal cell hyperplasia, clear cell cribriform hyperplasia, mucinous metaplasia and variants of atrophy are well described and can constitute a challenge in clinical practice (42).

It has long been known that prostate cancer is very common and that most affected men do not have any symptoms of the disease. In a study by Franks in 1954, prostates were removed from 220 males at autopsy (43). In all cases death had been sudden or unexpected and the patients had no history of prostate cancer. The prostates were fully embedded and prostate cancer was microscopically diagnosed in 69 cases. Of men in the sixth and seventh decades of their life about a third of had prostate cancer, in the eighth decade nearly half, in the ninth more than three quarters and in the tenth all had cancer. Approximately 80% of patients are diagnosed with organ-confined disease, 15% with regional metastases and 5% with distant metastases (44). Patients who are diagnosed with a late-stage, distant metastatic, disease have a markedly worse prognosis with an overall five-year survival of 30% (44). With 13 years follow up of clinically localized prostate cancer, prostate cancer specific mortality has been reported to be 13%. Patients who had received surgical treatment showed better survival (45). Early detection of tumors, before they have reached an advanced stage, as well as improved treatment algorithms for deciding which patients should receive radical treatment, is of great importance for improving patient survival and reducing overtreatment.

1.7 TREATMENT

Patients with localized prostate cancer, without evidence of distant metastases, can be managed according to three different approaches: expectant management, radical prostatectomy or radiotherapy (46). For patients with limited life expectancy and/or cancers with low Gleason score expectant management may be preferred. Expectant management avoids the risks of complications that are related to curative strategies while ensuring that the risk for prostate cancer specific death remains low (46). Expectant management includes watchful waiting and active surveillance. In watchful waiting patients are followed and clinical symptoms are managed if they arise during the course of the disease. Active surveillance is a more active approach that aims to monitor the disease and treat patients who develop significant cancer. Active surveillance includes regular PSA testing, clinical examination, repeated prostate biopsy investigations, or a combination of these (47).

In intermediate and high-risk cancers, where active treatment is judged to be beneficial for the patient, radical prostatectomy or internal/external radiotherapy can be performed (48). Both surgery and radiotherapy can be complicated by side effects that lower the quality of life, such as erectile dysfunction and urine leakage (49). Androgen deprivation therapy alone or in combination with chemotherapy or other drugs inhibiting the synthesis of or the receptors for androgens is usually used in metastatic, castration-sensitive prostate cancer (50). However, most patients with advanced prostate cancer will in the end develop resistance to hormonal treatment, metastatic castration-resistant prostate cancer (mCRPC), making it important to develop new treatment strategies. The rapidly increasing understanding of the biology and genetics of cancer has led to the insight that cancer can be divided into different molecular subgroups. This deepened understanding has awakened the hope that we are entering an era of personalized cancer treatment, where the treatment of each patient will be guided by tumor specific genetic characteristics rather than merely by its tissue of origin or anatomical location. Especially in prostate cancer, where many tumors are relatively indolent, there is a great need to develop better predictive tools to select which patients will benefit from radical treatment. Currently, patients with low-risk tumors may be overtreated with accompanying risk of complications following radiation therapy or operation. Prostate cancer risk stratification and treatment is still based on the traditional parameters serum-PSA, tumor grade and tumor stage. Based on this, patients are stratified into low, intermediate and high risk groups to predict the risk of biochemical recurrence (48). At present, specific genetic characteristics of individual tumors are not analyzed in clinical routine practice. Active surveillance is an option for patients with a low risk disease but there is a need to develop new prognostic markers to better determine which patients may develop aggressive disease (51). This is important for avoiding overtreatment while still treating all patients with aggressive disease.

In patients with mCRPC, where the prognosis is dismal, there is a movement towards a more personalized treatment approach (52). Currently, studies investigating treatment with PARP (Poly (ADP-ribose) polymerase)-inhibitors for mCRPC with BRCA mutations are ongoing (53,54). Due to the milder side effects and promising results in other cancer forms, treatment with immune checkpoint inhibitors has gained attention in treatment of prostate cancer and several clinical trials are ongoing with special focus on mCRPC exhibiting microsatellite instability (MSI) and deficient DNA mismatch repair (dMMR) (55). The introduction of new oncological treatments in the mCRPC patient group will require pathology laboratories to perform molecular analyses on tumor samples to determine which patients are suitable for treatment. During the last decade, several tumor types, such as breast and lung cancer, have moved towards establishing prognostic and predictive biomarker panels, something that has still not happened for prostate cancer. To develop a more tailored approach to patient management and pharmaceutical interventions, new predictive and prognostic biomarkers need to be developed and validated. In order to develop new biomarkers and oncological drugs for prostate cancer, ample access to biobanked prostate cancer tissue of high quality is

essential. Harvesting of prostate cancer tissue for biobanking purposes is usually performed by pathologists from radical prostatectomy specimens. It is of great importance that the biobanking is performed in a way that enables comprehensive studies of prostate cancer without jeopardizing the histopathological diagnosis.

1.8 BIOBANKING OF PROSTATE CANCER

Collecting biological tissue and/or fluids in biobanks constitutes a cornerstone in facilitating the development of precision medicine. Access to biobanked material is crucial for research in basic tumor biology as well as in the development of new oncological drugs and prognostic/predictive biomarkers. It has been reported that harvesting of fresh tumor tissue from radical prostatectomy specimens for research is performed in 55.4% of academic institutions and 7.2% of non-academic laboratories in Europe (56). Even though novel molecular techniques have been introduced that enable analysis of formalin-fixed paraffin-embedded tissues, biobanked frozen tumor tissue is still often preferred. In most organs, biobanking of tumor tissue and normal tissue is based on visual inspection of operation specimens. However, since prostate cancer is more heterogeneous and multifocal than tumors of other organs, visual identification of cancer in prostatectomy specimens is challenging.

In the literature, various techniques for harvesting of fresh tumor tissue from prostatectomy specimens are described (57). A few protocols describe biobanking through multiple punch biopsies from sliced prostatectomy specimens (58–61). This technique preserves the surgical margins but has several drawbacks. Punch biopsies only contain limited amounts of tumor and identifying where the punch biopsies have been taken requires multi-colored ink labelling. Other biobanking protocols includes the removal of the central regions of prostatectomy specimens (62,63). This approach leaves the peripheral tissues and the surgical margin of the prostate untouched but may lead to insufficient sampling of the PZ, the region where most prostate cancers are located. Many biobanking protocols are based on macroscopic identification of cancer and benign tissue in radical prostatectomy specimens (64–66). A significant disadvantage of this approach is that identifying prostate cancer visually can be challenging or impossible, as prostate cancer is notoriously difficult to see with the naked eye (15). Because of earlier detection, there has been a stage shift towards smaller tumors at diagnosis (67). This trend has increased the number of cases in which the tumors are difficult, if not impossible, to identify grossly. Since the largest and most advanced tumors are most easily identified this technique also introduces a sampling bias.

A cytology-based technique for sampling cells from prostatectomy specimens has earlier been reported by our group (68). With this technique a scalpel blade is used to scrape cells

from the cut surfaces of macroscopically visible cancer and presumably benign regions of the prostatectomy specimen. A cytological smear is produced, stained, and used for morphological investigation. Harvesting locations are noted on a specimen map for correlation with histopathological slides. The specimen's resection margins are not compromised, but an experienced cytologist is required for interpreting the cytological smears and the cell yield is limited. Tumor tissue can also be sampled by core needle biopsies from the outside of prostatectomy specimens (69). Using this technique, the capsular plane and the surgical margins of the specimen remain intact. However, it is often very difficult to identify cancers by palpation of the prostatectomy specimen. This, together with the fact that core needle biopsies yield limited amounts of tissue, makes it very difficult to harvest substantial amounts of representative tumor material using this technique. Other biobanking methods retrieve one or several full slices from radical prostatectomy specimens. In a method described by Bertilsson *et al.*, a complete transversal slice is harvested, using a double-bladed knife, and snap-frozen without being cut into smaller pieces (70). Jhavar *et al.* reports a protocol where the entire prostatectomy specimen is sliced fresh and a single slice harvested for research (71). Dev *et al.* suggested that the entire prostatectomy specimen be sliced fresh. Every second transverse slice is divided in four segments, coated in OCT gel, and snap-frozen (72). The advantage of harvesting a complete transverse slice of prostatic tissue is that it facilitates the identification and microdissection of cancer as well as other tissue components, such as tumor-associated stroma and precursor lesions. The disadvantage of the technique is that it removes a large part of the prostate from histopathological specimen and it can cause deformation of the prostate. The downside of snap-freezing a full transversal prostate tissue slice without dividing it into smaller pieces is that the full tissue slice will need to be thawed every time that new sections are taken for research. This recurring thawing may jeopardize the RNA integrity of the tissue.

1.9 PROGRAMMED DEATH LIGAND-1

Programmed Cell Death Protein-1 (PD-1), a T cell regulator, is expressed on the surface of activated T cells, pro-B cells or macrophages and regulates the immune system by acting as an immune checkpoint. This is done by inducing apoptosis in antigen-specific T cells and reducing apoptosis in regulatory T cells. This immunoregulatory function is important in reducing the risk of autoimmunity (73). Programmed Death-Ligand-1 (PD-L1), the ligand of PD1, is expressed on tumor cells, macrophages and T cells (74). The interaction between PD1 and PD-L1 reduces the immunological response. PD-L1 has been shown to be overexpressed in several cancer types. The PD-L1 - PD1 interaction is thought to be a way for cancer cells to escape immune surveillance, also known as cancer immune evasion (75). The expression of PD-L1 in tumor or immune cells in cancer specimens has been shown to correlate with treatment response to anti-PD1 or anti-PD-L1 treatment in many advanced tumors (76). Immune checkpoint inhibitors, drugs that inhibit PD1 or PD-L1, aim at exposing tumor cells to the antitumoral effects of the immune system by inhibiting the PD-L1-PD1

interaction. Several immune checkpoint inhibitor drugs are now U.S. Food and Drug Administration (FDA) approved for use in various cancer types. In 2017 FDA gave accelerated approval of anti-PD-1 cancer treatment (pembrolizumab) of cancers with MSI or dMMR, regardless of the tumor's original location (77). It can be presumed that this decision will make testing for MSI and dMMR in pathology laboratories more common in the future.

Many studies have analyzed the PD-L1 expression in prostate cancer by IHC (78–84). A major problem with these studies is that many different PD-L1 antibodies have been used, some of them research antibodies and others FDA-approved antibody clones available as prepackaged kits. Different staining platforms and immunostaining protocols have been used for different antibody clones. Adding to the difficulty of comparing PD-L1 expression in different studies, the scoring systems and cut offs for a positive PD-L1 test also differ depending on antibody clone. The wide range of reported PD-L1 expression in prostate cancer can probably to a large part be attributed to different IHC protocols and interpretation approaches (85). In initial reports of PD-L1 expression in prostate cancer, the results varied widely. In some studies, most tumor cells were shown to express PD-L1. Gevensleben *et al*. reported high PD-L1 expression in 61.7% of cases in a cohort of 611 prostate cancers (78). In another study of 535 radical prostatectomy specimens, PD-L1 staining in tumor cells was seen in 92% of cases (79). Massari *et al*. immunohistochemically investigated tumor material from 16 patients with castration-resistant prostate cancer and reported PD-L1 expression in 50% of cases (80). Other studies have shown distinctly lower PD-L1 expression in prostate cancer. Martin *et al*. showed focal areas of PD-L1 positivity (defined as 5% membrane staining) in cancer cells in 10% of prostatectomy specimens (81). Baas *et al*. investigated PD-L1 expression in tissue samples from prostatectomy or biopsy specimens of 25 men with high-grade prostate cancer. PD-L1 scoring of both cancer cells and inflammatory cells showed high expression in only two of 25 cases (82).

In three recently published papers, immunohistochemical testing for PD-L1 in prostate cancer has been performed using the validated antibody clone Ventana SP263, which is the complementary predictive test for the PD1 inhibitor pembrolizumab. In one study, PD-L1 IHC (antibody clone SP263) was performed on a TMA with 82 castration-resistant prostate cancer specimens and 96 localized prostate cancers. Only 3.7% of castration-resistant prostate cancer specimens were positive in tumor cells while 14.6% showed PD-L1 expression in tumor infiltrating immune cells. All localized prostate cancers were negative (83). In another study, tumor material from patients who had undergone radical prostatectomy after neo-abiraterone acetate plus prednisone and leuprolide (Neo-AAPL) treatment and 44 matched controls, were stained for PD-L1 (antibody clone SP263). Neo-AAPL–treated tumors showed less PD-L1 than matched controls (7% and 21%, respectively). Loss of MSH2 expression was observed in one of the 21 PD-L1-positive tumors (86). PD-L1 protein expression has also been investigated (antibody clone SP263) in a cohort of 539 primary prostate cancers,

prostate cancer metastases and castration-resistant prostate cancers (84). In primary prostate cancer PD-L1 staining was seen in 7.7% of cases. Interestingly, PD-L1 expression was much higher in prostatic small cell carcinoma (42.9%) and castration-resistant prostate cancer (31.6%).

1.10 PROSTATE CANCER GENETICS

The understanding of cancer biology has been revolutionized during the last few decades by the understanding that cancer is a genetic disease with abnormal and uninhibited growth of a clonal cell line (87). Rapid technological development in the fields of genetics and genomics has allowed us to study the stepwise development of cancer, key mechanisms that allow cancer to metastasize and invade surrounding tissues and how they evade and become resistant to therapeutic interventions. These techniques have also shed light on the heterogeneity of malignant tumors and has led to reconsiderations of the histogenesis, the tissue/cell of origin, of various tumors (88). The development of prostate cancer is related to the accumulation of genetic alterations in the luminal cells of prostatic glands during the patient´s life. Prostate cancer is considered to have a limited mutation burden and most genetic changes are copy number variations and structural gene rearrangements (89). In localized prostate cancer, TMPRSS2-ERG gene fusions are detected in almost 50% of cases (90). This fusion can occur through deletions on chromosome 21 or through a translocation and results in overexpression of ERG, a transcription factor (91). The TMPRSS2 gene is regulated by androgens. Many studies have evaluated if TMPRRSS2-ERG gene fusion influence patient prognosis and some have reported it to be associated with shorter survival (92,93). Whole-genome sequencing has shown loss-of-function mutations in SPOP in 11% and gain-of-function of FOXA1 in 3% of cases (94). In patients with localized disease, it has been difficult to differentiate low-risk cancers from high-risk cancers based on specific genetic abnormalities. Metastatic prostate cancer includes castration-sensitive prostate cancer (mCSPR) and metastatic castration-resistant prostate cancer (mCRPC). In mCRPC copy number variation and defects in DNA damage repair genes are more often seen resulting in a higher tumor mutation burden than in localized prostate cancer (4). mCRPC often show amplification of regulators of AR transcription or amplification of AR (95). In mCRPC, gain-of-function mutations of CTNNB1 has been reported in 4% of cases and TP53 and RB1 alterations in 50% and 21%, respectively (96).

The genetic profile of ductal adenocarcinoma is not fully characterized. It has been reported that copy number alteration levels are similar to those seen in high-grade prostate cancer (Gleason score 8-9) but lower than in metastatic prostate cancer (97). An increased number of mutations in DNA damage repair genes has been reported in ductal cancer compared to acinar cancer (98). TMPRSS2-ERG gene fusions have been reported to be less common in ductal cancer than in acinar cancer (98–100). In a recent study by Schweitzer *et al.*, using targeted next-generation DNA sequencing, 4 out of 10 (40%) patients with ductal cancer

showed signs of MSI-H. This is a small and preliminary study, but the authors suggested that MSI may be more common in ductal cancer than in acinar prostate cancer (101). The presence and characteristics of MSI in ductal prostate cancer is not yet well described in larger studies.

Every time a cell divides, approximately 100,000 polymerase errors occur. These are normally corrected through proofreading by polymerase enzymes (102). Still, some errors escape proofreading, and these are normally corrected through the mismatch repair (MMR) system. Tumors with defects in the mismatch repair system will develop numerous frameshift mutations in coding and non-coding microsatellites and at other genetic loci beyond the microsatellites, so called hypermutability (103). This is also known as the microsatellite instability-high (MSI-H) phenotype, and it is closely related to the carcinogenesis of hereditary and sporadic tumors (103). Malignant tumors with MSI occur as sporadic cancer in acquired (somatic) MSI and as hereditary cancer in patients with a germline mutation in one of the MMR genes. Deficient DNA mismatch repair (dMMR) secondary to germline mutation (Lynch Syndrome) results from mutational inactivation of both alleles of one or more of the genes involved in the MMR system (103). Epigenetic inactivation of MMR genes is more common in sporadic tumors with MSI (104). The epigenetic inactivation of MMR genes in sporadic MSI is usually caused by epigenetic inactivation of the MLH1 promoter (105). The numerous frameshift mutations in coding sequences seen in MSI gives rise to the production of abnormal proteins in the tumor, ”neoantigens” (106). Neoantigens can be recognized as ”non-self” molecules by the immune system through expression of neoantigens on cell surface HLA-complexes interacting with antigen-specific T cells (107). This leads to an anti-tumoral immune response through the infiltration of activated neoantigen-specific cytotoxic and T helper cells (108).

The genetic instability of MSI can be detected by using PCR to compare the length of nucleotide repeats in tumor cells and cells from normal tissue (103). Immunohistochemical analysis of four MMR proteins (MLH1, MSH2, MSH6 and PMS2) is often used as an alternative to PCR-based MSI-detection methods to detect MMR deficiency in a routine clinical setting (109). IHC for MMR proteins has a performance similar to MSI testing with a concordance rate of 100% specificity in MSI-H tumors (110). Deficient expression of one or more of MMR proteins on IHC indicates dMMR. IHC measures the protein expression which allows identification of which MMR genes are likely mutated, but it can also identify inactivation through hypermethylation. This is an advantage of IHC to PCR-based MSI testing which only detects DNA mutations. However, in around 5% to 11% of MSI cases, IHC will not show loss of MMR proteins. This is because missense mutations in the MMR gene will cause the production abnormal MMR-proteins that are expressed but functionally inactive (110). Next-generation sequencing (NGS) has emerged as a new technique to

assess MSI. It uses targeted gene sequencing or whole exome/genome sequencing techniques and compares sequencing reads from microsatellite regions in the tumor and matched normal tissue, or counts mutations identified in exons (111,112).

In prostate cancer, MSI and dMMR have been reported in a subset of tumors ranging from around 1% of primary cancers to up to 12% of metastatic cancers (111). In a meta-analysis including 23 studies, the risk of developing prostate cancer in patients with Lynch syndrome was investigated. The meta-analysis reported the risk ratio estimated for prostate cancer in Lynch syndrome patients to be 3.67, thus indicating that men carrying a DNA mismatch repair (MMR) gene mutation have an increased risk for prostate cancer (113). In a study by Rosty *et al.* dMMR was observed in 69% of prostate cancers from patients with Lynch syndrome with the highest prevalence of tumors in MSH2 mutation carriers (114). Other studies have investigated MSI/dMMR in sporadic prostate cancer. Kumar *et al.* described three hypermutated cancers in a series of 23 prostate cancer cases using gene sequencing (115). In a multi-institutional sequencing study investigating mCRPC, hypermutation and alterations in the mismatch repair pathway genes MLH1 or MSH2 were identified in four of 150 cases (116). Pritchard *et al.* has described hypermutation in 11.6% of advanced prostate cancers. The mutations were frequently MSH2 or MSH6 structural rearrangements rather than MLH1 epigenetic silencing (117). Nghiem *et al.* has reported deficient staining for MMR-proteins in primary prostate cancers in 5.0%, 15.0%, 17.8% and 11.2 % for MLH1, MSH2, MSH6 and PMS2, respectively (118). Guedes *et al.* investigated 1113 primary prostatic adenocarcinomas and 43 prostatic small cell carcinomas with screening for defect MSH2 using IHC followed by next-generation sequencing for confirmation. They showed that 1.2% (14/1176) of cases had MSH2 loss. Of cases with primary Gleason grade 5 (Gleason score 9-10), 8% (7/91) had MSH2 loss compared with only 0.4% (5/1042) of adenocarcinomas with other Gleason scores. Of prostatic small cell carcinomas, 5% (2/43) had MSH2 loss (119). In a recent study, molecular tumor profiling was performed on 1033 prostate cancers. 3.1% of cases showed MSI-H/dMMR while an additional 2.2% showed only MSI-H. 21.9% of MSI-H/dMMR cases had Lynch syndrome. The authors concluded that the MSI-H/dMMR molecular phenotype is uncommon but could be relevant for potential anti–PD-1/PD-L1 treatment (120).

2 RESEARCH AIMS

In Study I the aim was firstly to describe systematically how often prostate cancers can be identified macroscopically on the cut surface of bisected unfixed prostatectomy specimens. Secondly, to investigate how well macroscopic findings of cancer correlate to microscopic findings. Thirdly, to describe the features of macroscopically identified tumors such as color, size, localization and tumor demarcation.

In Study II the aim was to establish and validate a new biobanking technique for harvesting of unfixed prostate cancer and benign prostate tissue from radical prostatectomy specimens. The aim was also to validate that the biobanking technique did not jeopardize reporting of clinical histopathological parameters. Furthermore, we wanted to evaluate if the application of cryogel to tissues before snap freezing would improve the histomorphological quality of frozen sections. Finally, we wanted to evaluate if the harvested tissue was of high quality, as measured by RNA integrity number (RIN).

In Study III the aim was firstly to investigate and describe how common PD-L1 expression in tumor cells and tumor infiltrating immune cells is in acinar and ductal adenocarcinoma of the prostate. Secondly, we wanted to determine the frequency of deficient mismatch repair (dMMR) in acinar and ductal adenocarcinoma. Lastly, we wanted to describe the characteristics of tumor infiltrating immune cells in acinar and ductal adenocarcinoma.

The aim of Study IV was to investigate if acinar and ductal adenocarcinoma in mixed prostate cancer had arisen from a common somatic denominator or if they were clonally independent. Also, we wanted to compare the genetic characteristics of acinar and ductal adenocarcinoma.

3 MATERIALS AND METHODS

"The finest and healthiest thing about science is, as in the mountains, the brisk air blowing around in it"

—Friedrich Nietzsche

3.1 TISSUE COLLECTION AND PREPARATION

In Study I, 514 unfixed radical prostatectomy specimens accessioned at the Karolinska University Hospital during the period 2002-2010 were included. None of the patients had received pre-operative hormone treatment. In Study II, 20 consecutive radical prostatectomy specimens accessioned at the Karolinska University Hospital during a period of one month from December 2011 to January 2012 were included. In Study III, a tissue microarray (TMA) including 76 prostate cancer cases from Karolinska University Hospital and Aquesta Uropathology, Brisbane, Australia was utilized. In Study IV, 51 prostatectomy specimens with ductal adenocarcinoma from Aquesta Uropathology, Brisbane, Australia were included in the initial morphological assessment.

Study I

The prostatectomy specimens were received at the pathology laboratory directly from the operation theatre. The specimens were bisected at the level of palpable nodules, positive pre-operative biopsies or at the junction between the mid and apical third of the prostate. Photographs of the cut surfaces were taken. The cut surface was inspected by a single investigator (Lars Egevad) and if lesions were visually identifiable, they were categorized as conclusive or suspicious for cancer. The size, color and demarcation of the identified lesions were described.

Study II

Directly upon arrival to the department of pathology, a full thickness horizontal tissue slice was cut through the prostatectomy specimens utilizing a custom made two-bladed knife. When a nodule was palpable, the cut was made at the level of the nodule. Otherwise, the cut was made at the level between the lower and middle thirds of the prostate. The horizontal slice was then cut into 4-12 smaller tissue segments. The tissue segments were numbered according to location. The tissue segments were embedded in Tissue-Tek OCT compound (Sakura Finetek, AJ Alphen aan den Rijn, The Netherlands), *Figure 2*. Following this, the tissue blocks were snap frozen in liquid nitrogen and stored in -80°C. Frozen sections were cut and stained with HE. The microscopic slides were scanned, and entire horizontal tissue slices were digitally reconstructed. The cutting time for the frozen sections was noted. Microscopic examination followed and tumors, positive surgical margins, perineural invasion, HGPIN and areas of extraprostatic extension were outlined with India ink.

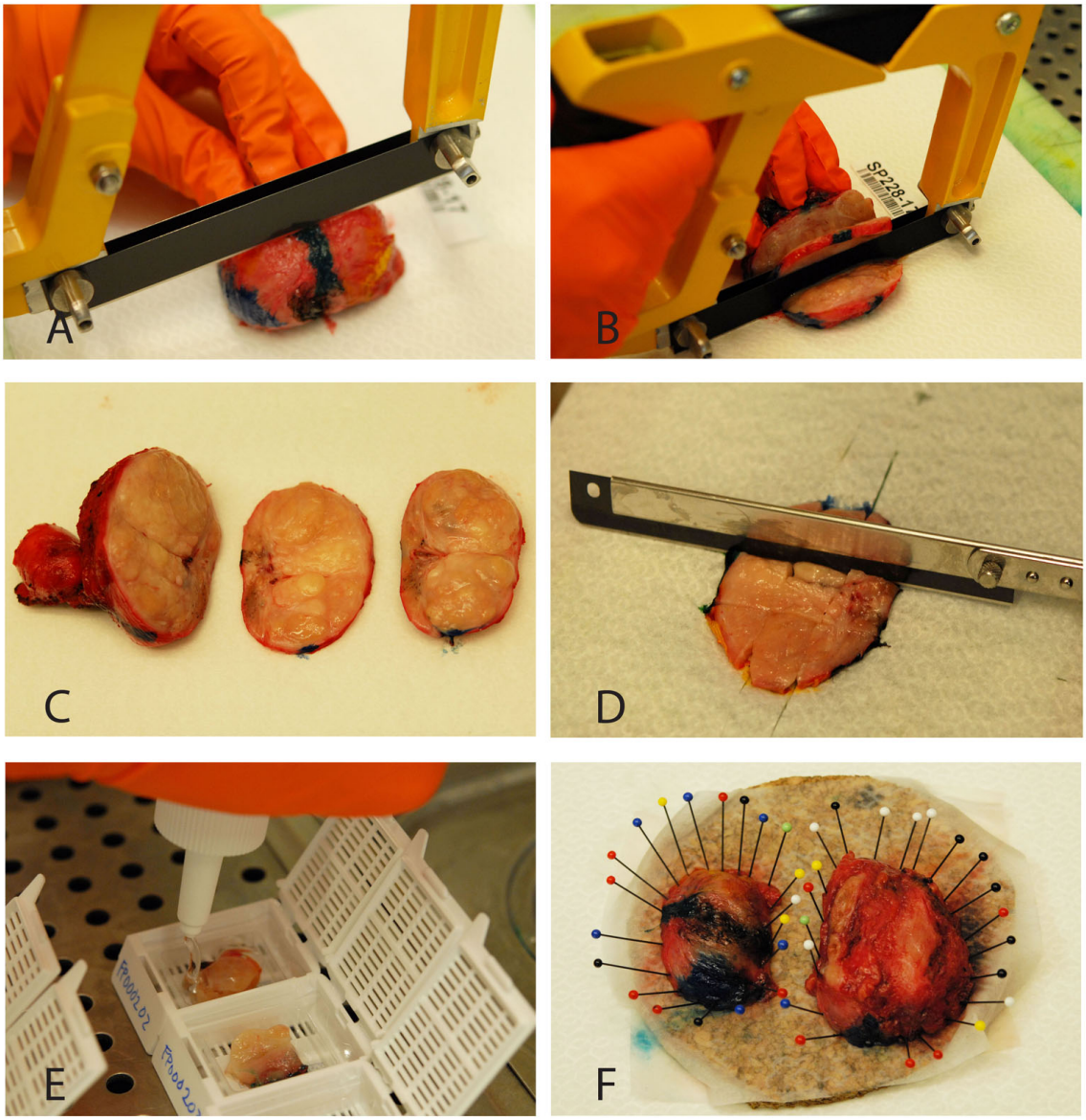


Figure 2. Using a double-bladed knife, a 4-mm-thick full horizontal tissue section was cut at the level between the lower and middle thirds of the prostate or at the level of a palpable nodule (A–C). The horizontal slice was divided into 4–12 smaller segments (D), the tissue was embedded in OCT gel in plastic cups fitted in separate cassettes and snap-frozen in liquid nitrogen (E). The remaining bisected prostatectomy specimen was mounted by pinning the edges to cork plates, and fixed in formalin (F).

Study III

A tissue microarray (TMA) consisting of 115 formalin-fixed tumors (34 ductal adenocarcinomas, 42 acinar adenocarcinomas and 39 malignant tumors from other organs) was used for producing immunohistochemical and HE-stained slides.

Study IV

Laboratory records from 2012 to 2019 at Aquesta Uropathology, Brisbane, Australia, were

searched for radical prostatectomy specimens with ductal adenocarcinoma. In total, 51 prostatectomy specimens with ductal adenocarcinoma were identified. Microscopic slides from these cases were reviewed. After a first selection, sections were recut, stained by HE and reviewed again by two pathologists who assessed if there were sufficient volumes of acinar and ductal cancer tissue for genetic investigations. The criteria for classifying a tumor as ductal adenocarcinoma were: tall columnar tumor cells with prominent nucleoli, pseudostratification, elongated nuclei and cribriform or papillary architecture. Also, in order to enable microdissection of pure tumor tissue from the acinar and ductal components, only cases where the two cancer components were spatially separated in mixed tumors were selected for the study. Cases where the acinar and ductal cancers intermingled were excluded from the study to avoid contamination in the genetic analysis. Semiquantitative assessment of the tumor purity of the acinar and ductal cancer components was performed in tiers of 10%. Samples with a cancer cell fraction larger than 20% was accepted for the study and microdissected, *Figure 3*. 17 cases remained for DNA extraction, but two of the cases were excluded due to low DNA yield, leaving 15 cases for analysis.

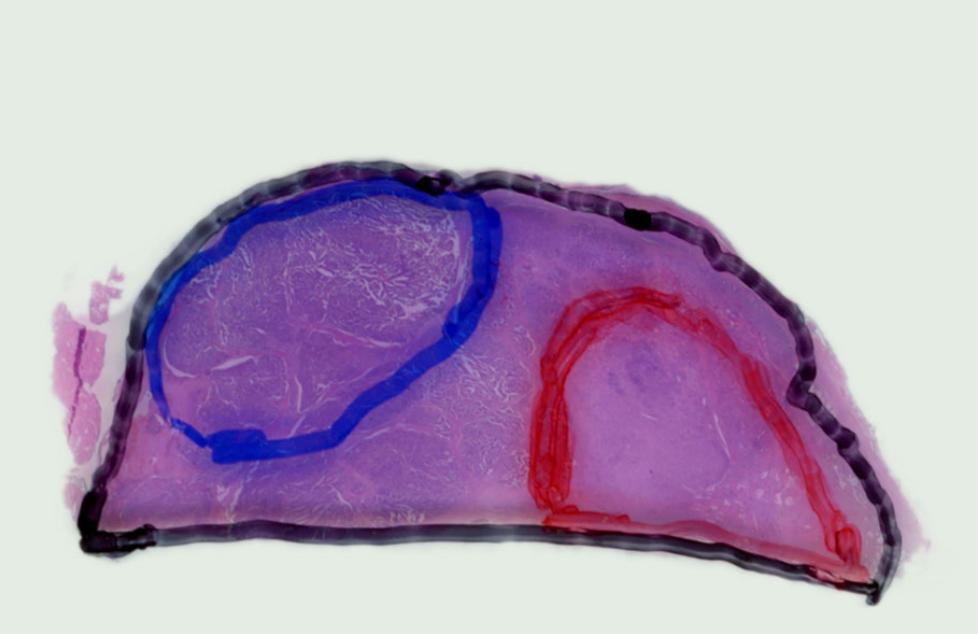


Figure 3. The acinar and ductal adenocarcinoma components were outlined with India ink. Only cases with spatially separated and morphologically distinct areas of acinar and ductal adenocarcinoma were included. Black: cancer; blue: ductal adenocarcinoma; red: acinar adenocarcinoma. Hematoxylin and eosin.

Handling of prostatectomy specimens

In all studies the prostatectomy specimens were formalin fixed by injecting at least 20 ml of formalin as previously described (121). After fixation, the prostates were completely inked with four colors (left, anterior, right, and posterior). The surfaces where the seminal vesicles had been attached were not inked. The halves of the bisected prostate were mounted by pinning the edges to cork plates (*Figure 2F*) followed by overnight fixation in formalin which

was kept circulating by a magnetic stirrer. After completed fixation, the prostatectomy specimens were sliced at 4-mm intervals, totally embedded, cut at 4 µm, whole-mounted, and stained with HE.

3.2 TISSUE MICROARRAY

Tissue microarray (TMA) is a method that facilitates immunohistochemical investigations of a large number of tumors in a single paraffin block. The technique was first described in 1986 and has the advantages of being cost efficient and allowing a standardized approach (122). In a TMA all tissue cores are exposed to the same immunohistochemical staining conditions. The disadvantage of the technique is that it only allows analysis of limited parts of tumors and that assessment of, for example, tumor heterogeneity, different tumor components and tumor stroma is difficult. When constructing a TMA, investigators start by reviewing HE-stained slides to identify and outline tissue components of interest, such as cancer. The outlined areas are then punched out from the donor tissue blocks and transferred to holes in recipients blocks. The cores are arranged in asymmetrical rows to allow identification of individual tissue cores. Histological sections are cut from the TMA blocks and stained histochemically or immunohistochemically.

For Study III we used a TMA that was constructed by our group for a previous study (123). One core of tumor tissue had been sampled from each case, in total 115 tumors were included, comprising 34 prostatic ductal adenocarcinomas, 42 prostatic acinar adenocarcinomas and 6 colorectal, 7 endometrial, 7 pulmonary, 5 pancreatic, 5 gastric and 9 urinary bladder adenocarcinomas. The cores were arranged in two paraffin blocks.

3.3 IMMUNOHISTOCHEMISTRY

In Study III, IHC was used to investigate protein expression in tumor cells and tumor infiltrating immune cells. Sections from a TMA were stained using an automated IHC system (Ventana Medical Systems, Tucson, AZ, USA). Staining with antibodies to MLH1, MSH2, MSH6, PMS2, PD-L1 (Clone SP263, Ventana Medical Systems Inc., Tucson, AZ, USA), PD-L2 (Clone D7U8C, Cell Signaling Technology, Inc., Danvers, MA, USA), CD4 and CD8 (Agilent Technologies, Inc., Santa Clara, CA, USA) was performed following the manufacturer's protocol for each antibody. Sections from normal tonsillar tissue were used as external controls for PDL-L1 and PD-L2 IHC. All antibodies were processed using a CC1 agent, a Tris-based buffer with pH 8.5 and heating for 32 (CD4, CD8), 40 (MLH1, MSH2), 64 (PD-L1, PD-L2), 72 (PMS2) or 80 (MSH6) minutes for deparaffinization and antigen retrieval. For visualization of immune binding, sections were incubated using an OptiView DAB IHC Detection Kit, according to the manufacturer's instructions (Ventana Medical Systems Inc., Tucson, AZ, USA). An amplification kit (Ventana Medical Systems Inc.,

Tucson, AZ, USA) was used to increase the signal for MLH1, MSH2, MSH6, PMS2, CD8 and PD-L2 stains. All sections were counterstained with hematoxylin.

Immunohistochemical stains for MMR proteins (MLH1, PMS2, MSH2, MSH6) were graded as positive or negative nuclear staining on microscopic evaluation. If the immunohistochemical staining was negative in both tumor cell nuclei and in the surrounding epithelial/stromal/endothelial/inflammatory cell nuclei, the staining was judged as indeterminate, indicating that it could not be ruled out that the staining was technically unsatisfactory. The PD-L1 and PD-L2 stains were semiquantitatively scored as negative or positive in 0-5% of cells, 5-10% of cells and thereafter in 10% intervals. The number of CD4 and CD8 positive immune cells per tissue core was counted and reported in the intervals <50 cells/core, 50-99 cells/core, 100-199 cells/core and >200 cells/core.

3.4 RNA INTEGRITY NUMBER ANALYSIS

The quality of unfixed tissue harvested for biobanking purposes is often assessed through RNA Integrity Number analysis (RIN) analysis and assigned a RIN value from 1-10 (124). If the RNA is completely degraded the value will be 1 and if it is completely intact the RIN value will be 10. The higher the tissue quality is, the higher the RIN value will be. In Study II we analyzed the RIN values of biobanked prostate tissue in order to safeguard that our novel biobanking technique supplied tissue material of high quality. We analyzed RINs in 26 samples of formalin-fixed paraffin-embedded tissue, 197 unfixed samples that had been biobanked with an earlier used biobanking technique based on visual sampling, and 41 unfixed samples that had been biobanked using the new whole-slice technique (including the 20 cases described in Study II). RNA was extracted from snap frozen or paraffin-embedded tissue with the Allprep kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. 1 µl of extracted RNA was analyzed and RIN values were retrieved by use of an Agilent 2100 Bioanalyser and RNA LabChips (Agilent, Santa Clara, CA, USA).

3.5 GENETIC STUDIES

In Study IV, DNA was extracted from acinar and ductal cancer components using AllPrep FFPE kit (Qiagen, Venlo, Netherlands). Kapa DNA hyper (Roche) was used to construct libraries for sequencing. Targeted sequencing, with a design optimized for prostate cancer, was performed. Baits (120 bp oligos) were obtained from Twist Bioscience (San Francisco, USA). The baits were designed to target unique regions in the human genome commonly altered in prostate cancer. The design targeted approximately 3000 common SNPs enabling genome wide copy-number alteration profiling and ploidy assessment. The panel had a genomic footprint of 1.39 Mb and allowed identification of genomic structural

rearrangements, somatic and germline small variants, genome-wide copy-number alterations, assessment of ploidy, cancer cell purity, DNA repair and homologous recombination repair (HRR) genes, tumor mutation burden and microsatellite instability. An AutoSeq pipeline was applied for bioinformatic processing and manual curation of data. Visualization of gene variants was performed in an integrated genomics viewer (125). For two cases, germline DNA could not be isolated and white blood cell DNA from an anonymous healthy donor was used as germline DNA reference.

3.6 STATISTICAL ANALYSIS

Statistical analyses were performed using R statistics software version 3.5.2 (The R Project for Statistical Computing, Vienna, Austria) or SPSS software version 23.0.0.0 (SPSS, USA). In Study I, differences in proportions were compared using a chi-square test. To compare means, paired t-tests were used. In Study II confidence intervals for the means were calculated using one-sample t-tests. Unpaired t-tests were used to compare differences in means. In Study III, results were reported in contingency tables. Formal comparisons for 2 x 2 and 2 x k contingency tables were based on Fisher's exact test. In Studies I, II and III significance was defined as a $p < 0.05$. In Study IV, bioinformatics was performed in an integrated genomics viewer by specialized bioinformaticians. Integrated with this process, statistical analysis was performed in R (126).

3.7 ETHICAL CONSIDERATIONS

Study I-IV were approved by the Regional Ethic Review Board, Stockholm (Dnr 2001/353, 2006/1014-31, 2009/780-31, 2010/710-31/2, 2013/1451-32, 2018/827-32) and the Aquesta Ethics Committee, Brisbane, Australia (AQ376421, AQ421010).

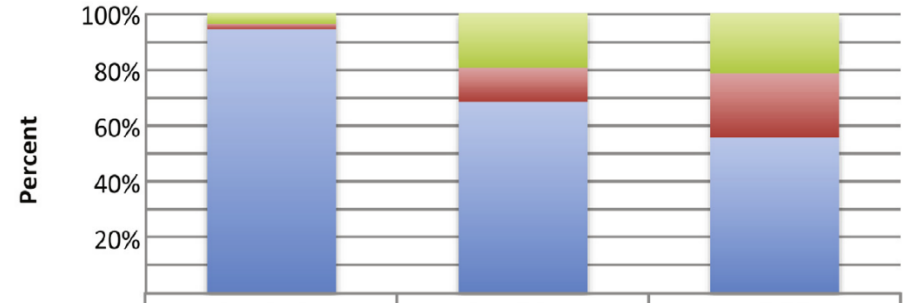
4 RESULTS AND DISCUSSION

"I am turned into a sort of machine for observing facts and grinding out conclusions"

—Charles Darwin

4.1 PAPER I: MACROSCOPIC FEATURES OF PROSTATE CANCER

In Study I we investigated the macroscopic features of 514 unfixed prostatectomies. Of the 514 cases in the study, 52% had macroscopic findings conclusive for cancer and 24% had findings suspicious for cancer. Of cases with gross findings conclusive for cancer, 94% showed microscopically substantial cancer, *Figure 4*. Among cases with findings suspicious for cancer on macroscopic examination, 69% showed substantial cancer on microscopic examination. Of substantial prostate cancers (≥2 mm diameter), 42% could be confidently identified macroscopically. In cases without macroscopic findings of cancer, 56% still revealed substantial tumors on microscopic evaluation. Microscopic examination showed substantial cancer at the level of bisection in 84% of all cases and minimal cancer in 10%.



	Conclusive	Suspicious	Invisible
No cancer	3.7%	19.4%	21.3%
<2 mm	1.9%	12.1%	23.0%
≥2 mm	94.4%	68.5%	55.7%

Figure 4. Distribution of microscopic cancer in cases with macroscopic findings conclusive or suspicious for cancer or no visible tumor.

Of lesions conclusive for cancer on macroscopic examination with microscopic findings of cancer, 30% were tan, 30% white, 16% yellow and 24% orange, *Figures 5-8.* Prostate cancers located in the TZ were more likely to be yellow or orange than cancers of the PZ ($p < 0.001$), *Figure 8*. Ten of the cases with macroscopic findings conclusive for cancer did not show any tumor on microscopic examination. In these false positive cases microscopy revealed inflammation, postatrophic hyperplasia or stromal hyperplasia. Of substantial tumors not identified macroscopically, 57% were located in the PZ and 35% in the TZ compared to 73% and 18%, respectively in the whole series. Thus, substantial TZ cancers were less

frequently identified compared with substantial PZ cancers. High-grade cancers (Gleason score ≥4+3=7) were more likely to be identified macroscopically than low-grade tumors (Gleason score ≤3+4=7) ($p = 0.001$). All identifiable tumors in the study were poorly demarcated and assessment of the extension of macroscopically identified tumors underestimated true, microscopic tumor size ($p < 0.001$).

The number of large systematic studies of the macroscopic features of prostate cancer in prostatectomy specimens is very limited (13,14). A reason for this could be that radical prostatectomy surgery rarely was performed before the 1980s. Even after the introduction of radical surgery as a standard therapy, only a few studies have been published reporting the frequency of macroscopically visible tumors in unfixed prostatectomy specimen. The few descriptions in the literature lack detailed, systematic reporting of tumor characteristics such as tumor color, zonal distribution, tumor demarcation and correlation between gross and microscopic findings (14–16,25). This is all the more surprising taking into consideration that many biobanking protocols for harvesting of unfixed prostate cancer tissue is based on macroscopic identification of tumors (66). Macroscopic findings conclusive for cancer, assessed by an experienced urological pathologist, in unfixed prostatectomy specimens was highly predictive for microscopic findings of cancer in the same foci. 52% of cases showed macroscopic findings conclusive for cancer and microscopic examination confirmed prostate cancer in 96% of these lesions. Prostate cancer typically shows a highly infiltrative growth pattern. In all macroscopically identified cancers the tumors were poorly demarcated on gross examination. The infiltrative growth pattern of prostate cancer, often growing between and around benign glands, also corresponds well with the findings that macroscopic examination underestimated the true tumor size. The poor demarcation of prostate cancer and the fact that tumors often are tan, similar to surrounding benign tissue, could explain why prostate cancer can be difficult to identify macroscopically.

Cancers with higher Gleason scores (≥4+3=7) were more likely to be identified macroscopically compared to cancers with lower Gleason scores (≤3+4=7). High-grade cancers are more cellular with more desmoplastic stroma reaction (13). This could explain why high-grade tumors are more readily identified on visual inspection, *Figure 6*. Tumors in the TZ were less likely to be identified on gross examination compared to tumors in the PZ. Patients undergoing prostatectomy are often of an age where benign prostatic hyperplasia is common. Benign prostate hyperplasia could mask TZ-cancers on gross examination through its nodular structure. We noted cases of TZ cancer growing within nodules of benign hyperplasia, making macroscopic detection very difficult, *Figure 5*. Macroscopically identified tumors in the TZ were more often orange or yellow compared to tumors in the PZ. A possible explanation may be that tumors in the TZ often display tall, columnar tumor cells with abundant cytoplasm, *Figure 8*.

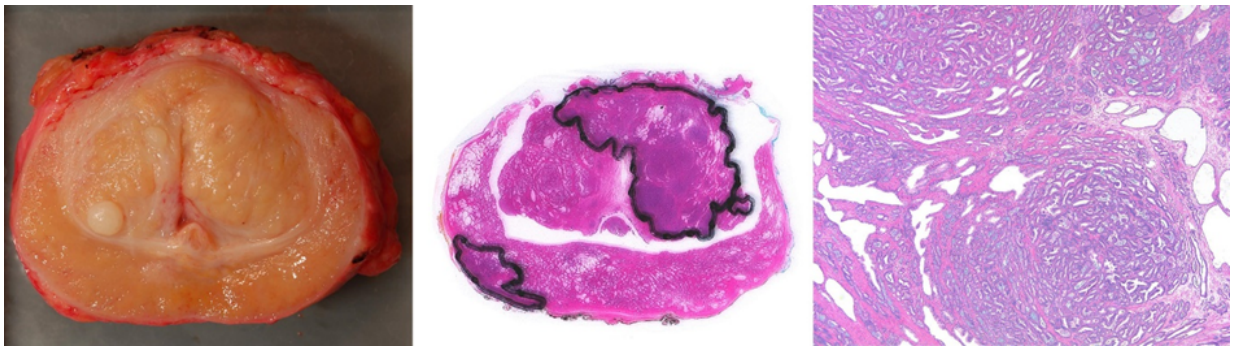


Figure 5. Macroscopic identification of transition zone cancers is often difficult. Nodular growth pattern within pre-existing benign prostatic hyperplasia. Gleason score 3+4=7.

Figure 6. Peripheral zone prostate cancer with tan cut surfaces. Gleason score 4+5=9.

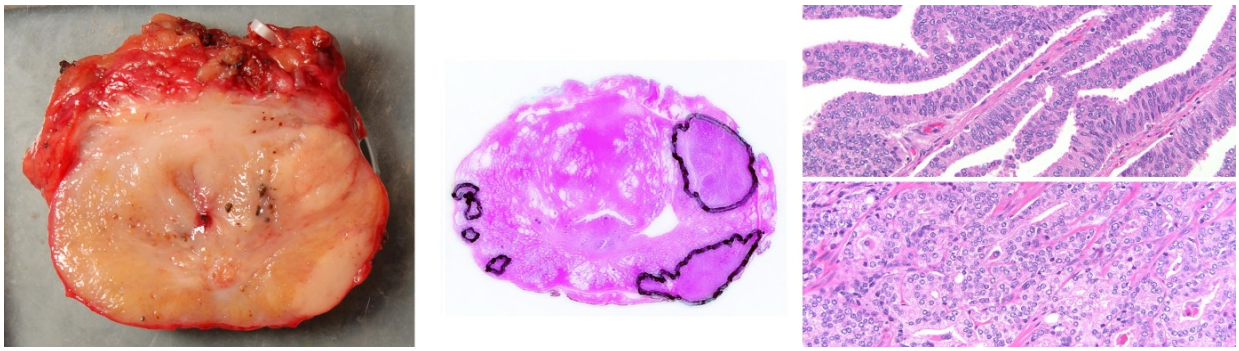


Figure 7. Prostate cancers with white cut surfaces. Posterolateral, right: Acinar adenocarcinoma, Gleason score 4+3=7. Anterior, right: Ductal adenocarcinoma, Gleason score 4+4=8.

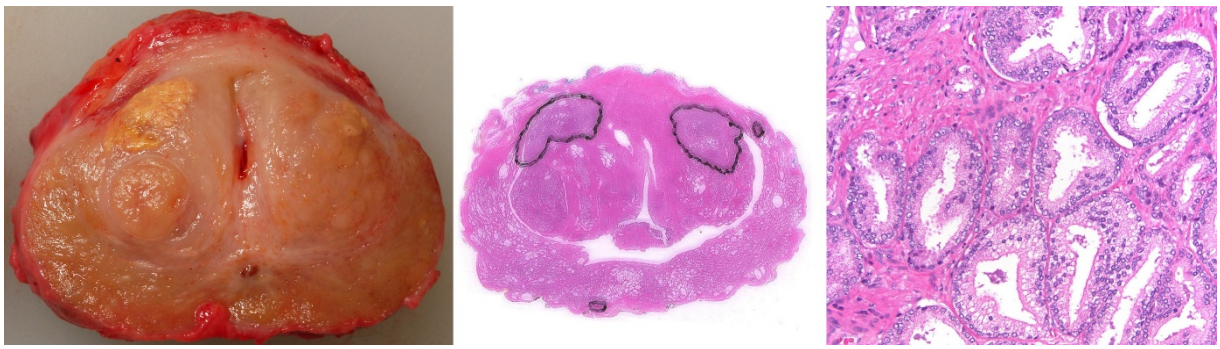


Figure 8. Prostate cancer with orange cut surfaces. Transition zone cancer with abundant cytoplasm, Gleason score 3+3=6.

A limitation of the study is that the gross examination was performed by a single, expert urological pathologist. In the routine laboratory setting, biobanking would probably be performed by multiple residents and technicians. It is possible that the accuracy of gross examination could be worse under such circumstances. The findings that 42% of cancers 2 mm or larger were not conclusively identified at macroscopic examination, that high-grade tumors were easier to identify and that TZ tumors were less likely to be identified, suggest that biobanking based on visual inspection may lead to a sampling bias. This finding is directly related to our novel biobanking technique that is described in Study II.

4.2 PAPER II: A NOVEL TECHNIQUE FOR BIOBANKING OF LARGE SECTIONS OF RADICAL PROSTATECTOMY SPECIMENS

In Study II we described a new biobanking protocol that facilitates harvesting of a large volume of tumor and benign tissue from prostatectomy specimens by utilizing a custom-made double-bladed knife. In 18 of 20 cases in the study, cancer was sampled in the biobanked tissue slice. Prostate cancer was present in 72 of 155 of tissue blocks. The morphological quality of the frozen sections produced with the protocol was excellent, *Figure 9*. We attributed the well-preserved morphology to the coating of tissue segments in Tissue-Tek OCT compound before freezing. Histological details such as HGPIN (14 cases), perineural invasion (seven cases), extraprostatic extension (four cases), positive margins (one case) and multifocal tumors (seven cases) were identifiable in frozen sections. By combining this novel biobanking method with laser capture microscopy or other dissection techniques it could be possible to correlate reginal morphology and genetic alterations. The high morphological quality also enables identification and microdissection of cancer precursors and specific tumor components. When harvesting large amounts of prostatic tissue for biobanking purposes, it is important that reporting of clinical parameters such as surgical margins, is not imperiled. With this protocol, the entire surgical margin and extraprostatic tissue was sampled. It was possible to produce frozen sections from the individual tissue segments and reconstruct a complete horizontal slice to report Gleason grade, tumor location, multifocality, tumor stage and surgical margins, *Figure 10*. Also, in cases where no cancer can be identified in the non-biobanked part of a prostatectomy specimen, pT0-tumors, frozen sections could be produced from all tissue sections for tumor identification.

Numerous biobanking protocols have been described in the literature (57). The most traditional way to biobank tumor tissue in pathology is based on visual inspection and identification of tumors. In cases where the investigator can identify tumor foci, the technique usually supplies enough tumor tissue. But prostate cancer can be difficult to identify grossly, and this approach most often samples only the largest tumor focus. This means that multifocal tumor foci and precursor lesions are not sampled. Other published prostate cancer biobanking protocols includes multiple punch or core needle biopsies, the removal of the central regions of prostatectomy specimens and cytology-based approaches (59–

63,68,69,127). Biobanking protocols where a full slice of prostatic tissue is harvested and snap frozen have also been published (70–72).

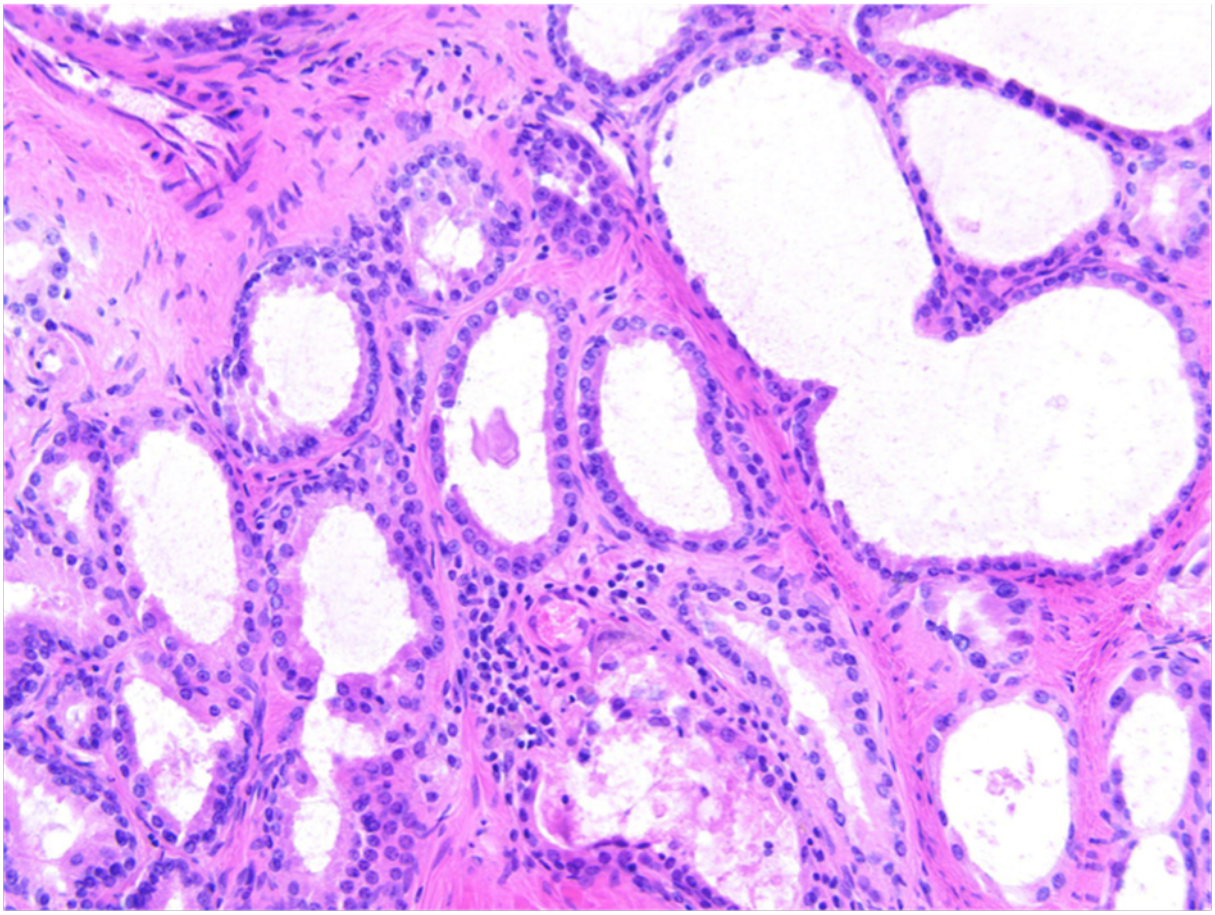


Figure 9. Frozen section from Study II. The micromorphological quality and morphology was excellent. The high quality of the frozen sections allowed detected of various clinically relevant histological details.

With our new technique, the full slice of prostatic tissue was divided into smaller pieces that were snap frozen individually. This facilitates that individual tissue blocks can be used for research studies without the need to bring out the entire tissue material from the freezer with the accompanying risk of thawing and DNA/RNA degradation. The technique facilitates the study of multifocal tumors, tumor heterogeneity, precursor lesions and allows the identification of index tumors. Also, the novel protocol allows in-depth studies of tumor associated stroma and tumor infiltrating immune cells. With this technique we will be able to sample small, multifocal tumors that would normally not be identified and sampled in biobanking techniques based on macroscopic identification of tumors. The quality of the harvested tissue was excellent with high RNA quality (RIN), making it optimal for research studies. A challenge with the protocol was that cutting frozen sections from numerous tissue blocks was time-consuming. On the other hand, the protocol is standardized and can be performed by a trained laboratory technician.

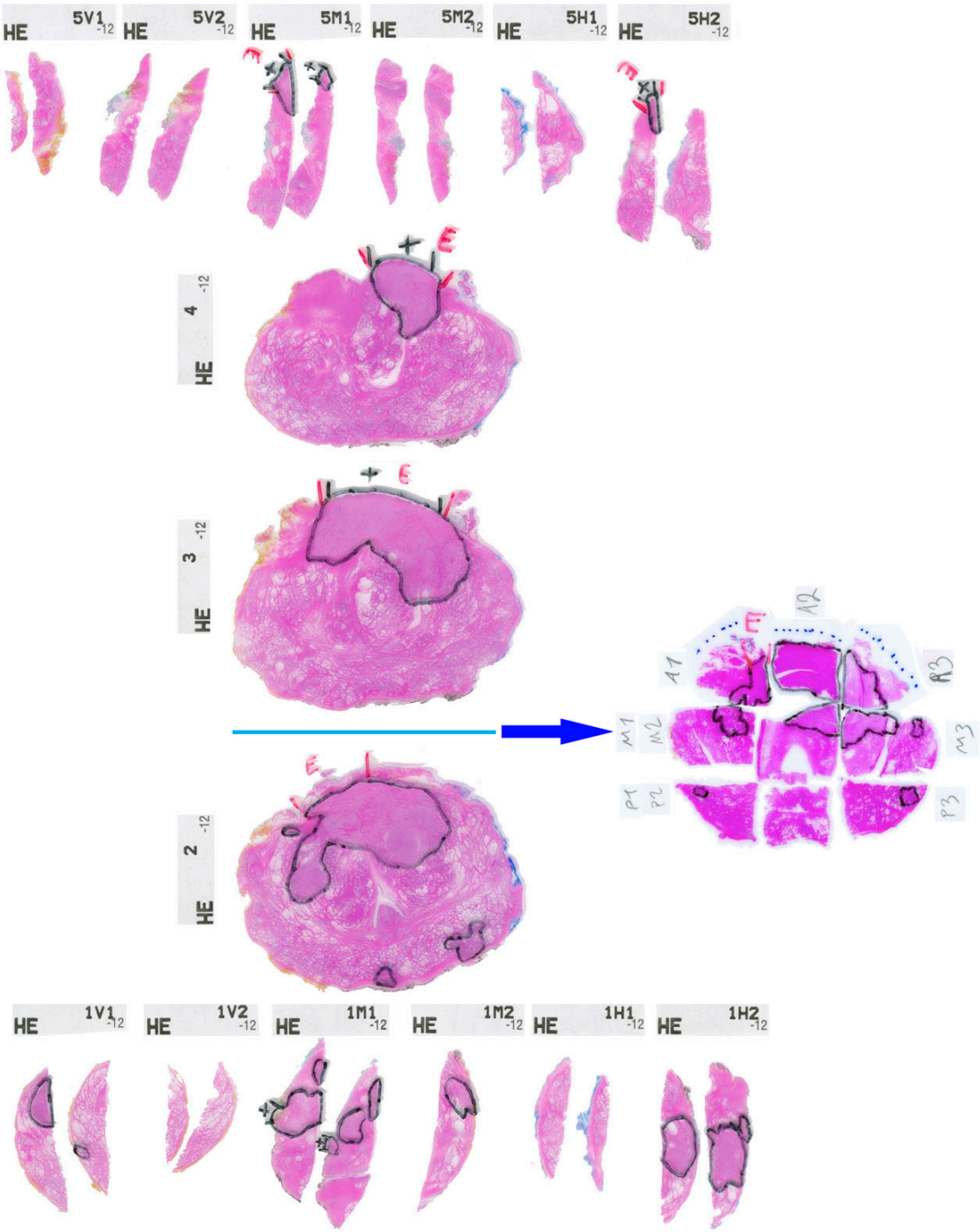


Figure 10. A scanned tumor map of a prostatectomy specimen from Study II. A full horizontal tissue slice had been biobanked between sections 2 and 3. The biobanked slice was divided in nine tissue segments, snap-frozen, frozen-sectioned and reassembled. A large anterior tumor with anterior extraprostatic extension and positive surgical margins is outlined with India ink.

4.3 PAPER III: PD-L1 EXPRESSION AND DEFICIENT MISMATCH REPAIR IN DUCTAL ADENOCARCINOMA OF THE PROSTATE

In Study III we showed that PD-L1 expression in acinar and ductal prostate cancer cells was a rare event with expression in only 4% (3/76) of cases. PD-L1 positivity in tumor infiltrating immune cells was more common. It was identified in 21% of cases (16/76), in 29% of ductal adenocarcinomas and 14% of acinar adenocarcinomas, *Figure 11A-C, E, Table 1*. The number of cases with deficient mismatch repair (dMMR) was investigated using IHC for MLH1, PMS2, MSH2 and MSH6. dMMR, defined as the loss of one or more MMR-proteins, was seen in 5% (4/73) of cases, three in acinar adenocarcinoma (in all cases loss of MSH6) and one ductal adenocarcinoma (loss of both MSH2 and MSH6), *Figure 11 F, Table 2*. In one ductal adenocarcinoma, the immunohistochemical stain for MSH6 was indeterminate and in another the stains for both MSH2 and MSH6 were indeterminate. One case of ductal adenocarcinoma and two cases of acinar adenocarcinoma were excluded due to insufficient tissue material. PD-L1 positivity was identified in one of the cases that exhibited dMMR. No significant association between PD-L1 expression and ISUP grade was identified. Immunohistochemical analysis of tumor infiltrating lymphocytes showed an increased number of CD8+ lymphocytes in ductal adenocarcinoma compared with acinar adenocarcinoma ($p = 0.04$), *Figure 11 D*. In contrast, there was no significant difference in the number of CD4+ lymphocytes in ductal vs acinar adenocarcinoma ($p = 0.28$).

In 1957 Sir Macfarlane Burnet formulated "The Concept of Immunosurveillance "- the idea that the immune system eliminates malignant cells and thereby protect the body from tumor development (128). Thus, tumors need to develop mechanisms to evade immune surveillance in order to successfully grow and spread in the host. The ability of cancers to avoid destruction of the immune system has received increased attention lately due to the introduction of immune checkpoint inhibitors in oncological treatment. Treatment response to immune checkpoint inhibitors has been shown to be associated to high expression of PD-L1 in several types of solid cancer (129) and if high expression of PD-L1 in primary or castration-resistant prostate cancer would be demonstrated, it could be hypothesized that also prostate cancer may be targeted with this treatment strategy.

Comparing studies of PD-L1 expression in prostate cancer is problematic because there are several validated, commercial antibodies but also various research developed antibodies in use. Differences in laboratory IHC protocols and staining platforms can affect the outcome of the staining process. To complicate matters more, there is as a lack of standardization of scoring systems and cut off criteria for what constitutes a 'positive' PD-L1 test. Taken together, it is very difficult to make any far-reaching conclusions when comparing reported staining results. Pre-analytic variables such as tissue fixation can also influence immunohistochemical staining results (130).

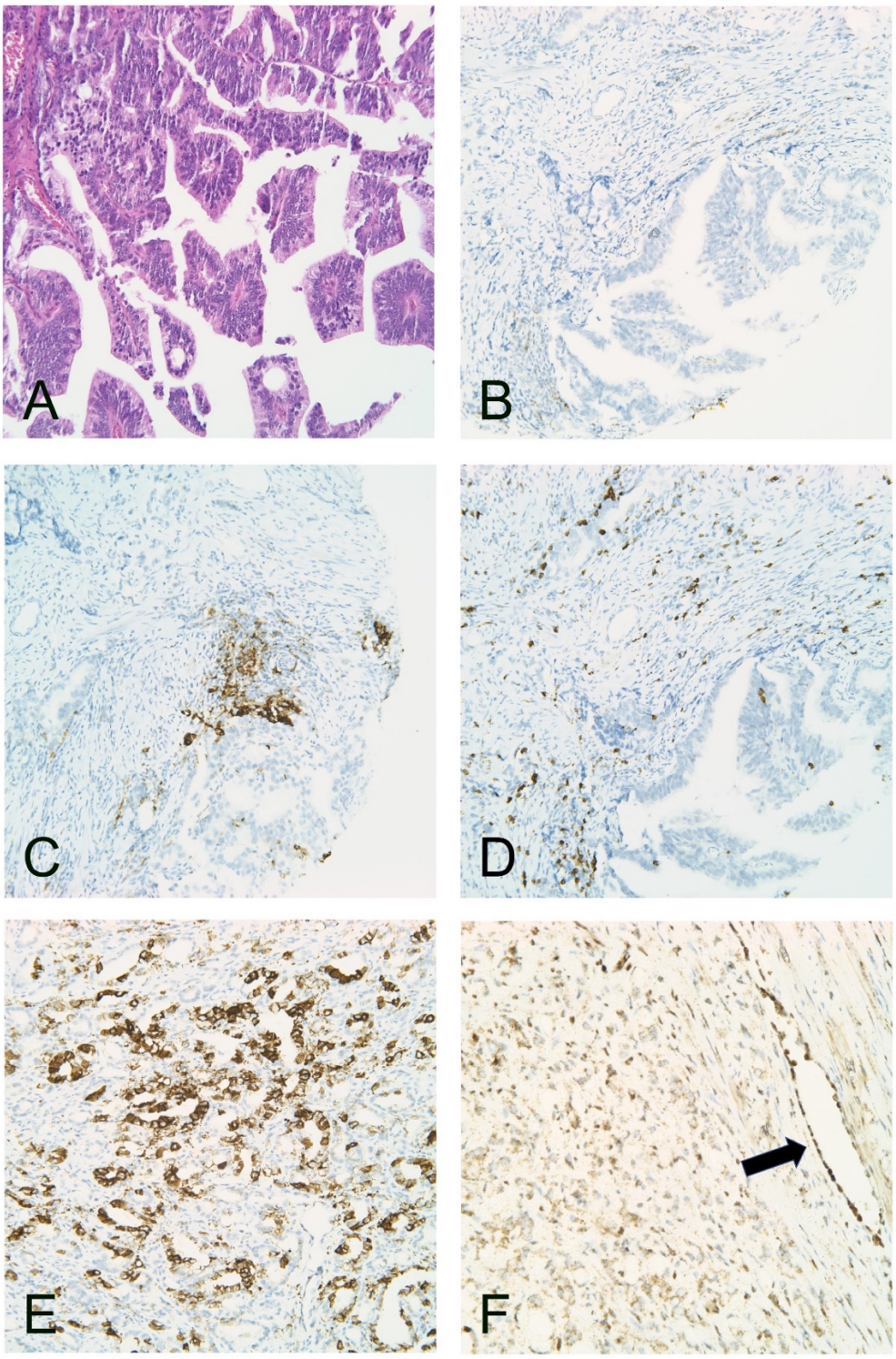


Figure 11. (A) Ductal adenocarcinoma of the prostate with papillary architecture and pseudostratified, columnar tumor cells with elongated nuclei. Hematoxylin and eosin. (B) Ductal adenocarcinoma without PD-L1 expression in tumor cells. IHC for PD-L1. (C) PD-L1 expression in tumor-infiltrating immune cells in ductal adenocarcinoma. (D) Tumor-infiltrating CD8+ lymphocytes in ductal adenocarcinoma. IHC for CD8. (E) Strong membranous expression of PD-L1 in acinar adenocarcinoma. IHC for PD-L1. (F) Acinar adenocarcinoma with complete loss of nuclear staining. This indicates dMMR (MSH6). Positive nuclear staining in endothelial cells (arrow) as positive internal control. IHC for MSH6. Lens magnification 20x.

Table 1. PD-L1 expression in tumor cells and PD-L1 expression in tumor-infiltrating immune cells

	Ductal adenocarcinoma	Acinar adenocarcinoma	p value
% of tumors with PD-L1 expression in cancer cells			
All tumors	2.9% (1/34)	4.8% (2/42)	p = 1.00
% of tumors with PD-L1-positive infiltrating immune cells			
ISUP grade 2	0/4	1/7	p = 0.27
ISUP grade 3	2/10	1/15	
ISUP grade 4	5/14	4/14	
ISUP grade 5	3/6	0/6	
All tumors	29.4% (10/34)	14.3% (6/42)	p = 0.16

Table 1. PD-L1 expression in tumor cells and tumor infiltrating immune cells in acinar and ductal adenocarcinoma.

Table 2. Number of cases with loss of MMR proteins (dMMR) in ductal and acinar adenocarcinoma

	Ductal adenocarcinoma	Acinar adenocarcinoma	p value
% of tumors with deficient MMR/microsatellite instability			
MSH2	3.0% (1/33)	0% (0/40)	
MSH6	3.0% (1/33)	7.5% (3/40)	p = 0.62
MLH1	0% (0/33)	0% (0/40)	
PMS2	0% (0/33)	0% (0/40)	

[1]Loss of MSH2 and MSH6 in the same case.

Table 2. Number of cases with loss of MMR proteins (dMMR) in acinar and ductal adenocarcinoma.

In prostate cancer, studies of PD-L1 expression have shown inconsistent findings and few reports investigating the expression of PD-L1 in ductal adenocarcinoma have been published (78–84,86).

In this study we showed that PD-L1 was expressed in tumor infiltrating immune cells in 21% of prostate cancers but in tumor cells in only 3% of cases when using a validated prepackaged kit (SP263) (Ventana Medical Systems). A higher expression of PD-L1 in tumor infiltrating immune cells was seen in ductal adenocarcinoma compared to acinar adenocarcinoma but the difference was not statistically significant. These findings are well in line with those of other studies that have utilized the same commercially available antibody clone (SP263) (83,84,86). All cases in our study fulfilled the minimum requirement of at least 100 tumor cells for evaluation of PD-L1 expression but since the samples were part of a TMA, large areas of tumor could not be examined. It could be argued that assessment of PD-L1 expression in full sections of the tumors could better visualize intratumor expression heterogeneity. In clinical practice, pathologists most often prefer to evaluate PD-L1 expression in slides from surgical specimens or large biopsies since it reduces the risk of under- or overestimating expression in heterogenous tumors. Numerous clinical trials using PD1 and PD-L1 inhibitors are ongoing which will direct the future for immune checkpoint inhibitors in prostate cancer. It still remains to be elucidated how to best identify which prostate cancer patient subgroups could benefit from therapy. Many different biomarkers for tumor response to immune checkpoint inhibitors has been suggested, such as PD-L1

expression, tumor-infiltrating lymphocytes, tumor mutation burden and dMMR (131). At present, the only indication for treatment with immune checkpoint inhibitors for prostate cancer supported by the FDA is treatment of MMR-deficient or MSI-H advanced prostate cancer with pembrolizumab. Further clinical trials and possibly the development of new biomarkers will determine the role of immune checkpoint inhibitors in prostate cancer (55). The results of more recent studies of PD-L1 expression in prostate cancer, using validated PD-L1 antibodies, show somewhat more uniformity than in earlier reports. Further studies should aim at standardizing the choice of antibody clone and tissue pretreatment protocols but also tissue handling such as fixative agent and fixation time (130).

In our series three acinar adenocarcinomas showed deficient expression of MSH6 and a single case of ductal adenocarcinoma showed deficient expression of both MSH2 and MSH6. The method for identifying dMMR in Study III was IHC but MSI-H was also investigated in Study IV. In that study, 15 acinar and ductal adenocarcinoma samples were investigated with genomic sequencing without any case exhibiting MSI-H. In the literature, the frequency of MSI-H and dMMR in prostate cancers ranges from around 1% of primary cancers up to 12% of metastatic cancers (111). Some studies have reported that dMMR/MSI-H is more common in high-grade prostate cancer (132). In a small study from 2016, using targeted next-generation DNA sequencing, 4 out of 10 patients with ductal adenocarcinoma showed signs of MSI (101). The authors hypothesized that MSI may be more common in ductal adenocarcinoma compared to acinar adenocarcinoma, although the size of the study was small. In our study we were not able to reproduce these results. On the contrary, dMMR was identified in only one of 33 ductal adenocarcinomas. Furthermore, in Study IV we reported that sequencing data from 15 ductal adenocarcinomas did not identify any case of MSI-H. Thus, in Study III and IV, we have shown by both IHC and genomic sequencing, that dMMR/MSI-H is a rare event in ductal adenocarcinoma in contrast to the data from Schweizer *et al.* (101). dMMR/MSI-H has been reported to be more common in high-grade prostate cancer (132). With that in mind, the higher rate of dMMR/MSI-H in the study by Schweizer *et al.* could be caused by a selection of high-grade cancers.

Extensive lymphocytic infiltration in malignant tumors has been described to be associated with a more favorable clinical outcome for several cancer types, including urothelial, colorectal, head and neck, breast, pulmonary and prostatic cancer (133,134). In the literature it has been proposed that high level of tumor infiltrating CD3+ T cells and CD8+ cytotoxic T cells is associated with a better prognosis, longer disease-free survival and overall survival for cancer patients (135). It has been proposed that the mechanism behind the increased number of tumor infiltrating immune cells in tumors with MSI is that tumor cells, due to their high tumor mutation burden, express more neoantigens which attracts antitumoral immune cells. PD-L1 overexpression on the surface of tumor cells or in tumor-infiltrating immune cells

could suppress this antitumoral immune response. In our study one of four dMMR tumors expressed PD-L1. There was a statistically significant increase in the number of CD8+ tumor-infiltrating lymphocytes in ductal adenocarcinoma when compared to acinar adenocarcinoma. For CD4+ tumor infiltrating lymphocytes no statistically significant difference was identified. The exact prognostic and predictive value of the quantitative/qualitative characteristics of tumor infiltrating immune cells in prostate cancer remains to be described in future research.

4.4 PAPER IV: DUCTAL AND ACINAR COMPONENTS OF MIXED PROSTATIC ADENOCARCINOMA FREQUENTLY HAVE A COMMON CLONAL ORIGIN

In Study IV we showed that acinar and ductal components in mixed adenocarcinoma most often shared a common somatic denominator. In 12 out of 15 cases genomic analysis indicated that the tumor components had a common clonal origin while they were clonally independent in three cases. TMPRSS2-ERG gene fusions were detected in 53% (8/15) of cases, *Figure 12*. The fusions were detected in both tumor components in 6 of cases, only in the acinar component in one case and only in the ductal component in one case. In 33% (5/15) of cases, clonal FOXA1 alterations were detected: in both components in three cases and only in the ductal component in two cases. SPOP mutations were seen in 27% (4/15) of cases: in three of the cases in both components and only in the ductal component in one case. Six of the ductal adenocarcinomas had CTNNB1 hotspot mutations or PTEN alterations that were mutually exclusive, three cases had CTNNB1 hotspot mutations, and three cases had PTEN alterations. KIAA1549−BRAF fusion was identified in both tumor components in one case. 53% of ductal adenocarcinomas showed gene duplication events, resulting in increased ploidy, while this was not observed in any of the acinar cancer samples. None of the cases showed signs of microsatellite instability or increased tumor mutation burden (defined as ≥15 mutations per megabase of the coding DNA). No HRR gene alterations were noted. On microscopic evaluation, no obvious morphological differences were observed between the clonal and non-clonal cancers, *Figure 13.*

In 1967, Melicow *et al.* first reported, what they called, “endometroid carcinoma of the prostatic utricle” (30). Later, this tumor would be renamed ductal adenocarcinoma of the prostate. At the time, it was believed that the tumor originating from a remnant of a Mullerian duct structure (31). In the 1970s a case of co-existing acinar and ductal adenocarcinoma in the same prostate specimen was described. The authors concluded that the two tumor components should be classified as neoplasms of different histogenesis, biological potential and histopathological features (136). Later it was shown that the tumor had a prostatic origin and the theory of an Mullerian histogenesis was abandoned (137,138).

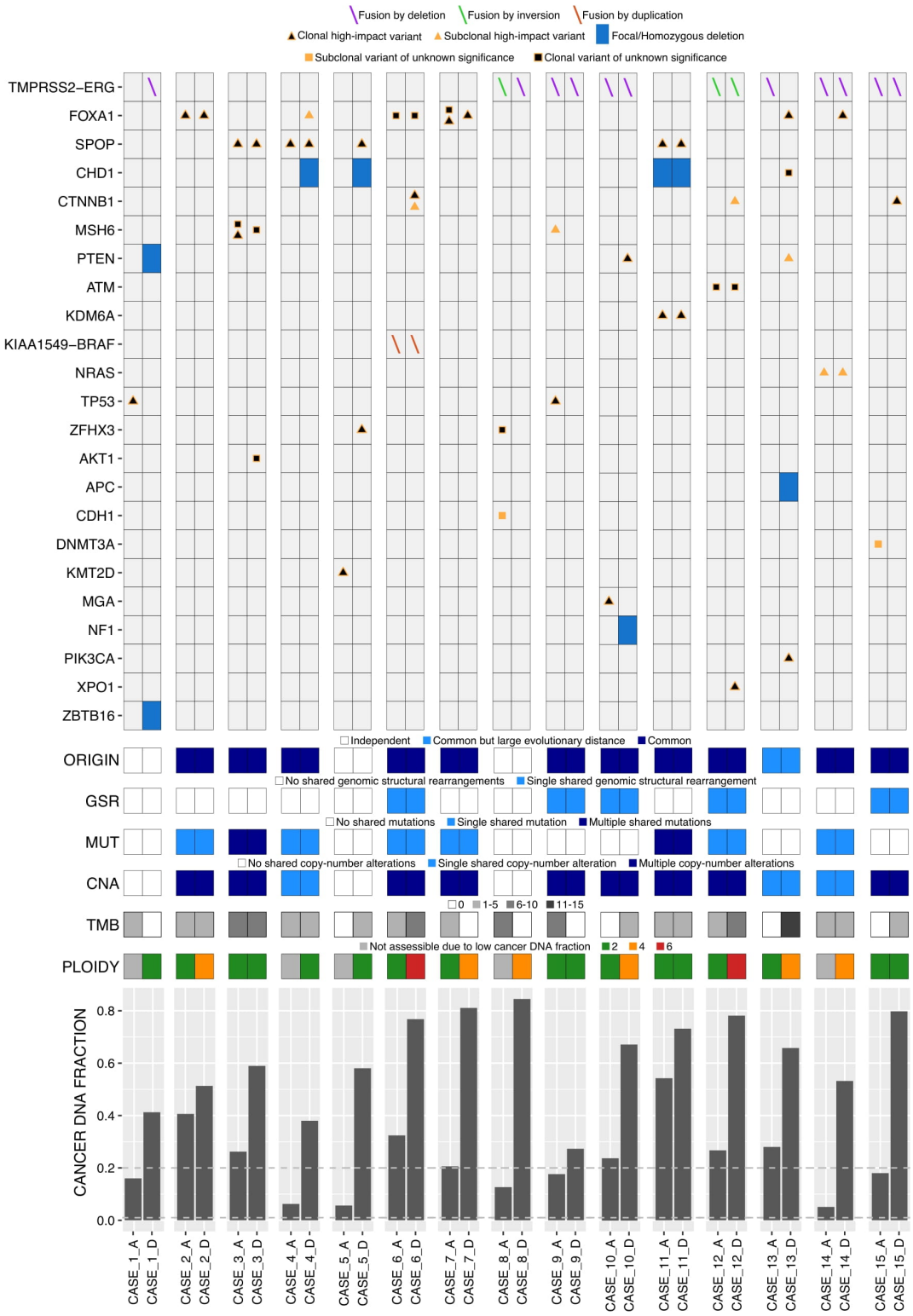


Figure 12. Genomic profiling of paired samples of tissues from acinar and ductal adenocarcinoma components. The top heatmap shows the somatic alterations detected from tumor tissue profiling. The type of alteration is coded according to the top legend. The middle heatmaps gives information on clonal origin, shared variants by variant type, tumor mutation burden and ploidy. The bottom panel displays the estimated fraction of cancer DNA in each sequenced tissue sample. The dashed lines at 0.01, 0.10, and 0.20 denote the cutoffs for reliable detection of point mutations, loss of heterozygosity, and homozygous deletions, respectively.

Tumors with endometroid-like features located both centrally in the prostate, in and around the verumontanum, and in the large ducts of the prostate were grouped together under the term ductal adenocarcinoma of the prostate (32).

Ductal adenocarcinoma rarely exists in a pure form, in most cases it presents in a mixed form with acinar adenocarcinoma (26). This fact has indicated that the two tumor components could share a common origin. The alternative would be that mixed tumors represent collision tumors of admixed, but independently arisen, acinar and ductal adenocarcinoma. Prostate cancer is typically heterogenous with varying tumor grade and morphological differentiation. Molecular studies have shown great genetic diversity between different regions and tumors in the same prostatectomy specimen (139,140). Based on the observation that ERG expression and loss of phosphatase and tensin homolog (PTEN) is reported to be less common in both acinar and ductal adenocarcinoma components in mixed prostate cancers compared to conventional acinar cancer, it has been suggested that both components could share a clonal origin (100). In a study from 2019, Gillard *et al.* investigated the clonal relationship between acinar and ductal adenocarcinoma foci in ten prostatectomy specimens (141). Their results indicated that coincident acinar and ductal adenocarcinomas shared a clonal relationship. In nine of ten cases mutually exclusive CTNNB1 hotspot mutations or PTEN alterations were identified in the ductal component, but this was not seen in the acinar component. Ploidy was not analyzed.

In our study, acinar and ductal adenocarcinoma components from mixed prostate cancers shared a common somatic denominator in a majority of cases. Hence, most acinar and ductal adenocarcinoma components of mixed prostate cancers should be viewed as divergent patterns of differentiation of the same tumor. In three cases the acinar and ductal cancer components showed no signs of a clonal relationship. It could be hypothesized that these cases represent true collision tumors. On microscopic examination, no morphological differences were identified that could separate clonal from non-clonal mixed prostate cancers, *Figure 13*. Great attention was put into selecting only cases and areas of tumor that unequivocally fulfilled the morphological criteria of ductal adenocarcinoma (14), *Figure 3*. By selecting ductal adenocarcinoma samples carefully, we wanted to avoid adding acinar adenocarcinomas with ductal features to the study. In clinical practice it is well known that there is a morphological continuum between the morphology of acinar and ductal cancer. Acinar cancer can exhibit certain morphological features of ductal cancer without fulfilling all criteria for ductal adenocarcinoma (26). Studies have shown that it can be difficult for pathologists to agree on where to draw the morphological line between acinar and ductal adenocarcinoma (37). The existence of prostatic adenocarcinomas with ductal features corresponds well with our findings, i.e., the tumor components represent patterns of differentiation within the same tumor.

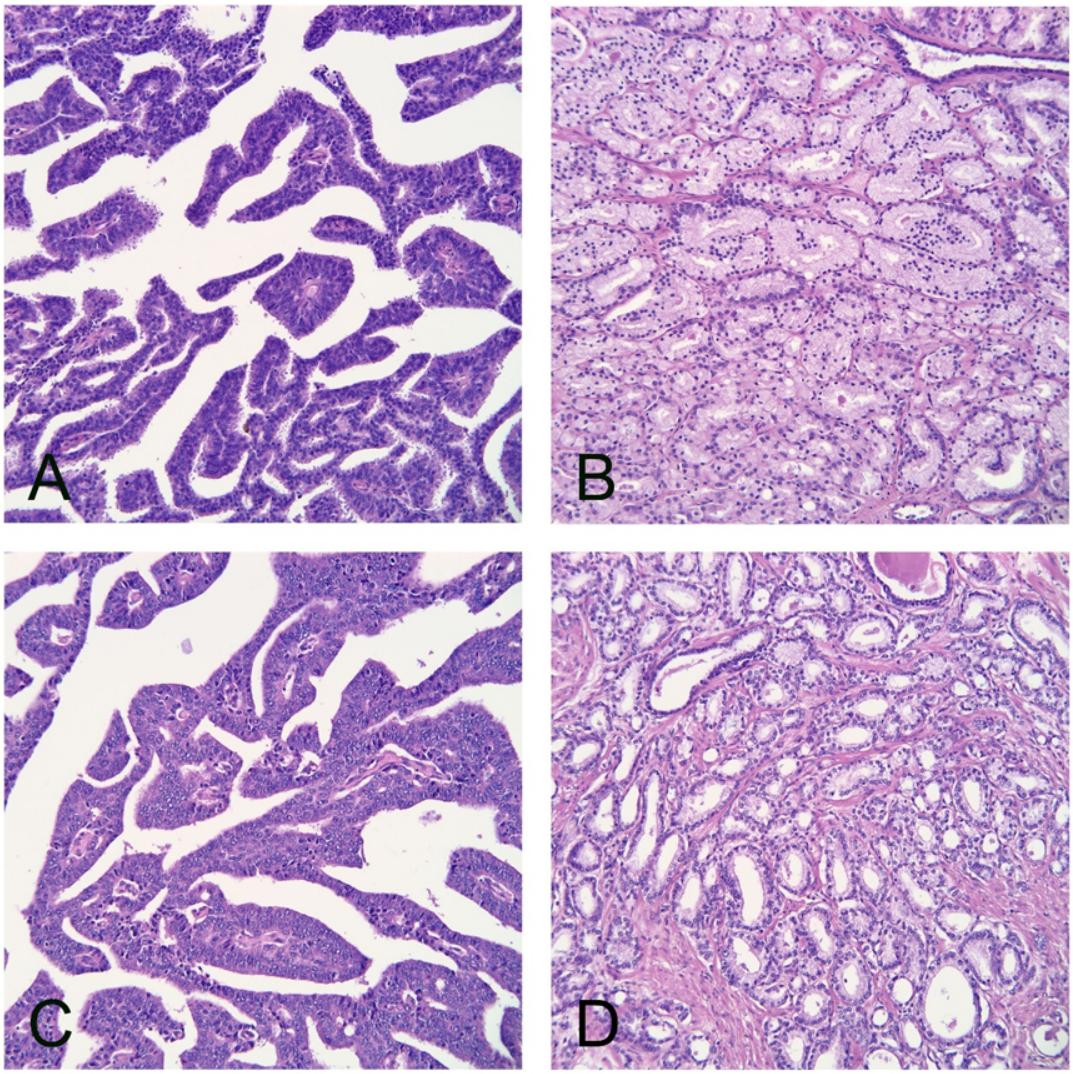


Figure 13. Mixed prostate cancer with ductal and acinar adenocarcinoma components. No specific histological characteristics separating clonal and non-clonal cancers could be identified. A+B. Mixed adenocarcinoma. Ductal (A) and acinar (B) adenocarcinoma components with clonal relationship. C+D: Mixed adenocarcinoma. Ductal (C) and acinar (D) adenocarcinoma components without clonal relationship. Hematoxylin and eosin, 20x lens magnification

One of the most striking findings of our study was that 8/15 cases of ductal adenocarcinoma showed genome doubling events resulting in aneuploidy. In six of these cases, ploidy increase was absent in the acinar component. In the remaining two cases ploidy in the acinar component could not be assessed. No acinar adenocarcinoma exhibited genome doubling events. Genome doubling events has been described to be the strongest genomic feature associated with advanced prostate cancer and our findings are well in concordance with the description of ductal adenocarcinoma as an aggressive, high-grade prostate cancer (142). TMPRSS2-ERG gene fusions were detected in 7/15 cases. In five of these cases the fusion was present in both the acinar and ductal components. The finding, that TMPRSS2-ERG

fusions are common in mixed prostate cancers, contrasts with earlier reports where fusions have been described as uncommon in ductal prostate cancers (98,99). The presence of mutually exclusive PTEN and CTNNB1 mutations in the ductal cancer component of mixed prostate cancers has, in a small study, been reported to be very common, seen in 9/10 cases (141). In our study these mutations were identified in ductal cancer at a much lower rate. Mutually exclusive PTEN and CTNNB1 mutations were seen in the ductal cancer component in 6/15 cases. It is difficult to explain these differences in mutation frequency but since both studies are rather small, it cannot be excluded that the differences are random. Nevertheless, in our study we reproduced the findings that PTEN and CTNNB1 alterations are enriched in the ductal and absent in the acinar cancer components of mixed prostate cancer.

5 CONCLUSIONS

We conclude that macroscopic findings conclusive for cancer predict the identification of prostate cancer on microscopic examination in most cases. Transition zone cancers are more difficult to identify on gross examination. High-grade prostate cancers are more likely to be identified macroscopically than low-grade cancers.

We conclude that our new protocol for biobanking of fresh tissue from prostatectomy specimens provides ample tumor material for research purposes. The technique also enables reporting of clinical parameters from the biobanked tissue. The harvesting of a full tissue slice facilitates studies of tumor multifocality and heterogeneity.

We conclude that PD-L1 expression is rare in both acinar and ductal adenocarcinoma of the prostate while PD-L1 expression in tumor infiltrating immune cells is more common. dMMR is uncommon in both acinar and ductal adenocarcinoma.

We conclude that acinar and ductal adenocarcinoma components in mixed prostate cancers share a common somatic denominator in most cases. Ductal adenocarcinoma shows a high rate of genome doubling events, consistent with aggressive prostate cancer.

6 FUTURE PERSPECTIVES

The future of prostate cancer research will move along several different paths. Increased understanding of the biology and genetics of prostate cancer can lead to better diagnostics and improved tumor surveillance, but also towards a more personalized, targeted treatment. Developments in imaging technologies such as magnetic resonance imaging (MRI) and positron emission tomography–computed tomography (PET/CT) will give detailed information about primary tumors and metastases. These techniques can facilitate targeted biopsies but also increase the accuracy of staging and detection of early tumor relapse. Performing MRI before invasive investigations to select patients for biopsy can reduce the number of diagnosed prostate cancer without missing clinically significant high-risk tumors. Digital pathology with the application of an artificial intelligence (AI) system has shown a good ability to identify and grade prostate cancer (143). These findings have raised the hopes that AI could reduce subjectivity in prostate cancer diagnostics and tumor grading. It could also improve overall efficiency and cost-effectiveness in prostate cancer diagnosis which would be beneficial since there is a worldwide shortage of pathologists. It may also in the future become possible to develop machine learning algorithms that, independent of traditional histopathological parameters, could add prognostic and predictive information for patient management (144).

The addition of MRI to preoperative prostate cancer diagnostics has received a lot of attention. MRI can be used to identify areas suggestive for cancer, enabling targeted core-needle biopsies. Biopsies in patients with MRI results suggestive of prostate cancer are not inferior to standard systematic biopsies for the detection of clinically significant prostate cancer (145). A reduction in the number of diagnosed low-risk prostate cancers would be beneficial by reducing overtreatment and healthcare costs. I would also reduce procedure-related complications such as pain, hemorrhage and post-biopsy infections. Reducing the detection of small, low-grade prostate cancers that can be managed conservatively would reduce the negative psychosocial aspects of a cancer diagnosis (146). At present, patients with high-risk prostate cancer undergo a CT-scan and a bone scintigraphy to detect lymph node or distant metastases. Recently imaging by PSMA-PET has been introduced for the detection of metastatic prostate cancer (147). This method combines CT and PET scans. The patient is intravenously injected with a radioisotope attached to a molecule that selectively binds to prostate-specific membranous antigen (PSMA), which is often overexpressed on the cell surface of prostate cancer. The radioactive isotope will accumulate in PSMA-expressing metastatic lesions and highlight them in the PET-scan. Studies have shown that PSMA PET-CT is more accurate for lymph node and distant metastases than conventional imaging techniques. Also, radiation exposure is lower with PET-CT than with the conventional CT

scan/bone scintigraphy approach. Circulating tumor DNA is a new tool in the emerging concept of precision medicine also known as liquid biopsies. By analyzing tumor DNA, circulating in the blood, targetable mutations and treatment induced adaptations in tumor cells can be identified (148). The clinical implementation of this technique could make a more personalized treatment approach to prostate cancer possible in the future. Conventional tissue biopsies are invasive and sometimes difficult to obtain. In contrast, through the liquid biopsies a blood test will be sufficient to map the tumor DNA and follow genetic changes during the different phases of the disease.

A targeted treatment for prostate cancer, aimed at PSMA has been developed, Lutetium-177 PSMA. Patients with mCRPC are injected with a radioligand that delivers beta-particle radiation specifically to PSMA-expressing cells and the surrounding microenvironment. In this way a high level of radiation can be achieved in areas of tumor growth without negative side effects in the rest of the body. Investigators have reported improved progression-free survival and overall survival using this treatment approach (149). The successful introduction of immune checkpoint inhibitors in various cancer types has spurred interest in their use in prostate cancer, especially mCRPC. Multiple studies on metastatic prostate cancer have shown some promising results. In particular, there are indications that the response rate is higher in tumors harboring MSI or DNA Damage Response (DDR) gene defects. Further studies are ongoing where it hopefully will be elucidated which patient categories could benefit from immune therapy (120,150). Clinical trials have also shown that prostate cancer with defect DDR pathways can be treated with PARP-inhibitors (151) and it has been purposed that all metastasized prostate cancers should undergo molecular investigations to identify DDR alterations (152).

A deepened understanding of prostate cancer biology, better imaging techniques, digital pathology including artificial intelligence, personalized treatment and improved surveillance of patients in remission will hopefully lead to improved patient management, less overtreatment and longer overall survival. A future prostate cancer management could include PSA-testing, predictive biomarker testing and MRI to decide which patients should undergo biopsy. This would be followed by targeted biopsies based on MRI findings. PSMA-PET would then be used to identify patients with regional and/or distant metastatic disease. Patients that have undergone radical prostatectomy, are in active surveillance or active antitumoral drug treatment would take regular liquid biopsies to detect relapse, signs of more aggressive disease or signs of treatment response/treatment failure. By using this type of surveillance is would be possible to rapidly change treatment strategy and thereby possibly improve quality of life and overall survival.

7 ACKNOWLEDGEMENTS

My supervisor, **Lars Egevad**, for your enthusiastic and encouraging support during the process. Also, for sharing your immense clinical and scientific knowledge about prostate cancer. Finally, for all our many conversations on science, pathology and things in general.

My co-supervisor, **Johan Lindberg**, for patiently introducing me to the world of genetics.

Hemamali Samaratunga and **Brett Delahunt. Dr Delahunt** for your masterful command of the English language in proof reading my manuscripts. **Dr Samaratunga** for supplying our projects with a cornucopia of prostatic ductal adenocarcinomas.

Co-authors, **John Yaxley, Mark Clements, Peter Wiklund, Jona Gudjónsdóttir, Rebecka Bergström, Venkatesh Chellappa.** Thank you for your many contributions in making this project possible.

My PhD-project mentor, **Jonas Hydman**, for all the pleasant discussions about research, sailing and music.

Hans Hamberg, for sharing your vast knowledge of the noble art of general histopathology.

"The good old team", **Lorand, Robert, Joe, Christofer, Felix, Caroline, Oskar** and all the other colleagues at the Department of Pathology, Karolinska University Hospital Solna.

Childhood friends, **Gustaf, Niklas and David**. For all the fun years and good friendship.

Old KI-friends, **Stig and Johan,** for the various adventures from the student years onwards.

My mother, **Anna Marit**, and my late father, **Hans**, for always supporting me in my interests and goals in life. For inspiring me to read and seek knowledge. Finally, for always being there for me. My sister, **Helena**, for all the good times and experiences together.

Sakura and **Hugo**, for your unstoppable good spirit and for making every day an adventure!

Rika, my dear, fantastic and capable wife. Thank you for all your initiatives, support and love that has made this possible!

8 REFERENCES

1. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Dicker D, Pain A, Hamavid H, Moradi-Lakeh M, et al. The Global Burden of Cancer 2013. JAMA Oncol. 2015 Jul;1(4):505–27.

2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018 Nov;68(6):394–424.

3. Socialstyrelsen. Socialstyrelsen. Cancer i siffror 2018. Available from: https://www.socialstyrelsen.se/globalassets/sharepoint-dokument/artikelkatalog/statistik/2018-6-10.pdf.

4. Rebello RJ, Oing C, Knudsen KE, Loeb S, Johnson DC, Reiter RE, et al. Prostate cancer. Nat Rev Dis Primer. 2021 Feb 4;7(1):9.

5. Quinn M, Babb P. Patterns and trends in prostate cancer incidence, survival, prevalence and mortality. Part I: international comparisons. BJU Int. 2002 Jul;90(2):162–73.

6. Grönberg H. Prostate cancer epidemiology. Lancet Lond Engl. 2003 Mar 8;361(9360):859–64.

7. Johns LE, Houlston RS. A systematic review and meta-analysis of familial prostate cancer risk. BJU Int. 2003 Jun;91(9):789–94.

8. Merriel SWD, Funston G, Hamilton W. Prostate Cancer in Primary Care. Adv Ther. 2018 Sep;35(9):1285–94.

9. Nadler RB, Humphrey PA, Smith DS, Catalona WJ, Ratliff TL. Effect of inflammation and benign prostatic hyperplasia on elevated serum prostate specific antigen levels. J Urol. 1995 Aug;154(2 Pt 1):407–13.

10. Datta K, Muders M, Zhang H, Tindall DJ. Mechanism of lymph node metastasis in prostate cancer. Future Oncol Lond Engl. 2010 May;6(5):823–36.

11. Litwin MS, Tan HJ. The Diagnosis and Treatment of Prostate Cancer: A Review. JAMA. 2017 Jun 27;317(24):2532–42.

12. Nativ O, Bergstralh EJ, Boyle ET, Zincke H. Transurethral resection versus needle biopsy prior to radical prostatectomy for stage C prostate cancer. Influence on progression and survival. Urology. 1991 Jan;37(1):22–7.

13. Humphrey PA. Prostate Pathology. Chicago: ASCP Press; 2003.

14. Moch H, Humphrey PA, Ulbright TM, et al. WHO Classification of Tumours of the Urinary System and Male Genital Organs. Vol. 2016. Lyon: WHO Press,;

15. Renshaw AA. Correlation of gross morphologic features with histologic features in radical prostatectomy specimens. Am J Clin Pathol. 1998 Jul;110(1):38–42.

16. Hall GS, Kramer CE, Epstein JI. Evaluation of radical prostatectomy specimens. A comparative analysis of sampling methods. Am J Surg Pathol. 1992 Apr;16(4):315–24.

17. McNeal JE, Redwine EA, Freiha FS, Stamey TA. Zonal distribution of prostatic adenocarcinoma. Correlation with histologic pattern and direction of spread. Am J Surg Pathol. 1988 Dec;12(12):897–906.

18. Grignon DJ. Unusual subtypes of prostate cancer. Mod Pathol Off J U S Can Acad Pathol Inc. 2004 Mar;17(3):316–27.

19. Epstein JI. Diagnostic criteria of limited adenocarcinoma of the prostate on needle biopsy. Hum Pathol. 1995 Feb;26(2):223–9.

20. Varma M, Lee MW, Tamboli P, Zarbo RJ, Jimenez RE, Salles PGO, et al. Morphologic criteria for the diagnosis of prostatic adenocarcinoma in needle biopsy specimens. A study of 250 consecutive cases in a routine surgical pathology practice. Arch Pathol Lab Med. 2002 May;126(5):554–61.

21. Vesalainen S, Lipponen P, Talja M, Syrjänen K. Mitotic activity and prognosis in prostatic adenocarcinoma. The Prostate. 1995 Feb;26(2):80–6.

22. De Vivar AD, Sayeeduddin M, Rowley D, Cubilla A, Miles B, Kadmon D, et al. Histologic features of stromogenic carcinoma of the prostate (carcinomas with reactive stroma grade 3). Hum Pathol. 2017 May;63:202–11.

23. Varma M, Jasani B. Diagnostic utility of immunohistochemistry in morphologically difficult prostate cancer: review of current literature. Histopathology. 2005 Jul;47(1):1–16.

24. Brimo F, Epstein JI. Immunohistochemical pitfalls in prostate pathology. Hum Pathol. 2012 Mar;43(3):313–24.

25. Humphrey PA. Histological variants of prostatic carcinoma and their significance. Histopathology. 2012 Jan;60(1):59–74.

26. Seipel AH, Wiklund F, Wiklund NP, Egevad L. Histopathological features of ductal adenocarcinoma of the prostate in 1,051 radical prostatectomy specimens. Virchows Arch Int J Pathol. 2013 Apr;462(4):429–36.

27. Meeks JJ, Zhao LC, Cashy J, Kundu S. Incidence and outcomes of ductal carcinoma of the prostate in the USA: analysis of data from the Surveillance, Epidemiology, and End Results program. BJU Int. 2012 Mar;109(6):831–4.

28. Brinker DA, Potter SR, Epstein JI. Ductal adenocarcinoma of the prostate diagnosed on needle biopsy: correlation with clinical and radical prostatectomy findings and progression. Am J Surg Pathol. 1999 Dec;23(12):1471–9.

29. Amin A, Epstein JI. Pathologic stage of prostatic ductal adenocarcinoma at radical prostatectomy: effect of percentage of the ductal component and associated grade of acinar adenocarcinoma. Am J Surg Pathol. 2011 Apr;35(4):615–9.

30. Melicow MM, Pachter MR. Endometrial carcinoma of proxtatic utricle (uterus masculinus). Cancer. 1967 Oct;20(10):1715–22.

31. Melicow MM, Tannenbaum M. Endometrial carcinoma of uterus masculinus (prostatic utricle). Report of 6 cases. J Urol. 1971 Dec;106(6):892–902.

32. Wernert N, Lüchtrath H, Seeliger H, Schäfer M, Goebbels R, Dhom G. Papillary carcinoma of the prostate, location, morphology, and immunohistochemistry: the histogenesis and entity of so-called endometrioid carcinoma. The Prostate. 1987;10(2):123–31.

33. Samaratunga H, Duffy D, Yaxley J, Delahunt B. Any proportion of ductal adenocarcinoma in radical prostatectomy specimens predicts extraprostatic extension. Hum Pathol. 2010 Feb;41(2):281–5.

34. Christensen WN, Steinberg G, Walsh PC, Epstein JI. Prostatic duct adenocarcinoma. Findings at radical prostatectomy. Cancer. 1991 Apr 15;67(8):2118–24.

35. Morgan TM, Welty CJ, Vakar-Lopez F, Lin DW, Wright JL. Ductal adenocarcinoma of the prostate: increased mortality risk and decreased serum prostate specific antigen. J Urol. 2010 Dec;184(6):2303–7.

36. Epstein JI. Prostatic ductal adenocarcinoma: a mini review. Med Princ Pract Int J Kuwait Univ Health Sci Cent. 2010;19(1):82–5.

37. Seipel AH, Delahunt B, Samaratunga H, Amin M, Barton J, Berney DM, et al. Diagnostic criteria for ductal adenocarcinoma of the prostate: interobserver variability among 20 expert uropathologists. Histopathology. 2014 Aug;65(2):216–27.

38. Bock BJ, Bostwick DG. Does prostatic ductal adenocarcinoma exist? Am J Surg Pathol. 1999 Jul;23(7):781–5.

39. Epstein JI, Allsbrook WC, Amin MB, Egevad LL. Update on the Gleason grading system for prostate cancer: results of an international consensus conference of urologic pathologists. Adv Anat Pathol. 2006 Jan;13(1):57–9.

40. Partin AW, Kattan MW, Subong EN, Walsh PC, Wojno KJ, Oesterling JE, et al. Combination of prostate-specific antigen, clinical stage, and Gleason score to predict pathological stage of localized prostate cancer. A multi-institutional update. JAMA. 1997 May 14;277(18):1445–51.

41. Kench JG, Judge M, Delahunt B, Humphrey PA, Kristiansen G, Oxley J, et al. Dataset for the reporting of prostate carcinoma in radical prostatectomy specimens: updated recommendations from the International Collaboration on Cancer Reporting. Virchows Arch Int J Pathol. 2019 Sep;475(3):263–77.

42. Srigley JR. Benign mimickers of prostatic adenocarcinoma. Mod Pathol Off J U S Can Acad Pathol Inc. 2004 Mar;17(3):328–48.

43. Franks LM. Latent carcinoma. Ann R Coll Surg Engl. 1954 Oct;15(4):236–49.

44. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018 Jan;68(1):7–30.

45. Albertsen PC, Hanley JA, Penson DF, Barrows G, Fine J. 13-year outcomes following treatment for clinically localized prostate cancer in a population based cohort. J Urol. 2007 Mar;177(3):932–6.

46. Hamdy FC, Donovan JL, Lane JA, Mason M, Metcalfe C, Holding P, et al. 10-Year Outcomes after Monitoring, Surgery, or Radiotherapy for Localized Prostate Cancer. N Engl J Med. 2016 Oct 13;375(15):1415–24.

47. Filson CP, Marks LS, Litwin MS. Expectant management for men with early stage prostate cancer. CA Cancer J Clin. 2015 Aug;65(4):265–82.

48. D'Amico AV, Moul J, Carroll PR, Sun L, Lubeck D, Chen MH. Cancer-specific mortality after surgery or radiation for patients with clinically localized prostate cancer managed during the prostate-specific antigen era. J Clin Oncol Off J Am Soc Clin Oncol. 2003 Jun 1;21(11):2163–72.

49. Hugosson J, Stranne J, Carlsson SV. Radical retropubic prostatectomy: a review of outcomes and side-effects. Acta Oncol Stockh Swed. 2011 Jun;50 Suppl 1:92–7.

50. Attard G, Parker C, Eeles RA, Schröder F, Tomlins SA, Tannock I, et al. Prostate cancer. Lancet Lond Engl. 2016 Jan 2;387(10013):70–82.

51. Hugosson J, Carlsson S, Aus G, Bergdahl S, Khatami A, Lodding P, et al. Mortality results from the Göteborg randomised population-based prostate-cancer screening trial. Lancet Oncol. 2010 Aug;11(8):725–32.

52. Leung DKW, Chiu PKF, Ng CF, Teoh JYC. Novel Strategies for Treating Castration-Resistant Prostate Cancer. Biomedicines. 2021 Mar 27;9(4):339.

53. Messina C, Cattrini C, Soldato D, Vallome G, Caffo O, Castro E, et al. BRCA Mutations in Prostate Cancer: Prognostic and Predictive Implications. J Oncol. 2020;2020:4986365.

54. de Bono J, Mateo J, Fizazi K, Saad F, Shore N, Sandhu S, et al. Olaparib for Metastatic Castration-Resistant Prostate Cancer. N Engl J Med. 2020 May 28;382(22):2091–102.

55. Isaacsson Velho P, Antonarakis ES. PD-1/PD-L1 pathway inhibitors in advanced prostate cancer. Expert Rev Clin Pharmacol. 2018 May;11(5):475–86.

56. Egevad L, Algaba F, Berney DM, Boccon-Gibod L, Griffiths DF, Lopez-Beltran A, et al. Handling and reporting of radical prostatectomy specimens in Europe: a web-based survey by the European Network of Uropathology (ENUP). Histopathology. 2008 Sep;53(3):333–9.

57. Egevad L. Handling of radical prostatectomy specimens. Histopathology. 2012 Jan;60(1):118–24.

58. Gill PS, Roberts ISD, Browning L, Perera R, Warren AY, Hamdy FC, et al. The handling and sampling of radical prostatectomy specimens for reporting and research: the Oxford approach. J Clin Pathol. 2012 Dec;65(12):1057–61.

59. Sooriakumaran P, Henderson A, Denham P, Langley SE. A novel method of obtaining prostate tissue for gene expression profiling. Int J Surg Pathol. 2009 Jun;17(3):238–43.

60. Warren AY, Whitaker HC, Haynes B, Sangan T, McDuffus LA, Kay JD, et al. Method for sampling tissue for research which preserves pathological data in radical prostatectomy. The Prostate. 2013 Jan;73(2):194–202.

61. Wheeler TM, Lebovitz RM. Fresh tissue harvest for research from prostatectomy specimens. The Prostate. 1994 Nov;25(5):274–9.

62. Schäfer SC, Pfnür M, Yerly S, Fandel TM, Jichlinski P, Lehr HA. Cryopreservation of prostate cancer tissue during routine processing of fresh unfixed prostatectomy specimen: demonstration and validation of a new technique. The Prostate. 2009 Feb 1;69(2):191–7.

63. Morrison C, Cheney R, Johnson CS, Smith G, Mohler JL. Central quadrant procurement of radical prostatectomy specimens. The Prostate. 2009 May 15;69(7):770–3.

64. Riddick ACP, Barker C, Sheriffs I, Bass R, Ellis V, Sethia KK, et al. Banking of fresh-frozen prostate tissue: methods, validation and use. BJU Int. 2003 Mar;91(4):315–23; discussion 323-324.

65. Vlajnic T, Oeggerli M, Rentsch C, Püschel H, Zellweger T, Thalmann GN, et al. Ceramic foam plates: a new tool for processing fresh radical prostatectomy specimens. Virchows Arch Int J Pathol. 2014 Dec;465(6):637–42.

66. Egevad L, Engstrom K, Busch C. A new method for handling radical prostatectomies enabling fresh tissue harvesting, whole mount sections and landmarks for alignment of sections. J Urol Pathol. 1998(9):17–28.

67. Knoedler JJ, Karnes RJ, Thompson RH, Rangel LJ, Bergstralh EJ, Boorjian SA. The association of tumor volume with mortality following radical prostatectomy. Prostate Cancer Prostatic Dis. 2014 Jun;17(2):144–8.

68. Lexander H, Hellman U, Palmberg C, Auer G, Hellström M, Franzén B, et al. Evaluation of two sample preparation methods for prostate proteome analysis. Proteomics. 2006 Jul;6(13):3918–25.

69. Walton TJ, McCulloch TA, Rees RC, Bishop MC. Obtaining fresh prostate cancer tissue for research: a novel biopsy needle and sampling technique for radical prostatectomy specimens. The Prostate. 2005 Sep 1;64(4):382–6.

70. Bertilsson H, Angelsen A, Viset T, Skogseth H, Tessem MB, Halgunset J. A new method to provide a fresh frozen prostate slice suitable for gene expression study and MR spectroscopy. The Prostate. 2011 Apr;71(5):461–9.

71. Jhavar SG, Fisher C, Jackson A, Reinsberg SA, Dennis N, Falconer A, et al. Processing of radical prostatectomy specimens for correlation of data from histopathological, molecular biological, and radiological studies: a new whole organ technique. J Clin Pathol. 2005 May;58(5):504–8.

72. Dev H, Rickman D, Sooriakumaran P, Srivastava A, Grover S, Leung R, et al. Biobanking after robotic-assisted radical prostatectomy: a quality assessment of providing prostate tissue for RNA studies. J Transl Med. 2011 Jul 26;9:121.

73. Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. Immunol Rev. 2010 Jul;236:219–42.

74. He J, Huo L, Ma J, Zhao J, Bassett RL, Sun X, et al. Expression of Programmed Death Ligand 1 (PD-L1) in Posttreatment Primary Inflammatory Breast Cancers and Clinical Implications. Am J Clin Pathol. 2018 Feb 17;149(3):253–61.

75. He J, Hu Y, Hu M, Li B. Development of PD-1/PD-L1 Pathway in Tumor Immune Microenvironment and Treatment for Non-Small Cell Lung Cancer. Sci Rep. 2015 Aug 17;5:13110.

76. He J, Hu Y, Hu M, Li B. Development of PD-1/PD-L1 Pathway in Tumor Immune Microenvironment and Treatment for Non-Small Cell Lung Cancer. Sci Rep. 2015 Aug 17;5:13110.

77. U.S. Food and Drug Administration. FDA approves first cancer treatment for any solid tumor with a specific genetic feature. 2017. https://www.fda.gov/newsevents/newsroom/pressannouncements/ucm560167.htm. Accessed 10 Dec 2017.

78. Gevensleben H, Dietrich D, Golletz C, Steiner S, Jung M, Thiesler T, et al. The Immune Checkpoint Regulator PD-L1 Is Highly Expressed in Aggressive Primary Prostate Cancer. Clin Cancer Res Off J Am Assoc Cancer Res. 2016 Apr 15;22(8):1969–77.

79. Ness N, Andersen S, Khanehkenari MR, Nordbakken CV, Valkov A, Paulsen EE, et al. The prognostic role of immune checkpoint markers programmed cell death protein 1 (PD-1) and programmed death ligand 1 (PD-L1) in a large, multicenter prostate cancer cohort. Oncotarget. 2017 Apr 18;8(16):26789–801.

80. Massari F, Ciccarese C, Caliò A, Munari E, Cima L, Porcaro AB, et al. Magnitude of PD-1, PD-L1 and T Lymphocyte Expression on Tissue from Castration-Resistant Prostate Adenocarcinoma: An Exploratory Analysis. Target Oncol. 2016;11(3):345–51.

81. Martin AM, Nirschl TR, Nirschl CJ, Francica BJ, Kochel CM, van Bokhoven A, et al. Paucity of PD-L1 expression in prostate cancer: innate and adaptive immune resistance. Prostate Cancer Prostatic Dis. 2015 Dec;18(4):325–32.

82. Baas W, Gershburg S, Dynda D, Delfino K, Robinson K, Nie D, et al. Immune Characterization of the Programmed Death Receptor Pathway in High Risk Prostate Cancer. Clin Genitourin Cancer. 2017 Oct;15(5):577–81.

83. Fankhauser CD, Schüffler PJ, Gillessen S, Omlin A, Rupp NJ, Rueschoff JH, et al. Comprehensive immunohistochemical analysis of PD-L1 shows scarce expression in castration-resistant prostate cancer. Oncotarget. 2018 Feb 13;9(12):10284–93.

84. Haffner MC, Guner G, Taheri D, Netto GJ, Palsgrove DN, Zheng Q, et al. Comprehensive Evaluation of Programmed Death-Ligand 1 Expression in Primary and Metastatic Prostate Cancer. Am J Pathol. 2018 Mar 22;

85. Hutarew G. PD-L1 testing, fit for routine evaluation? From a pathologist's point of view. Memo. 2016;9(4):201–6.

86. Calagua C, Russo J, Sun Y, Schaefer R, Lis R, Zhang Z, et al. Expression of PD-L1 in Hormone-naïve and Treated Prostate Cancer Patients Receiving Neoadjuvant Abiraterone Acetate plus Prednisone and Leuprolide. Clin Cancer Res Off J Am Assoc Cancer Res. 2017 Nov 15;23(22):6812–22.

87. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011 Mar 4;144(5):646–74.

88. Yazawa T. Recent advances in histogenesis research of lung neuroendocrine cancers: Evidence obtained from functional analyses of primitive neural/neuroendocrine cell-specific transcription factors. Pathol Int. 2015 Jun;65(6):277-85.

89. Baca SC, Prandi D, Lawrence MS, Mosquera JM, Romanel A, Drier Y, et al. Punctuated evolution of prostate cancer genomes. Cell. 2013 Apr 25;153(3):666–77.

90. Weier C, Haffner MC, Mosbruger T, Esopi DM, Hicks J, Zheng Q, et al. Nucleotide resolution analysis of TMPRSS2 and ERG rearrangements in prostate cancer. J Pathol. 2013 Jun;230(2):174–83.

91. Hermans KG, van Marion R, van Dekken H, Jenster G, van Weerden WM, Trapman J. TMPRSS2:ERG fusion by translocation or interstitial deletion is highly relevant in androgen-dependent prostate cancer, but is bypassed in late-stage androgen receptor-negative prostate cancer. Cancer Res. 2006 Nov 15;66(22):10658–63.

92. St John J, Powell K, Conley-Lacomb MK, Chinni SR. TMPRSS2-ERG Fusion Gene Expression in Prostate Tumor Cells and Its Clinical and Biological Significance in Prostate Cancer Progression. J Cancer Sci Ther. 2012 Apr 26;4(4):94–101.

93. Hägglöf C, Hammarsten P, Strömvall K, Egevad L, Josefsson A, Stattin P, et al. TMPRSS2-ERG expression predicts prostate cancer survival and associates with stromal biomarkers. PloS One. 2014;9(2):e86824.

94. Barbieri CE, Baca SC, Lawrence MS, Demichelis F, Blattner M, Theurillat JP, et al. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nat Genet. 2012 May 20;44(6):685–9.

95. Ku SY, Gleave ME, Beltran H. Towards precision oncology in advanced prostate cancer. Nat Rev Urol. 2019 Nov;16(11):645–54.

96. Cancer Genome Atlas Research Network. The Molecular Taxonomy of Primary Prostate Cancer. Cell. 2015 Nov 5;163(4):1011–25.

97. Seipel AH, Whitington T, Delahunt B, Samaratunga H, Mayrhofer M, Wiklund P, et al. Genetic profile of ductal adenocarcinoma of the prostate. Hum Pathol. 2017;69:1–7.

98. Schweizer MT, Antonarakis ES, Bismar TA, Guedes LB, Cheng HH, Tretiakova MS, et al. Genomic Characterization of Prostatic Ductal Adenocarcinoma Identifies a High Prevalence of DNA Repair Gene Mutations. JCO Precis Oncol. 2019;3:1–9.

99. Lotan TL, Toubaji A, Albadine R, Latour M, Herawi M, Meeker AK, et al. TMPRSS2-ERG gene fusions are infrequent in prostatic ductal adenocarcinomas. Mod Pathol Off J U S Can Acad Pathol Inc. 2009 Mar;22(3):359–65.

100. Morais CL, Herawi M, Toubaji A, Albadine R, Hicks J, Netto GJ, et al. PTEN loss and ERG protein expression are infrequent in prostatic ductal adenocarcinomas and concurrent acinar carcinomas. The Prostate. 2015 Oct;75(14):1610–9.

101. Schweizer MT, Cheng HH, Tretiakova MS, Vakar-Lopez F, Klemfuss N, Konnick EQ, et al. Mismatch repair deficiency may be common in ductal adenocarcinoma of the prostate. Oncotarget. 2016 Dec 13;7(50):82504–10.

102. Preston BD, Albertson TM, Herr AJ. DNA replication fidelity and cancer. Semin Cancer Biol. 2010 Oct;20(5):281–93.

103. Vilar E, Gruber SB. Microsatellite instability in colorectal cancer-the stable evidence. Nat Rev Clin Oncol. 2010 Mar;7(3):153–62.

104. Baretti M, Le DT. DNA mismatch repair in cancer. Pharmacol Ther. 2018 Apr 15;

105. Liu B, Parsons R, Papadopoulos N, Nicolaides NC, Lynch HT, Watson P, et al. Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients. Nat Med. 1996 Feb;2(2):169–74.

106. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology. 2010 Jun;138(6):2073-2087.e3.

107. Sim MJW, Sun PD. T Cell Recognition of Tumor Neoantigens and Insights Into T Cell Immunotherapy. Front Immunol. 2022;13:833017.

108. Zou W, Wolchok JD, Chen L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: Mechanisms, response biomarkers, and combinations. Sci Transl Med. 2016 Mar 2;8(328):328rv4.

109. Baudhuin LM, Burgart LJ, Leontovich O, Thibodeau SN. Use of microsatellite instability and immunohistochemistry testing for the identification of individuals at risk for Lynch syndrome. Fam Cancer. 2005;4(3):255–65.

110. Shia J. Immunohistochemistry versus Microsatellite Instability Testing For Screening Colorectal Cancer Patients at Risk For Hereditary Nonpolyposis Colorectal Cancer Syndrome. J Mol Diagn JMD. 2008 Jul;10(4):293–300.

111. Hempelmann JA, Lockwood CM, Konnick EQ, Schweizer MT, Antonarakis ES, Lotan TL, et al. Microsatellite instability in prostate cancer by PCR or next-generation sequencing. J Immunother Cancer. 2018 Apr 17;6(1):29.

112. Salipante SJ, Scroggins SM, Hampel HL, Turner EH, Pritchard CC. Microsatellite instability detection by next generation sequencing. Clin Chem. 2014 Sep;60(9):1192–9.

113. Ryan S, Jenkins MA, Win AK. Risk of prostate cancer in Lynch syndrome: a systematic review and meta-analysis. Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol. 2014 Mar;23(3):437–49.

114. Rosty C, Walsh MD, Lindor NM, Thibodeau SN, Mundt E, Gallinger S, et al. High prevalence of mismatch repair deficiency in prostate cancers diagnosed in mismatch repair gene mutation carriers from the colon cancer family registry. Fam Cancer. 2014 Dec;13(4):573–82.

115. Kumar A, White TA, MacKenzie AP, Clegg N, Lee C, Dumpit RF, et al. Exome sequencing identifies a spectrum of mutation frequencies in advanced and lethal prostate cancers. Proc Natl Acad Sci U S A. 2011 Oct 11;108(41):17087–92.

116. Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, et al. Integrative Clinical Genomics of Advanced Prostate Cancer. Cell. 2015 Jul 16;162(2):454.

117. Pritchard CC, Morrissey C, Kumar A, Zhang X, Smith C, Coleman I, et al. Complex MSH2 and MSH6 mutations in hypermutated microsatellite unstable advanced prostate cancer. Nat Commun. 2014 Sep 25;5:4988.

118. Nghiem B, Zhang X, Lam HM, True LD, Coleman I, Higano CS, et al. Mismatch repair enzyme expression in primary and castrate resistant prostate cancer. Asian J Urol. 2016 Oct;3(4):223–8.

119. Guedes LB, Antonarakis ES, Schweizer MT, Mirkheshti N, Almutairi F, Park JC, et al. MSH2 Loss in Primary Prostate Cancer. Clin Cancer Res Off J Am Assoc Cancer Res. 2017 Nov 15;23(22):6863–74.

120. Abida W, Cheng ML, Armenia J, Middha S, Autio KA, Vargas HA, et al. Analysis of the Prevalence of Microsatellite Instability in Prostate Cancer and Response to Immune Checkpoint Blockade. JAMA Oncol. 2019 Apr 1;5(4):471–8.

121. Jonmarker S, Valdman A, Lindberg A, Hellström M, Egevad L. Tissue shrinkage after fixation with formalin injection of prostatectomy specimens. Virchows Arch Int J Pathol. 2006 Sep;449(3):297–301.

122. Battifora H. The multitumor (sausage) tissue block: novel method for immunohistochemical antibody testing. Lab Investig J Tech Methods Pathol. 1986 Aug;55(2):244–8.

123. Seipel AH, Samaratunga H, Delahunt B, Wiklund F, Wiklund P, Lindberg J, et al. Immunohistochemical profile of ductal adenocarcinoma of the prostate. Virchows Arch Int J Pathol. 2014 Nov;465(5):559–65.

124. Schroeder A, Mueller O, Stocker S, Salowsky R, Leiber M, Gassmann M, et al. The RIN: an RNA integrity number for assigning integrity values to RNA measurements. BMC Mol Biol. 2006 Jan 31;7:3.

125. Robinson JT, Thorvaldsdóttir H, Winckler W, Guttman M, Lander ES, Getz G, et al. Integrative genomics viewer. Nat Biotechnol. 2011 Jan;29(1):24–6.

126. Ripley BD. The R project in statistical computing. MSOR Connect. 2001 Feb;1(1):23–5.

127. Gill PS, Roberts ISD, Browning L, Perera R, Warren AY, Hamdy FC, et al. The handling and sampling of radical prostatectomy specimens for reporting and research: the Oxford approach. J Clin Pathol. 2012 Dec;65(12):1057–61.

128. Burnet FM. The concept of immunological surveillance. Prog Exp Tumor Res. 1970;13:1–27.

129. Davis AA, Patel VG. The role of PD-L1 expression as a predictive biomarker: an analysis of all US Food and Drug Administration (FDA) approvals of immune checkpoint inhibitors. J Immunother Cancer. 2019 Oct 26;7(1):278.

130. Palicelli A, Bonacini M, Croci S, Magi-Galluzzi C, Cañete-Portillo S, Chaux A, et al. What Do We Have to Know about PD-L1 Expression in Prostate Cancer? A Systematic Literature Review. Part 1: Focus on Immunohistochemical Results with Discussion of Pre-Analytical and Interpretation Variables. Cells. 2021 Nov 14;10(11):3166.

131. Gibney GT, Weiner LM, Atkins MB. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. Lancet Oncol. 2016 Dec;17(12):e542–51.

132. Guedes LB, Antonarakis ES, Schweizer MT, Mirkheshti N, Almutairi F, Park JC, et al. MSH2 Loss in Primary Prostate Cancer. Clin Cancer Res Off J Am Assoc Cancer Res. 2017 Nov 15;23(22):6863–74.

133. Clark WH, Elder DE, Guerry D, Braitman LE, Trock BJ, Schultz D, et al. Model predicting survival in stage I melanoma based on tumor progression. J Natl Cancer Inst. 1989 Dec 20;81(24):1893–904.

134. Schalper KA, Brown J, Carvajal-Hausdorf D, McLaughlin J, Velcheti V, Syrigos KN, et al. Objective measurement and clinical significance of TILs in non-small cell lung cancer. J Natl Cancer Inst. 2015 Mar;107(3):dju435.

135. Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer. 2012 15;12(4):298–306.

136. Rotterdam HZ, Melicow MM. Double primary prostatic adenocarcinoma. Urology. 1975 Aug;6(2):245–8.

137. Shapiro E, Huang H, McFadden DE, Masch RJ, Ng E, Lepor H, et al. The prostatic utricle is not a Müllerian duct remnant: immunohistochemical evidence for a distinct urogenital sinus origin. J Urol. 2004 Oct;172(4 Pt 2):1753–6; discussion 1756.

138. Zaloudek C, Williams JW, Kempson RL. 'Endometrial' adenocarcinoma of the prostate: a distinctive tumor of probable prostatic duct origin. Cancer. 1976 May;37(5):2255–62.

139. Cheng L, Song SY, Pretlow TG, Abdul-Karim FW, Kung HJ, Dawson DV, et al. Evidence of independent origin of multiple tumors from patients with prostate cancer. J Natl Cancer Inst. 1998 Feb 4;90(3):233–7.

140. Wei L, Wang J, Lampert E, Schlanger S, DePriest AD, Hu Q, et al. Intratumoral and Intertumoral Genomic Heterogeneity of Multifocal Localized Prostate Cancer Impacts Molecular Classifications and Genomic Prognosticators. Eur Urol. 2017 Feb;71(2):183–92.

141. Gillard M, Lack J, Pontier A, Gandla D, Hatcher D, Sowalsky AG, et al. Integrative Genomic Analysis of Coincident Cancer Foci Implicates CTNNB1 and PTEN Alterations in Ductal Prostate Cancer. Eur Urol Focus. 2019 May;5(3):433–42.

142. Bielski CM, Zehir A, Penson AV, Donoghue MTA, Chatila W, Armenia J, et al. Genome doubling shapes the evolution and prognosis of advanced cancers. Nat Genet. 2018 Aug;50(8):1189–95.

143. Bulten W, Kartasalo K, Chen PHC, Ström P, Pinckaers H, Nagpal K, et al. Artificial intelligence for diagnosis and Gleason grading of prostate cancer: the PANDA challenge. Nat Med. 2022 Jan;28(1):154–63.

144. Acs B, Rantalainen M, Hartman J. Artificial intelligence as the next step towards precision pathology. J Intern Med. 2020 Jul;288(1):62–81.

145. Eklund M, Jäderling F, Discacciati A, Bergman M, Annerstedt M, Aly M, et al. MRI-Targeted or Standard Biopsy in Prostate Cancer Screening. N Engl J Med. 2021 Sep 2;385(10):908–20.

146. Parker PA, Davis JW, Latini DM, Baum G, Wang X, Ward JF, et al. Relationship between illness uncertainty, anxiety, fear of progression and quality of life in men with favourable-risk prostate cancer undergoing active surveillance. BJU Int. 2016 Mar;117(3):469–77.

147. Farolfi A, Calderoni L, Mattana F, Mei R, Telo S, Fanti S, et al. Current and Emerging Clinical Applications of PSMA PET Diagnostic Imaging for Prostate Cancer. J Nucl Med Off Publ Soc Nucl Med. 2021 May 10;62(5):596–604.

148. Boerrigter E, Groen LN, Van Erp NP, Verhaegh GW, Schalken JA. Clinical utility of emerging biomarkers in prostate cancer liquid biopsies. Expert Rev Mol Diagn. 2020 Feb;20(2):219–30.

149. Sartor O, de Bono J, Chi KN, Fizazi K, Herrmann K, Rahbar K, et al. Lutetium-177-PSMA-617 for Metastatic Castration-Resistant Prostate Cancer. N Engl J Med. 2021 Sep 16;385(12):1091–103.

150. Antonarakis ES, Piulats JM, Gross-Goupil M, Goh J, Ojamaa K, Hoimes CJ, et al. Pembrolizumab for Treatment-Refractory Metastatic Castration-Resistant Prostate Cancer: Multicohort, Open-Label Phase II KEYNOTE-199 Study. J Clin Oncol Off J Am Soc Clin Oncol. 2020 Feb 10;38(5):395–405.

151. Antonarakis ES, Gomella LG, Petrylak DP. When and How to Use PARP Inhibitors in Prostate Cancer: A Systematic Review of the Literature with an Update on On-Going Trials. Eur Urol Oncol. 2020 Oct;3(5):594–611.

152. Merseburger AS, Waldron N, Ribal MJ, Heidenreich A, Perner S, Fizazi K, et al. Genomic Testing in Patients with Metastatic Castration-resistant Prostate Cancer: A Pragmatic Guide for Clinicians. Eur Urol. 2021 Apr;79(4):519–29.