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ACTIVE TREATMENT OF PROSTATE CANCER

Greetta Hackman

DOCTORAL DISSERTATION

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ABSTRACT

Prostate cancer is the second most common cancer worldwide and the most common cancer in Finland among men. While the prognosis of low-risk localised prostate cancer is excellent, a substantial proportion of prostate cancer patients experience disease progression after first-line treatment. This doctoral dissertation includes four studies that focused on active treatment options for prostate cancer patients with adverse pathologic features or risk factors associated with increased disease recurrence and/or mortality.

Epidermal growth factor receptor (EGFR) overexpression is associated with poor prognosis in prostate cancer and resistance to radiotherapy in several solid tumours. In the first two non-randomised trials, the objective was to evaluate the safety and efficacy of 250 mg once daily gefitinib, an orally active EGFR inhibitor, in prostate cancer patients. In the first (phase I/II) trial, 42 patients with nonmetastatic prostate cancer received gefitinib in combination with radical radiotherapy as the first-line treatment. In the second (phase II) trial, 30 patients with biochemical recurrence following radical treatment received gefitinib monotherapy.

The third study was a retrospective patient series of 46 patients with previously untreated metastatic prostate cancer—a diagnosis that continues to have poor overall survival. This study evaluated the safety and efficacy of multimodal treatment, including androgen deprivation and radical radiotherapy. In addition, the patients received various individually planned treatments.

The fourth study was a multicentre trial that randomised 250 radical prostatectomy-treated patients into an adjuvant radiation (126 patients) or observation (124 patients) group. All patients had positive surgical margins or extracapsular extension, both of which have been associated with increased prostate cancer progression; however, it was unclear whether these patients benefit from adjuvant radiation after surgery.

While most of the adverse events in the first study were mild to moderate, the toxicity of gefitinib in combination with radiation was unacceptable, considering that most patients had low-risk prostate cancer with a favourable prognosis even without any active treatments. In studies II–IV, the toxicity was acceptable.

The efficacy of gefitinib in prostate cancer patients was modest, both in combination with radical radiotherapy and as a monotherapy. The multimodal treatment approach in metastatic prostate cancer was promising but requires further confirmation in randomised trials. Adjuvant radiotherapy following radical prostatectomy resulted in significant improvement in patients' biochemical recurrence-free survival when compared to surgery alone. However, salvage radiation upon biochemical recurrence following surgery appeared equally effective in terms of overall survival.

Prostate cancer patients with adverse pathologic features or risk factors form a heterogeneous group of patients with different prognoses. To balance the subjective experience of treatment toxicity and the treatment's expected efficacy on survival, the patient must be adequately informed about the toxicity profiles of the treatments available as well as the risk for later disease progression. The aims of future research include more accurate risk-profiling for each prostate cancer patient, a better understanding of individual disease characteristics, and, thus, the identification of optimal treatments.

TIIVISTELMÄ (ABSTRACT IN FINNISH)

Eturauhassyöpä on miesten toiseksi yleisin syöpä maailmassa ja yleisin syöpä Suomessa. Vaikka paikallisena todetun matalan riskin eturauhassyövän ennuste on nykyään erinomainen, huomattavalla osalla eturauhassyöpäpotilaista tauti uusiutuu ensilinjan hoidosta huolimatta. Tämä väitöskirja koostuu neljästä osatyöstä, jotka keskittyivät aktiivihoitoihin eturauhassyöpäpotilailla, joilla oli todettu syövän uusiutumiseen ja/tai syöpäkuolleisuuteen liittyviä epäsuotuisia patologisia piirteitä tai riskitekijöitä.

Epidermaalisen kasvutekijän reseptorin (EGFR) poikkeavan runsas esiintyminen on yhdistetty eturauhassyöpäpotilaiden huonoon ennusteeseen sekä sädehoitoresistenssiin monissa kiinteissä kasvaimissa. Kahden ensimmäisen satunnaistamattoman tutkimuksen tavoitteena oli arvioida suun kautta otettavan EGFR:n estäjän, gefitinibin, turvallisuutta ja tehoa eturauhassyöpäpotilailla annoksella 250 mg kerran päivässä. Ensimmäisessä (vaiheen I/II) tutkimuksessa, 42 potilasta, joilla oli metastasoimaton eturauhassyöpä, sai gefitinibi-hoidon ja radikaalin sädehoidon ensilinjan yhdistelmähoitona. Toisessa (vaiheen II) tutkimuksessa, 30 potilasta, joilla oli radikaalihoidon jälkeen todettu eturauhassyövän biokemiallinen uusiutuminen, sai gefitinibi-hoidon monoterapiana.

Kolmas tutkimus oli retrospektiivinen potilassarja 46 miehestä, joilla oli vastikään todettu metastaattinen eturauhassyöpä—diagnoosi, jonka elinajanodote on edelleen huono. Kaikki potilaat saivat multimodaalihoidon, johon sisältyi antiandrogeeni-hoito ja radikaali sädehoito. Lisäksi potilaat saivat useita yksilöllisesti suunniteltuja hoitoja.

Neljäs tutkimus oli monikeskustutkimus, jossa satunnaistettiin 250 radikaalilla prostatektomialla hoidettua potilasta liitännäissädehoitoon (126 potilasta) tai seurantaan (124 potilasta). Potilailla oli todettu positiivinen leikkausmarginaali tai eturauhaskapselin läpi tunkeutuva tauti, joihin liittyy kohonnut eturauhassyövän uusiutumisriski. Oli kuitenkin epäselvää, hyötyisivätkö nämä potilaat liitännäissädehoidosta leikkauksen yhteydessä.

Vaikka suurin osa ensimmäisen tutkimuksen haittavaikutuksista oli mietoja tai kohtalaisia, gefitinibin ja sädehoidon haittavaikutusprofiilia ei voitu pitää hyväksyttävänä, kun otetaan huomioon, että suurimmalla osalla potilaista oli matalan riskin hyväennusteinen tauti ilman aktiivista hoitoakin. Tutkimuksissa II-IV annettujen hoitojen haittavaikutusprofiili oli hyväksyttävä.

Gefitinibin tehokkuus eturauhassyövän hoidossa oli vaatimaton sekä yhdistelmähoitona radikaalin sädehoidon kanssa että monoterapiana. Multimodaalihoito metastaattisessa eturauhassyövässä vaikutti lupaavalta, mutta tämän tuloksen vahvistamiseksi tarvitaan satunnaistettuja tutkimuksia. Liitännäissädehoito radikaalin prostatektomian jälkeen paransi merkittävästi biokemiallista uusiutumisvapaata elinaikaa verrattuna

pelkkään kirurgiseen hoitoon. Kun katsotaan kokonaiselinaikaa, leikkauksen jälkeen biokemiallisesti uusiutuneeseen tautiin annettu toisen linjan (salvage) sädehoito vaikutti kuitenkin yhtä tehokkaalta hoidolta.

Eturauhassyöpäpotilaat, joilla on epäsuotuisia patologisia piirteitä tai riskitekijöitä, muodostavat heterogeenisen ryhmän potilaita, joiden ennusteet poikkeavat toisistaan. Tasapainottaakseen subjektiivisen kokemuksensa hoidon haitoista ja hoidon odotettavissa olevasta vaikutuksesta elinaikaan, potilaan on saatava riittävästi tietoa sekä saatavilla olevien hoitojen haittavaikutusprofiileista että taudin etenemisriskistä. Tulevaisuuden tutkimustavoitteita ovat yksittäisen eturauhassyöpäpotilaan riskien tarkempi profilointi, eturauhassyövän yksilöllisten ominaisuuksien parempi ymmärtäminen ja siten optimaalisten hoitojen tunnistaminen.

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LIST OF ORIGINAL PUBLICATIONS

This doctoral dissertation is based on the following original publications:

I G. Joensuu, T. Joensuu, P. Nokisalmi, C. Reddy, J. Isola, M. Ruutu, M. Kouri, P. A. Kupelian, J. Collan, S. Pesonen, A. Hemminki. A phase I/II trial of gefitinib given concurrently with radiotherapy in patients with nonmetastatic prostate cancer. *Int. J. Radiat. Oncol. Biol. Phys.* 78, 42–49 (2010).

II G. Joensuu, T. Joensuu, N. Nupponen, M. Ruutu, J. Collan, S. Pesonen, A. Hemminki. A phase II trial of gefitinib in patients with rising PSA following radical prostatectomy or radiotherapy. *Acta Oncol.* 51, 130–133 (2012).

III T. Joensuu, G. Joensuu, K. Kairemo, T. Kiljunen, M. Riener, A. Aaltonen, M. Ala-Opas, A. Kangasmaki, T. Alanko, L. Taipale, P. Hervonen, A. Butzow, I. Virgolini, A. Hemminki. Multimodal Primary Treatment of Metastatic Prostate Cancer with Androgen Deprivation and Radiation. *Anticancer Res.* 36, 6439–6447 (2016).

IV G. Hackman, K. Taari, T. L. Tammela, M. Matikainen, M. Kouri, T. Joensuu, T. Luukkaala, A. Salonen, T. Isotalo, A. Pétas, N. Hendolin, P. J. Boström, S. Aaltomaa, K. Lehtoranta, P. Hellström, J. Riikonen, M. Korpela, H. Minn, P. L. Kellokumpu-Lehtinen, E. Pukkala, A. Hemminki. Randomised Trial of Adjuvant Radiotherapy Following Radical Prostatectomy Versus Radical Prostatectomy Alone in Prostate Cancer Patients with Positive Margins or Extracapsular Extension. *Eur. Urol.* 76, 586–595 (2019).

The publications are referred to in the text by their roman numerals.

ABBREVIATIONS

cT	Clinical tumour stage
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CYP17	Cytochrome P450 17α–hydroxy/17,20-lyase enzyme
DNA	Deoxyribonucleic acid
EAU	The European Association of Urology
EGFR	Epidermal growth factor receptor
GLMM	Generalised linear mixed model
Gy	Gray
IIEF-5	The International Index of Erectile Function questionnaire
IMRT	Intensity-modulated radiation therapy
IPSS	The International Prostate Symptom Score questionnaire
IQR	Interquartile range
ISUP	International Society of Urological Pathology
KRAS	Kirsten rat sarcoma viral oncogene homolog
LENT-SOMA	The Late Effects Normal Tissue Task Force-Subjective, Objective, Management, Analytic questionnaire
LHRH	Luteinising hormone-releasing hormone
M	Metastasis stage
N	Nodal stage
NCCN	The National Comprehensive Cancer Network
PET	Positron emission tomography
PSA	Prostate-specific antigen
PSMA	Prostate-specific membrane antigen
pT	Pathologic tumour stage
T	Tumour stage
ULN	Upper limit of normal
VMAT	Volumetric modulated arc therapy

1 INTRODUCTION

1.1 BACKGROUND

Worldwide, prostate cancer is the second most common cancer and the fifth most common cause of cancer mortality among men (*1*). Specifically, in Finland, an annual 5,500 new diagnoses and 900 prostate cancer deaths make prostate cancer the most common cancer and the second most common cause of cancer mortality among Finnish men (*2*).

Due to advancements in diagnostics and treatment, increased awareness, and early detection, the prognosis of localised prostate cancer has improved significantly during the past decades (*3*). Active definitive treatment options for clinically localised prostate cancer are radical radiotherapy and radical prostatectomy, with a 10-year cancer-specific survival rate commonly approaching 100% (*4*). Other emerging treatment options include cryotherapy and high-intensity focused ultrasound (*5*, *6*). However, therapy with curative intent fails to achieve long-term disease-free survival in some cases, especially in patients with high-risk features (*7*, *8*). For patients with locally advanced prostate cancer, there is a lack of evidence on optimal treatment options. Furthermore, there is no consensus on how to manage patients with disease recurrence following definitive treatment.

In diagnostics and treatment planning, it is equally essential to identify high-risk patients often requiring a combination of treatments to achieve cure, as well as low-risk patients to avoid overtreatment and thus treatment-related long-term adverse effects. Currently, the risk stratification for prostate cancer patients varies in different guidelines and studies. Three established prognostic factors—tumour, node, metastasis (TNM) stage, Gleason score/International Society of Urological Pathology (ISUP) grade group, and initial prostate-specific antigen (PSA)—remain, while several other risk features and nomograms have been investigated to identify patients at an increased risk for disease recurrence, with varying results (*9*, *10*). In addition, it is necessary to recognize active treatment options for these patients, including mono- and multimodal therapies, aiming at maximal long-term survival with an acceptable adverse effect profile.

With regard to newly diagnosed metastatic prostate cancer, there has been a significant shift toward more active treatment in the past decade due to the discovery of several novel treatment options (*11*). Despite improved survival rates, the overall survival of metastatic prostate cancer remains poor, ranging from 34 to 62 months (*12–15*). Clearly, the metastatic cancer requires the identification of new, active treatment options and their combinations to achieve better cancer control and improved survival.

1.2 FOCUS OF THE DISSERTATION AND DEFINITION OF ACTIVE TREATMENT

The patients included in the studies of this dissertation had adverse pathologic features or risk factors associated with prostate cancer recurrence and/or mortality. These include epidermal growth factor receptor (EGFR) expression, positive surgical margins (following radical prostatectomy), an extracapsular extension of the cancer, and metastatic prostate cancer. The active treatment options studied included 1) gefitinib alone and in combination with radical radiotherapy in nonmetastatic prostate cancer; 2) multimodal treatment in metastatic prostate cancer; and 3) adjuvant radiotherapy following radical prostatectomy in nonmetastatic prostate cancer. The original studies presented in this dissertation include prostate cancer patients with localised, locally advanced, or metastatic prostate cancer, and patients with biochemical recurrence following therapy with curative intent. In this dissertation, the concept of active treatment rules out watchful waiting, active surveillance, and palliative care. Thus, this paper emphasizes active treatment, with the aim to cure (in nonmetastatic prostate cancer) or achieve disease remission (in metastatic prostate cancer).

2 REVIEW OF THE LITERATURE

2.1 RISK STRATIFICATION

Most guidelines classify prostate cancer into risk groups based on three established prognostic factors: 1) clinical/pathological TNM staging, 2) histologic Gleason score/ISUP grade group, and 3) PSA at the time of diagnosis (*16–18*). The aim of classification is to group patients with similar prognostic impacts and then recommend treatment options depending on their risk group (*19*, *20*). Imaging, genomic profile, and/or molecular analysis can provide additional information regarding risk evaluation; however, they are not routinely recommended.

2.1.1 STAGING

TNM staging describes the expansion of the original tumour (T), cancer's possible dissemination to the regional lymph nodes (N), and metastatic status (M).

The clinical T-stage (cT) is commonly based on digital rectal examination, pathologic findings from prostate biopsies, and, in some cases, imaging. However, some guidelines base the cT-stage on digital rectal examination alone (*16*). The pathologic T-stage (pT), on the other hand, is based on pathologic findings of the removed prostate identified via radical prostatectomy, providing a more accurate evaluation of the cancer's expansion compared to the cT.

2.1.2 LOCALISED AND LOCALLY ADVANCED PROSTATE CANCER

Generally, clinically localised (cT1-2) prostate cancer is a low-risk disease with 10-year cancer-specific mortality ranging between 1 to 3% (*21*, *22*). However, when classified as a high-risk disease, the risk of cancer recurrence increases, and the 10-year cancer-specific mortality rises to 7% (*8*, *22–24*).

Extracapsular extension (T3-4) is an independent risk factor of biochemical recurrence, metastatic cancer, and prostate cancer death (*25*, *26*). Further invasion into the seminal vesicles is strongly associated with an increased risk of biochemical recurrence and prostate cancer mortality (*27*).

A locally advanced prostate cancer generally refers to nonmetastatic T3-4 prostate cancer. However, some studies even consider T1-2 cancer as locally advanced if the patient has other high-risk features, such as PSA > 20 μg/l or a Gleason score of 8 to 10, indicating possible disease progression through the prostate (*23*, *28*). For locally advanced

prostate cancer, the 10-year cancer-specific mortality is between 14 to 22% (*29*).

2.1.3 POSITIVE SURGICAL MARGINS

Cancer cell expansion to the border of a surgically removed prostate gland indicates *positive surgical margins*. In a meta-analysis of 141,222 radical prostatectomy-treated patients, positive surgical margins significantly decreased biochemical recurrence-free, cancer-specific, and overall survival compared to patients with negative margins (*30*). In the same meta-analysis, patients with positive margins had a significantly higher risk for cancer-specific and overall mortality (*30*).

Although positive margins are associated with an increased risk of biochemical recurrence, in some patients, the risk seems to be relatively low (*20*, *31–33*). Positive margins appear to predict prostate cancer mortality in some studies, particularly in pT3 disease, yet these findings are inconsistent (*27*, *34*). In a population-based study of 13,198 patients, positive surgical margins appeared as an independent predictor of secondary treatment (indicating biochemical recurrence) and palliative radiotherapy (*35*). However, there was no significant association between positive margins and prostate cancer mortality (*35*). The risk for palliative radiotherapy and cancer-specific mortality was highest among patients with pT3b and a Gleason score of 9 to 10, while, for patients with pT2 and a low Gleason score, the risk for palliative radiotherapy appeared low (*35*).

In the ProtecT trial, patients who experienced disease progression following radical prostatectomy were more likely to have positive surgical margins as well as higher Gleason score, pathological staging, larger tumours, and positive lymph nodes compared to patients without progression (*36*). Marchetti et al. reported significantly higher Gleason scores, higher pT-stages, higher PSA values, and smaller prostates in pT2-3 patients with positive surgical margins compared to patients with negative margins (*37*).

Thus, positive surgical margins appear to predict poor outcomes, especially in patients with high-risk features, whereas, in patients with low-risk features the importance of positive margins seems controversial. Altogether, the role of positive surgical margins as an independent risk factor, especially in pT2 patients, continues to be unestablished.

2.1.4 LYMPH NODE INVASION AND METASTATIC PROSTATE CANCER

Generally, patients with intermediate/high-risk features require imaging to detect possible lymph node invasion and metastases. The mainstays of conventional imaging are bone scan and computed tomography (CT), although research shows promising results for magnetic resonance imaging

(MRI), choline positron emission tomography (PET)/CT, and prostate-specific membrane antigen (PSMA) PET/CT imaging as well (*14*, *15*, *38–41*).

The number of positive lymph nodes and the lymph node density are prognostic factors in prostate cancer (*42*, *43*). The presence of two or more positive lymph nodes, lymph node diameter > 10 mm, and a lymph node density ≥ 20 to 30% decreases cancer-specific survival (*43–45*).

Prostate cancer primarily metastasizes to the bone. Regardless of its poor survival rate, metastatic prostate cancer includes a heterogeneous group of patients with different disease loads (*12*, *15*, *46*). In the SWOG trial, PSA after seven months of androgen deprivation therapy turned out to be as a strong independent predictor of survival in patients with newly diagnosed metastatic prostate cancer (*47*). The CHARTEED trial defined high-volume metastatic disease as "*the presence of visceral metastases or at least four bone lesions with at least one beyond the vertebral bodies and pelvis*" (*46*). In the LATITUDE trial, high-risk features of metastatic disease included a Gleason score of 8, at least three bone metastases, and/or visceral metastasis (*48*). Nevertheless, there are no established prognostic factors for survival in newly diagnosed metastatic prostate cancer.

2.1.5 PSA

Initial PSA (taken before treatment) is a significant prognostic factor in prostate cancer (*49–51*). The common threshold for a high-risk disease is PSA > 20 μg/l (*52*). A high initial PSA increases the risk for biochemical recurrence, metastatic disease, and cancer-specific mortality (*53*, *54*).

2.1.6 GLEASON SCORE/ISUP GRADE GROUP

The Gleason score, defined using prostate biopsy samples, describes prostate cancer cell differentiation of a higher grade as indicating poor differentiation and a more aggressive disease. It is the sum of the most predominant histologic pattern and the second most common pattern in the prostate biopsy. The original Gleason grading from 1966 used scores from 2 to 10; however, it has since undergone several modifications (*55*). The latest ISUP consensus from 2014 recommends five grade groups: 1) group 1 (Gleason score ≤ 3+3), 2) group 2 (Gleason score 3+4), 3) group 3 (Gleason score 4+3), 4) group 4 (Gleason score 4+4, 3+5, and 5+3), and 5) group 5 (Gleason score 9 to 10) (*56*). The ISUP grade groups correlate better with survival rates compared to the traditional Gleason scores and reduce overtreatment and unnecessary concern in low-risk, Gleason score ≤ 6 disease (*57–59*).

2.1.7 EGFR EXPRESSION

EGFR, also known as ErbB1 or HER1, is a transmembrane glycoprotein with an intracellular tyrosine kinase domain. It was the first discovered member

of the ErbB (erythroblastic viral leukaemia oncogene) receptor tyrosine kinase family, the signalling pathways of which contribute significantly to cell survival and proliferation in various normal tissues and body fluids. The overexpression of EGFR, on the other hand, is one of the distinct features of several solid tumours including prostate cancer. EGFR overexpression is associated with tumour growth, the inhibition of apoptosis, the promotion of angiogenesis, and metastatic disease, although its complete pathophysiology remains unsolved (*60*, *61*).

In prostate cancer, EGFR overexpression is strongly associated with biochemical relapse, castration-resistant prostate cancer, and metastatic disease (*60–63*). In addition, patients with metastatic prostate cancer appear to have EGFR expression in their circulating tumour cells (*61*). However, EGFR expression is not a strong prognostic factor in prostate cancer (*60*).

The association between EGFR overexpression and the established prognostic factors of prostate cancer is controversial. In a study by Shah et al., there was no association between EGFR expression and prognostic factors such as Gleason score, seminal vesicle invasion, or preoperative PSA (*62*). Schlomm et al., however, found a significant association between EGFR expression and high Gleason scores, advanced pT-stage, and preoperative PSA (*60*). Yet, in both studies, EGFR expression was not an independent predictor of biochemical recurrence (*60*, *62*).

In a study of 2,497 patients, Schlomm et al. detected 18% EGFR expression in radical prostatectomy specimens mostly from localised or locally advanced prostate cancer (*60*). In different studies, EGFR expression appears lower in localised, hormone-naïve prostate cancer and increased in castration-resistant and metastatic cancer (*60*, *64*, *65*). Di Lorenzo et al. detected 41% EGFR expression in hormone-naïve radical prostatectomy-treated patients, 76% in patients who received hormonal therapy prior radical prostatectomy, and 100% in patients with castration-resistant metastatic cancer (*66*). These results indicated that EGFR expression increases as the cancer progresses and finally converts into difficult-to-treat castration-resistant prostate cancer (*62*, *66*, *67*). In addition, EGFR overexpression is associated with resistance to radiotherapy and chemotherapy (*68–71*). In preclinical and clinical studies, the inhibition of EGFR in a combination with radiotherapy appears to result in better tumour control and survival compared to irradiation alone (*72*, *73*).

2.1.8 CLASSIFICATION AND STAGING

The D'Amico classification, first presented in 1998, is probably the most well-known approach to categorizing patients with similar risk for PSA failure following radical radiotherapy or radical prostatectomy (Table 1) (*18*, *74*). The classification is confined to localised disease alone, and it is based on the clinical tumour stage (TNM), biopsy Gleason score, and pretreatment PSA, and, consequently, classifies patients into low-, intermediate-, and high-risk groups (*74*). However, due to earlier detection of the cancer, improved diagnostics and cancer treatments over the past decades, the number of low-risk prostate cancer cases is pronounced and the related outcomes have

improved (*75*). Hence, the relevance of the D'Amico classification seems to be decreasing, although, it still significantly predicts biochemical recurrence-free survival among patients with localised prostate cancer (*75*).

Table 1. Risk stratification according to the D'Amico classification (*74*).

	Risk group		
	Low	**Intermediate**	**High**
TNM stage	T1c-2a	T2b	≥ T2c
Gleason score	≤ 6	7	≥ 8
PSA	< 10 μg/l	10–20 μg/l	> 20 μg/l

PSA = prostate-specific antigen, TNM = tumour, node, metastasis.

Different prognostic models aim to identify patients of different risk group in terms of disease recurrence or survival. In addition to the D'Amico classification, other well-known models include: 1) the Cambridge Prognostic Groups, and 2) the University of California San Francisco (UCSF) Cancer of the Prostate Risk Assessment (CAPRA) Score (*9*). The Cambridge Prognostic Groups criteria is based on T-stage, ISUP grade group, and PSA, and it stratifies patients into five risk groups predicting their cancer-specific mortality (*76*). The UCSF-CAPRA score is based on T-stage, Gleason score, PSA, percentage of positive biopsies, and age (*77*). It gives the patient a score from 0 to 10, with a higher score indicating a higher disease recurrence rate. However, critics of these models emphasize their inability to take into account non-cancer mortality and treatment effects (*9*).

As the prognosis of localised, low-risk prostate cancer continues to be excellent, research is more focused on identifying patients with localised or locally advanced yet not metastasized cancer with risk features predicting an increased risk of disease progression. Currently, the definition of *high-risk* prostate cancer varies among different studies. Generally, however, the features of high-risk disease include c/pT ≥ 3, Gleason score > 7, and PSA > 20 μg/l (*78*, *79*). Patients who experience disease progression following treatment with curative intent have more likely intermediate/high-risk than low-risk prostate cancer at baseline (*36*).

Currently, the European Association of Urology (EAU) classifies localised and locally advanced prostate cancer into three risk groups—low-, intermediate-, and high-risk—according to the cTN-stage, Gleason score/ISUP grade group, and PSA (*16*). The National Comprehensive Cancer Network (NCCN), on the other hand, uses five risk groups for clinically localised prostate cancer—very low-, low-, intermediate-, high-, and very high-risk—according to cT-stage, Gleason score/ISUP grade group, number/percentage of positive biopsy cores, and PSA (*17*).

2.1.9 BIOCHEMICAL RECURRENCE

A persistently rising PSA following initial prostate cancer treatment is commonly treated as a *biochemical recurrence* (*80*). As the natural history of prostate cancer is prolonged, a rising PSA may represent the only detectable manifestation of prostate cancer progression—even several years before clinical relapse (*81–83*). When the studies included in this dissertation were initiated, the PSA threshold for biochemical recurrence was higher (commonly PSA ≥ 0.4 μg/l), but then it decreased over time due to improved laboratory techniques. While the exact definition of biochemical recurrence varies, currently, the common threshold is PSA ≥ 0.2 μg/l (*84*).

Generally, biochemical recurrence precedes local progression, metastatic disease, and, eventually, prostate cancer death, usually within five years after initial treatment (*81*, *85*). However, not all patients with biochemical recurrence experience the further progression of the disease (*82*). A rapid increase of PSA and PSA relapse shortly after definitive treatment is associated with an increased risk of metastases and cancer death (*24*, *86*). PSA doubling time less than one year and a Gleason score between 8 to 10 appear to be strong predictors of clinical relapse following biochemical recurrence (*86*).

The progression from biochemical recurrence to clinically evident relapse, which is usually detected through imaging, can occur even 20 years after diagnosis (*81*). Yet, approximately only one third of patients with biochemical recurrence experience clinically evident disease progression (*87*, *88*).

In prostate cancer research, biochemical recurrence is one of the most used endpoints to evaluate treatment outcomes. Of patients with low-risk, localised cancer, a minority with biochemical recurrence experience clinically evident disease progression. Thus, other endpoints, such as cancer-specific or overall survival might have higher relevance as an outcome measure. However, biochemical recurrence continues to be an independent risk factor for metastatic disease and prostate cancer death (*86*). Especially in patients with several risk features and locally advanced or high-risk disease, the relevance of biochemical recurrence as a prognostic factor is pronounced, indicating further disease progression and the need for active treatment.

2.2 ACTIVE TREATMENT OPTIONS IN PROSTATE CANCER

2.2.1 LOCALISED AND LOCALLY ADVANCED PROSTATE CANCER

2.2.1.1 LOCAL TREATMENTS WITH CURATIVE INTENT

Radical prostatectomy includes the surgical removal of the entire prostate gland. Generally, it is also accompanied by the removal of seminal vesicles and, depending on the risk features of the cancer, lymph node dissection.

The concept of radical radiotherapy covers external beam radiotherapy and brachytherapy. Intensity-modulated radiation therapy (IMRT) provides high doses of radiation delivered within the treatment field from a limited number of beam angles. In addition, external beam radiotherapy can be delivered with volumetric modulated arc therapy (VMAT) through a linear accelerator that rotates continuously around the patient in contrast to fixed beam IMRT. Compared to IMRT, VMAT provides higher doses in shorter treatment durations.

In low-dose-rate brachytherapy, the patient receives permanent radioactive seed implants directly to his prostate. In high-dose-rate brachytherapy, the prostate is internally radiated as well but in the form of radioactive sources through implant catheters placed into the prostate gland through the perineum.

2.2.1.2 COMPARISON OF RADICAL RADIOTHERAPY, RADICAL PROSTATECTOMY, AND ACTIVE SURVEILLANCE

Radical radiotherapy and radical prostatectomy are well-established active treatment options for clinically localised prostate cancer (*4*, *89*, *90*). When comparing long-term results in non-randomised studies, radical prostatectomy often appears superior to radical radiotherapy (*91*). However, many of these studies seem to have a selection bias in favour of surgery with men who are physically more fit and have lower risk disease among prostatectomy-treated patients compared to radiation-treated patients (*18*, *92*).

While the focus of this dissertation is on active treatment options, it is equally important to acknowledge the role of active surveillance as an alternative to local treatments in newly diagnosed prostate cancer. When taking into account the various and potentially long-term adverse events of local treatments, active surveillance appears to be a well-established option, especially in patients with low-risk prostate cancer.

The randomised PIVOT trial, which had a follow-up of 19.5 years (median 12.7 years), compared radical prostatectomy and observation in

localised prostate cancer and showed similar survival between the treatments among patients with low-risk disease (*90*). Intermediate- and high-risk patients seemed to benefit a bit more from radical treatment in terms of all-cause mortality (intermediate-risk) and disease progression (high-risk), albeit these findings were statistically insignificant (*90*). Furthermore, regardless of the risk group, there was no statistically significant difference in prostate cancer-specific survival or overall survival between the treatments (*90*).

The SPCG-4 trial, which had a follow-up of 29 years (median 23.6 years), randomised patients with localised disease to radical prostatectomy or watchful waiting (*93*). It showed significantly improved prostate cancer-specific survival, overall survival, and metastatic survival among surgery-treated patients when compared to watchful waiting (*93*). Risk of death from prostate cancer started to increase from Gleason score 3+4 (*93*). When compared to patients with a Gleason score ≤ 6, patients with a Gleason score of 3+4 had similar risk of death from prostate cancer, while for patients with a Gleason score of 4+3 the risk was five times higher (*93*).

In the randomised ProtecT-trial, which compared external beam radiotherapy in combination with androgen deprivation, radical prostatectomy, and active monitoring, the local treatments appeared more effective in terms of clinical progression and cancer-specific mortality (*4*). Out of the 545 patients who were assigned to active monitoring, 25% received radical treatments within three years, and, more than 50% within 10 years following their initial assignment (*4*). There were no statistical differences between the treatments regarding prostate cancer-specific or overall survival (*4*). Considering that most of the patients enrolled in the ProtecT-trial had a low-risk disease and the number of events was low, a survival longer than the median 10 years would be needed to draw conclusions about the efficacy of local treatments (*94*).

PIVOT, SPCG-4, and ProtecT all included high-risk patients (*4*, *90*, *93*). According to current knowledge, high-risk prostate cancer represents an aggressive disease and, thus, active surveillance is not recommended for these patients. Consequently, today, enrolment of high-risk patients in the observation group would be unethical. In addition, inclusion of high-risk disease may have increased the number of events in all three trials, especially in the active surveillance groups, which may have favoured radical treatments over active surveillance (*4*, *90*, *93*).

In a randomised study by Giberti et al., the biochemical recurrence-free survival between radical prostatectomy and brachytherapy was similar but did not have statistical significance in low-risk prostate cancer patients (*95*).

In a Cochrane meta-analysis that compared transrectal ultrasonography-guided prostate biopsies to multiparametric MRI-targeted biopsies, the latter showed more favourable diagnostic accuracy in terms of sensitivity and specificity in the detection of Gleason score ≥ 7 prostate cancer (*96*). Thus, since the accuracy of MRI-targeted biopsy in clinically significant prostate cancer is excellent, active surveillance can be considered as a safe option even for patients with Gleason score 6 disease when MRI-targeted biopsy is available (*96*).

For patients with low-risk localised prostate cancer, active surveillance appears equally effective when compared to radical treatments. With regard to radical treatments, there is still a lack of randomised trials with long-term follow-up comparing the efficacy of radical prostatectomy and radical radiotherapy.

2.2.1.3 RADICAL RADIOTHERAPY IN HIGH-RISK LOCALISED AND LOCALLY ADVANCED PROSTATE CANCER

Traditionally, hormonal therapy or radiotherapy alone was the standard of care for locally advanced prostate cancer. Later, in the late 2000s, several randomised trials proved the superiority of radiotherapy and hormonal therapy given in combination, which made the treatment of these patients into more active mode and improved their prognosis significantly (*28*, *29*, *97*). Currently, the recommendations for locally advanced prostate cancer emphasize multimodal therapies including radical prostatectomy (*16*, *17*). As high-risk localised and locally advanced prostate cancer cases have similar prognostic profiles, the same treatment recommendations commonly apply for both (*98*).

Based on several randomised trials, external beam radiation therapy in combination with long-term androgen suppression was found superior to radiation alone in high-risk localised and locally advanced prostate cancer in terms of disease progression, cancer-specific survival, and overall survival (*23*, *28*, *29*, *97*, *99*, *100*). In the ASCENDE-RT trial, the addition of brachytherapy to external beam radiation and androgen deprivation therapy resulted in even better biochemical-free survival compared to combination treatment without brachytherapy (*101*). Docetaxel, on the other hand, failed to improve the biochemical disease-free survival when given after radiation for intermediate- and high-risk prostate cancer patients when compared to radical radiotherapy alone in the SPCG-13 trial (*102*).

2.2.1.4 RADICAL PROSTATECTOMY

2.2.1.4.1 POSITIVE SURGICAL MARGINS FOLLOWING RADICAL PROSTATECTOMY

The incidence of positive margins is associated with surgical experience, although the impact of experience on cancer-control is controversial (*103*). In a multinational study of 22,393 patients, Sooriakumaran et al. reported a positive surgical margin rate of 14% in robotic, 16% in laparoscopic, and 23% in open surgery (*104*). After the stratification of the risk groups, however, there seemed to be no difference between open and robotic surgery in the low- and intermediate-risk patients, whereas, in the high-risk patients, the robotic surgery significantly decreased the risk of positive margins (*105*).

In a study by Keller et al., 23% of ≤ pT2 and 54% of ≥ pT3 radical prostatectomy-treated patients had positive surgical margins (*106*). Usually, as nerve-sparing surgery is associated with an increased risk of positive margins, it is not recommended for high-risk patients with an increased risk of disease progression (*107*). Due to the increased use of preoperative MRI and, thus, optimized nerve-sparing, and improved surgical techniques, including the increased use of robotic surgery, the rate of positive surgical margins seems to be decreasing (*108*).

2.2.1.4.2 ORGAN CONFINED PROSTATE CANCER AND EXTRACAPSULAR EXTENSION

A common approach to identifying radical prostatectomy patients with different outcomes is to distinguish between organ-confined and non-organ-confined prostate cancer, as the former has significantly better outcomes. However, non-organ-confined cancer includes a heterogeneous group of patients whose prognosis is not uniformly poor. Thus, it would be advantageous to identify patients at an increased risk of cancer-specific mortality in this group.

In a study of 11,521 prostatectomy-treated patients, Eggener et al. reported a 15-year prostate cancer-specific mortality of 0.8 to 1.5% among organ-confined patients, 3 to 10% among extracapsular extension patients, 15 to 27% among seminal vesicle invasion patients, and 22 to 30% among patients with lymph node metastases (*27*). For patients with a Gleason score between 8 to 10, the 15-year prostate cancer-specific mortality was 26 to 37%. Only the primary and secondary Gleason score, seminal vesicle invasion, and year of surgery were significantly associated with prostate cancer-specific mortality (*27*).

Out of high-risk radical prostatectomy-treated patients, 25 to 37% have a specimen-confined cancer (*109*, *110*). For these patients, radical prostatectomy appears to be an excellent treatment providing long-term cancer control compared to high-risk patients with non-specimen-confined cancer (*109*, *110*). As for patients with locally advanced prostate cancer, especially in the presence of other high-risk features, the risk of cancer-specific mortality increases, and, thus, adjuvant therapies and a multimodal approach could provide better outcomes compared to radical prostatectomy alone.

2.2.1.4.3 RADICAL PROSTATECTOMY AND PELVIC LYMPH NODE DISSECTION

Pelvic lymph node dissection is commonly recommended upon radical prostatectomy for intermediate- and high-risk prostate cancer patients, although there is no evidence of its survival benefit (*111*). In addition, pelvic lymph node dissection associates with higher morbidity and exposes patients to a higher risk of operative complications when compared to surgery without pelvic lymph node dissection (*111*). A common justification for pelvic

lymph node dissection is its superior accuracy in prostate cancer staging (*111*). However, both MRI and PSMA PET/CT have appeared as promising staging tools in the detection of lymph node metastases (*112*, *113*). Compared to MRI, PSMA PET/CT seems to have a slightly better accuracy, especially in terms of sensitivity (*41*, *114*). In the future, an MRI or PSMA PET/CT that shows no lymph node metastases could spare patients from pelvic lymph node dissection and its potential complications without compromising the therapeutic effect of the primary treatment. Yet, as the extended pelvic lymph node dissection continues to represent the most accurate modality in detecting lymph node metastases among high-risk prostate cancer patients, pelvic lymph node dissection could be reserved for patients with high-risk features/high risk of lymph node metastases (*115*, *116*).

2.2.1.4.4 RADICAL PROSTATECTOMY AND ADJUVANT THERAPIES

Before study IV, there were three previous randomised studies that compared adjuvant radiotherapy to observation in radical prostatectomy-treated patients: the Southwest Oncology Group (SWOG 8794), the European Organization for Research and Treatment of Cancer (EORTC 22911), and the German Cancer Society (ARO 96-02/AUO AP 09/95) (*117–119*). All three trials included pT3 patients with or without positive margins. In addition, the EORTC included pT2 patients with positive margins, and the ARO included pT4 patients. While all trials detected a significant improvement in biochemical progression-free survival in the adjuvant radiotherapy-group, only the SWOG found significant improvements in the metastasis-free and overall survival (*117–119*). Of note, in the SWOG, the majority of events in the metastasis-free and overall survival analyses were unrelated to prostate cancer (*117*).

Out of the 1,005 EORTC patients, 163 (16%) had pT2 prostate cancer with positive surgical margins. In the first EORTC publication (2005), which had a median follow-up of five years, the researchers detected a statistically significant benefit from adjuvant radiation in patients without extracapsular extension and without seminal vesicle invasion (*120*). However, in the latest EORTC publication, which had a median follow-up of 10.6 years, corresponding results are not mentioned (*118*). In a subgroup analysis, positive surgical margins and age < 70 years were associated with greater adjuvant radiotherapy benefits (*118*). Similarly, in the ARO, patients with positive margins benefitted the most from adjuvant radiotherapy (*119*). The SWOG reported no subgroups that benefitted from adjuvant radiotherapy (*117*, *121*).

Still, it remains unclear which radical prostatectomy-treated patients benefit from adjuvant radiotherapy following surgery. Out of the patients with seminal vesicle invasion, 80–86% suffer from biochemical recurrence and, thus, they commonly receive adjuvant radiotherapy following radical prostatectomy (*122*, *123*). In patients with positive margins or extracapsular extension following radical prostatectomy, there is a lack of consensus regarding the optimal treatment. For these patients, the latest guidelines recommend adjuvant radiotherapy or observation (*16*, *17*). With regard to high-risk cancer, recent studies promote radical prostatectomy

combined with adjuvant treatment over radical radiotherapy alone as well as a multimodal treatment approach (*10*, *124*). Postoperative nomograms aim to identify patients who benefit the most from adjuvant radiotherapy (*10*). These studies underline the impact of adjuvant radiotherapy following radical prostatectomy in patients with high-risk disease and adverse pathologic features (*124*).

For radical prostatectomy-treated patients with biochemical recurrence, salvage radiation is the standard of care. The question is whether radical prostatectomy-treated patients with an increased risk for disease progression should receive irradiation in the form of routine adjuvant radiotherapy following surgery or in the form of salvage radiotherapy not given until the possible biochemical recurrence occurs. Retrospective studies suggest that early salvage radiation given at low PSA (< 0.5 μg/l) levels results in similar survival rates as adjuvant radiation, proposing that radiation-related toxicity and overtreatment could be minimized by only treating only patients with progressing cancer (*125*, *126*). However, in other studies, adjuvant radiotherapy is associated with longer freedom from biochemical recurrence, fewer cases of distant metastases, and better overall survival compared to salvage radiation (*127*, *128*). Results from several ongoing randomised trials comparing adjuvant and salvage radiotherapy (RADICALS, RAVES, GETUG-17) are awaited.

In a prospective randomised RTOG 9601 trial, the addition of antiandrogen therapy and bicalutamide to salvage radiation following radical prostatectomy significantly improved overall and metastasis-free survival compared to salvage radiation with a placebo (*26*). In this trial, the patients received bicalutamide 150 mg once daily for 24 months from the beginning of radiation (*26*). In another randomised trial, GETUG-AFU 16, combining androgen suppression with salvage radiation significantly improved the progression-free survival compared to salvage radiation alone in radical prostatectomy-treated patients (*129*).

In a randomised SPCG-12 trial of high-risk localised and locally advanced prostate cancer by Ahlgren et al., docetaxel given after radical prostatectomy showed no significant effect on biochemical disease progression compared to surveillance (*130*).

2.2.1.5 COMPARISON OF LOCAL TREATMENTS IN HIGH-RISK LOCALISED AND LOCALLY ADVANCED PROSTATE CANCER

Regarding the treatment of locally advanced prostate cancer, there are no randomised controlled trials on radical prostatectomy. Observational studies comparing radical radiotherapy to radical prostatectomy in high-risk localised or locally advanced prostate cancer at diagnosis have resulted in similar outcomes between the treatments or they support for radical prostatectomy over radiation (*131–134*). When compared to the combination of radiotherapy and androgen suppression, radical prostatectomy alone appears equally effective (*134–137*). However, the quality of evidence from retrospective studies is low and they tend to have a selection bias of younger

prostatectomy-treated patients with lower tumour load (*98*). The results from the SPCG-15, a randomised trial comparing radical prostatectomy with the combination of radiation and androgen suppression, are awaited (*138*).

In a non-randomised study of Gleason score 9–10 prostate cancer comparing a combination of radical prostatectomy, adjuvant radiotherapy, and androgen suppression with a combination of external beam radiotherapy, brachytherapy, and androgen suppression, the outcomes between these two multimodal treatments were similar (*139*). In another retrospective study of Gleason 9–10 disease, patients who received external beam radiation, brachytherapy boost, and androgen suppression had significantly lower prostate-cancer mortality and longer metastatic-free survival compared to patients receiving radiation together with androgen suppression or radical prostatectomy alone (*140*).

2.2.1.6 TOXICITY OF LOCAL PROSTATE CANCER TREATMENTS

2.2.1.6.1. TOXICITY OF RADICAL RADIOTHERAPY

Bowel dysfunction, commonly reported as bloody stools, bowel urgency and incontinence, is more common among prostate cancer patients who receive radical radiation than those who undergo surgery (*141–144*). However, the rate of bowel dysfunction after radiation is generally low and remains so several years following initial treatment (*141*, *143*, *145*). With regard to urinary symptoms, several non-randomised studies have reported lower rates of urinary incontinence but higher rates of urinary irritation symptoms in radical radiotherapy-treated patients compared to those who undergo surgery (*144–146*). The randomised ProtecT-trial reported similar results regarding urinary incontinence between local treatments; however, urinary irritation was higher six months following initial treatment in the patients receiving radiation, after which the rates between the treatments became similar and remained so for several years (*142*). Although most of the patients receiving radical radiotherapy experienced erectile dysfunction in the ProtecT-trial, the potency rate was significantly lower among radical prostatectomy-treated patients six months and six years after the initial treatment (*141*).

2.2.1.6.2 TOXICITY OF RADICAL PROSTATECTOMY

Radical prostatectomy-treated patients have significantly more sexual dysfunction and urinary incontinence compared to radical radiation-treated patients six months as well as several years after initial treatment (*142*, *145*, *147*, *148*). However, regarding hormone and bowel function as well as health-related quality of life, the treatment outcomes appear similar (*145*). With regard to high-risk patients, the radical treatment is often more aggressive, requiring more extensive surgical resection with a risk of nerve damage,

while nerve-sparing surgery is associated with better outcomes in terms of sexual function and quality of life (*144*). When stratified to risk groups, low- and intermediate-risk prostatectomy-treated patients seem to have more sexual dysfunction compared to radiotherapy-treated patients (*148*). Yet, for high-risk patients, sexual function appears similar between prostatectomy and radiotherapy groups three years after treatment (*148*).

2.2.1.7 HORMONAL THERAPY

2.2.1.7.1 TYPES OF HORMONAL THERAPIES

Prostate cancer is an androgen-dependent disease. Thus, hormonal treatments are initially highly effective by decreasing the androgen levels in prostate cancer patients. However, the duration of their response is variable and eventually lost, leading to currently incurable castration-resistant prostate cancer. The previous conception was that castration-resistant cancer, which progresses regardless of androgen deprivation therapy and the castration levels of serum testosterone, is an androgen-independent or hormone-refractory disease. Later, several studies overruled this misconception and proved the significance of androgen-receptor signalling in castration-resistant cancer as well (*149–151*).

The use of hormonal treatments at different stages of prostate cancer is common. Today, hormonal treatments play a significant role in the treatment of high-risk and locally advanced cancer, in biochemical recurrence following local therapy, and in metastatic cancer. While their use as a monotherapy was common in the past decades, at present, their use as a part of combination treatments is increasing (*13*, *97*).

Androgen deprivation therapy refers to surgical castration through orchiectomy or to chemical castration through luteinising hormone-releasing hormone (LHRH) agonists or antagonists. LHRH agonists, including leuprolide and goserelin, are long-acting, which is why their clinical use is often preferred over short-acting LHRH antagonists, such as degarelix. LHRH agonists bind to specific pituitary receptors, causing the continuous production of luteinising hormone, which stimulates the testicles to release androgens. This can cause a transient surge in serum testosterone levels, leading to a *flare* phenomenon at the beginning of treatment (*152*). Although there has been questioning over the linear relationship between clinical flare and prostate cancer growth, LHRH agonists are often accompanied by first-generation antiandrogens to prevent the flare symptoms including bone pain, spinal cord compression, and urinary obstruction (*153*). Following the continuous presence of LHRH agonists, the pituitary gland ceases to produce luteinising hormone leading to decreased stimulation of the testicles and, thus, the castration levels of serum testosterone. LHRH antagonists, on the other hand, bind directly to the LHRH receptors, causing an immediate decrease in luteinising hormone, follicle-stimulating hormone, and testosterone levels without the flare.

First-generation antiandrogens, including non-steroidal bicalutamide, flutamide, and nilutamide, bind to androgen receptors, blocking the activity of androgens and thus inhibiting the tumour growth. Bicalutamide is well-tolerated, the most investigated, and widely used first-generation antiandrogen (*154*). The current use of antiandrogens is primarily at the beginning of LHRH agonist-treatment to prevent the flare symptoms, and in combination with LHRH agonists or antagonists to achieve complete androgen blockade (*155*).

Compared to bicalutamide, the novel second-generation non-steroidal antiandrogens, enzalutamide, darolutamide, and apalutamide, have higher binding affinity to androgen receptors and inhibit the nuclear translocation of the androgen receptors (*156–159*). In addition, enzalutamide and apalutamide inhibit the binding of the androgen receptor to deoxyribonucleic acid (DNA). Abiraterone, also a non-steroidal second-generation antiandrogen, prevents intracellular androgen biosynthesis through the inhibition of the cytochrome P450 17α–hydroxy/17,20-lyase enzyme (CYP17) (*160*).

2.2.1.7.2 TOXICITY OF HORMONAL THERAPY

Hormonal treatments can cause various detrimental short- and long-term side effects depending on the treatment used. Typical acute adverse events related to LHRH agonists are fatigue, hot flushes, decreased libido, erectile dysfunction, gynecomastia, skin disorders, and headache (*161*, *162*). Other adverse events, such as reduced muscle mass and decreased bone density, which expose patients to later bone fractures, develop slowly (*162*). LHRH agonists and antagonists have rather similar safety profiles; however, in studies comparing LHRH agonists and degarelix (an LHRH antagonist), the latter caused more injection-site reactions and less back pain, urinary tract infections, and arthralgia (*163*, *164*). The long-term use of LHRH agonists results in hypogonadism, leading to metabolic side effects, such as metabolic syndrome, increasing the risk of cardiovascular diseases (*165*). It remains unclear whether there is an association between cardiovascular disease and LHRH antagonists, although studies generally suggest the better cardiovascular profile of degarelix compared to that of LHRH agonists (*166*, *167*).

The main adverse events related to bicalutamide are gynecomastia and breast pain (*168*). Compared to LHRH agonists and other first-generation non-steroidal antiandrogens, bicalutamide has a more favourable toxicity profile (*154*). Also, unlike LHRH agonists, bicalutamide has a protective effect on bone (*169*).

In the PREVAIL trial, the most clinically relevant enzalutamide-related adverse events were fatigue and hypertension (*170*). Other commonly reported adverse events are hot flushes and diarrhoea (*150*). Enzalutamide exposes patients to seizures due to its ability to penetrate through the blood-brain barrier; thus, researchers recommend caution with to patients with a high-risk for seizures, although the incidence of convulsions is low (*150*). Adverse events related to mineralocorticoid excess (i.e., hypertension, hypokalaemia, fluid retention) and liver enzyme increase occur more often

among patients receiving abiraterone compared to placebo (*171*). Apalutamide-treated patients experience more rash, hypothyroidism, and fractures, while darolutamide-treated patients have been shown to experience more fatigue when compared to a placebo (*159*, *172*).

Multiple prostate cancer treatments, especially those with the addition of androgen deprivation therapy, expose patients to a higher risk of long-term adverse events when compared to a single curative treatment (*173*). Thus, patients receiving multimodal treatment have an increased risk for poor functional outcomes regarding sexual, urinary, and bowel function (*173*).

2.2.1.8 GEFITINIB

2.2.1.8.1 GEFITINIB AND PROSTATE CANCER

The tyrosine kinase domain of EGFR is a target for several EGFR tyrosine kinase inhibitors such as orally active gefitinib (*174*). In advanced non-small-cell lung cancer, gefitinib given as second- or third-line treatment improves progression-free survival and relieves symptoms (*175–177*). In some studies, gefitinib has shown improved overall survival in subgroups of non-smokers and patients of Asian origin (*176*, *178*). In addition, compared to chemotherapy, gefitinib prolongs progression-free survival as a first-line treatment in non-small-cell cancer patients with EGFR mutations (*179*). It is generally well-tolerated, with the most common adverse events including skin rash, diarrhoea, and changes in the liver transaminases (*175*). Consequently, gefitinib is an approved treatment option in advanced non-small-cell lung cancer.

Based on promising results from preclinical studies, gefitinib has appeared to be an attractive treatment option for prostate cancer as well (*180*). However, the results from randomised trials are more or less disappointing. In a randomised trial of 40 patients with castration-resistant prostate cancer by Canil et al., gefitinib showed no effect in terms of PSA response or objectively measurable disease (*181*). In another phase II trial of 58 patients with castration-resistant prostate cancer receiving gefitinib, Small et al. detected no PSA response (*182*). In a phase II study by Pezaro et al., one out of 51 patients with castration-resistant cancer experienced a confirmed PSA response following gefitinib treatment (*183*). Given together with antiandrogen and a luteinizing-hormone-releasing hormone analogue, gefitinib showed no PSA response or objectively measurable response in castration-resistant prostate cancer in a study by Curigliano et al. (*184*). Gefitinib given in combination with prednisone in castration-resistant prostate cancer showed small activity in terms of PSA response; however, there was no benefit in terms of survival or time to progression when compared to a placebo and prednisone (*185*).

2.2.1.8.2 EGFR MUTATIONS

In non-small-cell lung cancer, the presence of mutations in the kinase domain of EGFR predicts the efficacy of the tyrosine kinase inhibitors (*176*, *186*). Thus, EGFR-targeted agents, including gefitinib, are the approved first-line treatment for non-small-cell lung cancer patients with an EGFR mutation. In prostate cancer, however, similar findings have been unconfirmed. In a study of 23 patients with castration-resistant prostate cancer receiving gefitinib, Curigliano et al. detected no EGFR mutations concluding that tyrosine kinase inhibitors are unlikely to be effective in these patients (*184*). Later, researchers detected EGFR mutations in prostate cancer, similar to those in non-small-cell lung cancer, although the prevalence of mutations in prostate cancer seems to be lower (*63*). In a small study by Peraldo-Naia et al., there was no correlation between EGFR mutations and EGFR overexpression nor between EGFR mutations and time to biochemical relapse. In another study by Cho et al., there was no correlation between EGFR mutations and hormone-sensitive or castration-resistant status (*187*). However, the time to progression from hormone-sensitive to castration-resistant prostate cancer was significantly shorter in patients with EGFR mutations (*187*).

2.2.1.8.3 EGFR EXPRESSION AND RESISTANCE TO CHEMO- AND RADIOTHERAPY

Docetaxel fails to achieve any response in a substantial proportion of metastatic prostate cancer patients, and patients who primarily show a response, will ultimately develop resistance to docetaxel (*188*, *189*). Furthermore, as EGFR overexpression is associated with resistance to chemotherapy, one can assume that the inhibition of EGFR kinase activity together with docetaxel could present an effective treatment option (*70*). In phase I-II trials, this combination treatment has acceptable tolerability; however, its efficacy in prostate cancer has been modest (*71*, *190–192*). In addition, the neoadjuvant combination of gefitinib and docetaxel followed by radical prostatectomy showed no efficacy in a phase II trial (*193*).

EGFR is associated with resistance to radiotherapy as well, and the inhibition of EGFR activity seems to have a radiosensitizing effect when given together with radiotherapy (*194*). However, in several clinical trials, gefitinib in combination with radiation showed no significant efficacy in different solid tumours (*195–199*). In non-small-cell lung cancer, the results have been promising, although, given the lack of strong evidence, this combination continues to be experimental (*199*, *200*).

2.2.2 METASTATIC PROSTATE CANCER

2.2.2.1 TREATMENT OF NEWLY DIAGNOSED METASTATICT PROSTATE CANCER

Androgen deprivation therapy was the standard treatment for castration-naïve newly diagnosed metastatic prostate cancer for decades (*11*). While hormonal therapy is efficient in the majority of patients at the beginning of treatment, its effect is eventually lost, which leads to castration-resistant prostate cancer (i.e., disease recurrence after first-line androgen deprivation ceases to work). Until the beginning of the 21st century, docetaxel was the only treatment proven to improve overall survival in metastatic castration-resistant prostate cancer and, thus, the only recommended treatment option for these patients (*201–203*).

Not until recently, following the GETUG-AFU 15, CHARTEED, and STAMPEDE trials, did docetaxel in combination with androgen depression become the recommended first-line treatment option for physically fit patients with newly diagnosed metastatic prostate cancer (*13*, *46*, *204*). All trials showed improved progression-free survival with the addition of docetaxel to androgen deprivation compared to androgen deprivation alone. CHARTEED and STAMPEDE showed significantly improved overall survival as well (*13*, *46*). At present, the median survival of newly diagnosed metastatic cancer ranges between 34 to 62 months (*14*, *15*).

After the emergence of several new treatments, docetaxel no longer represents the only relevant therapeutic option for metastatic castration-resistant prostate cancer. Two oral androgen-receptor axis targeted agents, abiraterone and enzalutamide; an autologous vaccine, sipuleucel-T; a bone-targeting radiopharmaceutical, radium-223; and a taxane, cabazitaxel, all showed improved survival in metastatic castration-resistant disease (*150*, *151*, *170*, *171*, *189*, *205–207*). Currently, regardless of the wide use of these promising treatments for metastatic castration-resistant prostate cancer, its median survival remains to be 14 to 35 months (*151*, *206*).

Following good results in metastatic castration-resistant prostate cancer, abiraterone proved its efficacy in newly diagnosed castration-naïve metastatic disease as well (*208*). In the LATITUDE and STAMPEDE trials, abiraterone given in combination with androgen deprivation therapy improved overall survival in a newly diagnosed metastatic setting (*48*, *209*). Later, enzalutamide as well as apalutamide in combination with androgen depression showed improved overall survival in metastatic castration-naïve prostate cancer (*210*, *211*).

With regard to nonmetastatic castration-resistant prostate cancer, enzalutamide, apalutamide, and darolutamide all showed improved metastatic-free survival when compared with a placebo in randomised trials (*159*, *172*, *212*). Later, these trials proved an overall survival benefit in patients receiving enzalutamide (PROSPER), apalutamide (SPARTAN), or darolutamide (ARAMIS) (*213–215*).

2.2.2.2 LOCAL THERAPIES IN METASTATIC PROSTATE CANCER

Traditionally, there has been a strong preference for systemic therapies over local treatments in the metastatic setting, and the role of radiotherapy has been one of palliative treatment for local disease progression and distant metastases. However, observational data supports local therapies as a primary treatment for newly diagnosed metastatic prostate cancer, and, in subgroup analyses, patients with low-risk metastatic disease appear to benefit the most from local therapies (*115*, *216*, *217*). Not until recently did the randomised HORRAD and STAMPEDE trials compare radiotherapy to the prostate in combination with androgen depression to androgen depression alone in metastatic castration-naïve prostate cancer (*38*, *218*). In favour of radiotherapy, HORRAD detected a statistically significant difference in PSA progression, and STAMPEDE in failure-free survival (*38*, *218*). Even though HORRAD detected no significant difference regarding overall survival between the treatments, it hypothesized that patients with low tumour burden would benefit the most from radiotherapy (*218*). STAMPEDE reported no significant difference between the treatments in unselected patients; however, in a prespecified subgroup of patients with low tumour burden, radiotherapy significantly improved overall survival (*38*). Consequently, both the NCCN and EAU suggested radiotherapy to the prostate in low-volume metastatic cancer (*16*, *17*).

Although there are no randomised trials of radical prostatectomy in the metastatic setting, observational data supports surgery as a local therapy when compared to non-local therapies as well as surgery over radiation (*219–221*). Regardless of the promising outcomes, radical prostatectomy in the metastatic setting appears controversial and there is a clear need for prospective randomised trials (*217*).

2.2.2.3 TREATMENT OF BONE METASTASES

Bone metastases are a significant cause of morbidity among patients with metastatic prostate cancer (*222*). Prostate cancer metastasizes primarily the bone, which can cause bone pain, pathological fractures, and spinal cord compression (*222*). Also, androgen suppression exposes patients to secondary osteoporosis and, thus, skeletal-related complications. Zoledronic acid, a third-generation bisphosphonate, reduces skeletal-related complications in castration-resistant metastatic prostate cancer; however, no trials have reported survival benefits caused by bisphosphonates (*223*). In a randomised trial by Fizazi et. al, denosumab, a human monoclonal antibody that inhibits receptor activator of nuclear kappa-B ligand, prolonged the time to the first skeletal-related event compared to zoledronic acid in metastatic castration-resistant prostate cancer (*224*). Nevertheless, neither denosumab nor zoledronic acid showed a significant effect on survival (*224*). Radium-223, on the other hand, is a radioactive isotope that significantly delays symptomatic skeletal events, and prolongs overall survival when compared with a placebo in metastatic castration-resistant prostate cancer (*206*).

3 AIMS

The aims of this doctoral dissertation are listed according to the original publications:

I To evaluate the safety (phase I) and feasibility (phase II) of gefitinib 250 mg once daily in combination with radical radiotherapy in nonmetastatic prostate cancer.

II To evaluate the activity of gefitinib 250 mg once daily in prostate cancer patients with biochemical recurrence following radical prostatectomy or radiotherapy with curative intent.

III To evaluate the safety and efficacy of multimodal primary treatment, including radical radiotherapy and androgen deprivation in patients with newly diagnosed metastatic prostate cancer.

IV To compare adjuvant radiotherapy following radical prostatectomy with prostatectomy alone in patients with positive margins or extracapsular extension.

4 MATERIAL AND METHODS

4.1 STUDY PROTOCOLS AND DESIGN

4.1.1 ETCHICS

All studies adhered to the principles of the Declaration of Helsinki. The Surgical Ethics Committee of the Hospital District of Helsinki and Uusimaa approved the trial protocols and informed consent forms for studies I, II, and IV. The Finnish National Institute for Health and Welfare, Data Protection Ombudsman, and the Population Register Centre authorized study number III.

4.1.2 STUDY DESIGN AND CENTERS

Studies I and II are open-label and non-randomised trials. All patients received study treatment at Helsinki University Central Hospital. Study I is a phase I/II trial, and study II is a phase II trial.

Study III is a non-randomised, retrospective patient series. This study is based on retrospectively analysed patient records from the Docrates Cancer Center (Helsinki, Finland), where all study patients received cancer treatment and/or were followed.

Study IV is a randomised, open-label, parallel-group, multicentre trial including eight Finnish hospitals (Helsinki University Hospital, Kuopio University Hospital, Mikkeli Central Hospital, North Carelia Central Hospital, Oulu University Hospital, Päijät-Häme Central Hospital, Tampere University Hospital, and Turku University Hospital). The trial was a collaboration between FinnProstate Group, a Finnish urologist-run group that promotes prostate cancer research, and the Finnish Radiation Oncology Group, a group of Finnish radiation oncologists.

4.2 STUDY I

4.2.1 INCLUSION AND EXCLUSION CRITERIA

The inclusion criteria of study I consisted of written informed consent as well as histologically confirmed localised (cT2) or locally advanced (cT3), lymph node-negative, and nonmetastatic (no metastases in radioisotope bone scan nor in computed tomography) prostate cancer in addition to PSA < 20 μg/l, good performance status (World Health Organization 0-1), and age 18 years or older.

The exclusion criteria consisted of Gleason score 2 to 4 localised (cT2) prostate cancer, known hypersensitivity to gefitinib, chronic toxicity greater than grade 2 (according to Common Toxicity Criteria version 2.0) from previous cancer therapy, prostatectomy, severe skin disorders, significant ocular abnormality, evidence of severe systemic disease, evidence of clinically active interstitial lung disease, and malignancies other than prostate cancer diagnosed within the previous five years (*225*). The use of LHRH analogus, antiandrogens, phenytoin, carbamazepine, barbiturates, rifampicin, or St. John's wort was forbidden. Furthermore, the exclusion criteria included the following laboratory results: absolute neutrophil count less than 1.5 x 10^9/L, platelet count less than 120 x 10^9/L, serum bilirubin above the upper limit of normal (ULN), aspartate aminotransferase level above 1.25 x ULN, alanine aminotransferase level above 1.25 x ULN, alkaline phosphatase level above 1.25 x ULN, and serum creatinine level above 1.5 x ULN.

4.2.2 TREATMENT SCHEDULE

Patients had at least one screening visit before gefitinib treatment and one visit when the treatment began, after which visits occurred once weekly for the first three months of the trial.

Patients received gefitinib 250 mg orally once daily from the first day of the trial treatment until the end of radiation therapy. The trial treatment duration was 60 days, including the first seven days of gefitinib as a monotherapy, and from day eight of gefitinib administration in combination with radiation therapy. The total radiation dose was 72.4 gray (Gy), given in 39 fractions in approximately 53 days. First, the prostate gland, tumour extensions outside the prostate, and the seminal vesicles received irradiation with a total dose of 50.4 Gy (1.8 Gy/day) in 28 fractions (5 days/week) with a 1 cm margin. Then, the prostate gland and tumour extensions received a 22 Gy booster (2 Gy/day) in 11 fractions (11 days) with a 1 cm margin, with the exception of a 0.6 cm margin toward the rectum.

In the case of patient withdrawal, loss to follow-up, death, or protocol noncompliance, the trial treatment was discontinued. In addition, at the discretion of the investigator, the trial treatment could be discontinued due to an adverse event.

PSA measurements occurred every four weeks, and other blood tests (including absolute neutrophil, haemoglobin, platelet, and white blood cell count as well as total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine, sodium, potassium, and calcium content) were conducted weekly. The definition of PSA relapse was PSA nadir + 2 μg/l (*84*).

After the end of follow-up, prostate cancer-free survival, PSA relapse-free survival, salvage-free survival, and overall survival were compared to data of 91 matched controls treated with radiotherapy alone at Cleveland Clinic (*226*). These control patients received a slightly higher total dose of 74 to 78 Gy given in 2 Gy fractions compared to patients who received radiation in combination with gefitinib.

4.2.3 TOXICITY

At every trial visit, the oncologist recorded the patient's adverse events, graded them according to the Common Toxicity Criteria version 2.0, and evaluated whether the adverse event was gefitinib-related (*225*).

The criteria for dose-limiting toxicity were: 1) gefitinib-related grade 4 haematological toxicity, 2) gefitinib-related grade 3 nonhematological toxicity, 3) any serious adverse event, 4) treatment interruption for longer than 14 days due to gefitinib-related toxicity, 5) more than three interruptions of treatment (excluding technical failure in delivering radiotherapy), or 6) death from any cause. The maximum tolerated dose was to be exceeded if three or more patients experienced dose-limiting toxicity.

4.2.4 EGFR ANALYSIS

EGFR expression analysis was performed immunohistochemically with the monoclonal NCL-EGFR antibody (Novocastra Laboratories, Newcastle, UK), which detects wild-type EGFR. Equal or higher staining intensity of the cell membrane, compared with normal prostate epithelial tissue, defined EGFR expression. Another immunohistochemical staining was done using the monoclonal NCL-EGF-RT antibody (Novocastra Laboratories, Newcastle, UK), which detects the EGFR variant III (*227*). Glioblastomas with known EGFR variant III status were used as a positive control. The amplification of EGFR was analysed with chromogenic in situ hybridisation (Zymed Inc., South San Francisco, CA, USA). The criteria for amplification were the same as for HER-2/neu in a similar assay (*228*). EGFR activation analysis was analysed immunohistochemically using a monoclonal antibody against phosphorylated EGFR1 (Santa Cruz Biotechnology, Inc., CA, USA). This antibody detects the tyrosine-phosphorylated (Tyr1173) form of EGFR in paraffin sections.

4.2.5 CYTOKINE ANALYSIS

Cytokine analysis was performed with a BD Cytometric Bead Array (CBA) Human Soluble Protein Flex Set (Becton Dickinson, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. Patients' serum samples were used for the measurements.

4.2.6 STATISTICS

Prostate cancer-free, biochemical recurrence-free, salvage therapy-free, and overall survival were calculated using the Kaplan-Meier method (SPSS version 15.0. software for Windows). Patients were censored at the time of the defined event or their last follow-up. These survival analyses were unplanned.

4.2.7 ENDPOINTS

The primary endpoint of phase I was the incidence of gefitinib-related dose-limiting toxicities. The primary endpoints of phase II were: 1) the number, nature, and severity of the adverse events; 2) the incidence of and reasons for gefitinib interruptions, dose reductions, and withdrawals; and 3) gefitinib exposure (duration of treatment), laboratory assessments, and physical examination.

Secondary endpoints were EGFR expression and activation status in both phases (I and II). In addition, the incidence of PSA relapse defined the preliminary efficacy of gefitinib in combination with radiotherapy.

4.3 STUDY II

4.3.1 INCLUSION AND EXCLUSION CRITERIA

The inclusion criteria were the biochemical recurrence of prostate cancer after radical prostatectomy or radical radiotherapy with curative intent and PSA below 10 μg/l. The definition of biochemical recurrence was two (following radical prostatectomy) or three (following radical radiotherapy) consecutively increasing PSA measurements at least four weeks apart. In addition, the inclusion criteria included written informed consent as well as lymph node-negative or unknown status (N0, NX) assessed via magnetic resonance imaging and computed tomography, nonmetastatic (M0) disease assessed via radioisotope bone scan, good performance status (World Health Organization 0-1), and age 18 years or older.

The exclusion criteria consisted of hormonal treatment within the previous six months; concomitant radiotherapy, surgery, and/or chemotherapy or the use of phenytoin, carbamazepine, rifampicin, barbiturates, or St. John's Wort; previous participation in a gefitinib study; treatment with a non-approved or investigational drug within previous 30 days; known hypersensitivity to gefitinib; other malignancies diagnosed within five years (except basal cell carcinoma); any unresolved chronic toxicity (except alopecia) greater than grade 2 (according to the Common Toxicity Criteria version 2.0) from previous cancer therapy; and any evidence of severe uncontrolled systemic disease or clinically active interstitial lung disease (*225*).

4.3.2 TREATMENT SCHEDULE

Patients had at least one screening visit before gefitinib treatment and one visit once the treatment began. Following the initiation of the gefitinib treatment, visits occurred every four weeks or until withdrawal.

Gefitinib 250 mg once daily was to be administered for a minimum of three months until PSA progression, detected metastases, unacceptable toxicity, protocol non-compliance, or patient withdrawal. The definition for PSA progression was the doubling of the PSA from its level upon study entry.

At every trial visit, the oncologist recorded the patient's adverse events, graded them according to the Common Toxicity Criteria version 2.0, and evaluated whether the adverse event was gefitinib-related (*225*). PSA measurements and other blood tests (including absolute neutrophil, haemoglobin, platelet, and white blood cell count as well as total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine, sodium, potassium, and calcium content) were taken on the first day of gefitinib treatment and every four weeks thereafter.

4.3.3 PSA RESPONSE AND PSA DOUBLING TIME

The definition of PSA response at study closure was the percentage of patients experiencing PSA normalization or more than a 50% decrease compared to their study entry levels sustained for three months (three consecutive measurements). The definition of PSA normalization was a PSA decrease to undetectable levels (< 0.05 μg/l or < 0.4 μg/l depending on the laboratory) following radical prostatectomy or below 4.0 μg/l following radical radiotherapy. PSA measurement occurred at screening and every month for a minimum of three months.

PSA doubling time was calculated using a nomogram provided by Pound et al. (*88*). In study II, this nomogram included all PSA values taken within 12 months prior (for PSA doubling time before gefitinib) and within (for PSA doubling time during gefitinib) the gefitinib treatment. The effect of treatment on the PSA doubling time was the number of patients experiencing an increase in their doubling time, and the change (%) in the PSA doubling time before and during gefitinib treatment.

4.3.4 TREATMENT FAILURE

The definition of time to treatment failure was the time between the first study date and the first date of any additional or alternative therapy due to PSA progression, metastases, or adverse events. The time from the first documented PSA response to PSA progression, death, or final on-study PSA measurement was defined as the duration of the PSA response. The time from the first study date until patient death, PSA progression, or final in-study PSA measurement defined the PSA progression-free survival.

4.3.5 TOXICITY

An oncologist reported all adverse events weekly for each patient during the first three months of the gefitinib treatment and graded them according to the Common Toxicity Criteria version 2.0 (*225*). In addition, the oncologist evaluated the relationship between the gefitinib and each adverse event (gefitinib-related vs. not gefitinib-related event).

4.3.6 SEQUENCE ANALYSES OF EGFR EXONS 18-21 AND KIRSTEN RAT SARCOMA VIRAL ONCOGENE HOMOLOG (KRAS) EXON 1

When formalin-fixed paraffin-embedded tumour tissues were available, sequence analyses were performed. DNA was extracted (GenElute Mammalian Genomic DNA Miniprep Kit, Sigma, St. Louis, MO) and amplified using the GeneomePlex Tissue WGA Kit (Sigma). Using standard

methods, 50 to 100 ng of DNA was amplified with polymerase chain reaction. The forward and reverse primers were used to amplify the exons of EGFR (18-21) and KRAS (exon 1). The primers were as follows: EGFR ex18f: CAAATGAGCTGGCAAGTGCCGTGTC; ex18r: GAGTTTCCCAAACACTCAGT GAAAC; ex19f: GCAATATCAGCCTTAGGTGCGGCTC; ex19r: CATAGAAAGT GAACATTTAGGATGTG; ex20f: CCATGAGTACGTATTTTGAAACTC; ex20r: CATATCCCCATGGCAAACTCTTGC; ex21f: CTAACGTTCGCCAGCCATAAGT CC; ex21r: GCTGCGAGCTCACCCAGAATGTCTGG; KRASf: AGGCCTGCTGA AAATGACTG; and KRASr: TCAAAGAATGGTCCTGCACC. The polymerase chain reactions were performed in a reaction volume of 50 µl with 35 cycles consisting of denaturation at 94 °C for 45 s, annealing at 59 °C for 45 s and elongation for two minutes at 72 °C for KRAS, and using a touchdown program (from 63.2 °C to 58.2 °C) for EGFR. A DNA sample from an anonymous blood donor was used as a control.

4.3.4 ENDPOINTS

The primary endpoint was the PSA response at study closure. The secondary endpoints were the time to treatment failure, the duration of the PSA response, PSA progression-free survival, the effect of the treatment on the PSA doubling time, and adverse events.

4.4 STUDY III

4.4.1 PATIENTS SELECTION

All patients had histologically confirmed prostate cancer and bone metastases with no previous prostate cancer treatment. The same oncologist provided the multimodal treatment, which included the primary treatment as well as radiotherapy planning and contouring, consecutively for all study patients. Most of the patients were diagnosed with prostate cancer at the Docrates Cancer Center. Patients who were diagnosed elsewhere, received the same multimodal treatment following their diagnosis.

All patients underwent screening before prostate cancer treatment. The screening included full-body CT and bone scintigraphy or (from 2010) PET/CT with ^{18}F-choline, ^{18}F-fluoride, or (from October 2015) a gallium-68-labelled prostate-specific membrane antigen (^{68}Ga-PSMA). Most of the patients underwent endorectal multiparametric MRI before their diagnostic biopsies. When there was a clinical suspicion of metastatic prostate cancer, MRI was done not only to achieve accurate staging but also to enable radical radiation of the primary tumour with adequate margins (*229*). All patients underwent biopsies once.

4.4.2 PROSTATE CANCER TREATMENTS

All patients received anti-androgen therapy as a primary treatment and radiotherapy with radical doses. In addition, the patients received several, individually chosen treatments, including targeted therapy, chemotherapy, and radiopharmaceuticals, in order to facilitate maximal cancer cell death (Table 2 in the original publication).

As a primary treatment, all patients received 1) luteinizing hormone-releasing hormone (LHRH) analogues or a LHRH antagonist, and 2) 150 mg daily of bicalutamide following a single 12 Gy (6 to 9 MeV) fraction for breasts in order to reduce and prevent bicalutamide-induced mastodynia and gynecomastia.

If the PSA decrease stopped following the primary treatment, the patient intravenously received docetaxel 75 mg/m^2 every three weeks or 50 mg/m^2 every two weeks. Prior to 2010, docetaxel was the only chemotherapy that had shown a survival benefit in metastatic castration-resistant prostate cancer. Before 2010, selected patients in this study received an experimental combination of gemcitabine 1000 mg/m^2 and oxaliplatin 85 mg/m^2 every two weeks. Following the approval of cabazitaxel in the treatment of castration-resistant metastatic prostate cancer in 2010, it was used in this study as a second-line chemotherapy with a dose of 25 mg/m^2 every three weeks.

After oral abiraterone (in 2011) and enzalutamide (in 2014) became available in Finland, the study patients received these treatments as

well. The dose for abiraterone was 1 g daily in combination with 10 mg oral prednisolone, and, for enzalutamide, it was 160 mg daily.

Following the occurrence of PSA nadir, all patients received radiation therapy with radical doses. The radiation therapy was comprised of 78 or 80 Gy (in 2 Gy fractions) external beam irradiation to the prostate. Depending on the location and number of bone metastases, patients received 1.8 to 3.5 Gy single fractions, with a total dose ranging from 38.6 to 76.5 Gy for the bone metastases. The radiotherapy technique used for concomitant bone metastases is presented in detail by Kiljunen et al. (*230*). Regional and retroperitoneal/para-aortic lymph nodes received 45 to 50 Gy irradiation, and, if the metastases were PET/CT-active, they received an increased dose of 59.4 to 76 Gy. The minimum total dose for the pelvic lymph nodes was 45 Gy in 25 fractions. Dose planning CT was registered with MRI, ^{18}F-choline-, ^{18}F-Fluoride-, or ^{68}Ga-PSMA-PET/CT for contouring the prostate and organs at risk. The radiation therapy technique used in this study was intensity-modulated radiation therapy (IMRT) until the year 2009, after which the patients received volumetric modulated arc therapy (VMAT, RapidArc).

Patients with relatively large and diffuse bone metastases received radiopharmaceuticals as well (Table 3 in the original publication). In this study, radium-223 (55 kBq/kg) replaced samarium-153 (1 mCi/kg) in February 2013. Na^{18}F-PET/CT-active bone metastases was a rationale for early radium-223 therapy despite an immeasurable PSA.

Other therapies used included zoledronate, ibandronate, denosumab, cyclophosphamide, doxorubicin, carboplatin in combination with etoposide, cetuximab, vinblastine in combination with estramustine, vinorelbine, and pembrolizumab (for one patient who participated in another study). One patient received high-density rate brachytherapy (3 x 9 Gy) due to local relapse.

4.4.3 TREATMENT SCHEDULE

Blood tests including PSA measurements occurred once a month and, later, once every three or six months. In the case of increasing PSA or any new, possibly cancer-related symptoms, the patient underwent PET/CT in order to localise the possible new relapse. Following PET/CT, it was considered whether the patient needed irradiation with VMAT RapidArc.

4.4.4 TOXICITY

During and one year after definitive radiotherapy, the clinician evaluated the adverse events and graded them according to the Common Terminology Criteria for Adverse Events version 4.03 (*231*).

4.4.5 SURVIVAL ANALYSES

Overall survival and progression-free survival analyses were measured from the date of the prostate cancer diagnosis and calculated using the Kaplan-Meier method. The definition of disease progression was a consecutive increase in the PSA or recurrent prostate cancer assessed via imaging. Patients were censored at the time of the defined event or their last follow-up.

4.5 STUDY IV

4.5.1 INCLUSION AND EXCLUSION CRITERIA

The inclusion criteria included written informed consent, pT2N0M0 with a positive margin or pT3aN0M0 with/without a positive margin prostate cancer, a Gleason score of 2 to 10, preoperative PSA $\leq$ 20 μg/l, postoperative PSA < 0.5 μg/l, World Health Organization performance status 0 to 2, and a life expectancy of at least three months.

The exclusion criteria included other simultaneous cancer therapy, including systemic endocrine therapy; more than 12 weeks since receiving radical prostatectomy; metastatic disease (N+ or M1); cancer invasion to the seminal vesicles; any other previous malignancy within the last five years, excluding basalioma or squamous cell carcinoma of the skin; any contraindication to irradiation; and any physical or mental condition that may interfere with the patient's compliance with the scheduled study visits.

4.5.2 TREATMENT SCHEDULE

The randomisation of the patients occurred following the radical prostatectomy, in which open, laparoscopic, and robot-assisted laparoscopic techniques were used. After the radical prostatectomy, the urologist screened each patient for eligibility. Following the patient's informed consent, the urologist called the Finnish Cancer Registry (Helsinki, Finland). The Cancer Registry stratified the patients into three groups based on their Gleason score (Gleason scores 2 to 6, 7, and 8 to 10), conducted the randomisation, and informed the urologist of the patient's treatment group (adjuvant or observation).

All patients visited their urologist/oncologist at randomisation as well as three, six, and 15 months after randomisation, after which the visits usually occurred annually. Patients receiving adjuvant radiotherapy visited their oncologist for radiotherapy planning after randomisation, during radiotherapy if acute reactions occurred, and once the radiotherapy ended.

The PSA was measured every three months for five years after which it was usually measured annually. The definition for biochemical recurrence was 1) PSA > 0.4 μg/l in two consecutive measurements at least four weeks apart, 2) metastatic prostate cancer, or 3) recurrent prostate cancer in imaging. In the case of biochemical recurrence in the observation group, the patient could be offered salvage radiotherapy.

If the urologists detected any symptoms or signs of clinical progression during the patient visits, an imaging was performed to detect metastases. The imaging approaches used to detect metastatic disease were a bone scan, computed tomography (CT), or positron emission tomography/CT. Castration-resistant prostate cancer was defined as

consecutive increases in the PSA within two successive PSA measurements at least four weeks apart, despite androgen deprivation therapy.

4.5.3 TOXICITY

Adverse events were scored from individual medical records using the Common Terminology Criteria for Adverse Events version 4.03 (*231*). The scoring of adverse events began at randomisation and ended when progression occurred or at the end of the follow-up period if the patient was free of biochemical recurrence. The relationship between the adverse event and the trial treatment was not evaluated (all adverse events were scored).

At seven visits, which occurred between 0 to 51 months from the radical prostatectomy, the patients filled out three questionnaires: 1) the International Index of Erectile Function (IIEF-5), 2) the International Prostate Symptom Score (IPSS), and 3) the Late Effects Normal Tissue Task Force-Subjective, Objective, Management, Analytic (LENT-SOMA) questionnaire, with intestinal and urinary questions from the subjective, objective, and management sections of the LENT-SOMA parameters. Incompletely filled out questionnaires were omitted from the analysis.

The IIEF-5 score defined the patient's evaluation of his erectile function. Erectile dysfunction was recorded as severe (IIEF-5 score 1 to 7), mild-moderate (IIEF-5 score 8 to 21), or no erectile dysfunction (IIEF-5 score 22 to 25).

The IPSS score defined the patient's evaluation of his urinary symptoms and his quality of life as affected by these urinary symptoms. Urinary symptoms were recorded as *mild* (IPSS score 0 to 7), *moderate* (IPSS score 8 to 19), or *severe* (IPSS score 20 to 35). Quality of life was recorded as *delighted, pleased, mostly satisfied* (IPSS quality of life score 0 to 2), *mixed* (IPSS quality of life score 3), or *mostly dissatisfied, unhappy, terrible* (IPSS quality of life score 4 to 6).

The LENT-SOMA toxicities were graded from 0 to 4 (grade 0 for no toxicity, grade 4 for the most severe toxicity). For one LENT-SOMA question regarding the management of dysuria, the answer option for surgical intervention (grade 4 toxicity) was unavailable, therefore, the answers for this question were graded from 1 to 3. In addition, the kidney-related toxicity was based on two questions: answering "*yes*" to "*Do you suffer from tiredness and headache?*" resulted in grade 3, while answering "*yes*" to "*Are you passing less urine than you usually do/are your feet swollen?*" resulted in grade 4 toxicity.

4.5.4 ADJUVANT RADIOTHERAPY

The total dose of adjuvant radiation was 66.6 Gy given in 37 fractions of 1.8 Gy per day five days per week. Patients received three-dimensional conformal radiation therapy (with linear accelerator > 10 MV) without pelvic lymph node irradiation. The clinical target volume (CTV) included the caudal wall of the bladder (cranial border), posterior edge of the symphysis (anterior

border), and anterior margin of the rectum (posterior border). The lower border was assessed indirectly in relation to the bulbus of the penis identified via computed tomography. CTV + 1 cm formed the planning target volume and after a total dose of 50.4 Gy the posterior marginal was reduced to CTV + 6 mm. The maximal dose was 50 Gy for both the posterior rectal wall and the femoral heads. Radiation was set to begin within 12 weeks from the radical prostatectomy.

4.5.5 STATISTICS

The study hypothesis was that two-year progression-free survival would be 80% in the adjuvant group and 60% in the observation group, with a power of ≥ 80% and a significance level of 5%. The required sample size, calculated using Fisher's exact test for two independent groups, was 90 patients/group. However, to avoid a loss of power due to possible loss to follow-up, the sample size was increased to 125 patients/group, resulting in the randomisation of 250 patients in total with a ratio of 1:1.

Ten-year survival analyses were calculated using the Kaplan-Meier method. Survival differences between the adjuvant and observation group, including hazard ratios with 95% confidence intervals and p-values, were calculated using the Cox proportional hazard regression analysis. The association between biochemical recurrence and treatment group was tested after adjusting for preoperative PSA, Gleason score (Gleason scores 5–6, 7, and 8–9), and pT stage (pT2, pT3) using the Cox multivariable regression. In addition, the interaction between the preoperative PSA and the treatment groups in terms of biochemical recurrence was tested using the Cox multivariable regression. In all analyses, patients were censored at the time of the defined event or their last follow-up. The program used for the statistical analyses was SPSS (IBM SPSS Statistics for Windows, version 25.0; IBM Corp., Armonk, NY, USA).

A generalised linear mixed model (GLMM) with a lmer function was used for the comparison of treatment groups in terms of the number of patients experiencing adverse events. In this model, a binary response (any adverse event regardless of the grade vs. no adverse event) was used for the adverse events. Similarly, GLMM was used for the comparison of the treatment groups in terms of the total number of adverse events, modelled as Poisson distribution. In both models, all adverse events were modelled regardless of the grade, the groups were modelled as a fixed effect; and the patients were modelled as a random effect (*232*).

The GLMM was used to compare the treatment groups in terms of 1) severe erectile dysfunction, 2) severe urinary symptoms, 3) LENT-SOMA urinary symptoms, and 4) LENT-SOMA intestinal toxicities. Respectively, a binary response was used in the aforementioned models as 1) IIEF-5 scores 1 to 7 vs. 8 to 25, 2) IPSS scores 20 to 35 vs. 0 to 19, 3) grade 3 to 4 vs. 1 to 2, and 4) grade 3 to 4 vs. 1 to 2. In all models, the explanatory variables (group and time in months) were modelled as fixed effects, and patient-specific effects were modelled as a random effect. Time was a continuous variable. The GLMM was applied using the R statistical software

package (version 3.5.2, lme4 function, R Core Team [2018]. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

4.5.6 ENDPOINTS

The primary endpoint was biochemical recurrence-free survival. The secondary endpoints were overall survival, cancer-specific survival, local recurrence, and adverse events. Additional, unplanned analyses included metastatic and castration-resistant survival. All survival analyses and follow-ups were measured from the date of the radical prostatectomy.

5 RESULTS

5.1 STUDY I

5.1.1 PATIENTS

This study enrolled a total of 42 patients diagnosed with T2-3N0M0, Gleason score 4–8 prostate cancer (Table 1 in the original publication). As the maximum tolerated dose was not exceeded during phase I (12 patients), the study enrolled 30 additional patients for phase II (Figure 1). The mean PSA before gefitinib was 8.4 μg/l (range 1.6–18.8 μg/l). The median time on trial was 154 days (range 19–197 days), and the median time on gefitinib was 55 days (range 19–71 days). Thirty (71.4%) patients experienced toxicity that caused at least one gefitinib interruption. One or more radiation interruptions occurred in 39 patients. Generally, there were one or two dose interruptions per patient, which were due to public holidays or machine malfunctions in most of the cases and due to toxicity in four cases.

5.1.2 WITHDRAWALS AND AND SERIOUS ADVERSE EVENTS

Thirty (71.4%) patients completed the trial, while 12 (28.6%) withdrew due to an adverse event (Figure 1). Out of the 12 discontinued patients, three experienced one or more serious adverse events (Table 2 in the original publication). One patient potentially suffered from gefitinib-related cardiomegaly, cardiac failure, and myocarditis, which led to patient's death. In addition, the same patient suffered from other serious adverse events, including gastroenteritis with fever, and renal insufficiency, both of which were possibly related to gefitinib. One patient suffered from bladder pain and pollakiuria, and one from ureteric stones, all of which were possibly gefitinib-related serious adverse events. For the remaining nine patients who withdrew from the trial, the reason for their withdrawal was grade 1–4 alanine transaminase alone (in two patients) or in combination with grade 2–4 aspartate transaminase increase (in seven patients). The maximum tolerated dose was not exceeded.

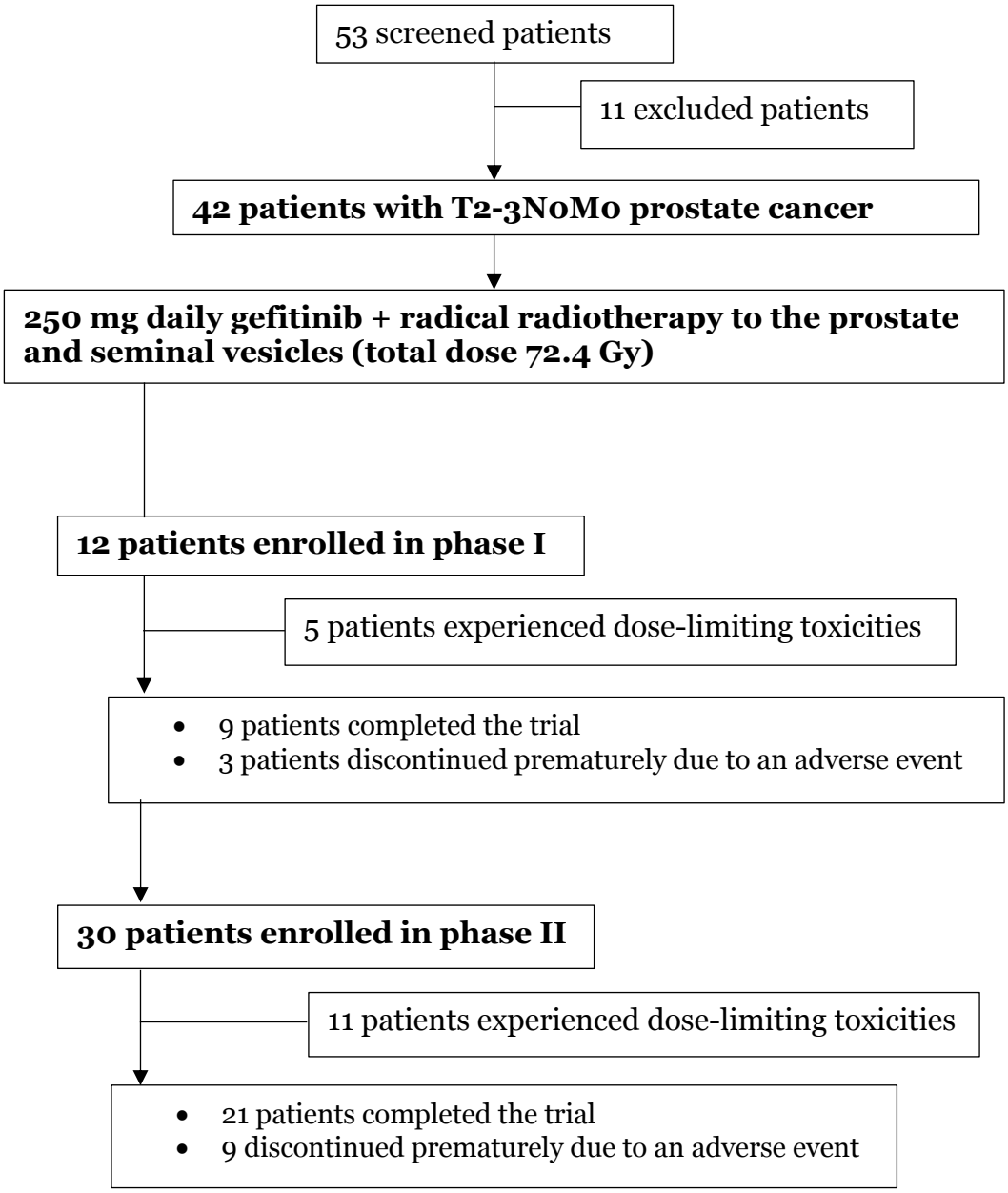
5.1.3 TOXICITY

All patients experienced one or more adverse events (Tables 2 and 3 in the original publication). These were most commonly gastrointestinal (40 [95.2%] patients), renal/urinary (36 [85.7%] patients), and

skin/subcutaneous tissue-related (34 [81.0%] patients). The most common adverse events were proctitis (31 [73.8%] patients), pollakiuria (30 [71.4%] patients), diarrhoea (27 [64.3%] patients), and dysuria (24 [57.1%] patients).

Fourteen patients experienced grade 3–4 adverse events, of which alanine (7 [16.7%] patients) and aspartate (6 [14.3%] patients) transaminase increases were the most common (both gefitinib-related). Transaminase increase caused frequently treatment interruptions as well. Among patients with higher transaminase levels, there was a tendency for a more notable PSA decrease during the gefitinib treatment; however, since the PSA decreased rapidly in all patients, no reliable conclusions could be drawn.

Figure 1. Flowchart of study I. *G. Hackman et al., modified from figure 1 of the original publication.*



5.1.4 DOSE-LIMITING TOXICITIES

A total of 16 (38.1%) patients suffered from dose-limiting toxicities, of which the most common were alanine and aspartate transaminase increases in nine patients (grade 3–4, gefitinib-related). The median time from the treatment initiation to the transaminase increase was 42 days (range 26–64 days). One patient suffered from urticaria as well as alanine and aspartate transaminase increases (all grade 3, gefitinib-related); one suffered from subdural hematoma (grade 4, gefitinib-related), and two suffered from pollakiuria (grade 3, gefitinib-related). The remaining three patients experienced the following serious adverse events: 1) cardiomegaly, cardiac failure, and myocarditis (grade 4, gefitinib-related); 2) bladder pain and pollakiuria (grade 3, gefitinib-related); and 3) ureteric stones (grade 3, gefitinib-related).

5.1.5 EGFR EXPRESSION

Immunohistochemistry testing detected high EGFR expression (in 100% of the cells) in 17 (40.5%) patients, elevated EGFR expression (in 50-80% of the cells) in 12 (28.6%) patients, and no EGFR expression in one patient (Table 4 in the original publication). Data regarding EGFR expression was unavailable for 12 (28.6%) patients. None of the samples showed EGFR amplifications, EGFR variant III or phosphorylated EGFR. The analyses were controlled with glioma specimens positive for EGFR variant III, and head and neck cancer specimens featuring phosphorylated EGFR. Chromogenic in situ hybridisation was internally controlled by the presence of normal signals in each sample.

5.1.6 CYTOKINE ANALYSES

Due to frequently seen transaminase increases among study I patients, the cytokines were tested, as these have been hypothesized to underlie under liver toxicity (*233*, *234*). However, cytokine levels were low, and there was no correlation between the serum cytokines (tumour necrosis factor, interleukin 1 alpha, interleukin 6) and alanine transaminase increases.

5.1.7 SURVIVAL ANALYSES

After five years (median 36.4 months) of follow-up, the cumulative recurrence-free survival was 100% in the patients treated with gefitinib in combination with radiotherapy compared to 96% in the patients treated with radiotherapy alone ($p = 0.27$). Respectively, in terms of cumulative PSA-relapse-free survival, the values were 97% compared to 79 % ($p = 0.06$), and, for cumulative salvage therapy-free survival, 61% compared to 89 % ($p = 0.93$). After five years of follow-up, 2/42 of the patients treated with gefitinib in combination with radiotherapy received salvage therapy (both

bicalutamide) compared to 17/91 matched controls. As for cumulative overall survival, the percentages were the same (87%) in both groups (p = 0.57). For comparison of baseline characteristics between the groups, see Table 2, and of survival analyses, see Figure 2 in the original publication.

Table 2. Patient characteristics at baseline. *G Hackman et al. unpublished data.*

		Gefitinib + radical radiation number of patients (%)	**Radical radiation alone (matched controls)** number of patients (%)
Total number of patients		42 (100%)	91 (100%)
Total dose of radiation		72.4 Gy	74-78 Gy
T-stage			
	T2	37 (88%)	83 (91%)
	T3	5 (12%)	8 (9%)
Gleason score			
	4-5	6 (14%)	11 (12%)
	6	17 (40%)	42 (46%)
	7	17 (40%)	32 (35%)
	8	2 (5%)	6 (7%)
PSA	mean (range)	8.4 μg/l (1.6-18.8 μg/l)	8.6 μg/l (1.0-18.8 μg/l)

Gy = Gray, PSA = Prostate-specific antigen, T = tumour.

5.2 STUDY II

5.2.1 PATIENTS

This study included 30 patients, of whom 19 underwent radical prostatectomy and 11 radical radiotherapy before trial enrolment (Figure 1 in this paper and Supplementary Table 1 in the original publication). One patient, who primarily underwent radical prostatectomy, also received radiotherapy with a radical dose three months after radical prostatectomy. For surgically treated patients, the range for initial PSA was 0.2 to 4.5 μg/l, and, for radiation treated patients, it was 1.1 to 8.5 μg/l.

5.2.2 TREATMENT

The median time spent on gefitinib treatment was 145.5 days (range 33–600 days). A total of 12 (40.0%) patients had one or more interruptions in their gefitinib treatment. The interruptions occurred due to transaminase increases in 11 (36.7%) patients and a lapse of memory in one (3.3%) patient.

5.2.3 TREATMENT FAILURES

Three months after the initiation of gefitinib, seven (23.3%) patients had discontinued the treatment, while 23 (76.7%) were free of treatment failure (Figure 2 in this paper and Supplementary Figure 1 in the original publication). Reasons for treatment failure included PSA progression (five patients, 16.7%) and adverse events (two patients, 6.7%). These adverse events included grade 2 nausea and a grade 2 ocular adverse event (flashing lights).

5.2.4 TOXICITY

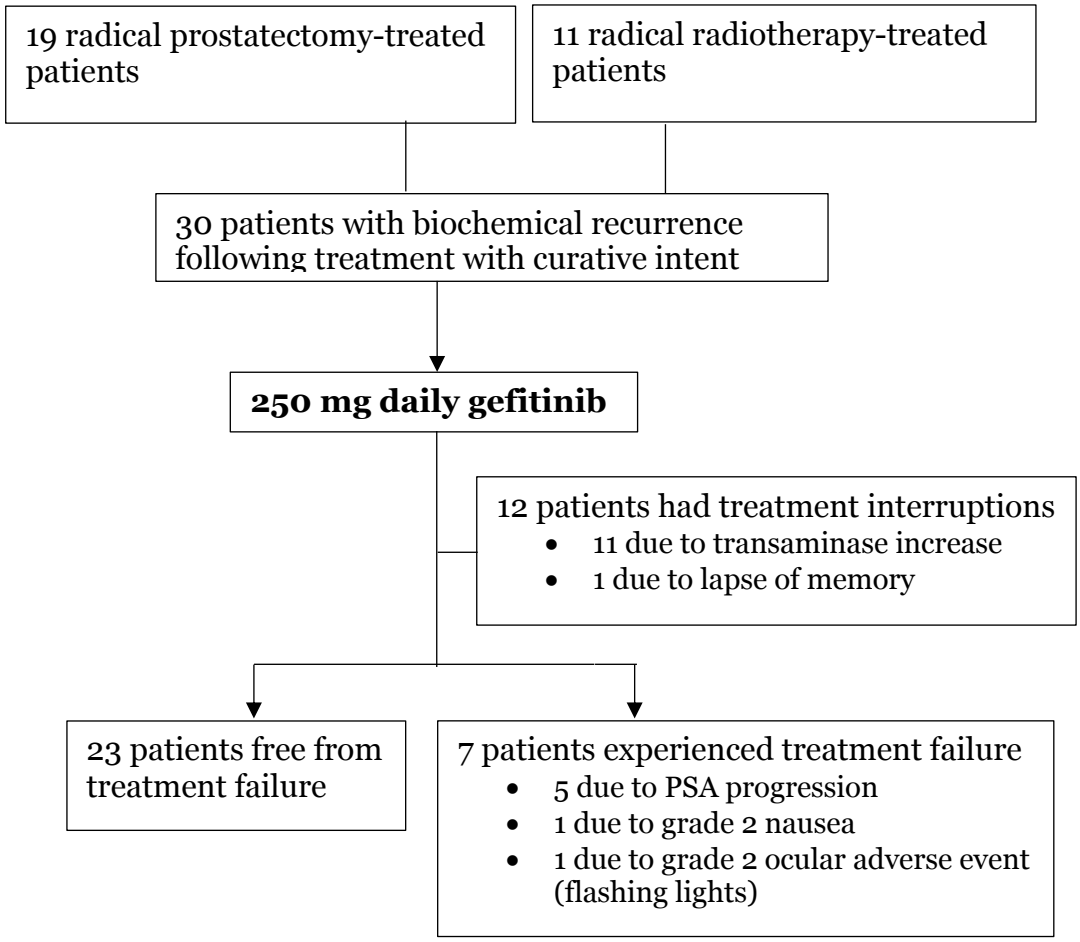
During the three months of gefitinib treatment, 28 (93.3%) patients suffered from grade 1–3 adverse events potentially related to gefitinib (Supplementary Table 2 in the original publication). Of these patients, only three experienced grade 3 gefitinib-related adverse events. No grade 4–5 adverse events occurred.

The most common events were gastrointestinal disorders (23 [76.7%] patients), skin/subcutaneous tissue-related disorders (22 [73.3%] patients), and infections (12 [40%] patients). Acne (19 [63.3%] patients) and diarrhoea (15 [50.0%] patients) were the most common individually reported adverse events and considered to be gefitinib-related.

A total of four (13.3%) patients suffered from a grade 3 adverse event during the three months of gefitinib treatment. Out of these patients,

three experienced potentially gefitinib-related (two alanine transaminase increases, one alanine and aspartate transaminase increase) events and one experienced a non-gefitinib-related (syncope) grade 3 adverse event. One adverse event (urinary calculus) was first recorded as *serious* due to the hospitalisation of the patient. However, the final grade of this event, which was unrelated to gefitinib, was 1, and the patient continued normally in the trial.

Figure 2. Flowchart of study II. Three months of gefitinib treatment. *G Hackman et al. unpublished data.*



PSA = prostate-specific antigen.

5.2.5 EFFICACY

No PSA responses occurred among study II patients. During three months of gefitinib-treatment, seven (23.3%) patients experienced PSA progression, with a median time of 60 days (range 27–90 days) to that progression (Supplementary Table 3 and Figure 1 in the original publication). Hence, 23 (76.7%) patients were progression-free, out of which 20 continued with the gefitinib and two withdrew due to an adverse event.

The change in the PSA doubling time was unavailable for three patients, since they had only one PSA measurement during the gefitinib treatment. Out of the 27 patients, the PSA doubling time decreased in 10 patients during gefitinib when compared to the PSA doubling time before the gefitinib treatment; however, in eight of these patients, the decrease was less than 50% (Supplementary Figure 2 in the original publication). Seventeen patients experienced an increase in the PSA doubling time during gefitinib, and, in six of these patients, this increase was 100% or more.

5.2.6 GENE MUTATION ANALYSES

Four patients' samples were available for gene mutation analyses. Single nucleotide polymorphisms were found in two patients' samples: 1) in *EGFR* exon 20: substitution of guanine for adenine in Gln787; and 2) in *EGFR* exon 21: substitution of adenine for guanine in Thr854. However, neither one of these polymorphisms changed the amino acids, and, thus, no activating mutations were found.

5.3 STUDY III

5.3.1 PATIENTS

Study III was a patient series of 46 patients with newly diagnosed metastatic prostate cancer treated consecutively with a multimodal approach from 2005 to 2016 (Figure 3). All patients had histologically confirmed prostate cancer with bone metastases (Table 1 in the original publication). Other metastatic sites included pelvic lymph nodes (in 24 patients) consisting of the obturator, parailiac, and presacral lymph nodes. Other metastatic lymph nodes (in 17 patients) included the inguinal, retroperitoneal, mediastinal, and supraclavicular lymph nodes. One patient had lung metastases. Additionally, the patients had T1–4, N0–1, M1 prostate cancer with a Gleason score of 7–10 (median Gleason score of 9). The initial median PSA was 98.5 μg/l (mean 658 μg/l, range 6.7–15500 μg/l). One patient had T1 disease; however, his initial PSA was 1000 μg/l, indicating an aggressive prostate cancer. PSA was ≤ 1 μg/l for 22 patients before prostate radiotherapy. The median age of the patients at diagnosis was 63 years (range 39–86 years). The median follow-up period was 4.38 years (mean 4.63 years, range 0.36–11.24 years).

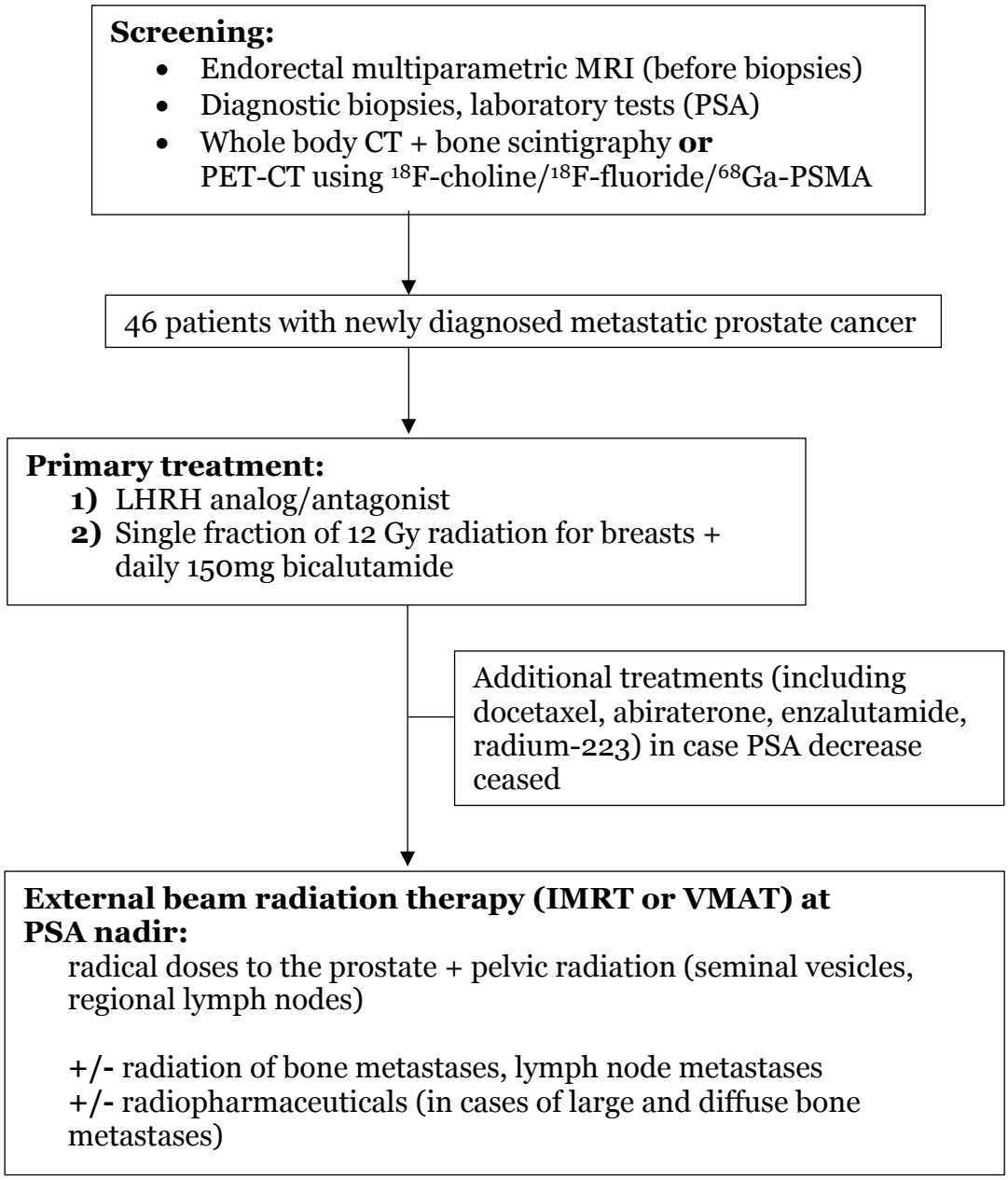
5.3.2 RADIOTHERAPY

The patients received radiotherapy from June 2005 to July 2016 (Table 4 in the original publication). All patients received a radical dose of radiation to the prostate. The mean dose for the prostate was 78.2 Gy for 44 patients (range 76–80 Gy). For two patients, the total dose of VMAT was 50 Gy, after which they received a high-dose-rate brachytherapy booster of 2 x 10 Gy. Two patients received IMRT, and 44 patients received VMAT.

All patients received radiation to the seminal vesicles (at least 50 Gy in 2 Gy fractions). The mean dose for the seminal vesicles was 52.2 Gy (range 50.0–80 Gy). All patients also received radiation to the regional lymph nodes, with a mean dose of 45.4 Gy (range 45–50.4 Gy).

Out of the 46 study patients, 23 received radiation to their bone metastases (mean dose 29.0 Gy, range 38.6–77.4 Gy), four to their lymph node metastases (mean dose 67.2 Gy, range 59.4–76 Gy), and 11 for their retroperitoneal/para-aortic lymph node metastases (mean dose 47.9 Gy, range 45–50.4 Gy).

Figure 3. Algorithm for screening and multimodal treatment of newly diagnosed metastatic prostate cancer used in study III. All patients received primary treatment (androgen deprivation) and radical radiotherapy. *G Hackman et al., modified from Figure 4 of the original publication.*



CT = computed tomography, IMRT = intensity-modulated radiation therapy, LHRH = luteinising hormone-releasing hormone, MRI = magnetic resonance imaging, PET = positron emission tomography, PSA = prostate-specific antigen, PSMA = prostate-specific membrane antigen, VMAT = volumetric modulated arc therapy.

5.3.3 TOXICITY

Adverse events were recorded for all patients during radiotherapy and, for 42 patients one year after radiotherapy (four patients had follow-up periods less than one year). Most adverse events were grade 1 and transient (Table 5 in the original publication). Grade 3 adverse events consisted of urinary and intestinal adverse events. Three grade 3 urinary retention events, one grade 3 urinary incontinence event, and one grade 3 diarrhoea event occurred during radiotherapy. One year after radiotherapy, one patient experienced grade 3 urinary incontinence, and no grade 3 intestinal adverse events were reported. There were no grade 4–5 adverse events. Since all patients underwent castration and experienced erectile dysfunction, this adverse event was unreported. All adverse events related to the treatment of metastases were grade 1.

5.3.4 SURVIVAL ANALYSES

Eleven study patients died from prostate cancer. Eight of these patients had a last PSA > 1 μg/l (median 3.2 μg/l, range 1.07-104 μg/l) before the beginning of the definitive radiotherapy. One 90-year-old patient died from pneumonia following a traumatic hip fracture. At the time of his death, the PSA was immeasurable. The five-year progression-free survival rate was 21.6% (Kaplan-Meier); the median progression-free survival was 3.03 years; the five-year overall survival was 81.3% (Kaplan-Meier); and the median overall survival was 8.35 years (Figures 2 and 3 in the original publication).

5.4 STUDY IV

5.4.1 PATIENTS

Study enrolment occurred between April 2004 and October 2012, and the follow-up ended in January 2017. A total of 250 patients were randomised into two groups: 126 in the adjuvant group and 124 in the observation group (Figure 4 in this paper and Table 1 in the original publication). Five patients from the adjuvant group and two patients from the observation group withdrew their consent shortly after the randomisation. The data is reported on an intent-to-treat basis.

5.4.2 ADJUVANT RADIOTHERAPY

Adjuvant radiotherapy began a median of 11.7 weeks after radical prostatectomy (range 7.6 before the beginning of definitive radiotherapy 30 weeks). A total of five patients had interruptions of their adjuvant irradiation due to adverse events. One patient had grade 1–2 increased defecation frequency, loose stools, pollakiuria, and nocturia; one patient had grade 2 viral flu with fever; one patient had grade 3 cholecystitis, and, for one patient, the reason for interruption was unknown. These four patients received the planned total dose of 66.6 Gy. One patient had grade 2 increased defecation frequency, and his dose was limited to 63 Gy.

5.4.3 SURVIVAL ANALYSES

The median follow-up time in the adjuvant group was 8.95 years (range 0.61–12.60 years), and, in the observation group, 8.41 years (range 1.24–12.07 years). For patients who were alive when the follow-up period ended, the median follow-up time in the adjuvant group was 9.3 years (range 3.3–12.6, interquartile range [IQR] 6.5–10.3) and it was 8.6 years (range 3.6–12.1, IQR 6.4–10.4) in the observation group.

One patient in each group died of prostate cancer (Table 2 and Figure 2 in the original publication). The 10-year overall survival was 91.6% in the adjuvant group and 86.5% in the observation group (HR 0.69 [95% CI 0.29–1.60], p = 0.4). The 10-year prostate cancer-specific survival was 98.8% in the adjuvant group and 98.9% in the observation group (HR 1.00 [95% CI 0.06–15.91], p = 1).

The prostate cancer metastasized in two patients in the adjuvant group and in four patients in the observation group (Table 2 and Figure 2 in the original publication). The 10-year metastatic-free survival was 97.7% in the adjuvant group and 96.3% in the observation group (HR 0.49 [95% CI 0.09–2.68], p = 0.4). Imaging detected no local recurrence, and all patients diagnosed with metastases experienced biochemical progression before the

occurrence of metastatic disease. Thus, for all patients who had disease progression, the first sign of this progression was biochemical recurrence.

Castration-resistant prostate cancer occurred in three patients in the adjuvant group and in six patients in the observation group (Table 2 in the original publication). The 10-year castration resistant-free survival was 96.1% in the adjuvant group and 92.4% in the observation group (HR 0.50 [95% CI 0.12–1.88], p = 0.3).

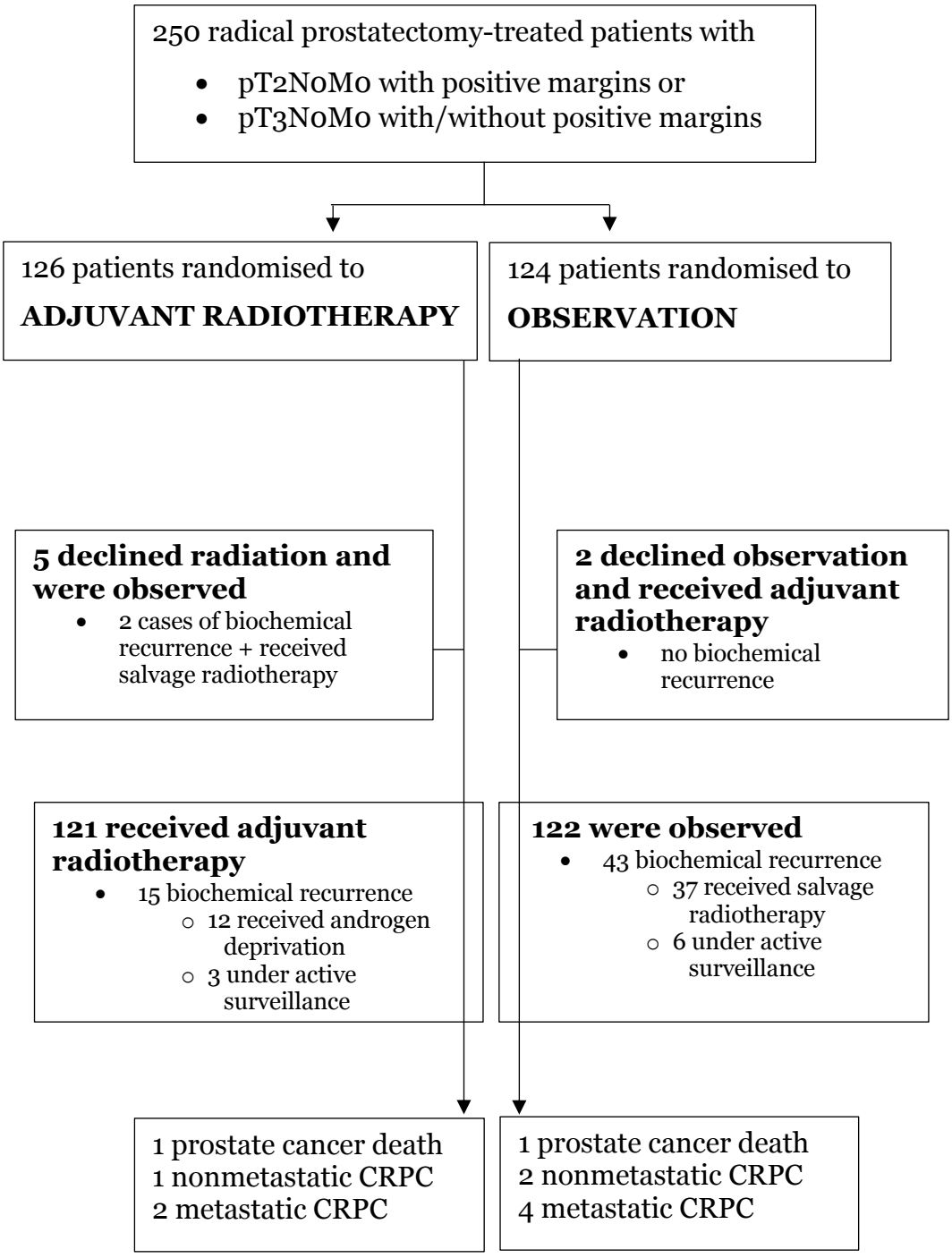
5.4.4 BIOCHEMICAL RECURRENCE

Fifteen (11.9%) patients in the adjuvant group and 43 (34.7%) in the observation group experienced biochemical recurrence as defined in the protocol (Figure 4 in this paper, Table 2 and Figure 2 in the original publication). The 10-year biochemical recurrence-free survival was 81.9 % in the adjuvant group and 60.6% in the observation group (HR 0.26 [95% CI 0.14–0.48], p < 0.001; and after adjusting for preoperative PSA, Gleason score, and pT stage: HR 0.30 [95% CI 0.16–0.54], p < 0.001). The number needed to treat was 4 (number of patients that need to be treated to prevent one biochemical recurrence). For the biochemical recurrence-free patients, the median follow-up time in the adjuvant group was 8.6 years, and, in the observation group, it was 8.2 years.

The median preoperative PSA for the patients with biochemical recurrence was 9.7 μg/l (IQR 0.1–14) in the adjuvant group and 8.9 μg/l (IQR 6.6–11) in the observation group. For the recurrence-free patients, the median preoperative PSA was 6.9 μg/l (IQR 5.1–9.7) in the adjuvant group and 6.9 μg/l (IQR 5.3–9.9) in the observation group. However, the interaction between the treatment group and preoperative PSA was not statistically significant (HR 0.95; 95% CI 0.88–1.05, p = 0.34).

Out of the pT2 patients with positive surgical margins, 3/73 in the adjuvant group compared to 21/63 in the observation group experienced protocol-defined biochemical recurrence. As for the pT3 patients, 12/53 in the adjuvant group versus 22/59 in the observation group experienced protocol-defined biochemical recurrence. Out of the Gleason score 5–6 patients, 2/38 in the adjuvant group compared to 12/33 in the observation group experienced protocol-defined recurrence. As for the Gleason score 7 patients, 11/81 in the adjuvant group versus 29/83 in the observation group experienced protocol-defined recurrence.

Figure 4. Flowchart of study IV. *G. Hackman et al., modified from Figure 1 of the original publication.*



CRPC = castration-resistant prostate cancer.

Biochemical recurrence (defined in the protocol) = 1) PSA > 0.4 μg/l in two consecutive measurements at least four weeks apart, 2) metastatic prostate cancer, or 3) recurrent prostate cancer in imaging.

5.4.5 TREATMENTS FOLLOWING BIOCHEMICAL RECURRENCE

Following biochemical recurrence as defined in the protocol of study IV, 12 patients received androgen deprivation therapy (median PSA 1.40 μg/l [range 0.44–32.20 μg/l), and three were under active surveillance in the adjuvant group (Figure 4). In the observation group, 37 received salvage radiotherapy, within a median of 19.9 weeks (range 4.6–152.4 weeks) measured from the date of the protocol-defined biochemical recurrence (median PSA 0.70 μg/l [range 0.42–8.20 μg/l, IQR 0.57–0.83 μg/l]), and six continued to be under active surveillance (Figure 4). The PSA was measured at a median of 4.71 weeks (range 0.0–11.9 weeks) before the beginning of radiation. Out of the 37 patients who received salvage radiotherapy, 28 achieved PSA remission, and nine required systemic therapies.

Ten patients with a median PSA of 0.40 μg/l (range 0.12–0.53 μg/l) received salvage radiotherapy for biochemical recurrence not defined in the protocol (Figure 4). Nine of these patients were from the observation group and one patient was from the adjuvant group. The latter declined adjuvant radiotherapy shortly after randomisation and, consequently, had undergone radical prostatectomy alone before the biochemical recurrence for which this patient received salvage radiation. Five of these patients (one from the adjuvant and four from the observation group) received salvage radiotherapy at PSA < 0.4 μg/l. Out of the 10 patients who received salvage radiotherapy for biochemical recurrence not defined in the protocol, all except one from the observation group achieved PSA remission. In addition, one patient in the observation group who had no biochemical recurrence or metastases received hormonal therapies

5.4.6 TOXICITY

5.4.6.1 ADVERSE EVENTS FROM MEDICAL RECORDS

A total of 125 patients in the adjuvant group and 123 in the observation group experienced adverse events (Table 3 in the original publication). Out of all the adverse events in the study IV, 70.1% occurred in the adjuvant group and 29.9% in the observation group. Most of these were grade 1–2 and transient. Erectile dysfunction and urinary incontinence were the most common grade 3 adverse events. In the adjuvant group, 37.3% of the patients experienced grade 3 erectile dysfunction compared to 28.2% in the observation group. For urinary incontinence, the numbers were 11.9% in the adjuvant group and 4.8% in the observation group. Twelve patients in the adjuvant group suffered grade 3 urethral stricture compared to three patients in the observation group. Moreover, grade 3 inguinal hernia occurred in eight patients in the adjuvant group and nine in the observation group.

One grade 4 adverse event, a compartment syndrome, occurred in the adjuvant group. The patient underwent a ventral hernia repair, after

which he suffered from urinary retention. The treatment for this postoperative complication was a transurethral incision of the vesicourethral anastomosis, which led to urinary incontinence. Nearly seven years after the radical prostatectomy, the urinary incontinence was treated with a cystectomy, which made the compartment syndrome as a postoperative complication.

5.4.6.2 PATIENT-REPORTED TOXICITY

Depending on the time of visit (between 0–51 months from the radical prostatectomy), a median of 86 (range 18–105) patients in the adjuvant group and a median of 71 (range 28–94) patients in the observation group filled out the LENT-SOMA questionnaire. Out of all the LENT-SOMA toxicities reported in study IV, 59.3% occurred in the adjuvant group and 40.7% in the observation group (Figure 3 in the original publication). The most common LENT-SOMA toxicities were urinary frequency (93.0% of patients in the adjuvant radiotherapy group and 91.6% of patients in the observation group who filled out the questionnaire), urinary incontinence (69.6%, 62.2%), decreased urinary stream (60.9%, 55.5%), and rectal tenesmus (63.5%, 42.0%). In the adjuvant group, 75 (65.2%) patients experienced grade 3 LENT-SOMA toxicity versus 64 (53.8%) in the observation group. For grade 4 LENT-SOMA toxicity, the numbers were 28 (22.2%) versus 20 (16.1%) patients. The most common grade 4 toxicities were kidney-related toxicity (18 patients in the adjuvant group, 15 in the observation group), urinary incontinence (seven and five patients) and urinary frequency (five and two patients).

Depending on the time of visit (between 0–51 months from the radical prostatectomy), a median of 93 (range 77–104) patients in the adjuvant group and a median of 100 (range 73–110) patients in the observation group filled out the IIEF-5 questionnaire. Patients most commonly reported their erectile dysfunction as *severe* in both groups throughout the follow-up period (Figure 3 in the original publication).

Depending on the time of visit (between 0–51 months from radical prostatectomy), a median of 92 (range 80–106) patients in the adjuvant group and a median of 99 (range 70–112) patients in the observation group filled out the IPSS questionnaire. Most of the patients reported their urinary symptoms as *mild* and their quality of life as affected by these urinary symptoms as *delighted, pleased, or mostly satisfied* throughout follow-up period (Figure 3 in the original publication).

6 DISCUSSION

6.1 STUDY I AND II

6.1.1 SAFETY AND TOLERABILITY

As there were no previous clinical prostate cancer studies of gefitinib in combination with radiotherapy at the time of the study initiation, the first phase of study I was to evaluate the tolerability of this treatment. Naturally, tolerability was one of the endpoints in the second phase as well. Study II evaluated adverse events as the secondary endpoint.

The original publication of study I evaluated the toxicity of gefitinib and radiation as acceptable. In study I, 88% of the patients had T2 and 55% had Gleason score ≤ 6 prostate cancer indicating a low-risk disease. At the initiation of this trial, the common treatment recommendation for these patients was radical treatment. Thus, the original publication of this trial compared the toxicity of gefitinib and radical radiation to the toxicity of radiation alone. However, according to current knowledge, active surveillance would be the standard of care for these patients. Even though most of the adverse events in this study were grade 1–2, all patients experienced at least one event and 33% experienced grade 3–4 event. Consequently, taking into account that the majority of the study patients had low-risk localised disease with an excellent prognosis even without the radical treatment, the toxicity profile of gefitinib in combination with radiation was unacceptable.

As expected, the combination of radiotherapy and gefitinib caused more adverse events and changed the profile of the adverse events when compared to gefitinib given as a monotherapy. In study I, the most common events were gastrointestinal, renal and urinary, and skin and subcutaneous tissue disorders, all of which are typical side effects of radiation. During the trial, one patient died due to cardiovascular problems. It was impossible to rule out the possible effect of gefitinib regarding the death; however, the patient had a history of aortic stenosis, intermittent calculation, and high blood pressure. The other serious adverse events were grade 3 cystitis, a typical side effect of radiation, and ureteric stones, which might have emerged by chance. However, both were considered to be as possibly gefitinib-related.

Surprisingly, as many as 74% of the study I patients experienced liver enzyme elevation, graded as 3 in six patients and 4 in one patient. Sixteen patients (38%) experienced dose-limiting toxicity, the most common of which were grade 3-4 transaminase increases in six patients. While an increase in transaminase levels is not uncommon for EGFR inhibitors, this occurs rarely if at all and is usually mild according to previous studies (*181*, *182*). In a study by Maurel et al., one out of six pancreatic cancer patients receiving gefitinib and radiation experienced grade 3 transaminitis (*196*). In

another study by Ma et al., gefitinib and the radiation of brain metastases from non-small-cell lung cancer resulted in grade 1 liver dysfunction in two out of 21 patients (*199*). In a study of 16 head and neck cancer patients Caponigro et al. reported three grade 1 and three grade 3 liver toxicities with a 250 mg dose of gefitinib in combination with radiation (*195*). Chen et al. reported only grade 1 elevated transaminases in a study of head and neck cancer where patients received gefitinib in a combination with radiotherapy or chemoradiation (*235*). In a study of Fu et al. one out 29 non-small-cell lung cancer patients receiving gefitinib and radiation experienced grade 3 liver dysfunction (*200*).

The release of cytokines upon radiation might have explained the unexpectedly high incidence of elevated transaminases in study I; however, there was no correlation between the cytokine and alanine aminotransferase levels. Moreover, all patients with liver enzyme elevation were asymptomatic and suffered no long-term liver-related side effects. High-dose brachytherapy might present one approach to shortening the duration of radiation, thus potentially reducing liver-related toxicity caused by gefitinib. In addition, since radiotherapy has improved significantly since the execution of study I, with modern techniques, there could be less treatment-related toxicity.

In study II, the safety and tolerability of gefitinib were good, as most of the adverse events were grade 1–2. The most common events were acne and diarrhoea. Two patients experienced grade 3 elevated alanine aminotransferase alone and one together with grade 3 elevated aspartate aminotransferase, all of which were possibly gefitinib-related. In addition, one patient suffered grade 3 syncope, which was considered to be non-gefitinib-related. There was one serious adverse event due to the hospitalization of one study II patient; however, this was eventually scored as grade 1 urinary calculus and not related to the gefitinib. Study II recorded no grade 4–5 adverse events.

6.1.2 EFFICACY

6.1.2.1 STUDY I

Primary local treatment with curative intent fails to achieve long-term cancer control in a substantial proportion of patients, especially if high-risk features are present. Currently, the standard of care for high-risk localised and locally advanced prostate cancer is a combination of local treatment and adjuvant therapy (*28*, *98*). The most common adjuvant therapy used in these patients is hormonal treatment, which causes significant morbidity and exposes patients to long-term side-effects (*165*, *200*).

While EGFR overexpression is associated with poor prognosis and resistance to radiotherapy, it appears to be a good target for gefitinib, which inhibits the tyrosine kinase activity of EGFR (*194*). In addition, gefitinib is a well-tolerated and approved treatment option for non-small-cell lung cancer (*175*). The hypothesis in study I was that gefitinib could improve the progression-free as well as cancer-specific survival of newly diagnosed

nonmetastatic prostate cancer with acceptable toxicity through the radiosensitization and inhibition of the antiproliferative mechanisms of the EGFR signalling pathways.

After four years of follow-up in study I, gefitinib in combination with radiation resulted in an estimated PSA relapse-free survival (Kaplan-Meier) of 97%, a salvage treatment-free survival of 91%, and an overall survival of 87%, all of which only compared favourably with the matched controls treated with radiation alone only at higher doses (biologically effective dose of 72.4 Gy/1.8 Gy plus gefitinib compared to 74-78 Gy/2 Gy radiation only). After five years of follow-up, the survival rates of gefitinib in combination with radiation were equally favourable compared to the matched controls, with the exception of salvage treatment-free survival, which decreased from 91% to 61%. This was due to a small number of patients and events (at four years one patient, and at five years two patients received salvage treatment) and a short follow-up period (median 34 months). However, there was no statistical significance between the treatments at four or at five years of follow-up.

After nearly 20 years of follow-up, the PIVOT-trial showed no statistically significant difference between radical prostatectomy and observation among localised prostate cancer patients, most of whom had low-risk features (*90*). Taking into account that the majority of the study I patients had low-risk localised disease and, due to increased knowledge, an excellent prognosis even without any active treatment, detecting a significant long-term efficacy seems unlikely regardless of the small survival differences between patients who received gefitinib in combination with radiation and patients who received radiation alone (matched controls).

6.1.2.2 STUDY II

The standard active treatment for biochemical recurrence after local therapy with curative intent is salvage radiation (following radical prostatectomy) or hormonal therapy (following radical radiotherapy). Eventually, the efficacy of hormonal therapy is lost, resulting in castration-resistant prostate cancer, which significantly decreases the survival of these patients. Salvage radiation generally provides good long-term cancer control in localised disease; however, approximately 50% of patients treated with salvage irradiation experience further disease progression, especially when they present with high-risk features (*236–238*).

Gefitinib has improved the progression-free survival in non-small-cell lung cancer (*175*). Meanwhile, in prostate cancer, EGFR overexpression is strongly associated with biochemical relapse, castration-resistant disease, and metastatic disease (*60–63*). Thus, the hypothesis of study II was, that gefitinib would prolong the biochemical recurrence-free survival in prostate cancer patients as well and, consequently, postpone the initiation of possible later hormonal treatment or salvage radiation.

The primary endpoint and statistical target for study II was PSA response in > 40% of the patients, which would have given a rationale to proceed to a phase III randomised trial. However, this was not achieved.

Following a protocol amendment, patients without a PSA response were able to continue the trial for a further three months. Regardless, no PSA responses occurred.

The study II protocol (written in 2005) defined biochemical recurrence for radiotherapy-treated patients as three consecutively rising PSA values within four weeks apart. Currently, the common PSA threshold for biochemical recurrence following radical radiotherapy with curative intent is PSA nadir + 2 μg/l (*84*). At the initiation of this trial the definition was variable, and the current PSA threshold was not as well-established as it is today. Considering the definition used in study II, there is a possibility that the study enrolled patients who would have stayed PSA progression-free regardless of the use of gefitinib. However, out of the radiation-treated patients in study II, only two had initial PSA < 2 μg/l (1.1 and 1.2 μg/l), and after initiation of gefitinib, both had PSA increase above 2 μg/l. Also, the median initial PSA among radiotherapy-treated patients was 4.35 μg/l (range 1.1–8.5 μg/l), commonly indicating biochemical recurrence following therapy with curative intent.

In contrast to other studies on gefitinib and prostate cancer, which enrolled patients with castration-resistant prostate cancer, study II patients had a hormone-naïve disease (*181*, *183–185*). While castration-resistant cancer progresses aggressively and has limited treatment options, hormone-naïve cancer has better survival outcomes and in addition to hormonal therapy is potentially sensitive to other treatment options as well. Therefore, one could assume, that study II patients' cancer was an optimal target for gefitinib monotherapy. However, by three months there were no PSA responses, although 23 patients (77% out of all study patients) remained PSA progression-free (free from treatment failure). As a possible sign of efficacy of gefitinib monotherapy, 17 patients (63% out of the study patients with PSA doubling time calculable) experienced a PSA doubling time increase, which, in different studies, has been significantly associated with prostate cancer mortality and overall survival (*239–243*).

6.1.3 SIGNIFICANCE OF EGFR EXPRESSION AND MUTATIONS

EGFR expression was not a prerequisite for enrolment in either of the studies. In the immunohistochemistry tests of study I, however, out of the 30 patients for whom the EGFR data was available, 29 showed EGFR expression. Of these patients, 17 had high (in 100% of cells) and 12 elevated (in 50-80% of cells) EGFR expression. In study II, the immunohistochemistry data was unavailable, and the number of study patients was small. Thus, there is a possibility that gefitinib was unlikely to exhibit any efficacy in cases where the EGFR expression would have been low.

In non-small-cell lung cancer, mutation in the kinase domain of EGFR is a strong predictor of the efficacy of gefitinib (*186*). Data regarding EGFR mutations was unavailable in studies I and II. It is conceivable, that

the study patients had no EGFR mutations, which could explain the modest activity of gefitinib. However, there is only one small study that has shown a significant association between EGFR mutations and prostate cancer in terms of the time to convert from hormone-sensitive to castration-resistant disease (*187*). Consequently, there is a lack of strong evidence of the importance of EGFR mutations in prostate cancer.

6.2 STUDY III

6.2.1 SAFETY

In study III, the combination of androgen deprivation therapy and radical radiotherapy in newly diagnosed metastatic prostate cancer was well-tolerated, as most of the adverse events during and one year after radiation were grade 1–2 and transient. Although 28% of the patients received docetaxel prior to radiation, which had the potential to cause the worsening of the adverse events, the incidence of grade 3 events was low and there were no grade 4–5 events. The tolerability of the androgen deprivation and radical radiation was as expected, as this combination treatment is known from several randomised trials on localised high-risk prostate cancer; yet, study III tested the treatment in the metastatic setting as well (*23*, *28*, *29*, *97*, *99*).

Treatment-related bone marrow toxicity caused concern over the possible damage it can cause to haematopoiesis, thus potentially limiting the use of some study III treatments, such as chemotherapies and radiopharmaceuticals. While 28% of the patients were ≥ 70 years or older, choosing the optimal multimodal treatment had to be done cautiously. The most common chemotherapies used were docetaxel (65% of the patients) and cabazitaxel (25% of the patients). In addition, the study patients received other chemotherapies that were not routinely used in the treatment of prostate cancer. Two patients were cytopenic at the time of their death, one of which had grade 3 leukocytopenia, and the other had grade 4 leukocytopenia and thrombocytopenia. A prolonged treatment could have caused these blood count changes; however, both had aggressive prostate cancer progression into the bone marrow, which commonly causes cytopenia as well. The survival of these patients was 7.1 and 8.1 years, respectively.

6.2.2 EFFICACY

The rationale behind the multimodal approach was to cause maximal cancer cell death by treating newly diagnosed metastatic prostate cancer as actively as possible from the very beginning of treatment. The cornerstone of this multimodal treatment was the combination of radical radiotherapy and androgen depression, which all patients received. In addition, the patients received several other, individually planned treatments, including hormonal therapy, chemotherapy, radiopharmaceuticals, and experimental approaches. The strategy was to use these additional treatments following androgen deprivation (primary treatment) to reach PSA nadir prior to radiation therapy.

Before the release of the study III results, there were no randomised trials regarding local therapies in metastatic prostate cancer. However, observational data supported radiotherapy, and, later, randomised HORRAD and STAMPEDE trials confirmed the effectiveness of radical

radiotherapy in combination with androgen depression in metastatic disease (*38*, *115*, *217*, *218*).

While the median survival of newly diagnosed metastatic prostate cancer is less than six years, patients can live more than 10 years following diagnosis (*14*, *15*, *244*). However, when the response to castration is lost as the castration-resistant disease emerges, the survival decreases significantly. In aggressive disease, this can occur even within a couple of months, as seen in 13 patients (28%) in study III. These patients received docetaxel as their PSA started to increase or the decrease stopped following androgen deprivation. Proving its significant survival benefit first in castration-resistant metastatic prostate cancer in several randomised trialsand later in newly diagnosed metastatic disease in the STAMPEDE and CHARTEED trials, docetaxel is an established treatment for the early phase of metastatic prostate cancer (*201–203*).

PSMA PET/CT appears to have superior diagnostic accuracy in detection of prostate cancer and its metastases when compared to conventional imaging (*112*). While PSMA is overexpressed in prostate cancer, it is present in several benign processes as well (*112*). Activity of osteoblasts leads to low to moderate PSMA expression seen in osteoarthritis, degenerative changes, and fractures (*112*). Consequently, oligometastases detected by PSMA PET/CT should be interpreted with caution. While study III included prostate cancer patients with metastases detected by PSMA PET/CT, a high initial PSA (median 98.5 μg/l, mean 658 μg/l) of the study patients was considered as a strong indication of metastatic disease. However, while high PSA significantly increases the risk of having an advanced disease, there is no well-established PSA cut-off for metastatic prostate cancer (*245*). Thus, there is a possibility that some of the study patients had oligometastatic disease in favour of overall survival. Nevertheless, the percentage of patients experiencing disease progression was similar among patients with bone-only metastases (61%) compared to patients with bone and lymph node metastases (65%, including one patient who had lung metastases as well) at diagnosis.

When comparing baseline characteristics of the patients who were alive at the end of the follow-up to those who died of prostate cancer, the initial median PSA was 103.4 μg/l versus 79 μg/l, respectively. Out of the patients who were alive at the end of the follow-up, 21% had Gleason score 7 prostate cancer versus 0% out of the patients who died of prostate cancer. For Gleason score 8, the percentages were 32% versus 18%, and, for Gleason score 9 to 10, 47% versus 82%, respectively.

Previously, two randomised studies showed an increased risk of Gleason score 8 to 10 prostate cancer among users of finasteride or dutasteride compared to placebo (*246–248*). In study III, three patients received these 5α-reductase inhibitors for benign prostatic hyperplasia. While all three had metastatic disease with an extracapsular extension, one patient had Gleason score 8 and two had Gleason score 9 cancer, and, thus, there is a possibility that the prior use of 5α-reductase inhibitors could have increased the Gleason score of these patients.

In addition to treating the primary tumour of metastatic prostate cancer with radical doses of radiotherapy and, thus, prolonging the survival

of patients with newly diagnosed metastatic disease, the hypothesis in study III was to simultaneously reduce any possible later dissemination of the cancer as well as to prevent possible later urinary retention and complications due to local disease progression. In prostate cancer, the usual cause of total urinary obstruction is the local progression of the castration-resistant disease (*249*). Thus, traditionally, patients do not receive palliative radiotherapy until this phase. In study III, the objective was to provide radical radiotherapy at an earlier phase of metastatic cancer, aiming for the beginning of remission, which was usually achieved with androgen deprivation alone.

The median follow-up time in study III was 4.38 years. The overall survival at five years was 81.3%, and the median overall survival was 8.35 years. STAMPEDE reported a five-year survival of 50% with hormonal therapy plus docetaxel group compared to 39% with hormonal therapy alone group (median follow-up 43 months) (*13*). In CHARTEED, the overall survival was 58 months with the combination of androgen deprivation and docetaxel compared to 47 months with androgen deprivation alone (median follow-up 54 months) (*15*).

As stated in observational studies as well as in the randomised STAMPEDE trial, radical radiotherapy with androgen deprivation appears to improve survival in metastatic prostate cancer patients with low tumour load (*38*, *115*, *216*). Although this combination failed to improve survival in high-volume disease in STAMPEDE, it significantly improved the failure-free survival of these patients as well as the biochemical progression-free survival in unselected patients in HORRAD (*38*, *218*). In contrast to these trials, study III patients received even more aggressive therapy in the form of additional treatments to achieve PSA nadir before the initiation of radical radiotherapy and thus to facilitate more cancer cell death and a longer survival. These treatments included docetaxel, abiraterone, enzalutamide, cabazitaxel, and radium-223, all of which have significantly improved the survival of newly diagnosed metastatic prostate cancer when given in combination with androgen deprivation in randomised trials (*15*, *48*, *189*, *206*, *209*, *210*). In addition, to decrease the possible further dissemination of metastatic disease, study III patients received irradiation to lymph node metastases and bone oligometastases as well as radiopharmaceuticals in cases of widely disseminated bone metastases.

6.2.3 LIMITATIONS OF THE STUDY

Study III had several limitations. Firstly, it was a retrospective patient series. Secondly, the patients received their treatment within a long follow-up period and, thirdly, the total number of patients was small. Due to the above, this study was prone to selection bias.

Fourthly, there was no centralised pathology review included in the study, which generally increases the quality of a study by standardizing the histopathologic evaluation of prostatic biopsies. Furthermore, as prostate pathology is strongly associated with prostate cancer survival, consistent pathologic accuracy has also a significant effect on treatment selection.

Fifthly, many treatments used in study III were experimental and exposed the patients to various adverse events in a nonrandomised setting without certainty of any survival benefit. At the beginning of this study, the only well-established treatment option for newly diagnosed prostate cancer was androgen deprivation therapy. Also, the first promising results of docetaxel in the treatment of metastatic prostate cancer emerged at the end of the follow-up of study III. Thus, the rationale behind the experimental approaches used in study III was the drastic need for new treatment options for newly diagnosed hormone-sensitive metastatic prostate cancer when taking into account its poor prognosis. Treating an 86-year-old prostate cancer patient with radical radiation can be considered experimental. However, all study patients were evaluated fit enough to receive the given treatments and expected to benefit from them not only in terms of survival but also in terms of their quality of life, as metastatic disease is a source of significant morbidity. With regard to toxicity, the multimodal treatment, including the experimental treatments, was as expected and well-tolerated as stated above. Since the release of study III results, there has been an emergence of several new treatment options for newly diagnosed hormone-sensitive prostate cancer with a significant survival benefit, albeit the prognosis of metastatic disease remains poor.

Seventhly, although all patients received androgen deprivation therapy in combination with radical radiation, the confounding factor in this study was the number of various other treatments the patients received. Therefore, no reliable conclusions can be drawn regarding the study outcomes. However, considering the excellent overall survival, an aggressive treatment approach in newly diagnosed metastatic prostate cancer seems appealing and deserves to be studied in a randomised setting.

6.3 STUDY IV

6.3.1 EFFICACY

In study IV, adjuvant radiotherapy following radical prostatectomy significantly prolonged the biochemical recurrence-free survival in pT2 patients with positive margins and in pT3 patients with or without positive margins compared to surgery alone. This finding supports earlier randomised trials (*117–119*). In study IV, there was no difference between the groups in terms of overall and metastatic-free survival. Out of these four randomised trials, only SWOG found significant improvement in terms of overall and metastatic-free survival in the adjuvant radiotherapy group.

Compared to study IV, the patients in the three previous randomised studies had predominantly higher risk features. All three included mainly ≥ pT3 cancer patients as well as patients with seminal vesicle invasion and postoperative PSA ≥ 0.2 μg/l. In study IV, pT2N0M0 with positive margins or pT3N0M0 (regardless of margins) prostate cancer was an optimal subject for adjuvant radiotherapy, as the patients had some adverse pathologic features (positive margins, extracapsular extension) increasing the risk of disease progression, but not risk features commonly treated as an 'automatic' indication for adjuvant radiotherapy. For example, most of the patients with seminal vesicle invasion experience biochemical recurrence following radical prostatectomy and therefore receive adjuvant therapies (*250*).

In the case of detectable postoperative PSA (≥ 0.2 μg/l), the primary radical treatment is generally deemed to be non-curative, and, thus, radiation after surgery should be considered a salvage treatment. In study IV, one of the inclusion criteria was postoperative PSA < 0.5 μg/l, as this was the common threshold at the initiation of the trial, although, according to current standards, the threshold should be < 0.2 μg/l. Nevertheless, in study IV, 46% of the patients in the adjuvant group and 52% in the observation group had preoperative PSA < 0.2 μg/l.

Instead of low-risk localised prostate cancer, for which surgery or radiation are well-established as a monotherapy, the study IV patients had a disease progressed to the border of the surgically removed prostate or through the prostate gland yet not to the lymph nodes or further. Thus, hypothetically, their cancer was still in an optimal phase to achieve cure with the primary treatment. Compared to SWOG, EORTC, and ARO, most of the patients' prostate cancer in study IV was lower-risk; yet irradiation significantly improved the biochemical recurrence-free survival—even in patients with Gleason score < 7.

6.3.2 ADJUVANT VERSUS SALVAGE RADIOTHERAPY

While the results of randomised trials comparing adjuvant and salvage radiation following radical prostatectomy (RADICALS, RAVES, GETUG-17) are awaited, retrospective studies report controversial results. In some studies, adjuvant radiotherapy appears superior to salvage radiation in terms of biochemical relapse-free survival, freedom from distant metastases, and overall survival (*127*, *128*, *251*). In other studies, adjuvant and salvage radiation appear equally effective and, thus, these studies tend to support salvage radiation due to the increased amount of adverse effects in adjuvant radiation and a fear of overtreatment (*125*, *126*, *252*). In these retrospective studies, patients had adverse pathologic features, such as ≥ pT3, positive margins, and seminal vesicle invasion (*127*, *128*, *251*).

The timing of salvage radiation is another matter of debate. In observational data, early irradiation with PSA levels ≤ 0.5 μg/l appears superior to late irradiation with PSA levels > 0.5 μg/l (*253*). In patients with more aggressive disease, early salvage radiation has resulted in better cancer control when compared to patients with less adverse pathologic features (*254*).

In study IV, 121 patients received adjuvant radiotherapy (five declined radiation after randomisation), of which 106 remained progression-free. In contrast, out of the 124 patients randomised in the observation group, 37 received salvage radiation for protocol-defined progression, out of which 28 achieved PSA remission. In addition, nine patients from the observation group received salvage radiation for a progression not defined in the protocol, with a median PSA of 0.4 μg/l (range 0.12–0.53 μg/l), after which eight achieved PSA remission.

6.3.3 TOLERABILITY

As expected, in study IV, patients in the adjuvant group experienced significantly more adverse events compared to patients in the observation group. Although most of these were grade 1–2 and transient, 56% of the patients in the adjuvant group experienced grade 3 events, compared to 40% in the observation group. In addition, it is notable that grade 3 urethral stricture was more than three times as common (12/126 vs. 3/124 patients), and urinary incontinence more than twice (15/126 vs. 6/124) as common in the adjuvant group compared to the observation group.

The findings were similar regarding patient-reported urinary and intestinal toxicities (LENT-SOMA), although the patients reported more grade 3-4 LENT-SOMA toxicities compared to CTCAE (Common Terminology Criteria for Adverse Events version 4.03) events (*231*). When comparing the toxicity gradings used in this study, LENT-SOMA showed a pattern of higher grades compared to CTCAE. For instance, urinary incontinence CTCAE grade 3 corresponds to LENT-SOMA grade 4, and urinary frequency CTCAE grade 2 corresponds to LENT-SOMA grade 4. The

results are in line with previous studies, in which patient-reported outcomes appear more severe when compared to adverse events recorded by physicians and nurses (*255*). Of note, the delivery of radiotherapy has constantly improved in its precision, allowing for speculation that, with modern techniques, treatment-related toxicity would be even lower.

Table 3. Study designs and main results of studies included in this dissertation. The toxicity was unacceptable in study I, and acceptable in studies II-IV.

Study	Study design (number of patients)	Adverse pathologic feature/ risk factor associated with prostate cancer recurrence and/or mortality	Stage of prostate cancer	Active treatment studied	Main result
I	Phase I/II non-randomised trial (42)	EGFR expression	cT2-3N0M0	Gefitinib in combination with radical radiotherapy	Modest efficacy (good PSA relapse-free and overall survival)
II	Phase II non-randomised trial (30)	Biochemical recurrence following radical prostatectomy or radical radiotherapy, EGFR expression	Non-metastatic cancer	Gefitinib monotherapy	Modest efficacy (in terms of increased PSA doubling time)
III	Retrospective patient series (46)	Metastatic prostate cancer	Newly diagnosed metastatic cancer	Multimodal treatment including androgen deprivation and radical radiotherapy	Excellent overall survival
IV	Randomised trial (250)	Positive surgical margins or extracapsular extension	pT2N0M0 with positive margins or pT3N0M0 with/ without positive margins	Adjuvant radiotherapy following radical prostatectomy compared to radical prostatectomy alone	Significant improvement in biochemical recurrence-free survival

7 SUMMARY

7.1 RISK FACTORS IN STUDIES I-IV

Risk stratification varies between different studies and guidelines, although it continues to be based on the three main established prognostic factors: TNM-stage, Gleason score/ISUP grade group, and initial PSA. EGFR overexpression (studies I and II) is associated with poor prognosis; however, it is not an independent prognostic factor in prostate cancer (*60*). Metastatic disease (study III), on the other hand, decreases the overall survival of newly diagnosed patients significantly (*12*). Locally advanced prostate cancer (extracapsular extension, study IV) is a significant risk factor for biochemical recurrence, metastatic disease, and prostate cancer death (*25*, *26*). Finally, while decision-making should not be based solely on other adverse pathologic features, such as positive margins (study IV), they can add important value when choosing an optimal treatment.

7.2 CONCLUSIONS REGARDING ACTIVE TREATMENT OPTIONS

Studies I and II

Regardless of the appealing concept behind EGFR tyrosine kinase inhibition, promising results from preclinical prostate cancer studies, and efficacy in non-small cell lung cancer, gefitinib showed only modest efficacy as both a first-line treatment in combination with radiotherapy (study I) and as a monotherapy upon biochemical recurrence following local treatment (study II) in nonmetastatic prostate cancer patients. Gefitinib had acceptable toxicity as a monotherapy. In combination with radiation, the toxicity was unacceptable when taking into account that most patients had low-risk disease with an excellent prognosis even without any active treatments, yet one third experienced grade 3-4 adverse event. To date, study I appears to be the only clinical trial of gefitinib in combination with radiotherapy in the context of prostate cancer, thus providing notable insight into the safety and activity of this combination. Potentially, in the future, a better understanding of the complexity of the EGFR family, its pathophysiology, and the downstream signalling pathways will also result in new treatment combinations with gefitinib.

Study III

In study III, all newly diagnosed metastatic prostate cancer patients received androgen deprivation followed by radical radiotherapy. In addition, several other, individually chosen treatments including hormonal therapy, chemotherapy, radiopharmaceuticals, and experimental approaches, complemented this active treatment approach. The multimodal treatment strived for aggressive therapy from the beginning of the diagnosis and led to good efficacy as well as safety results. Despite being a retrospective patient series, the excellent survival data encourages further research in the form of randomised trials.

Study IV

In study IV, adjuvant radiotherapy following radical prostatectomy caused more adverse events compared to surgery alone; yet, it was generally well-tolerated and significantly prolonged the biochemical progression-free survival in pT2N0M0 prostate cancer with positive surgical margins and in pT3N0M0 cancer regardless of margins. In terms of overall survival, adjuvant and salvage radiation appeared equally effective. While metastatic and castration-resistant disease occurred more frequently in the observation group, there were no statistically significant difference between the treatments.

8 FUTURE PERSPECTIVES

All study patients in this dissertation received local therapies. To date, the role of radical prostatectomy and radical radiotherapy as a cornerstone of nonmetastatic prostate cancer treatment appears strong regardless of due to the discovery of several novel systemic treatment options. In addition to radical treatments, active surveillance appears to be a well-established treatment option for localised prostate cancer, especially in low-risk cases (*4*, *93*, *256*). Patients who have intermediate- to high-risk yet nonmetastatic disease benefit from local therapies, commonly complemented with adjuvant treatments (*28*, *109*). Not until recently, did local therapies also show promising efficacy in metastatic prostate cancer (*38*, *218*, *221*).

One concern regarding local therapies is the several long-term side effects they can cause. Also, patients have to be physically fit enough to receive these treatments. While there has been an emergence of various new, systemic prostate cancer treatments during the past decades, at the same time, the development of local therapies has been constant. Modern radiotherapy techniques allow for higher and more accurate radiation volumes administered to tumours while simultaneously sparing the healthy tissues from unnecessary irradiation. One example is the golden fiducial markers placed in the prostate gland to ensure accurate targeting of the tumour and thus minimize the radiation of surrounding tissues (*257*). With regard to surgery techniques, robot-assisted radical prostatectomy appears to provide the best outcome in terms of negative margins as well as decreased toxicity compared to open or laparoscopic surgery (*104*, *258*).

Due to the excellent survival rate of low-risk localised prostate cancer, its current research focuses on reducing the toxicity of current treatment options and finding new focal therapies in order to preserve good cancer control with the minimised exposure of healthy tissues (*259*). At the same time, research on metastatic prostate cancer concentrates on aggressive therapies and multimodal treatments, with the aim of increasing the current poor survival of these patients. What lies between low-risk localised and metastatic prostate cancer is a heterogeneous group of prostate cancer patients with varying risk-profiles and prognosis. These patients have adverse pathologic features or risk factors that are associated with disease progression and/or mortality. Consequently, there is often a lack of consensus regarding optimal treatment as well.

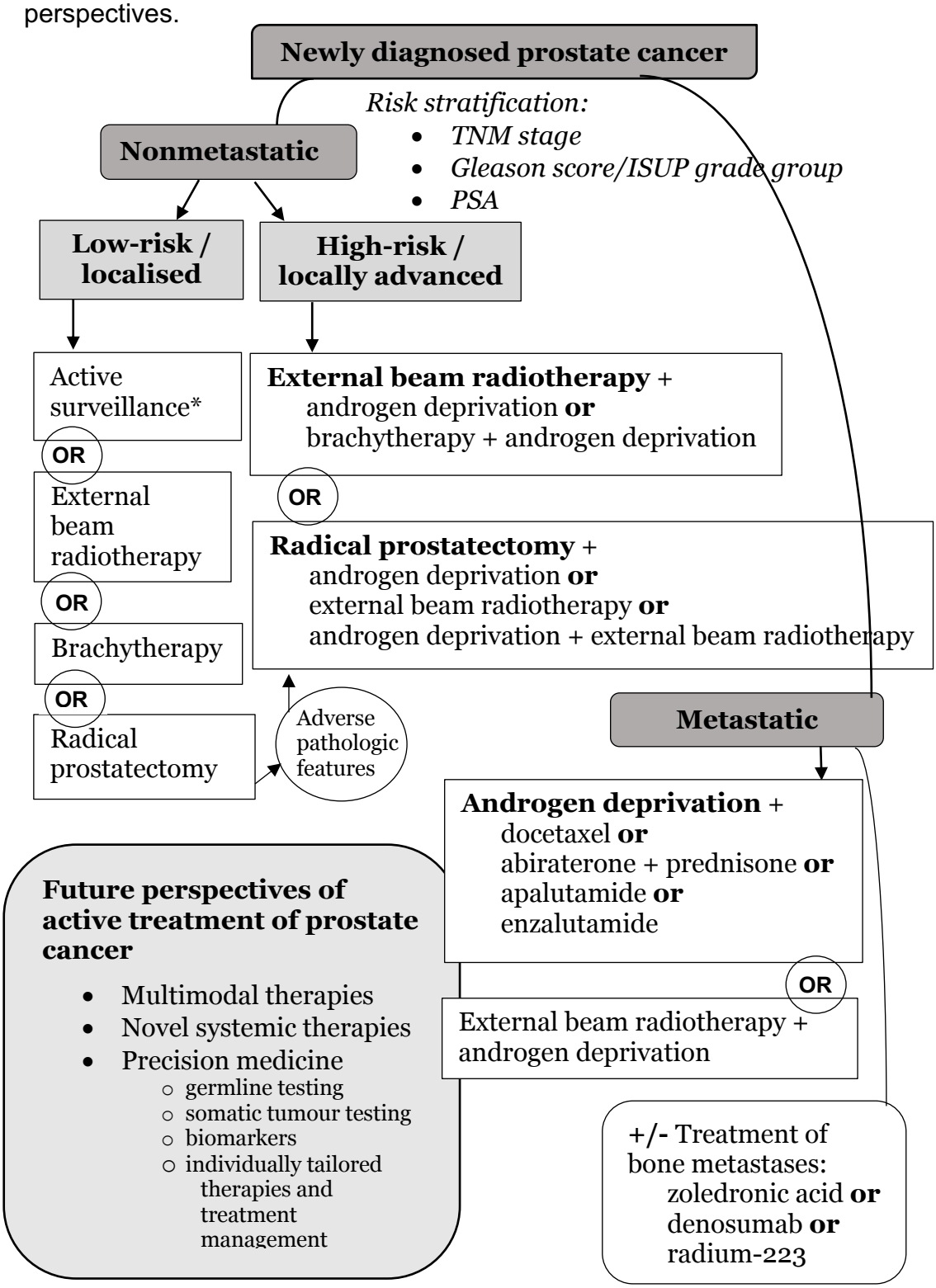
One of the aims of future cancer research is to achieve accurate risk-profiling of prostate cancer patients. Heredity and germline mutations contribute to the aggressiveness of prostate cancer; thus, genomic profiling can be recommended for patients with suspicious histology or family history, or those with known high-risk germline mutations in the family (*260–263*). The further genetic profiling of germline variants may enable more accurate prognostic evaluation and guide treatment decisions in all prostate cancer patients in the future (*264*, *265*).

Increased understanding of genetic variations and their signalling pathways in the progression of prostate cancer as well as the

identification of new genetic mutations will help provide targets for novel therapies (*266*). One of the most promising approaches to prostate cancer treatment is immunotherapy; however, it appears to show efficacy only in a proportion of patients (*267*). Enhancing knowledge of germline and somatic alterations and their impact on the development of prostate cancer into a lethal disease enables specific, treatment-dependent patient selection (*266*, *268*). This kind of treatment planning, based on patient's individual tumour characteristics, aims to improved cancer control among those who are expected to respond to the treatment and spares patients with no specified genetic alterations from overtreatment (*265*). Although the association between prostate cancer and EGFR mutations remains unclear, one example of personalised treatment is EGFR-targeted therapies, which serve as a first-line treatment option for non-small-cell lung cancer patients with detected EGFR mutations (*269*).

In conclusion, future active prostate cancer treatment will include local as well as systemic therapies, often complementing each other. The development of response and surrogate biomarkers has the potential to guide treatment decisions as well as post-treatment management (*270–273*). In addition, increasing understanding of the molecular biology of prostate cancer provides a pivotal platform for the ongoing development of novel therapies. Consequently, future treatment planning will be able to be based on individual disease characteristics and their known responsiveness to available treatments. Finally, the constant development of active prostate cancer treatment options enables decreasing treatment toxicity, better cancer control, and, hopefully, more curative options—even for patients with high-risk or advanced disease.

Figure 5. Current status and future perspectives of prostate cancer treatment. Simplified algorithm for choosing the treatment for newly diagnosed prostate cancer based on current research, and future research perspectives.



*In the case of Gleason score ≤ 6. Consider in the case of Gleason score 3+4 and ≤ T2.

ISUP = The International Society of Urological Pathology; PSA = prostate-specific antigen; TNM = tumour, node, metastasis

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Greetta Hackman

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