Effect of multipeptide vaccination therapy on disease outcome of patients with prostate cancer and biochemical recurrence

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Para mis padres, Anabela y Rafael (21.12.1984-18.10.1996)

No llores si me amas.
¡Si conocieras el don de Dios y lo que es el cielo!
Si pudieras oír el cántico de los ángeles
y verme en medio de ellos…

San Agustín

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Abbreviations

3D-CRT	three-dimensional conformal radiotherapy
ADT	androgen deprivation therapy
APCs	antigen presenting cells
approx.	approximately
BMI	body-mass-index
CD4+	cluster of differentiation 4 positive
CD8+	cluster of differentiation 8 positive
CRPC	castration resistant prostate cancer
CSPC	castration sensitive prostate cancer
CT	computed tomography
CTLA4	cytotoxic T-lymphocyte associated protein 4
CYP17	cytochrome P450 17
DCs	dendritic cells
DNA	deoxyribonucleic acid
DRE	digital rectal examination
EBRT	external beam radiotherapy
e.g.	exempli gratia (for example)
EMP	estramustine phosphate
FDA	Food and Drug Administration
FDG	fluordesoxyglucose
GM-CSF	granulocyte macrophage colony-stimulating factor
GS	Gleason Score
Gy	Gray
HDR	high dose radiation
HLA	human leukocyte antigen
IFN-γ	interferon gamma
IMRT	intensity-modulated external beam radiotherapy
LDR	low dose radiation
LHRH	luteinizing hormone-releasing hormone
MDSCs	myeloid-derived suppressor cells
MHC	major histocompatibility complex

MRI	magnetic resonance imaging
n.a.	not applicable
OS	overall survival
PAP	prostatic acid phosphatase
PC	prostate cancer
PCSA	prostate stem cell antigen
PD-1	programmed cell death protein 1
PET-CT	positron emission tomography–computed tomography
PFS	progression-free survival
PPV	personalized peptide vaccine
PSA	prostate specific antigen
PSMA	prostate specific membrane antigen
RCC	renal cell carcinoma
RNA	ribonucleic acid
RP	radical prostatectomy
STEAP1	six transmembrane epithelial antigen of the prostate 1
TAA	tumor associated antigen
TGF-ß1	transforming growth factor beta 1
TNF	tumor necrosis factor
TRP-P8	transient receptor potential p8
TRUS	transrectal ultrasound
TTP	time to progression
VEGF	vascular endothelial growth factor
vs.	versus

Introduction

1. Prostate cancer

1.1 Epidemiology

The medical community has increased the amount of attention paid to prostate cancer in recent decades. Due to its socioeconomic impact and the demographical development in industrial countries, the subject has gained much importance and interest in researching new therapies for this disease is rising. Prostate cancer is the leading tumor entity among men in Germany and the third most common cause of cancer related death. The incidence in Germany was 65 800 cases in 2010, for 2014 it has been calculated to be approximately 70 100 [1]. It is the second most common cause of cancer related death among men in the United States of America. In Europe it constitutes 11% of all cancer cases in men [2], with an estimated 2,6 million patients diagnosed yearly. The mean age for diagnosis is 69 years, which is also the mean age for cancer diagnosis in Germany [1].

The 5-year-survival rate for the disease has improved considerably in the last 35 years. Men diagnosed at the beginning of the 1980s had a five-year-survival rate of 70%, whereas men who were diagnosed between 2000 and 2004 had a five-year-survival-rate of 87% [3]. This improvement is most likely explained by the preventive measures for detecting the disease and as a result the now much earlier time of diagnosis.

1.2 Histology

Prostate cancer is in 95% of the cases an adenocarcinoma [4], which sprouts from the epithelial cells that make up the glandules. Non adenocarcinomas generally have their origin in the transitional epithelial cells from urothelium, the tissue that covers the inside of the urethra and the bladder. Other tumor types

like lymphoma, neuroendocrine tumors and sarcoma develop very rarely in the prostate [5].

1.3 Risk factors

Among the most important risk factors for prostate cancer is age. Men under the age of 40 rarely develop this tumor [6]. The hereditary factor also plays an important role. Provided a first-line relative suffers from the disease, the risk for the person in question to become sick is at least doubled. When two or more first-line relatives develop the disease, the risk rises to 5-11 times more than a person with no familial history of prostate cancer [7]. Other factors such as taller height, smoking history, high caloric diet and higher BMI elevate the risk for a fatal prostate cancer. On the other hand, high physical activity levels correlate with lower prostate cancer risk [8].

1.4 Diagnosis

1.4.1 Screening

PSA level measures and digital rectal examination (DRE) are established screening methods for prostate cancer in clinical practice. The goal of early detection measurements is to detect the disease in a local stage, since this is the only way to treat the patient with a curative approach. In Germany, men from the age of 40 and below 70 are advised to submit themselves to this screening [9]. Although it seems reasonable to perform these tests routinely it is important to point out that none of them have shown to reduce mortality from prostate cancer [10].

1.4.1.1 Prostate specific antigen

The prostate-specific antigen (PSA, also known as kallikrein-3 (KLK3) and gamma-seminoprotein) is a serine peptidase enzyme secreted almost exclusively by the epithelial cells of the prostate, and can be measured in low

quantities in healthy male serum. Its physiological function is to liquefy the semen. PSA is an organ-specific but not tumor specific marker; other processes in the prostate for example a prostatitis, prostate adenoma or rectal manipulation of the prostate in a DRE can elevate PSA levels. Therapies with steroids or 5-alpha-reductase inhibitors can create false low PSA levels.

A PSA value higher than 4 ng/ml is considered suspicious and requires a further test for confirmation before proceeding with more diagnostics. The probability of diagnosing prostate cancer through an elevated PSA level depends on how much this value has risen in the patient's serum. The positive predictive value of PSA measurements for prostate cancer is of approx. 10% at a PSA level of 0.6-1.0 ng/ml, of 27% at a PSA level of 3.1-4.0 ng/ml and 41% for a PSA level of 4.0-10.0 ng/ml [11]. Though very rare, it is worth mentioning that there are certain prostate cancers that do not present a PSA elevation and can therefore pass unnoticed when merely assessing the PSA blood level. The Prostate Cancer Prevention Trial from the National Cancer Institute showed that 15% of patients who presented normal PSA levels indeed had prostate cancer [12].

The majority of PSA is bound to serum-proteins, the so called total PSA. The fraction of PSA unbound is called free PSA. The ratio of free PSA to total PSA is important for evaluating the risk of the presence of prostate cancer. In the case of a free PSA/total PSA ratio of less than 20%, the probability of a tumor is elevated. This has proven to be useful to assess the risk for cancer when PSA levels are elevated in a range between 4-10 ng/ml: a study showed that 56% of men who had PSA levels between 4 and 10 ng/ml and a free PSA/total PSA ratio of > 0.1 were diagnosed with prostate cancer after submitting themselves to ultrasound-guided biopsies, whereas only 8% of the men with PSA levels in the same range and a ratio of > 0.25 were diagnosed with the disease [13]. How PSA levels behave is also interesting for predicting the development of the disease. Men who had a PSA level rise of more than 2,0 ng/ml during the year before the disease was diagnosed present a higher risk of death caused by prostate cancer. Undergoing radical prostatectomy had no influence on the prognosis of these patients [14].

Nowadays routine PSA measures have led to the detection of several prostate cancer cases which otherwise would have possibly gone unnoticed. According to estimations of the Robert Koch Institute, approximately 90% of the prostate cancer diagnoses are achieved throughout elevated PSA levels, and 50% of these would have probably remained undiscovered if not for PSA screening [3]. Before PSA was used as a screening method, 33% of the patients at the time of diagnosis had already developed metastases. This number has shrunk now to 7%. The use of PSA as a screening method has caused some controversy though, given that it elevates the risk of overdiagnosis, and as a consequence overtreatment. It is estimated that between 23-42% of the prostate cancers detected via elevated PSA levels would have otherwise remained unnoticed throughout the patient's life [15]. Since prostate cancer regularly develops slowly and the patients get sick from it at advanced ages, many cases would probably not cause any symptoms and remain undiagnosed, and these patients would die from a different cause. With routine PSA screening, these patients get treatment and suffer from a variety of side effects, most commonly urinary incontinence and erectile dysfunction, causing unnecessary decline in life quality [16].

1.4.1.2 Digital rectal examination

The digital rectal examination is a cost-efficient, easy to conduct and quick way to inspect the prostate. Its size and consistency can be examined and the examination provides an overview of the surrounding tissue and organs. The part of the prostate that can be palpated is the peripheral lobe. Nearly three quarters of the tumors generate in this area. However, the sensitivity of DRE for detection of PC is limited and below 40% [17]. A normal palpation result does not guarantee a tumor absence, since a part of the prostate cannot be felt by the examiner, and the ability to perform the examination thoroughly and accurately varies among the medical community [18]. Nevertheless, DRE is an important part of the early detection process for prostate cancer and it definitely cannot be replaced by merely examining PSA levels.

1.4.2 Imaging

1.4.2.1 Transrectal ultrasound

Transrectal sonography (TRUS) has become a standard procedure for the detection of prostate cancer. The TRUS provides a morphologic overview of the prostate and the surrounding tissues. However, it is not a very sensitive procedure, since the probability of identifying a tumor lies between 17 and 57%, and 39% of the prostate tumors cannot be differentiated from regular gland tissue [19]. In combination with DRE and PSA though, it can provide significant information on the potential presence of a tumor and the tumor extent. TRUS is an important tool for tumor staging as it allows the examiner to assess to what extent the tumor has infiltrated the prostate capsule. It also plays an vital role for therapeutic indications such as brachytherapy and cryotherapy.

1.4.2.2 Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) has a good sensitivity for detection of PC ranging between 60-90% for detecting a tumor [20]. New techniques such as dynamic contrast-enhanced MRI suggest that its specificity for detecting PC ranges 88% [21], though false positive results can occur due to bleeding after TRUS guided biopsy or prostatitis [22]. The most common indication for using MRI is a continuing rise of PSA values in spite of a negative result in the TRUS guided biopsy. Studies show that about a third of the patients with one or more negative biopsy results were finally diagnosed with prostate cancer through MRI [23, 24]. For staging purposes, it is also very accurate in showing extra-capsular growth of the tumor or seminal vesicle invasion and enlarged abdominal or pelvic lymph nodes. In some cases MRI can also be used for performing a MRI guided biopsy [25].

1.4.2.3 Biopsy

Nowadays there are several different ways to perform a prostate biopsy. The standard way for a first time prostate histologic examination is the ultrasound guided biopsy [26], which can be performed through the perineum, transrectal

or through the urethra, transrectal being the most common approach. The principle behind it is to identify the organ with the ultrasound, take systematically 12 samples from different parts of the organ which are then examined under the microscope. A study examining different biopsy schemes and their tumor detection rates indicated that the 12-sample biopsy was better than the other schemes with a detection rate of 44,4% [27]. The TRUS guided biopsy does not detect every tumor, so a negative biopsy does not guarantee the absence of a tumor. It is estimated that approximately a quarter of the initial TRUS guided biopsies present a false negative result [28]. These patients usually undergo second or further biopsies when PSA values continue to rise. To improve these numbers other methods have been developed that could provide results. The MRI-guided biopsy presents the advantage that the tumors are more likely identified with MRI than TRUS, so the biopsy can be taken from suspicious parts of the gland instead of “blindly”. There appears to be a higher correlation between MRI guided biopsy and the definite pathology results, than TRUS guided biopsy [29]. Fusion MRI which combines MRI with TRUS guided biopsy also seems to have promising results showing a high accuracy at detecting prostate cancer [30].

1.4.3 Staging and risk stratification

1.4.3.1 Gleason Score

The Gleason Score (GS) [31] has become the standard classification to determine the grade of differentiation of prostate cancer tissue. Moreover, it is one of the main prognostic parameters for prostate cancer. The prostate tissue is evaluated microscopically assessing the morphology and architecture of the glandular structures. The pathologist assesses the two most common patterns in the tissue and the numbers are added, so that the GS ranges between 2 and 10 possible points [32]. The Gleason score’s predictive value only applies to the naïve, primary prostate cancer (before radiation or hormone therapy) and is only partially applicable for metastases or secondary tumors.

1.4.3.2 TNM classification

The TNM classification allows an objective assessment of the local and systemic extent of prostate cancer. It can be differentiated between a clinical stage (cT, cN) and a pathologic stage (pT, pN) depending on the method applied to determine the T and N-stage. According to the National Comprehensive Cancer Network (NCCN) [33], the TNM classification for prostate cancer reads as follows:

Table 1. TNM classification for prostate cancer according to the National Comprehensive Cancer Network (NCCN) clinical practice guidelines in Oncology [33].

T			Primary Tumor
	TX		Tumor cannot be assessed
	T0		No evidence of primary tumor
	T1		Clinically unapparent tumor not palpable or visible by imaging
		T1a	Tumor incidental histological finding in 5% or less of tissue resected
		T1b	Tumor incidental histological finding in more than 5% of tissue resected
		T1c	Tumor identified by needle biopsy (e.g. because of elevated prostate-specific level)
	T2		Tumor confined within the prostate
		T2a	Tumor involves one half of one lobe or less
		T2b	Tumor involves more than half of one lobe, but not both lobes
		T2c	Tumor involves both lobes
	T3		Tumor extends through the prostatic capsule
		T3a	Extracapsular extension (unilateral or bilateral) including microscopic bladder neck involvement
		T3b	Tumor invades seminal vesicle(s)
	T4		Tumor is fixed or invades adjacent structures other than seminal vesicles: external sphincter, rectum, levator muscles, and/or pelvic wall

N			Regional lymph nodes
	NX		Regional lymph nodes cannot be assessed
	N0		No regional lymph node metastasis
	N1		Regional lymph node metastasis
M			Distant metastasis
	MX		Distant metastasis cannot be assessed
	M0		No distant metastasis
	M1		Distant metastasis
		M1a	Non-regional lymph node(s)
		M1b	Bone(s)
		M1c	Other site(s)

1.4.3.3 Risk stratification according to D'Amico

The risk stratification of D'Amico [34] separates PC into three groups using PSA-value, Gleason score and cT-stadium. These values are predictors for the outcome of PC, and determine the risk of death from prostate cancer after radical prostatectomy [35].

Table 2. Risk stratification for prostate cancer according to D'Amico [34].

Risk	PSA level	Gleason score	T-stadium
Low risk	PSA < 10 ng/ml	GS ≤ 6	cT1-2a
Intermediate risk	PSA 10-20 ng/m	GS 7	cT2b
High risk	PSA > 20 ng/ml	GS > 7	cT2c-3a

1.4.4 Imaging Methods for clinical staging

1.4.4.1 Bone scan

The bone scan is used for staging purposes in prostate cancer because bone metastases are most common for this particular tumor. This scan has a very high sensitivity and negative predictive value [36], but not a very good specificity. A meta-analysis performed by Abuzallouf et al. suggests that the diagnostic value of the bone scan greatly correlates with the PSA level [37]. Apparently its diagnostic value is also highly dependent on the Gleason Score and the clinical stage of the disease [38]. Thus, according to the literature, a bone scan should not be performed on asymptomatic patients if they do not meet following criteria: GS of minimum 8 or clinical stage above T3, PSA level >10 ng/ml [39] or even >20 ng/ml [40]. Nevertheless the presence of symptoms (such as pain or pathological fractures) is a definite indication for a screening via bone scan.

1.4.4.2 MRI

See 1.4.2.2 above.

1.4.4.3 Computer tomography and positron emission tomography–computed tomography scan

Both computer tomography (CT-scan) and positron emission tomography–computed tomography scan (PET-CT) play a secondary role for the initial diagnosis and staging of prostate cancer. Data support that in case of a PSA lower than 20 ng/ml and a Gleason score of less than 7, it is rare to identify any pathologies in the prostate using computed tomography. Therefore, CT is not recommended as a standard imaging method in the staging of clinically localized prostate cancer. Nowadays, hybrid imaging methods such as PET-CT are gaining increasing importance as they allow both a morphologic and a functional assessment. PET-CT presents a promising possibility to track down metastases. However, the majority of PET-CTs are performed in order to detect potential local tumor recurrences [41]. The several tracers used, for example

FDG (F-18-fluorodeoxyglucose) and 11-C-Choline[42] present different but promising results for improving the care of patients with PSA recurrence.

1.5 Therapy

1.5.1 Therapy of localized prostate cancer

1.5.1.1 “Active surveillance”

As mentioned before, the incidence of prostate cancer has risen mostly due to PSA screening, revealing prostate cancers that in many cases are overtreated. According to a study by Godtman et al. approximately 45% of the patients diagnosed with prostate cancer through PSA screening apply as candidates for conservative therapy [43]. The concept of active surveillance was developed in to prevent this excessive treatment and loss of life quality, but still remains a curative approach. Applicable only for men with low risk disease (according to D’Amico et al. [34, 44]), it involves sequential serum PSA assessments and clinical assessments (DRE). One year after diagnosis, patients undergo a repeat biopsy to detect any changes in tumor differentiation or extent. In case of a tumor progression, the patients are mostly advised to undergo curative treatment such as prostatectomy or radiotherapy. Current studies show that within 5 years of active surveillance, less than 50% of patients require definitive radical treatment [45].

1.5.1.2 Brachytherapy

LDR-brachytherapy is a procedure to implant a radioactive seed into the prostate through the perineum under ultrasound control. The dosage can be accurately calculated so that only the prostate gland receives radiation, and the rectum and urethra remain unaffected. Until now, this type of therapy has been only recommended for low risk prostate cancers (a PSA of under 10 ng/dl and a Gleason score of under 7 are mostly required as eligibility criteria) [46, 47], though some studies suggest that the outcome is equally effective for patients with a moderate or high risk prostate cancer. The LDR-brachytherapy shows a

recurrence-free survival of 71-93% after 5 years, and 65-85% after 10 years [48-50]. It is difficult to interpret these results since there are no randomized studies comparing LDR-brachytherapy with other treatment options for localized prostate cancer.

The high dose rate brachytherapy (HDR-brachytherapy) or non-permanent transperineal interstitial prostate brachytherapy is another option for the treatment of localized prostate cancer. After external radiation with a dose between 50-60 Gy, the patient receives extra radiation directly in the prostate using a needle with a highly radioactive nuclide, thus achieving a total radiation dose of 100-130 Gy. According to a trial by Hoskin et al. the combination of HDR-brachytherapy and external beam radiation therapy (EBRT) shows a significant improvement in the quality of life and the recurrence free survival compared to EBRT alone [51]. However, only a small proportion of patients with prostate cancer is treated by HDR brachytherapy.

1.5.1.3 Radical prostatectomy

Radical prostatectomy (RP) is considered the gold standard curative treatment for localized prostate cancer for patients with a life expectancy of at least 10 years. A radical prostatectomy can be performed through several ways: retropubic, perineal, laparoscopic and robot-assisted laparoscopic. Regardless of the approach, the procedure aims to remove the whole organ, the two seminal vesicles and surrounding tissue (often also with a bilateral pelvic lymph node dissection). It remains the only form of therapy that has proven to reduce the rate of death from prostate cancer compared to conservative treatment, according to Bill-Axelson et al. [52-54]. However, there is no clear evidence that it provides improved oncologic outcome compared to radiotherapy. The two most common complications of RP are incontinence and impotence. Data suggest that if performed correctly, 12 months post-surgery the mean continence rate is 80-97% [55] with a mean potency recovery rate of 26-63% [56]. It is still unclear whether robotic-assisted prostatectomy provides an advantage over open prostatectomy regarding functional outcome. Small cohort trials propose that robotic prostatectomy performs better with regards of blood

loss and hospitalization time [57]. According to a study by Bianco et al. though, RP does seem to improve patients' prognosis: a study analyzed the data of 1045 patients who had undergone radical prostatectomy and found that the 10-year survival rate for cancer-specific survival was of 92%, for metastasis-free-survival of 91% and for PSA progression-free-survival of 75% [58].

1.5.1.4 External radiotherapy

Radiotherapy is a curative treatment option for prostate cancer, though benefits regarding overall survival or decrease in mortality from prostate cancer have not yet been proven. Although there are no randomized studies, many data suggest that radiotherapy is equally effective as radical prostatectomy for treating PC and that the quality of life after treatment is also better after radiation than after surgery [59]. Several techniques, such as three-dimensional conformal radiotherapy (3D-CRT) and intensity-modulated external beam radiotherapy (IMRT), have been developed in order to minimize radiation of the prostate surrounding tissue, preventing the numerous gastrointestinal and genitourinary complications of this treatment. A meta-analysis comparing radical prostatectomy with several radiation forms showed that men who submitted themselves to radiation only in comparison to RP presented less erectile dysfunction problems [60]. Radiotherapy presents a good alternative for patients who do not wish to undergo surgery, or in cases where any contraindications for the operation exist.

1.5.2 Therapy of hormone sensitive prostate cancer

1.5.2.1 Hormonal therapy

Testosterone and other androgenic substances physiologically stimulate the growth and proliferation of prostate cells. In the presence of PC, testosterone has the same effect on tumorous cells. The majority of this hormone is produced in the testicles, a small amount in the adrenal gland. The aim of hormone therapy is to stop the stimulation of androgens on the malignant cells since without testosterone tumor cells undergo apoptosis. There are several

ways to accomplish this, but in general they can be summarized under the concept of androgen depriving therapy (ADT). Androgen deprivation can be performed by either inhibiting the secretion of the hormones from the testicles (surgical orchiectomy, LHRH-agonists, LHRH-antagonists), or preventing the hormones from docking on their respective receptors (steroidal- and non-steroidal-antiandrogens). The combination of both strategies is known as total androgen blockade.

The decision to begin with ADT must be thoroughly weighed and made cautiously. Until the present day no studies have been able to prove that ADT does indeed improve cancer specific survival [61]. The lack of evidence must be taken into account since ADT causes many adverse effects that have an impact on the patients' quality of life [62]. The different treatment plans have diverse side effects, but the most common are: hot flashes, erectile dysfunction and loss of libido [63]. Other side effects include cardiovascular diseases, metabolic syndrome, impaired glucose tolerance and accelerated loss of bone mineral density. There is also no significant gain in overall survival if ADT is begun at a point where metastases are asymptomatic in comparison to commencing when metastases are symptomatic [64]. Since there is no optimal timing to begin with ADT, the indication to proceed should be stated on an individual basis for each patient. There are different opinions as to what kind of scheme should be used as first-line therapy. Bilateral orchiectomy is under local anesthesia a relatively simple to perform procedure that quickly achieves androgen deprivation [65]. Many men do not feel comfortable with it though, so most patients prefer chemical castration. LHRH (luteinizing hormone-releasing hormone) agonists are currently the most frequently used for ADT. They present a great advantage which is that they can be applied as depot injections on a monthly up to yearly basis. Both orchiectomy and LHRH agonists are adequate first line treatments at the beginning of hormone therapy [64, 66]. LHRH antagonists such as Abarelix and Degarelix successfully achieve chemical castration, though they are less used than LHRH agonists. Non-steroidal antiandrogens (Bicalutamide, Flutamide, Nilutamide) do not suppress testosterone levels, so sexual

dysfunction and bone problems do not seem to be a trouble [67] compared to the above mentioned castration therapies and steroidal antiandrogens. The concept of complete androgen blockade includes both the inhibition of systemic testosterone by LHRH-agonists and the blockade of the androgen receptor by a steroidal or non-steroidal androgen-receptor-inhibitor (such as bicalutamide). The survival benefit achieved by this strategy is low and therefore it is not recommended as standard-of-care [68]. However, to prevent a flare phenomenon, most patients receive complete androgen blockade during the first weeks of androgen deprivation therapy [69].

1.5.2.2 Progression into castration resistant prostate cancer

ADT, although effective for prolonging PSA relapse free survival and delaying complications such as ureteral obstruction or skeletal related events, is not a curative treatment, and disease progression will happen after a certain amount of time. Estimations suggest that first line therapy in form of orchiectomy, LHRH agonists or antagonists, or antiandrogens has a response rate of a 100% and a mean response duration of 36-48 months [70]. Eventually, the effect of this therapy wears off, and secondary hormone manipulation strategies can be recommended. One option in these patiens is a maximal androgen blockade, which has a response rate of 60-80% and a response duration of approximately 6-8 months [71]. When a maximal androgen blockade ceases to work, the subsequent form of therapy varies from patient to patient since multiple facts should be taken into consideration (from the will of the patient, to symptoms and stage of the disease). The pathophysiology behind the progression from hormone sensitive into castration resistant PC has not been entirely clarified, though there are some approaches that could explain this development such as ligand independent activation of the androgen receptor [72, 73].

1.5.3 Therapy of castration resistant prostate cancer

Castration resistant prostate cancer (CRPC) is defined by a radiologic progression (new bone or soft tissue metastatic lesions) or multiple subsequent

increases of the PSA despite a testosterone level lower than 50 ng/dL [74]. The main endpoint in most phase III trials assessing new therapies for CRPC is overall survival. Improvement in quality of life, although not as easily measured, is also important for palliative treatment of PC.

1.5.3.1 Docetaxel and cabazitaxel chemotherapy

Docetaxel is currently the standard therapy for CRPC. It shows a moderate prolongation in overall survival of approximately 2-2,5 months against mitroxantone+prednisone [75, 76]. The most beneficial application seems to be docetaxel combined with prednisone every three weeks. Indications for a docetaxel chemotherapy are mostly tumor related pain and a rapid PSA-doubling time (< 3 months). This therapy seems more profitable for patients with a good Karnofsky-Index and no visceral metastases [77]. Adverse effects include neutropenia, skin conditions such as exanthema and nail malformations, neuropathy, among others.

The TROPIC trial was a randomized phase III trial that compared cabazitaxel plus prednisone against mitoxantrone + prednisone. The men engaged in the trial were all CRPC patients who had suffered progression under docetaxel treatment. The primary endpoint was overall survival, and the cabazitaxel group showed a benefit of 2,4 months. The cabazitaxel group also showed an improvement for the secondary endpoints which were progression free survival, PSA response and treatment response [78]. This group also showed more adverse effects than the control group, especially hematological side effects.

1.5.3.2 Enzalutamide

Enzalutamide works by blocking nuclear translocation and transcription processes and androgen-binding receptors. In 2012 data from the AFFIRM study were published with promising results for enzalutamide. The trial enrolled 1199 patients with CRPC who had progressed after docetaxel therapy. Its primary endpoint was overall survival, where the enzalutamide showed a

significant benefit of 18,4 months over 13,6 from the placebo arm [79]. It also showed better results for the enzalutamide group regarding PSA value, quality of life, time to PSA progression and soft tissue response.
In patients with chemotherapy-naïve CRPC, Enzalutamide has been shown to decrease the risk of radiographic progression and death [80].

1.5.3.3 Abiraterone

Arbiraterone acetate is a CYP17 inhibitor and thereby blocks both intraadrenal, intratesticular and intraprostatic androgen production and is applied once a day in combination to prednisone. Similarly to enzalutamide, it showed promising results in a phase III trial (COU-AA-301 trial) where 1195 patients were randomly divided into an abiraterone and a placebo group. In this trial all patients also had a CRPC and had suffered progression during or after docetaxel treatment. The study showed an overall survival of 15,8 months for abiraterone versus 11,2 for the placebo group [81, 82] and better results for the secondary endpoints, same as the enzalutamide trial.

Moreover, Abiraterone has proven to prolong progression free survival and time to initiation of chemotherapy in patients who have not received chemotherapy so far. In this study, patients with asymptomatic castration resistant prostate cancer were randomized either to receive 1000 mg of Abiraterone + 10 mg Prednisone daily or Placebo + 10 mg Prednisone [83].

1.5.3.4 Radium-223

Radium-223 is a newly developed radioactive medication to treat bone metastases caused by different types of cancer. It is injected intravenously and is taken up by the bone, since its chemical structure is similar to that of calcium. It emits up to 96% alpha radiation, which has a very short range, reaching mostly cancer cells and sparing the surrounding tissue. This presents a great advantage compared to external radiation which is normally used to treat pain from bone metastases, but has limited tissue selectivity, has a high toxicity and

causes bone marrow suppression. This advantage can also be seen compared to other bone seeking medication such as strontium-89, which emits beta radiation which has a wider range than alpha radiation [84]. In phase II studies it was demonstrated that radium-223 has a positive effect on bone metastases-related pain and biomarkers (PSA and bone alkaline phosphatase) [85, 86]. Moreover, the ALSYMPCA trial, a phase III double-blind, randomized study conducted with 921 CRPC men showed an improved overall survival for the radium-223 group compared to the placebo group [87]. Conclusively, it can be said that radium-223 can be safely utilized for treating bone metastases in CRPC even in combination with the above mentioned therapies, since its mechanism of action does not interfere with these other treatments. Currently radium-223 combined with docetaxel is being tested on a phase I-II trial [88].

There are no clear recommendations which therapy is the most appropriate in CRPC patients with disease progression after docetaxel therapy. Enzalutamide, arbiraterone, cabazitaxel have showed improvement of overall survival in these patients, but to date there are no data available from randomized trials comparing different therapy sequences. Also, the costs of these new treatments are very high which presents an immense challenge for the public health system.

1.5.4 Follow-up after treatment of localized prostate cancer

Even though most patients with localized prostate cancer get treated with a curative intention, some will have a disease relapse. The goal of follow-up is to identify treatment complications or disease relapse in time to proceed with a second-line curative therapy or delay clinical disease progression. For this purpose, two important concepts have been developed: biochemical disease relapse and clinical disease relapse. For the first one PSA value samples are routinely assessed; for the second one, the DRE and a detailed symptom history of the patient are indispensable. Imaging diagnostics are not used routinely for follow-up of patients treated with a curative intention; generally,

imaging techniques should be used for patients with biochemical or symptomatic disease recurrence, but only for those for whom the imaging results will provide useful information as to how to proceed therapeutically.

The risk for therapy failure and consequentially disease recurrence is highest during the first years after therapy. This is why follow up should be conducted more thoroughly at the beginning: according to the European Association of Urology guidelines, after three, six and twelve months after RP, every six months during the following two years, and then yearly. Taking into consideration that the patients in our study all underwent RP, a focus will be set on follow-up treatment for patients after RP.

1.5.4.1 PSA progression and PSA monitoring

For patients after RP, PSA progression is defined as at least two consecutive PSA measurements > 0,2 ng/mL [89] . It has been suggested though that for patients with a higher risk of disease relapse, PSA progression should be defined as two measurements >0,4 ng/mL [90] . Six weeks after a successful RP, PSA levels are expected to sink until PSA is no longer detectable [91]. The persistence of elevated PSA values is considered as a sign of local remaining tumorous tissue, or micrometastasis. At this point, the behavior of PSA can also bring information as to where the source of PSA might be; in the case of local disease recurrence, PSA tends to rise late and slowly, whereas by distant metastases, PSA rises rapidly and the PSA doubling time is rather short [92]. Biochemical relapse usually precedes clinical relapse, sometimes even for years. Although, not all patients with biochemical relapse develop automatically a clinical relapse. According to Pound et al., 34% of 1997 evaluated patients who had had biochemical recurrence eventually developed clinical recurrence [93]. Boorjian et al. presented similar results after analyzing the data of 2400 patients, from which only 23% proceeded to develop a clinical recurrence after initial biochemical recurrence [94].

1.5.4.2 Clinical recurrence and monitoring

Besides PSA monitoring, regular DRE remains the most important examination in the follow up of patients after prostatectomy. A recurrence without PSA increase is most probable in patients with low differentiated tumors [95]. If a local recurrence is expected, transrectal sonography may be indicated as further examination.

When speaking of tumor recurrence, there is a difference between a local and systemic recurrence. As mentioned above, a PSA increase early after prostatectomy indicates systemic disease relapse. Moreover, patients with low differentiated tumors (e.g. Gleason 9 or 10) or nodal involvement have an increased risk of systemic recurrence [96]. Clinical symptoms such as obstructive voiding symptoms or hematuria are present only in a low proportion of patients with local relapse. In case of distant recurrence and bone metastases, pathologic fractures or bone pain often present the first symptoms of metastatic bone disease. If systemic recurrence is suspected, bone scan and whole body computed tomography are indicated. A study showed that a bone scan of a patient with PSA relapse has a probability of <5% to show pathological results related to tumor relapse if the PSA level is lower than 7 ng/ml [97]. Choline-PET-CT is indicated in patients who are candidates for local salvage therapy to visualize the local situation and rule out systemic recurrence. However, in patients with a PSA <4.0 ng/ml, the value of Choline-PET-CT is considered critically [98].

1.5.5 Treatment of biochemical recurrence

The rate of biochemical recurrence among men after RP varies depending on the different pathological risk factors (surgical margins, postoperative lymph node status, Gleason score, postoperative PSA levels and behavior) [99]. The numbers slightly differ: Han et al. analyzed data of men who submitted themselves to RP and found that the biochemical recurrence free survival was approx. of 84% after 5, 74% after 10 and 66% after 15 years [100]. Porter and

his group showed in a similar analysis a biochemical recurrence free survival of 85%, 61% and 55% at 5, 15 and 25 years past RP time [101]. There are three options for the management of a patient with PSA relapse: radiation, ADT and a “wait and see” approach (parting from the already discussed fact that not every PSA-relapse evolves into a clinical relapse). Currently, there are no studies comparing these therapies with regards to overall survival after biochemical relapse. Mehta and his group examined data of 303 patients who had had a RP and biochemical relapse and stated that 43% of these men continued to be treated with radiation and 57% with hormone therapy [102]. According to their study, a high PSA level and seminal vesicle invasion were criteria common to those men who received ADT.

Radiation after surgical therapy can be given to the patient as adjuvant treatment (radiation three months postoperatively disregarding PSA levels) or as salvage radiation therapy (after a confirmed biochemical relapse). Radiation therapy for biochemical recurrence seems to be most effective, when performed at a time when the PSA level is <0,5 ng/ml [103, 104]. If done correctly, salvage radiation therapy can give patients up to 80% chances of living the next 5 years without disease progression [105]. A retrospective study showed that patients who had undergone salvage radiotherapy had a three times higher prostate cancer related survival compared to men who did not receive such treatment [106]. There are no data suggesting that adjuvant or salvage radiation have a benefit for survival [107]. Therefore, the decision to follow one or the other must be made between physician and patient. One reason that could lead physicians to wait for PSA relapse to proceed with radiation is the side effects of radiation. Though, the incontinence rate amongst men after second line radiation does not differ significantly from men without radiation [108].

The effect of ADT after disease relapse has already been discussed previously (see chapter 1.5.2.1). Moul et al. showed with a retrospective analysis that the time when ADT is started (after biochemical relapse or after relapse plus metastasis) did not make any difference in the time to distant metastasis

development [109], nor did it have a positive impact on PC related mortality. The “watch and see” strategy is most likely to be successful for men who present a PSA doubling time >12 months [94], but the indication is not stated easily. It is also a possibility for men with a life expectancy lower than 10 years or those who do not want to submit themselves to salvage radiation.

2. Immune therapy for prostate cancer

2.1 Cancer immunology

2.1.1 The role of the immune system in tumor development

The immune system plays as a crucial role as a guard in tumorigenesis. The theory of “immunoediting” states that the process from the transformation of regular cells into tumorous cells and their further development into a tumor happens in three phases: elimination, equilibrium, and escape. The first phase called “elimination” or “immunosurveillance" is described as the immune system’s ability to differentiate between the body’s own and foreign tissue and also of recognizing the host's own cells that degenerate (through chemical, genetic, viral alterations or spontaneously) and destroying these [110, 111]. According to this theory, a properly functioning immune system is able to counteract the emersion of tumors by eliminating malignant cells, thus protecting the host. The emerging tumorous cells generate a proinflammatory response and this leads to tumor elimination. However, if the host’s response is not successful in eradicating the malignant cells, they enter an “equilibrium” phase. In this phase the cells begin changing their qualities and the ones which present most resistance against the unfriendly environment created from the host’s immune reaction keep proliferating. If a cell variant succeeds to develop immune evading mechanisms, the immune system gets overrun, the balance can no longer be held and the tumor becomes clinically apparent (“escape”) [112, 113].

The immune system's response against the emerging tumor can be explained using the concept of an "adaptive immune response" [114], which describes the following: in the presence of a tumor associated antigen (TAA), immature antigen presenting cells (APCs), most importantly dendritic cells (DCs), are activated. The antigen is taken up and after a conversion process it is expressed on the surface of mature DCs in the form of smaller peptides on their MHC I and II molecules. The activated DCs migrate to the lymph nodes where the peptides together co-stimulatory molecules (B7 ligands interact with the CD28 receptor on T cells) are presented to CD4 + cells and CD8 + cytotoxic T-lymphocytes. This is a very complex process though, since antigen presentation from DCs and recognition from T cells is not enough for them to become active, but additional signals are just as important as antigen presentation itself. If T cells are activated, CD4 + cells produce cytokines which contribute to full CD8 + maturation. These mature CD8 + cells infiltrate the tumor tissue, where the same MHC-peptide complex that activated them is expressed and induce a lysis [115]. Not only do they lyse tumorous cells on their source, but the activated CD8 + cells search the host for remaining cells to eliminate [116]. However, when T cell activation occurs inhibitory pathways are also activated. Such innate processes play an important role in the decay of immune responses after infections for example or preventing autoimmunity, but can inhibit or abort the immune response against tumors, letting these proliferate and grow [114].

Unfortunately, tumors possess strategies to escape from the immune system and inhibit an immune response [117]. One of the main mechanisms is the production and expression of certain molecules which restrict the functions of T cells [118]. These molecules are called "immune checkpoints" [119, 120], among which are ligands for known receptors which regulate the T-lymphocytes: the inhibitory programmed death-1 (PD-1) and the cytotoxic T-lymphocyte associated receptor 4 (CTLA4) [114]. Stimulation of these receptors limits T cells. Preclinical studies showed that the blocking of CTLA4-receptor enhanced T cells to perform their function properly [121]. One known evasion

mechanisms of tumors is that their malignant cells can prevent the maturation process of dendritic cells (DC). DCs are the main representatives of the T-cell-stimulating APCs and they block the secretion of costimulatory molecules needed for the activation of T cells. Moreover, the conversion process of the TAAs to smaller presentable peptides and the expression of MHC molecules on the surface of APCs can be down-regulated or blocked, preventing T-cell activation [122, 123]. Another feature that some tumors possess in order to escape the host's immune protective strategies is the secretion of immunomodulatory factors such as GM-CSF, TNF, TGF-ß1 and vascular endothelial growth factor (VEGF). These factors are of great importance in the conversion of peripheral myeloid cells to so-called MDSCs (myeloid-derived suppressor cells and tumor-associated macrophages) [124], which play a crucial role in creating favorable conditions for the growth of the tumor by creating oxidative stress and producing nitric oxide, among others [125, 126]. MDSCs appear often in patients' blood who have metastatic bladder cancer or renal cell carcinoma (RCC) [127] and there appears to be a correlation between the MDSC rate in blood and the patient's disease stage.

2.1.2 The theory behind cancer vaccines

The goal of cancer vaccines is the stimulation of the cellular part of the human immune system, focusing on B- and T-cells, thus giving the body a chance to bring back to balance the tumor-immune system interaction. To generate a lymphocyte response, these cells have to be accurately activated by DCs through antigen presentation. DCs have to be stimulated first though, and that is the goal of cancer vaccines. For this stimulation to be effective, cancer vaccines until present time have been made out of two components: an adjuvant and the target peptide or protein that should be later presented to activate T and B lymphocytes. Adjuvants, also known as pathogen-associated molecular patterns [128], are substances that are intended to encourage DC activation, enlarging the vaccines immunogenicity. The peptide component is delivered to the host in different forms, but is customized to the tumor entity, to match the

same molecule expected to be found overly expressed in the tumorous cells. A lot of effort has been put on the identification of TAAs as a target for T-cells and on the characterization of the from these TAAs derived peptides that act as epitopes on HLA molecule presentation by APCs [129]. Most TAAs are proteins which are not specific to tumor tissue but already exist on regular prostate cells. On the tumor though, they are overly expressed or their structure is altered due to gene-mutation of the malign cells. In some cases completely new proteins result from the aberrations in tumorous cells. Some of these structures are not only expressed in prostate cancer, but in other tumors as well.

2.1.3 Immune therapy in prostate cancer

Up until now melanoma and renal cell carcinoma have been the key targets of immunotherapeutic approaches, given that spontaneous regressions of both of these tumors [130] have been observed and they both present a dense CD8+ cell infiltration in tumor tissue [131]. Even though for a long time prostate cancer was not considered an immunological tumor as were both the above mentioned cancers, there are many reasons to think otherwise and several arguments for considering immune therapy as a possibility for treating this disease. Patients with prostate cancer spontaneously develop antibodies against tumor antigens. This leads to the conclusion that this type of cancer does possess more immunogenicity than was previously attributed to it [132]. Many different TAAs and prostate specific antigens which can be used as targets have been identified, for example PSA (prostate specific antigen), PCSA (prostate stem cell antigen), PSMA (prostate specific membrane antigen), TRP-P8 (transient receptor potential p8), survivin and prostein [129, 133, 134]. Also, prostate cancer is a slow growing tumor which is favorable to the immune system because it has more time to generate a directed antitumor response. Furthermore, there is enough data proving that immune therapy has most success when combined with other therapies which reduce the tolerance of the immune system against the tumor, for example radiation and ablative hormone therapy [117, 135].

Because the prostate is a nonvital organ, immunotherapy has the other great advantage, that the potential target molecules on the tumor cells are not only tumor specific. In the case of a radical prostatectomy, since the goal is to remove the organ and possible metastatic cells in lymph nodes, if a patient has a biochemical disease relapse it is assumed that there are remaining tumorous cells. Having removed the healthy prostate cells the mutated cancer ones can easily be targeted or marked not only using the tumor proteins, but using the prostate specific tissue molecules as well.

2.1.4 Cancer vaccines as treatment for prostate cancer

There have been several immunological approaches for the development of a new prostate cancer therapy. The most notable advances have focused on ways to deliver tumor-associated-antigens to patients so as to stimulate a T-cell response. These include antigens with viral vectors, DNA-based and RNA-based vaccines, peptide vaccines among others.

One of the main agents of this group and the only FDA (Food and Drug administration) approved drug in the field is Sipuleucel-T (commercial name Provenge). It is an autologous (and therefor personalized) APC-based Vaccine which uses ex vivo antigen presentation for stimulating an immune response in vivo. The cells are gained from the patient's blood by leukapheresis. These spend 36-44 hours in a sterile culture with the protein PA2024, a fusion protein from PAP and GM-CSF [136]. The decision of using PAP was based on the fact that xenogeneic forms of PAP have been proven to induce prostate-specific immunity on rats and humans [137]. GM-CSF is thought to enhance dendritic cells maturation. After the in-vitro activation of the now antigen loaded cells, these are reinfused to the patient. The goal is that the treated APCs target PAP-carrying tumorous cells, thus inducing tumor inhibition. The therapy consists in three series of leukapheresis-infusion treatments, each round two weeks apart.

Three randomized controlled studies led to the approval of sipuleucel-T. In all three trials patients in the placebo group received an infusion of autologous cells cultured without PA2024 with the possibility of being vaccinated with sipuleucel-T in the case of disease progression. The first phase III trial included 127 men with asymptomatic metastatic CRPC. The distribution into the placebo and sipuleucel-T group was effected randomly at a ratio from 2:1 (82 patients received sipuleucel-T and 45 the placebo). The primary endpoint of the trial was not met, since there was no significant difference in the time to disease progression (TTP). However, the overall survival (OS) showed an improvement for sipuleucel-T of 4 months over placebo [138]. The second trial was similar to the first one, but did not demonstrate a benefit for sipuleucel-T, neither on the TTP nor on the OS. The third trial (IMPACT trial, Immunotherapy for Prostate Adenocarcinoma Treatment) enrolled 512 asymptomatic CRPC patients, also organized in a placebo and sipulecuel-t group on a ratio of 2:1. The primary end point was the overall survival which was significantly improved in the sipulecuel-T group (sipuleucel-T 25.8 months vs. 21.7 for placebo), the TTP showed no significant improvement [139]. Patients in the placebo arm who showed disease progression could be vaccinated with sipuleucel-T after a certain amount of time, which happened in 109 of 171 cases. A follow-up therapy with Docetaxel was allowed. This happened in 190 out of 341 cases of sipuleucel-T treated patients and in 86 of 171 placebo treated patients. The overall survival improvement in the sipuleucel-t group proved to be independent from the docetaxel treatment [139].

Another approach for treating prostate cancer is using tumor cells as immunological vectors. GVAX consists of allogeneic prostate tumor cell lines (LNCaP and PC3) cultured from numerous individuals (differing in MHC tissue type). These cells are treated with radiation in order to prevent further proliferation and are genetically altered through viral transfer for secretion of GM-CSF [140]. Two Phase II studies conducted with CRPC patients showed positive effects: one showed an OS of 26.2 months and the other one an OS between 20.0 and 29.1 months [141, 142]. This lead to the VITAL-1 and VITAL-

2 randomized phase III studies. VITAL-1 included 626 asymptomatic CRPC chemotherapy-naive patients, randomly divided into two groups, one being treated with GVAX and the other one with prednisone/docetaxel. The interim analysis showed the primary endpoint of the study (prolonged OS) was not likely to be achieved, and the study was interrupted. The final analysis after 279 of the 626 study-patients had died displayed an overall survival of 20.7 for the GVAX group vs 21.7 months for the prednisone/docetaxel group.

A further approach is PROSTVAC-VF. This vaccine is a mixture of recombinant fowlpox and vaccinia viruses encoding transgenes for PSA and three T-cell co-stimulatory substances (intercellular adhesion molecule, lymphocyte function-associated antigen 3 and B7-1, all three substances together are also known as TRICOM) which help to amplify the immune response [143]. A phase II, double-blind randomized study for PROSTVAC-VF was conducted with 125 CRPC men; the placebo group received an empty vector, the treatment group received PROSTVAC-VF. This trial did not meet its primary endpoint, a prolonged progression- free survival but an analysis of the same study done 3 years afterwards showed an improvement in the OS for the PROSTVAC-VF group (median OS 24,5 months) versus the placebo group (16,0 months) [144]. A Phase III trial of PROSTVAC-VF is now being conducted in men with asymptomatic or minimally symptomatic CRPC[145].

Another type of immunotherapy for prostate cancer is a DNA based vaccine encoding tumor antigens. A DNA plasmid is processed by the DCs and then expressed on their surface for further T cell activation. A phase I/II study was done using DNA plasmids encoding PAP and pro-inflammatory agents which recruit and activate DCs (TLR agonists and GM-CSF). The treatment was applied to men with a biochemically recurrent non-metastatic disease [146]. The results showed an antibody and T cell response against PAP [147], and a deceleration in PSA rate rise could also be observed.

Similar to DNA vaccines there has also been an attempt to stimulate an immune response with RNA. CV9103 is a vaccine that consists of full length mRNA encoding PSCA, PSMA, PSA and STEAP1 (six transmembrane epithelial antigen of the prostate 1) [148]. A phase I/IIa study included 38 patients with CRPC with and without metastasis; the patients were injected with CV9103 subcutaneously and monitored closely. The vaccine was generally well tolerated. Results reported a response against several antigens in 58% of the patients and 74% showed a T-cell response against minimum one of the antigens. In isolated cases, patients showed a stabilization of their PSA levels.

Noguchi et al. [149] performed a phase II randomized trial using a personalized peptide vaccination (PPV) in patients with CRPC. The trial included 57 patients, 28 of which received PPV combined with low dose estramustine phosphate (EMP). 27 patients in the control group received EMP therapy only. The patients in the PPV + EMP group were previously vaccinated with a mixture of up to 26 different peptides derived from different prostate cancer antigens. The immune response to this pre-vaccination was measured by IgG antibodies against the received peptides. During the actual vaccination therapy, the patients in the PPV arm only received a combination of up to 4 peptides, chosen carefully according to the strongest immune responses showed during pre-vaccination assessment. After a first round of treatment, patients were able to change groups in case of disease progression. The primary endpoint of the study was progression-free survival assessed by changes in PSA. The study showed an improvement in PFS for the PPV plus EMP group compared to the control group (median PFS was 8.5 vs 2.8 months, p=0.0012). Also a prolonged overall survival was observed for the PPV + EMP group compared to the EMP only group (p=0.03) [149].

The aim of this study was to assess the clinical outcome of patients who underwent multipeptide vaccination treatment and compare their disease development with patients who did not receive immune therapy. Multipeptide vaccination therapy stimulates the host’s immune system to target and destroy prostate cancer cells. The patients who received this treatment should present a

longer time to progression (longer time to subsequent cancer specific therapy, time to hormone therapy and time to clinical recurrence) and improvement in overall survival (OS) compared to patients who did not receive such vaccination treatment.

Materials and methods

1. Study approval

The study was approved by the local ethics committee of the medical faculty of the University of Tuebingen (358/2014BO2).

2. Patient collective

The patients included in this study all participated in a clinical trial conducted at the University hospital of Tuebingen between 2004 and 2014. The recruitment of patients took part between 2004 and 2009.

All patients were diagnosed with prostate cancer and submitted themselves to a radical prostatectomy (performed both at the University Hospital in Tuebingen or other clinic in Germany). After an initial postoperative PSA value decrease, the patients showed a biochemical PSA relapse. This was defined by the increase of two consecutive PSA values from the nadir after RP or radiation, evaluated by two measurements at least 14 days apart.

The collective of the present study consists of two groups: 36 patients who were included into the clinical trial and 65 patients that had a screening failure due to reasons stated below, 101 in total. For the clinical trial, 154 patients were screened in total (see study criteria below). HLA Status was determined for each of these patients. HLA-A2 status was the main inclusion criterion. Of the 154 patients screened, 55 patients were HLA A2 positive. Of these 55 patients 36 were included into the vaccination trial. The 19 patients who were HLA A2 positive were not included in the clinical trial because they rejected it, or they did not meet the inclusion criteria. Of these 19 HLA A2 positive patients, 16 were included in the control group. From the 99 HLA A2 negative patients, 49 were included into the study. The remaining 50 patients that were screened but not included in the clinical trial were not included in this study as they could not be contacted or they rejected the proposal of being part of this study (see Figure 1).

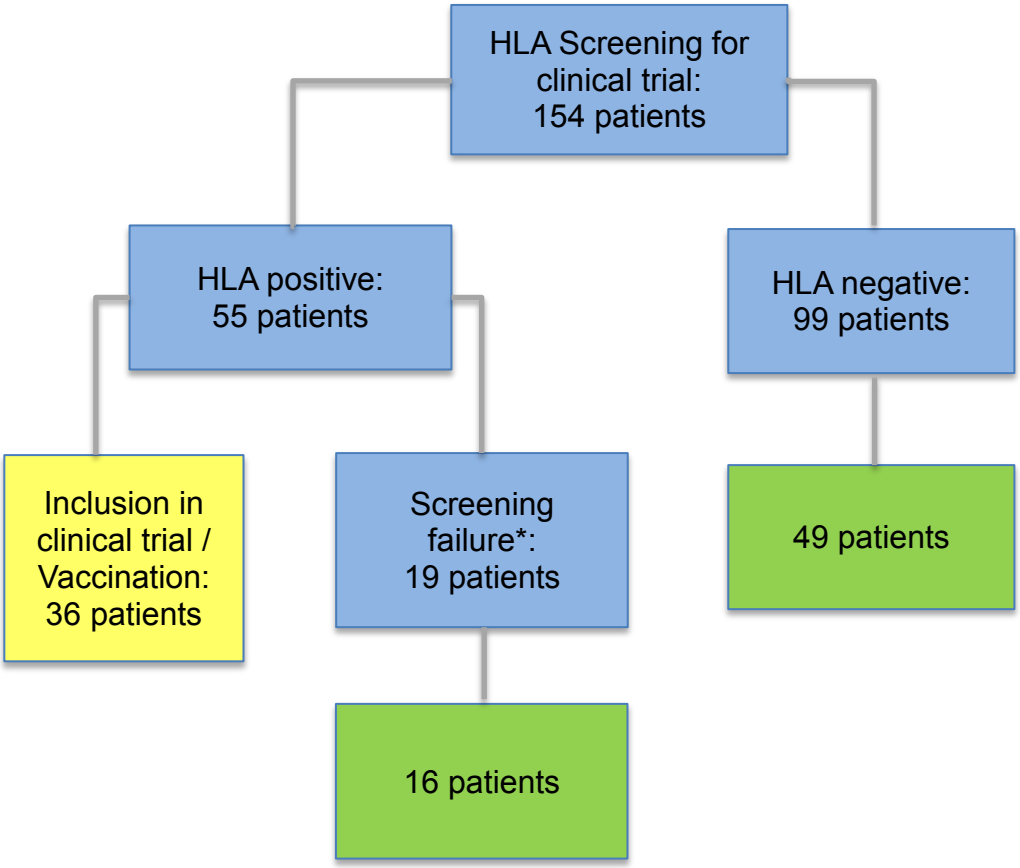


Figure 1: **Study Cohort**. Patients in green and yellow boxes were included in the present study. The patients in the green boxes served as control group whereas the patients in the yellow box formed the treatment group. * screening failure due to other reasons than HLA status.

3. Vaccination trial

The criteria for inclusion in the vaccination study were the following:

- Positive HLA A2 status
- Age between 45 and 80
- Biochemical disease defined by the increase of two consecutive PSA values from the nadir after RP or radiation, evaluated by two measurements at least 14 days apart.
- No therapy for treating biochemical disease recurrence (adjuvant therapies were allowed only in the case that they were performed before PSA recurrence).

- No local recurrence, nodal or distant metastases assessed by CT, MRI or bone scan
- No immune modulatory therapies, no corticoid therapy
- No simultaneous radiation, hormone deprivation therapy or chemotherapy
- No concomitant tumor, epileptic or pulmonary disease
- Eastern Cooperative Oncology Group performance status of 0 or 1 [150].

Exclusion criteria for the vaccination study:

- Karnofsky Index <70
- No consent from the patient
- Presence of a second tumorous disease
- Known obstructive lung disease and a predisposition for allergies
- Use of medication that alters or has an effect on the immune system
- Simultaneous radiation treatment
- Seizure disorder

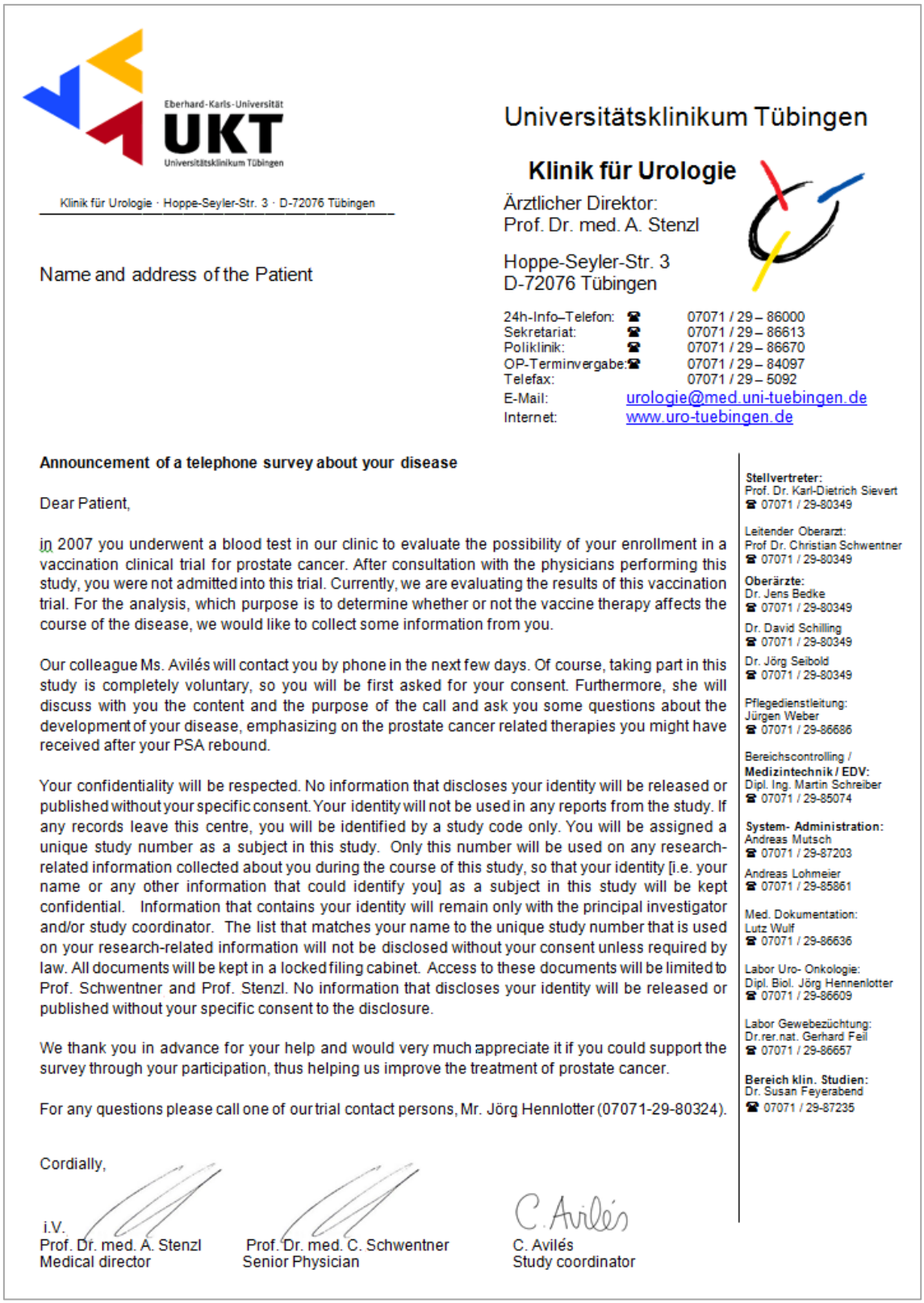
3.1 Clinical trial procedures

The vaccine consisted of 14 different, synthetic peptides, all of them HLA I and II binding molecules derived from prostate tissue specific antigens or prostate cancer associated antigens, aside from one derived from an influenza virus, which was thought as a marker peptide for memory T-cell activation [151]. These were selected to target a broad range of CD4+ and CD8+ T-cells, trying to prevent tumor escape. The peptide mixture in a Montanide ISA emulsion was injected subcutaneously to the patients, along to either one of four different adjuvants or no adjuvant at all. Between the possible adjuvants were GM-CSF, Imiquimod, a mucin-1-mRNA/protamine complex and hyperthermia which were thought to boost immune stimulation. The patients received the injections on the abdominal wall, always on the same area, on days 0, 7, 14, 28, 42 and 56. After each vaccination patients were thoroughly monitored and not discharged until adverse effects or toxicity were discarded. PSA was monitored at each

vaccination. After Day 56, if no PSA progression or clinical disease progress were observed, patients continued to be vaccinated every 4 weeks until day number 420 (approximately 18 months). After concluding with the vaccination program, patients were further monitored by their urologist or family doctor.

4. Assessment of follow-up

For the present study, follow-up of the 101 patients included was obtained either by in-house follow-up or by contacting the patient. To determine the latest survival status, cause of death, date and type of clinical progression and PSA measurements, the patients were contacted and/or the hospital's records were reviewed. Clinical endpoints were registered based on the latest available information before cut-off-date July 1st 2012. Clinical recurrence was defined as any comeback of the disease: local recurrence, nodal disease, bone or visceral metastasis. Distant metastasis was defined as bone or visceral metastasis. The term subsequent therapy includes all prostate cancer specific therapies which the patient received after the screening for the study or after the vaccination trial. Since the majority of the patients proceeded with their follow up treatment and monitoring outside of the hospital, the patients had to be contacted. Each man received an explanatory letter (see Figure 2) giving them the possibility of participating (clearly stated that it was voluntary to be included in the study). All patients were then contacted by phone and after verbally agreeing to taking part in the study, they were asked several questions about the course of their disease (see Figure 3). Patients who could not answer these questions by themselves allowed us to contact their family physician or urologist to get the information. An additional follow-up took place in March 2015 to update patients' data with regard to their subsequent therapy. The same steps as before were followed to contact the patients and collect the information.



Eberhard-Karls-Universität
UKT
Universitätsklinikum Tübingen

Klinik für Urologie · Hoppe-Seyler-Str. 3 · D-72076 Tübingen

Name and address of the Patient

Universitätsklinikum Tübingen

Klinik für Urologie

Ärztlicher Direktor:
Prof. Dr. med. A. Stenzl

Hoppe-Seyler-Str. 3
D-72076 Tübingen

24h-Info–Telefon: ☎ 07071 / 29 – 86000
Sekretariat: ☎ 07071 / 29 – 86613
Poliklinik: ☎ 07071 / 29 – 86670
OP-Terminvergabe: ☎ 07071 / 29 – 84097
Telefax: 07071 / 29 – 5092
E-Mail: urologie@med.uni-tuebingen.de
Internet: www.uro-tuebingen.de

Announcement of a telephone survey about your disease

Dear Patient,

in 2007 you underwent a blood test in our clinic to evaluate the possibility of your enrollment in a vaccination clinical trial for prostate cancer. After consultation with the physicians performing this study, you were not admitted into this trial. Currently, we are evaluating the results of this vaccination trial. For the analysis, which purpose is to determine whether or not the vaccine therapy affects the course of the disease, we would like to collect some information from you.

Our colleague Ms. Avilés will contact you by phone in the next few days. Of course, taking part in this study is completely voluntary, so you will be first asked for your consent. Furthermore, she will discuss with you the content and the purpose of the call and ask you some questions about the development of your disease, emphasizing on the prostate cancer related therapies you might have received after your PSA rebound.

Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent. Your identity will not be used in any reports from the study. If any records leave this centre, you will be identified by a study code only. You will be assigned a unique study number as a subject in this study. Only this number will be used on any research-related information collected about you during the course of this study, so that your identity [i.e. your name or any other information that could identify you] as a subject in this study will be kept confidential. Information that contains your identity will remain only with the principal investigator and/or study coordinator. The list that matches your name to the unique study number that is used on your research-related information will not be disclosed without your consent unless required by law. All documents will be kept in a locked filing cabinet. Access to these documents will be limited to Prof. Schwentner and Prof. Stenzl. No information that discloses your identity will be released or published without your specific consent to the disclosure.

We thank you in advance for your help and would very much appreciate it if you could support the survey through your participation, thus helping us improve the treatment of prostate cancer.

For any questions please call one of our trial contact persons, Mr. Jörg Hennlotter (07071-29-80324).

Cordially,

i.V.
Prof. Dr. med. A. Stenzl
Medical director

Prof. Dr. med. C. Schwentner
Senior Physician

C. Avilés
Study coordinator

Stellvertreter:
Prof. Dr. Karl-Dietrich Sievert
☎ 07071 / 29-80349

Leitender Oberarzt:
Prof Dr. Christian Schwentner
☎ 07071 / 29-80349

Oberärzte:
Dr. Jens Bedke
☎ 07071 / 29-80349

Dr. David Schilling
☎ 07071 / 29-80349

Dr. Jörg Seibold
☎ 07071 / 29-80349

Pflegedienstleitung:
Jürgen Weber
☎ 07071 / 29-86686

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Dipl. Ing. Martin Schreiber
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☎ 07071 / 29-86609

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☎ 07071 / 29-86657

Bereich klin. Studien:
Dr. Susan Feyerabend
☎ 07071 / 29-87235

Figure 2. Letter sent to patients before initiating survey for the study.

Phone call protocol for patient follow-up

Patient ID _____ Prostatectomy date _____

Known medical history until present date:

_____

_____

Telephone conversation/dialogue:

I. Introduction of oneself as member of the clinic for Urology at the Universtiy Hospital in Tuebingen. Explanation about current study, voluntary participation and absolute confidentiality. Question about patient's agreement to participate in the study.

Documentation of agreement: *Yes*_____ *No* _____

In case of "No": End of conversation.

II. In case of "Yes": Inquiry about course of disease since screening/last vaccination:

PSA level rise?

*No*_____ *Yes*_____ Date: _____

II.1 During the course of your disease, has there been a local recurrence or is there any knowledge of nodal or distant metastases?

*No*_____ *Yes*_____ *Date:* _____
Localisation: _____

II.2 After screening for the study/last vaccination, did you receive any kind of prostate cancer related therapy, for example radiation, hormone deprivation therapy or chemotherapy?

No _____ *Yes*_____

In case of yes, what kind of therapy and when?:
Therapy: _____ *Date*_____ *(eventually more than one)*

Thank you for participating

End of phone call

Figure 3. Telephone protocol for patient follow-up.

Gathered data:

Preoperative:

- Date of birth/age
- Preoperative PSA-value

Postoperative:

- Date of prostatectomy
- pTNM-Stadium
- Resection margins
- Gleason Score
- Date of screening for the study
- HLA-Status
- Start and end dates of vaccination study (when applicable)

After study/after screening for the study:

- PSA value trend
- Type of PC-related therapy prior to screening
- Type of PC-related therapy after screening
- Presence and date of local disease relapse (assessed by CT-Scan, bone scan or MRI)
- Presence and date of nodal and distant metastases (assessed by CT-scan, bone scan or MRI)
- Survival status/cause of death

5. Statistical analysis

Statistical analysis of data was performed using SAS JMP 10.0 (Cary, USA) and IBM SPSS 22 (Armonk, NY, USA).. For estimation of time to recurrence, time to metastasis, time to next therapy and time to androgen deprivation therapy, Kaplan Meier curves were used. For analysis of Kaplan-Meier curves, Log Rank tests were performed. In case of non-constant event rates, indicated

by a crossover of the curves in Kaplan-Meier estimates, Wilcoxon test was performed. Differences in clinicopathological parameters between the vaccinated and non-vaccinated group were assessed by Wilcoxon-Mann-Whitney tests, Wilcoxon-Kruskal-Wallis tests and Cochrane-Armitage tests for trend. A p value of less than 0.05 was considered statistically significant.

Results

1. Description of study cohort

1.1. General clinicopathological data.

In total, 101 male were included. The median age at time of randomization was 65 years (Range 45-81).

Tumor stage from prostatectomy samples was available in 85 patients. Of these, 42 (49.4%) had a locally advanced tumor (pT≥3). Nodal status from time of prostatectomy was available in 85 patients. Of these, 9 (10.6%) had nodal positive disease.

Preoperative PSA levels were available in 72 patients. The median preoperative PSA was 9.55 ng/ml (1.5-34.6).

Gleason Patterns and Surgical resection margin status are shown in Table 3.

1.2. HLA Status and vaccination group

Of 101 patients, 52 were HLA A2 positive (51.5%). Of the HLA A2 positive patients, 36 (69.2%) were treated with vaccination. There were no differences in clinicopathological parameters between vaccinated and non-vaccinated patients (see Table 3).

Table 3. Description of study cohort.

	Total (N=101)	Vaccinated = 1 (N=36)	Non-vaccinated = 0 (N=65)	p=
Age	Median= 65 Mean= 65.2 [45-81] (N=101)	Median = 65 Mean= 64.9 [52-81]	Median = 66 Mean= 65.3 [45-76]	0.67
HLA A2 +/ total	52/101 (51.4%)	36/36 (100%)	16/65 (24.6%)	< 0.0001
pT (TNM-Stadium)	2a= 5 2b= 11 2c= 27 3a=18 3b= 21 4= 3 (N=85)	2= 1 2a= 2 2b= 7 2c= 11 3a=4 3b= 10 4= 1 (N=36)	2a= 3 2b= 4 2c= 16 3a=14 3b= 11 4= 2 (N=50)	0.50 (Cochrane Trendtest)
Fraction of advanced stage tumors pT≥3	42 (49.4%) (N=85)	15 (42.8%) (N=35)	27 (54.0%) N=50	0.31
N positive (TNM-Stage)	9 (10.6%) (N=85)	2 (6.3%) (N=32)	7 (13.2%) (N=53)	0.31
M positive (TNM-Stage)	0 (N=75)	0	0	n.a.
R1 resection	24 (34.8%) (N=69)	10 (37.0%) (N=27)	14 (33.3%) (N=42)	0.75
Median Gleason-Score	7 [5-9] (N= 71)	7 [5-9] (N=29)	7 [5-9] (N=42)	0.10
Preoperative PSA-value	Median: 9.55 [1.5-34.6] (N=72)	Median: 8.85 [3.6-34.6] (N=32)	Median: 9.7 [1.5-26.8] (N=40)	0.68
Fraction of patients with therapy between RP and screening	37/101 (36.6%)	13 (36.1%)	24 (36.9%)	0.94

1.3 Treatment after prostatectomy and before vaccination/screening

Of 101 patients, 37 patients received a cancer-specific therapy between prostatectomy and inclusion into the study.

Table 4. Number of patients who received therapy after prostatectomy and before vaccination/screening

	Therapy				
	Radiation	ADT	Chemotherapy	Radiation + ADT	Radiation + Chemotherapy
Patients (whole cohort)	16	8	1	11	1
Vaccinated	4	4	0	4	1
Non-vaccinated	12	4	1	7	0

As a therapy before vaccination might have impacted the results of the study, subgroup analyses were performed for those patients, who did not receive any treatment between prostatectomy and inclusion into the study.

1.4 Course of disease

The mean follow up was 61.7 months (median 64 months [range: 31-91])..The mean time to clinical recurrence after inclusion in the study was 47.1 months (median 55 months [range 1-91]). The mean time to the development of distant metastases was 53.4 months (median 60 months [range 2-91]). During the follow-up, 13 patients (12.9%) had local recurrence or nodal disease and 21 patients (20.8%) a distant recurrence (distant metastasis). The proportion of patients who had no clinical recurrence after 2 and 5 years after screening was 83.0% and 70.7% (see figure 4). The proportion of patients without distant metastases after 2 and 5 years after screening was 89.0% and 82.6% (see

figure 5). Recurrence patterns in vaccinated and non-vaccinated patients are shown in table 5.

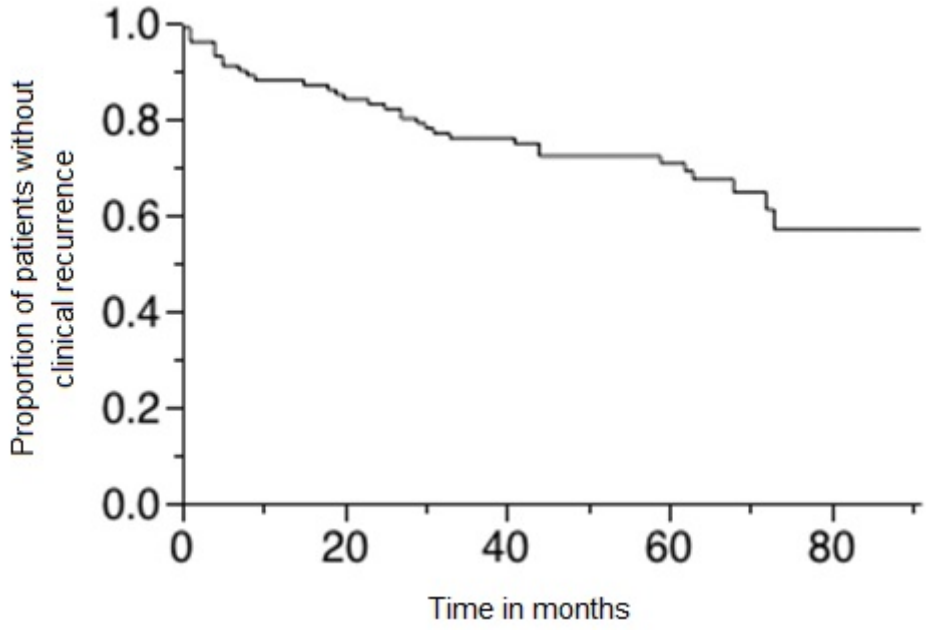

Figure 4: Proportion of patients without clinical recurrence.
Vaccinated and non-vaccinated patients combined.

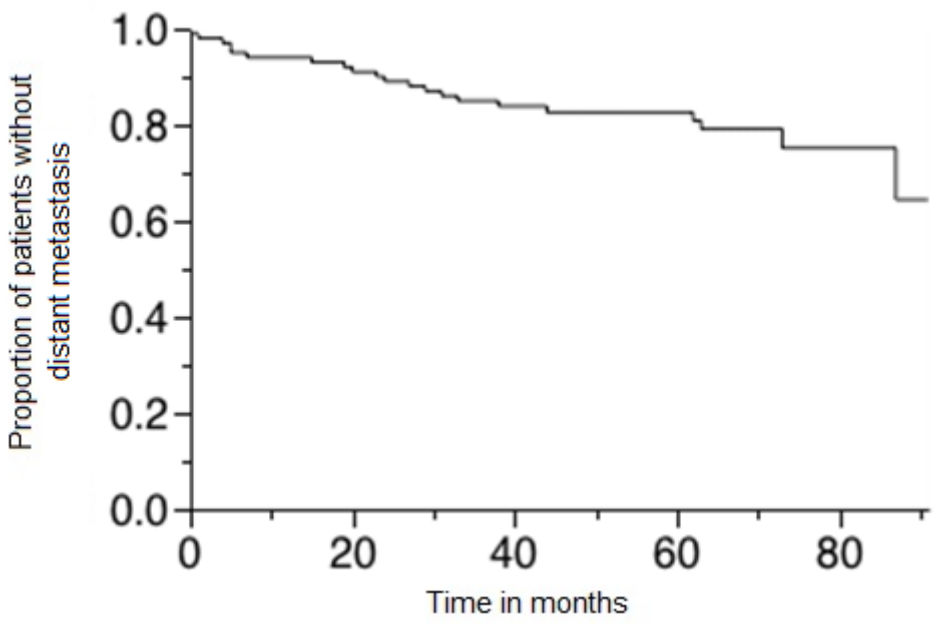

Figure 5: Proportion of patients without distant metastases.
Vaccinated and non-vaccinated patients combined.

Table 5. Local and distant disease recurrence

	Recurrence	
	Local/ regional recurrence	Distant metastasis
Patients (whole cohort)	13	21
Vaccinated	3	5
Non-vaccinated	10	16

1.5 Treatment after screening

The mean time to next therapy after inclusion in the study was 16.4 months (median 12 months [range 0-86]). The mean time to hormone therapy was 26.1 months (median 31 months [0-91]). During the follow-up, 83 patients received treatment after screening. 45 (44.6%) received radiotherapy, 34 (33.7%) patients had antihormonal treatments, 2 patients (2.0%) chemotherapy, 1 patient received both antihormonal therapy and radiation (1.0%) and 1 patient received surgery (1.0%). The rate of patients without subsequent therapy after screening after 2 and 5 years was 36.6% and 17.6% (see figure 6). The rate of patients without subsequent hormone therapy after screening after 2 and 5 years was 58.4% and 39.5% (see figure 7). Subsequent treatments in vaccinated and non-vaccinated patients are shown in table 6.

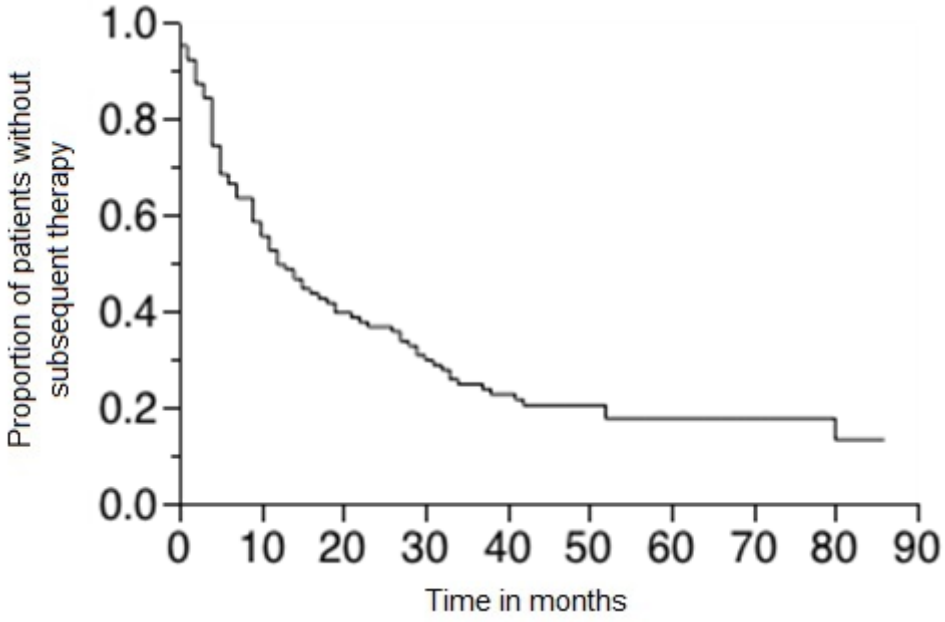

Figure 6: Proportion of patients without subsequent therapy.
Vaccinated and non-vaccinated patients combined.

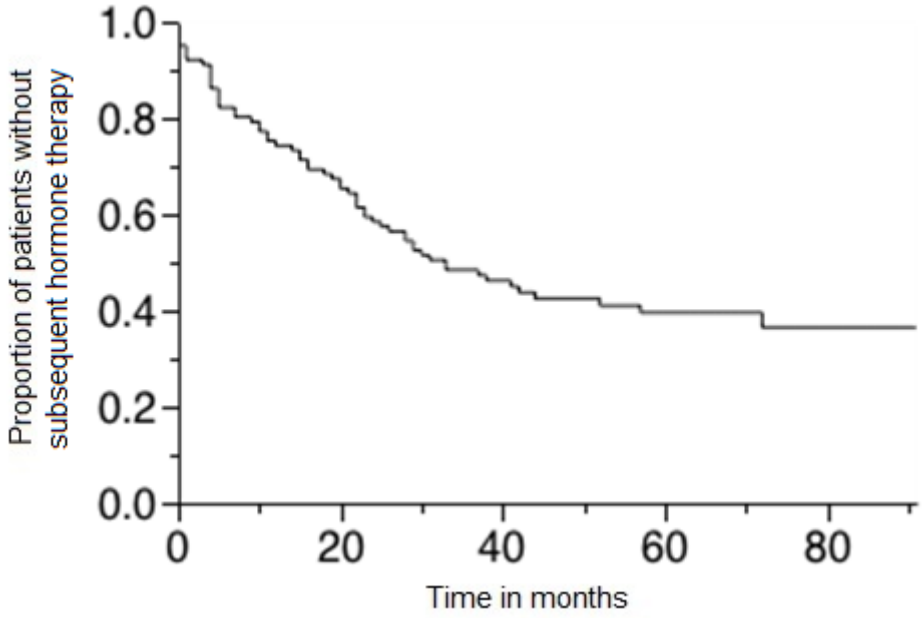

Figure 7: Proportion of patients without subsequent hormone therapy.
Vaccinated and non-vaccinated patients combined.

Table 6. Therapies received by the patients after vaccination/screening.

	Therapy				
	Radiation	ADT	Chemotherapy	Radiation + ADT	Surgery
Patients (whole cohort)	45	34	2	1	1
Vaccinated	13	11	1	2	1
Non-vaccinated	30	23	1	1	0

1.6 Overall survival and cancer specific survival

During the follow-up, 5 patients (5.0%) became deceased: 2 (5.5%) from the vaccinated group, and 3 (4.6%) from the non-vaccinated group. All deaths were caused by prostate cancer. After 2 and 5 years, the proportion of patients surviving was 100.0% (n=101) and 97.3% (n=98).

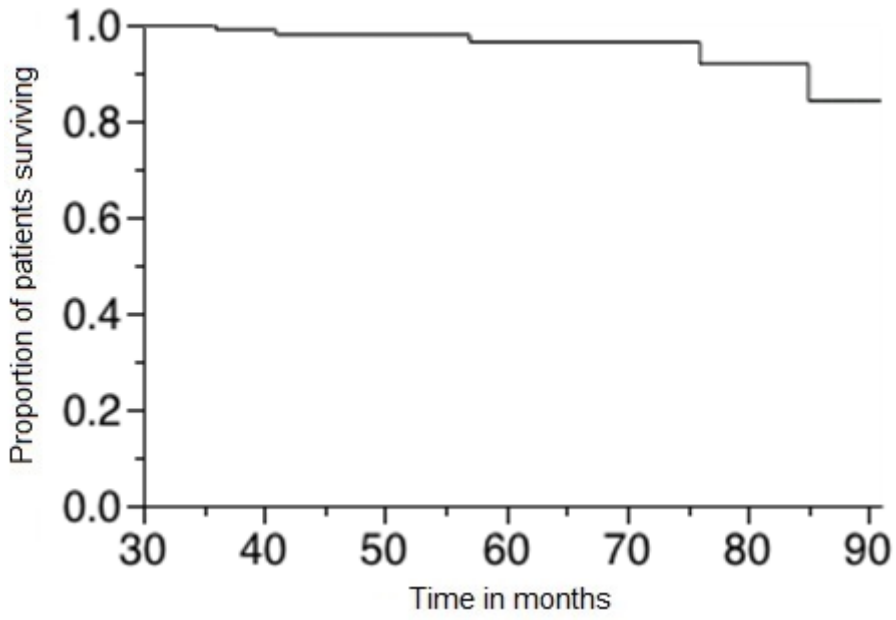

Figure 8: Proportion of patients surviving.
Vaccinated and non-vaccinated patients combined.

2. Outcome comparison between vaccinated and non-vaccinated patients

2.1 Recurrence free survival: any clinical recurrence (local, nodal or distant metastasis)

2.1.1 Time to clinical recurrence from screening (whole cohort)

The proportion of patients without clinical recurrence after 2 and 5 years after screening for the study was 91.4% and 82.1% for the vaccinated group and 78.5% and 64.3% for the non-vaccinated group (p=0.06).

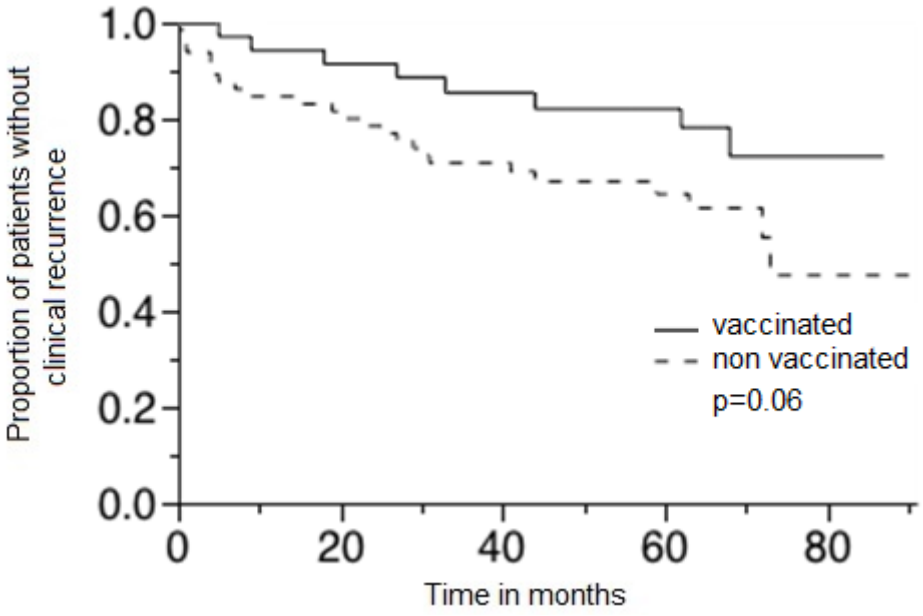

Figure 9: Proportion of patients without clinical recurrence after screening.
Comparing vaccinated group vs. non-vaccinated group. Whole cohort, n=101 (p=0.06 log-rank test)

2.1.2 Time to clinical recurrence from screening (patients without cancer-related therapy between prostatectomy and screening)

In the subgroup of patients without any therapy between prostatectomy and screening the proportion of patients without clinical recurrence 2 and 5 years after screening for the study was 91.3% and 81.2% in the group of vaccinated patients and 73.8% and 58.3% in the non-vaccinated patients group (p=0.05).

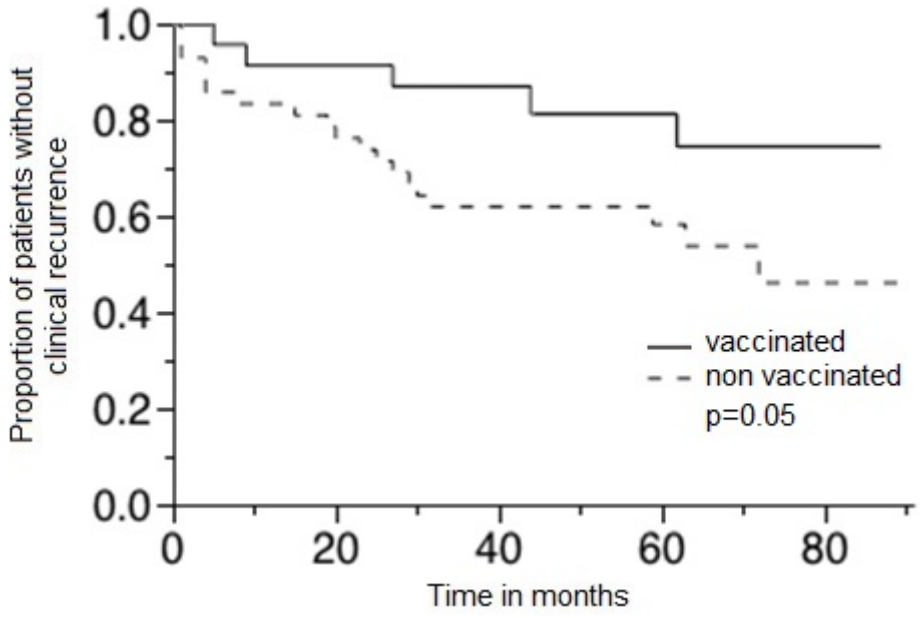

Figure 10: Proportion of patients without clinical recurrence after screening.
Comparing vaccinated group vs. non-vaccinated group. Included are only patients who did not become any prostate cancer specific therapy between prostatectomy and screening, n=64 (p=0.05 log-rank-test)

2.2 Recurrence free survival: distant metastasis

2.2.1 Time to development of distant metastasis (whole cohort)

The proportion of patients without distant metastases after 2 and 5 years after screening for the study was 97.1% and 87.4% for the vaccinated group and 84.6% and 80.0% for the non-vaccinated group (p=0.21).

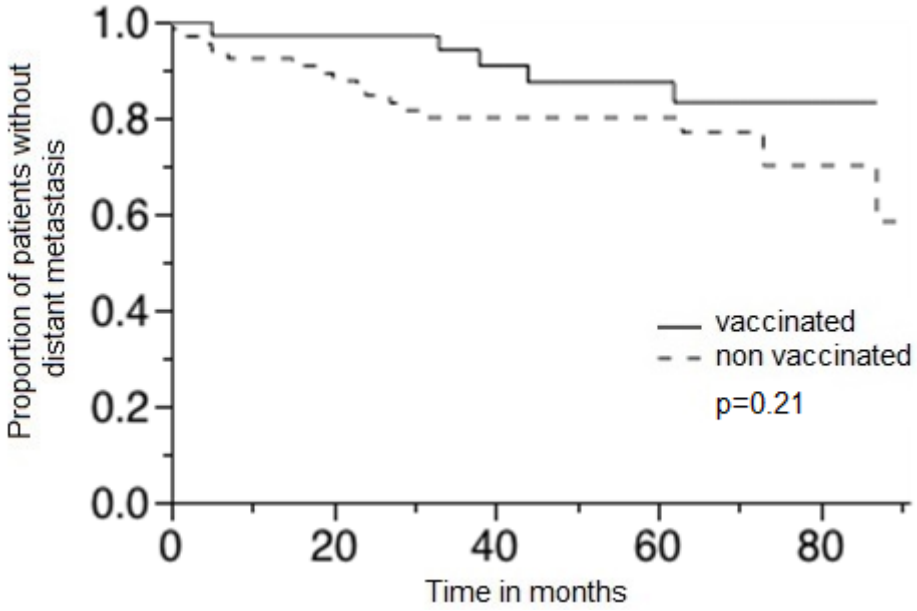

Figure 11: Proportion of patients without distant metastases after screening.
Comparing vaccinated group vs. non-vaccinated group. Whole cohort, n=101 (p=0.21 log-rank test)

2.2.2 Time to development of distant metastasis (patients without cancer-related therapy between prostatectomy and screening)

In the subgroup of patients without any therapy between prostatectomy and screening the proportion of patients without distant metastases 2 and 5 years after screening for the study was 95.7% and 89.7% in the group of vaccinated patients and 83.3% and 76.2% in the non-vaccinated patients group (p=0.19).

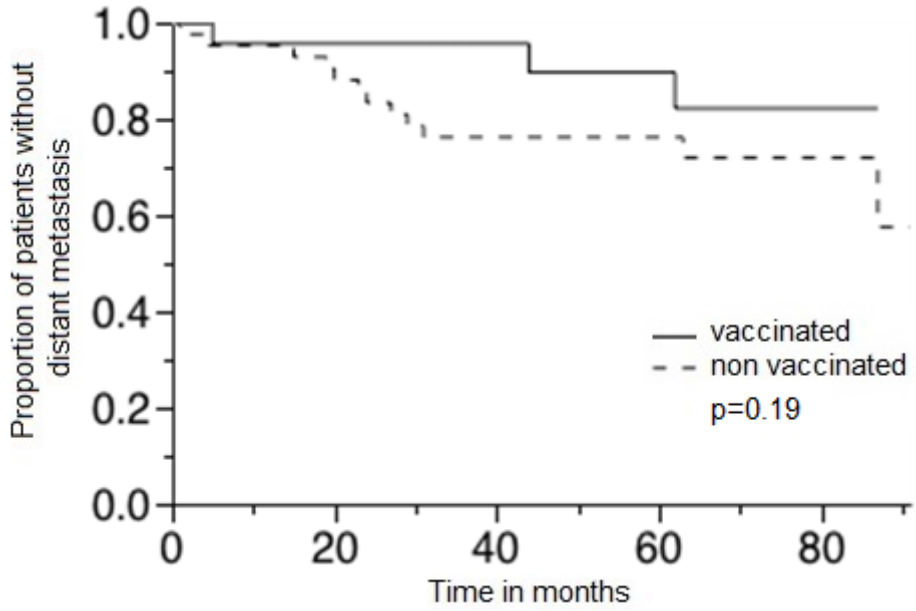

Figure 12: Proportion of patients without distant metastases after screening.
Included are only patients who did not become any prostate cancer specific therapy between prostatectomy and screening, n=64 (p=0.19 log-rank test)

2.3 Overall and cancer specific survival (whole cohort)

The proportion of patients surviving at the end of follow up was 97.3% for the vaccinated group and 95.4% for the non-vaccinated group.

The proportion of patients surviving after 2 and 5 years after screening for the study was 100% for both the vaccinated group and the non-vaccinated group after 2 years, and 100% and 93.3% after 5 years (p=0.89).

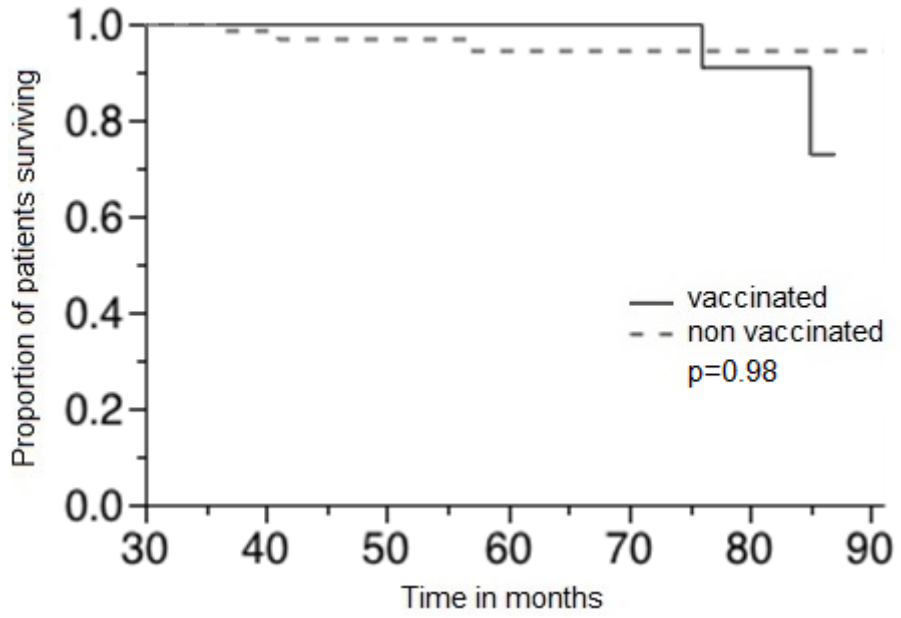

Figure 13: Overall Survival. Comparing vaccinated group vs. non-vaccinated group. Whole cohort n=101 (p=0.98 log-rank test)

2.4 Time to subsequent prostate cancer-specific therapy

2.4.1 Time to subsequent therapy after screening (whole cohort)

The proportion of patients without cancer-specific treatment 2 and 5 years after screening for the study was 41.7% and 21.9% in the group of vaccinated patients and 33.9% and 15.2% in the non-vaccinated patients group (log-rank test p=0.09. Wilcoxon test p= 0.01).

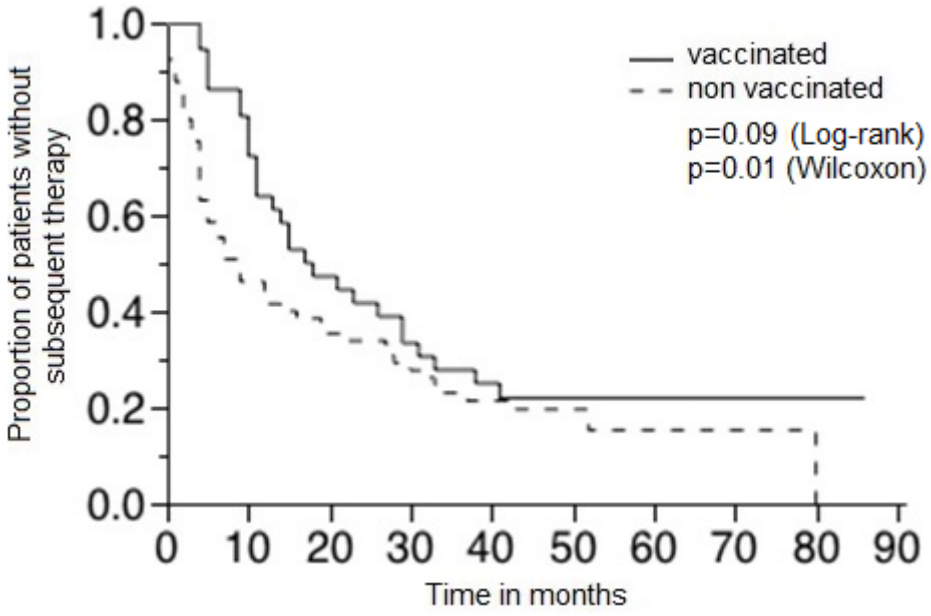

Figure 14: Proportion of patients without subsequent therapy after screening. Comparing vaccinated group vs. non-vaccinated group. Whole cohort, n=101 (p=0.09 log-rank test. p= 0.01 Wilcoxon test)

2.4.2 Time to subsequent prostate cancer specific therapy after screening (patients without cancer-related therapy between prostatectomy and screening)

In the subgroup of patients without any prostate cancer specific therapy between prostatectomy and screening the proportion of patients without cancer-specific treatment 2 and 5 years after screening for the study was 43.5% and 26.1% in the group of vaccinated patients and 33.3% and 11.3% in the non-vaccinated patients group (p=0.05).

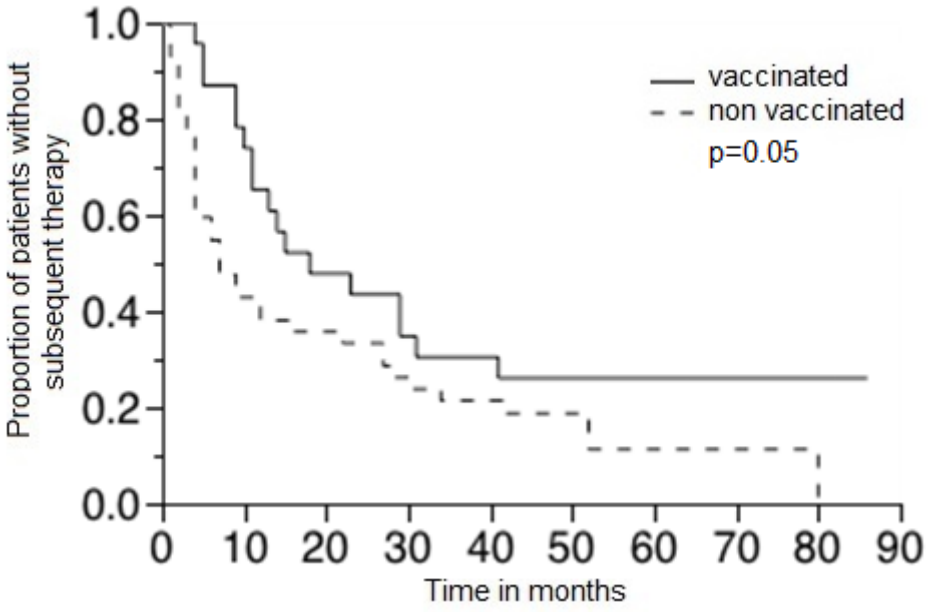

Figure 15: Proportion of patients without subsequent therapy after screening.
Comparing vaccinated group vs. non-vaccinated group. Included are only patients who did not become any prostate cancer specific therapy between prostatectomy and screening, n=64 (p=0.05 log-rank test)

2.4.3 Time to subsequent therapy after screening (whole cohort). Update 31.03.2015.

The mean time to subsequent therapy after screening was 26.53 months for the whole cohort, 29.75 months for the vaccinated patients and 24,75 months for the non-vaccinated patients (Breslow p=0.046).

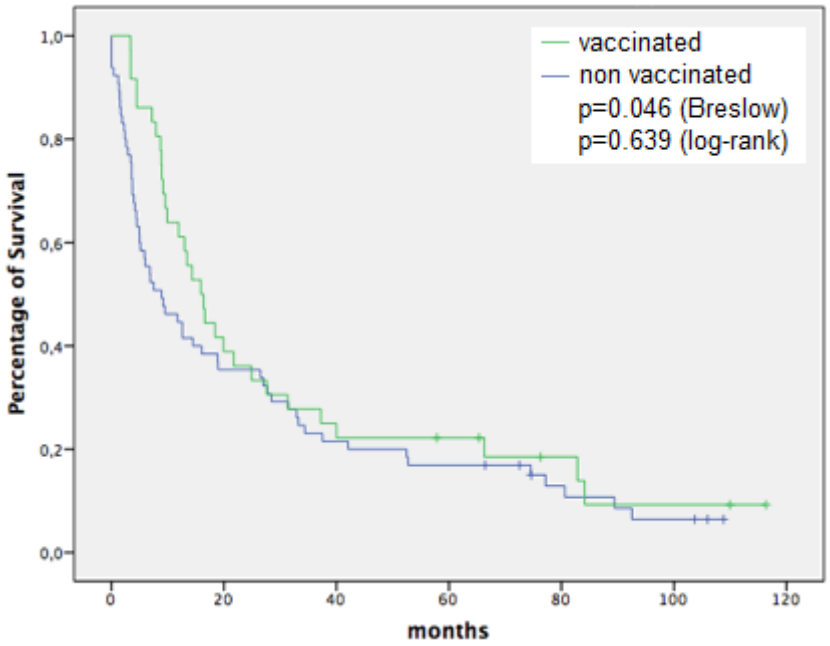

Figure 16: Proportion of patients without subsequent therapy after screening. Cut-off date 31.03.2015. Comparing vaccinated group vs. non-vaccinated group. Whole cohort n=101 (p=0.046 Breslow; log-rank test p=0.639).

2.4.4 Time to subsequent prostate cancer specific therapy from screening for non-vaccinated group and from last-vaccination for vaccinated group (patients without cancer-related therapy between prostatectomy and screening)

For the patients who did not receive any prostate cancer specific therapy between radical prostatectomy and screening, the data reads as follows: the proportion of patients without subsequent therapy after 2 and 5 years after last vaccination was both 30.4% and 25.4% for the vaccinated group. For the non-vaccinated group, as seen above, the proportion of patients without subsequent therapy after 2 and 5 years after screening for the study was 33.3% and 11.3% (log-rank test p=0.72. Wilcoxon test p=0.15).

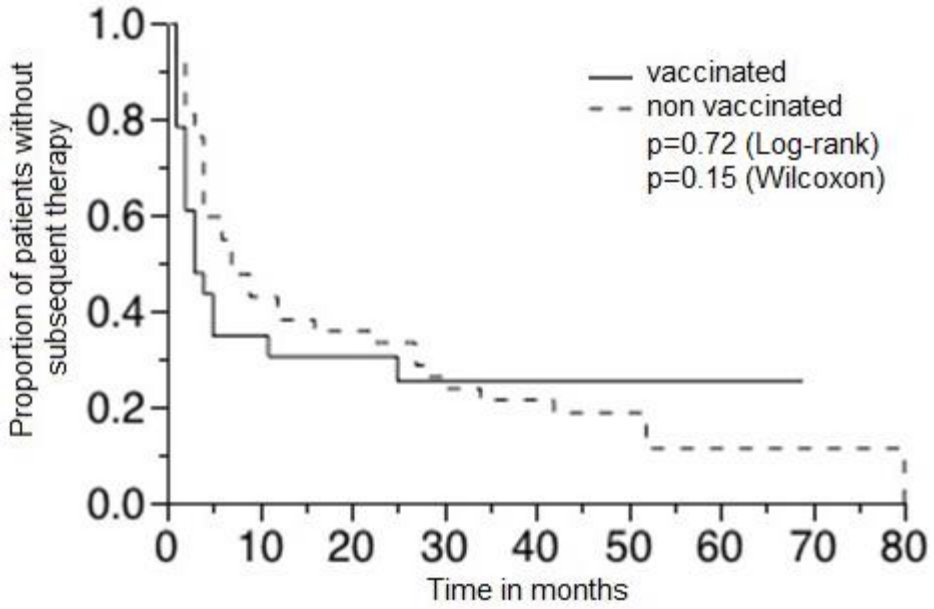

Figure 17: Proportion of patients without subsequent therapy. Comparing vaccinated vs. non-vaccinated group. Included are only the patients who did not receive any prostate cancer-specific treatment between prostatectomy and screening, n=64. Follow-up time starts for the vaccinated group after last vaccination, for the non-vaccinated group after screening (p=0.72 log-rank test, p=0.15 Wilcoxon test)

2.5 Time to subsequent hormone deprivation therapy

2.5.1 Time to androgen deprivation therapy from screening (whole cohort)

The proportion of patients without androgen deprivation therapy 2 and 5 years after screening for the study was 63.9% and 38.9% in the group of vaccinated patients and 55.4% and 39.7% in the non-vaccinated patients group (Log-Rank p=0.66. Wilcoxon test p=0.42).

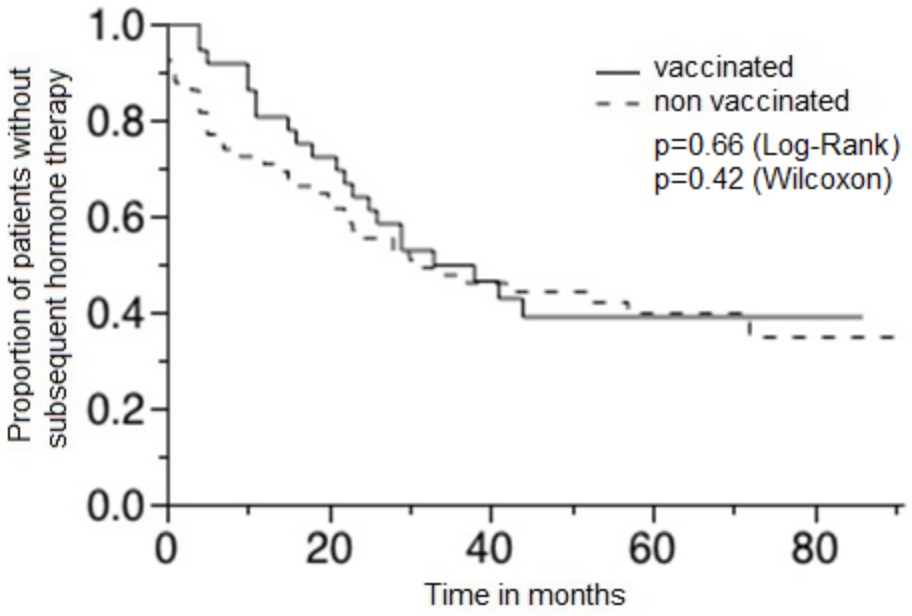

Figure 18: Proportion of patients without androgen deprivation therapy after screening. Comparing vaccinated vs. non-vaccinated group. Whole cohort, n=101 (p=0.66 log-rank test, p=0.42 Wilcoxon test)

2.5.2 Time to hormone deprivation therapy from screening (patients without cancer-related therapy between prostatectomy and screening)

In the subgroup of patients without any therapy between prostatectomy and screening the proportion of patients without hormone deprivation therapy 2 and 5 years after screening for the study was 78.3% and 53.8% in the group of vaccinated patients and 64.3% and 48.1% in the non-vaccinated patients group (p=0.46).

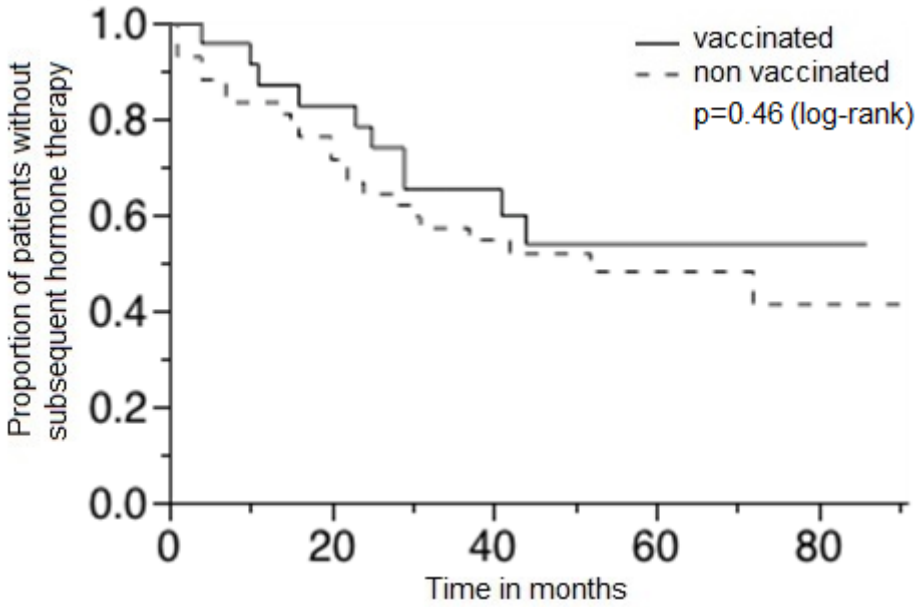

Figure 19: Proportion of patients without androgen deprivation therapy after screening. Comparing vaccinated vs. non-vaccinated group. Included are only patients who did not become any prostate cancer specific therapy between prostatectomy and screening, n=64 (p=0.46 log-rank test)

2.5.3 Time to subsequent hormone deprivation therapy from screening (whole cohort). Update 31.03.2015.

The mean time to androgen deprivation therapy was 45.50 months for the whole cohort, 43.49 months for the vaccinated patients and 46,57 months for the non-vaccinated patients (Mann-Whithney-U-test p=0.91).

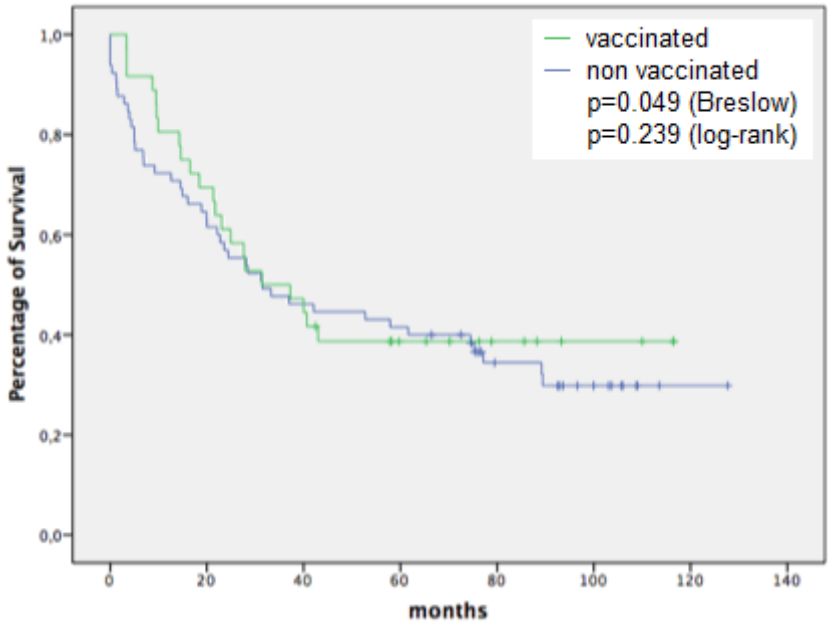

Figure 20: Proportion of patients without androgen deprivation therapy after screening. Cut-off date 31.03.2015. Comparing vaccinated vs. non-vaccinated group. Whole cohort, n=101 (Breslow test p=0.049; log-rank test p=0.239).

Discussion

Prostate cancer is the most common malignancy in men [115]. In case of a localized disease, radical prostatectomy and radiotherapy are available as treatment options with a curative intent. The outcome of patients with prostatectomy mainly depends on clinicopathological risk parameters such as T-stage, Gleason-Score, PSA value and surgical resection margin status [93]. Mainly in the group of patients with high risk features, a significant proportion experiences biochemical disease relapse. Not all of the patients with biochemical relapse will develop clinical disease recurrence or metastases. However, the rise of the serum PSA after prostatectomy is considered as an event often preceding recurrence and dissemination of tumor disease. The optimal treatment of these patients with biochemical recurrence is an issue of ongoing controversies. In the case of a slow PSA rise years after prostatectomy and low risk features in the prostatectomy sample, a local recurrence is most probable. Patients suspected to have local recurrence are generally treated with radiotherapy. Patients with PSA values >0.5 ng/ml, a fast increase of serum PSA and high risk features in prostatectomy are at increased risk of having a systemic disease relapse [92]. The gold-standard for metastatic disease is antihormonal treatment. Current imaging methods are often unable to detect systemic disease even though the tumor might indeed have spread systematically. The optimal moment for starting hormone deprivation therapy in patients with biochemical relapse but no disease spots detected in imaging is critically discussed, since there is no evidence that an early beginning of hormone deprivation treatment improves overall survival. Moreover, the side effects of antihormonal treatment may significantly impact the patients' quality of life and therefore should be taken into account when choosing this treatment option.

Cancer is considered to be associated with significant changes in the immune system. Moreover, it is broadly accepted that modulation of the immune system might be a promising approach to modify disease biology. Vaccination-based therapies have shown outcome improvements in various malignancies including

prostate cancer. Sipuleucel-T is an autologous APC-based vaccine approved for the treatment of castration resistant prostate cancer. As many of the vaccination based treatments have only limited side effects, it has been recently discussed whether vaccination based treatment might be a promising option for the treatment of biochemical relapse of prostate cancer. At the University hospital of Tuebingen, a phase I/II trial was conducted evaluating the effectiveness of a multi-peptide vaccination for patients presenting with biochemical recurrence but no visible disease recurrence. The aim of the present study was to compare the oncologic outcome of those patients who received vaccination within the trial with the outcome of the patients screened for the trial but not receiving vaccination.

In total, 101 patients with biochemical recurrence after prostatectomy were included in the study. Of these, 36 patients received vaccination treatment within the phase I/II trial. The proportion of patients with a cancer specific treatment between prostatectomy and screening for the trial was 36.6%. The five year recurrence free survival and overall survival rates were as high as 70.7% and 95.8%. In patients receiving vaccination treatment, the time to clinical disease recurrence tended to be longer compared to the control group not receiving vaccination treatment. Moreover, the time to first cancer-specific therapy after screening for the study was significantly longer in those patients receiving vaccination treatment. No differences in overall survival and cancer specific survival were observed between the two groups after a median follow-up of 64 months. The time to initiation of androgen deprivation therapy did not differ between the two groups in the primary analysis and demonstrated a slight and significant improvement for the vaccinated patients in the re-analysis at March 2015, .

The minority of patients with biochemical recurrence experiences clinical disease relapse and cancer specific death

One of the major observations of the present study is that the proportion of patients developing clinical recurrence, metastasis or cancer related death after biochemical recurrence is relatively low regardless of the treatment offered following biochemical recurrence. This is in accordance with other studies showing low rates of clinical recurrence and cancer specific mortality in patients with biochemical recurrence after radical prostatectomy. Roberts et al. did a retrospective analysis of 2809 patients who underwent RP between 1987 and 1993. PSA values were routinely measured after surgery; the clinical disease course was assessed through bone scan, DRE and ultrasound. They observed that 31% of the patients had a biochemical recurrence. For these patients the systemic progression-free survival rate was 94% after 5 years and 91% after 10 years from the time of biochemical relapse [152]. Freedland et al. performed a retrospective analysis of 379 patients with biochemical recurrence after RP. They observed a 10-year cancer specific mortality rate of 27% [153]. In contrast to the above mentioned studies our cohort included a significant proportion of patients having received adjuvant cancer specific treatment. The outcome of patients with biochemical recurrence after adjuvant radiation therapy has been reported in two studies. In a cohort of 134 men with biochemical recurrence following RP plus adjuvant radiation 31.5% experienced systemic progression and 14.2% died of prostate cancer after a median follow-up of 13.1 years [154]. Abdollah et al. observed a 10-year cancer specific mortality rate of 21.5% in a cohort of 336 patients with biochemical recurrence after adjuvant radiotherapy and RP [155]. These studies showing a favorable outcome after adjuvant therapy following prostatectomy concur with the results of the present study.

Time to clinical recurrence is delayed in patients receiving multipeptide vaccination

In the present study, the time to clinical recurrence was longer in patients who received multipeptide vaccination compared to the control group. This was statistically significant in the group of patients who did not receive any additional treatment between prostatectomy and screening. We consider this group to be

more homogenous than the remainder of the patients. The delayed time to clinical recurrence indicates that radiological non-visible tumor foci causing an increase of the serum PSA are affected by vaccination treatment which may cause growth inhibition. The fact, that the time to occurrence of distant metastases was not statistically different between the vaccinated patients and the non-treated control group indicates that the effect of vaccination might be different on cancer cells residing in the former surgical bed than disseminated tumor cells. However, several studies have indicated that disseminated tumor cells in the blood stream or bone marrow are also responsible for local recurrence [156, 157]. Therefore, this statement has to be considered with caution.

Most of the previous vaccination trials for prostate cancer have been conducted with CRPC patients and the clinical endpoint of time to progression was not affected by the treatment. All three trials that led to the approval of sipulecuel-T did not show any improvement in TTP for the vaccinated group compared to the placebo group [138, 139]. The phase II PROSTVAC-VF trial showed equal results, since it did not meet its primary endpoint, a prolongation of TTP [144]. The effect of vaccination in these trials was observed through the improvement in survival endpoints such as overall survival and cancer specific survival (see discussion below). It must be said that tumor dynamics while progression of the disease are different between CRPC patients and patients who have recently experienced biochemical tumor relapse, so the comparability of the data between the sipuleucel-T and PROSTVAC-VF studies and our study is limited.

.

Effect of vaccination on time to subsequent cancer specific therapy

In our study, patients receiving no vaccination treatment were set earlier on a further anti-cancer treatment than patients receiving vaccination. In the primary analysis including follow up data until 2012, this difference was statistically significant for the whole cohort only when applying the Wilcoxon test for analyzing the Kaplan Meier estimates. In this case the Wilcoxon test is more appropriate than the log-rank test since the crossover of the Kaplan-Meier

curves indicates a non-constant event rate in both groups [158]. In the secondary analysis the significant this difference persisted comparing mean time span to the subsequent therapy.

There are several potential causes for the delay of subsequent therapies. The first reason could be that the shorter time to clinical recurrence may have led to earlier initiation of an anticancer treatment in non-vaccinated patients. The majority of patients received either hormone deprivation therapy or radiation therapy as first subsequent anticancer treatment. The fact that the time to hormone deprivation treatment was not significantly affected by vaccination indicates that vaccination may have rather delayed the first treatment in patients receiving radiation treatment. This is in accordance with the above mentioned observation that vaccination seems to have a different effect on local and systemic recurrences. Regardless of these facts, the secondary analysis showing a significant difference between the two groups after more than two years suggests a profit of the patients from the vaccination therapy. This was observed in the first months of follow-up reflected by the significant difference as measured by the Breslow test, which is more sensitive to detect early differences in survival between the groups while the log-rank test did not show a significant difference.

However, the delay in the time to subsequent therapies may be also caused by differing criteria used by the treating urologists to initiate another treatment in patients already receiving vaccination treatment and those without vaccination treatment. The optimal time point of both radiation therapy and hormone deprivation therapy in patients with relapse after prostatectomy is still a current topic of controversy [109]. Whereas some patients receive treatment at a low level of PSA, others are set on treatment only in the case of visible recurrence or metastases. In our study, five patients of the control group received hormone deprivation therapy immediately after screening failure for the clinical trial. Other patients received hormone therapy only several months after screening, even though the performance status and disease stage did not differ significantly from the other patients. The vaccination treatment may have led to a later

initiation of radiation or antihormonal therapy as the treating urologists may have already considered the vaccination as a treatment with an anti-cancer-specific effect, although it was applied in the framework of a clinical trial and although the trial was designed not to affect further indicated treatments. To partially control for this bias, we performed an analysis where we compared the time to next therapy from last vaccination (in the case of vaccinated patients) and time to next therapy from screening (in the case of non-vaccinated patients). Since in this analysis we observed no difference between both groups, this might indicate that the application of vaccination treatment may indeed have influenced the decision-making process regarding subsequent treatments.

Hence, the initiation of another treatment has to be considered as an endpoint that is strongly influenced by individual differences in the management of relapse of prostate cancer. As mentioned above, criteria to start a subsequent treatment (including radiation and hormone deprivation therapy) vary broadly between different urologists.

Effect of vaccination treatment on time to hormone deprivation therapy

We did not observe a significant difference in the intervals between screening and initiation of hormone deprivation therapy in any of the two groups. The secondary analysis demonstrated a significant difference between the groups if the Breslow test was used, which is more sensitive to detect early differences. Nevertheless in the long-term the mean time to the initiation of ADT was not different between the groups. This concurs with the observation that time to occurrence of metastases did not differ between vaccinated and non-vaccinated patients, since the occurrence of metastases generally serves as a trigger for initiation of antihormonal treatment [159]. As previously mentioned, there is no clear evidence showing that a treatment delaying initiation of ADT affects overall survival of patients with prostate cancer [61]. Furthermore, hormone deprivation therapy is generally considered as a treatment with a negative impact on the quality of life of patients with prostate cancer. The most important

side effects include loss of libido, hot flushes, fatigue, osteoporosis and metabolic disorders [63]. Therefore, there is an important unmet need for therapies potentially delaying or replacing hormone deprivation therapy without impairing oncologic outcome. The endpoint of time to initiation of hormone deprivation treatment should not be considered as a strong surrogate marker for oncologic outcome as the optimal time point for initiation of antihormonal treatment is a controversial issue in urology.

Effect of vaccination treatment on overall and cancer specific survival

Our results indicate that multipeptide vaccination does not lead to improved cancer-specific and overall survival in patients with biochemical recurrence. This differs to other trial results in prostate cancer observing a significant improvement of survival in patients with CRPC. The IMPACT trial (Immunotherapy for Prostate Adenocarcinoma Treatment) enrolled 512 asymptomatic CRPC patients. Patients were either treated with sipulecuel-T or placebo in a ratio of 2:1. The primary end point was overall survival, which was significantly improved in the sipulecuel-T group (sipuleucel-T 25.8 months vs. 21.7 for placebo). A phase II, double-blind randomized study for PROSTVAC-VF was conducted with 122 CRPC men. Although the study failed to meet its primary endpoint of prolonged time to progression, an improvement in the OS for the PROSTVAC-VF group versus the placebo group could be observed (median OS 24.5 vs. 16.0 months)

There are several potential reasons for the discrepancy of the results of our study and these trials. First, in our study the follow-up was limited and the overall rate of deaths was low. This is in accordance with prior studies showing low cancer-specific mortality rates in patients with biochemical relapse. We cannot exclude that after a longer follow-up and an increased rate of events the survival differs between both groups. Second, the clinical setting was different in our setting compared to other studies. Phase II and III trials for Sipuleucel-T and Prostvac-VF were all performed in patients with castration resistant prostate

cancer. Castration resistant prostate cancer is considered to have significant biologic differences compared to castration sensitive prostate cancer (CSPC). Castration resistant cancer cells have been shown to exhibit various alterations compared to castration sensitive cells [160]. Although to date no evidence exists showing differences in tumor-immunology relevant aspects between CRPC and CSPC, the results of the present study, among other studies, indicate that CSPC might be more suitable for application of immune-system-targeting treatments.

The observed differences in survival benefits in the present study and other phase II/III trials may be also related to different efficacies of different vaccination strategies.

Differences in immunotherapy based approaches for prostate cancer.

Although considerable advances have been made in the field of tumor immunology in the last decade, the optimal application of tumor vaccines is still unclear. From what is known until now though, it seems clear that when it comes to cancer vaccines it is of the outmost importance to select adequate immunological targets in order to create a sufficiently large immune response and achieve long term immunological memory [161] to accomplish a positive effect. In the current study, a multipeptide vaccination was applied. The peptides composing this vaccine were carefully chosen on the basis that these epitopes would most likely create an adequately large, PC specific immune response and that the combination would counteract possible tumor evasion mechanisms. Because the majority of the peptides were HLA-A2-restricted epitopes, the vaccinated patients had to be HLA A2 positive. This limits the applicability of the peptide vaccine, since only estimated 40 to 50% of the german population are HLA A2 positive. The present phase I/II study had the evaluation of the drug tolerance and immune response as primary endpoint and PSA response as secondary endpoint. The multipeptide vaccine was well tolerated with only two grade 3 adverse effects.

The personalized peptide vaccine from Noguchi et al. showed positive results regarding progression-free survival and overall survival after the first round of treatment. Also, there was a significant immune response after the vaccination treatment measured by IgG titers and IFN-*γ* release essay, though there is not a clear correlation to a clinical benefit. Treating the patients only with those peptides which caused a strong immune reaction during pre-vaccination is probably crucial for the patients to achieve an efficient immune response. However, it makes the production of this vaccine, similar to sipuleucel-T, costly and more difficult. This study was conducted with CRPC patients so the question about how CSPC patients would respond remains open. The study was also limited to HLA A2 and HLA A24 patients, since the peptides were restricted to these HLA molecules respectively, which also reduces the number of patients that can be treated.

Sipuleucel-T treatment uses PAP loaded tumor cells which induce a tumor targeted T cell response. The immune response induced by this treatment was measured by titers of antibodies against the antigen PA2024. The patients treated with the vaccine showed elevated antibody titers and higher T-cell proliferation rates than the patients in the placebo group [138]. Of note, there was no correlation between survival and T-cell response. Sipuleucel-T was approved by the FDA since it was shown to provide a benefit in OS for the treated group and was well tolerated by the patients. However, several issues still have to be considered while using it as a standard therapy. The identification of patients with clinical benefit is difficult as patients continued to have disease progression. Moreover, the optimal timing for further therapies following Sipuleucel-T treatment is challenging due to the lack of surrogate parameters of clinical benefit. The target group of the trial was CRPC patients and therefore it is unknown how patients in a different disease stage would react. However, a tendency towards benefit could be observed, independent from Gleason Score and other risk factors, suggesting sipuleucel-T could be applicable for other patients as well. Furthermore, it is an individualized therapy which makes the production expensive and time consuming.

The combination in the PROSTVAC-VF treatment presents several advantages in promoting tumor immunity: first, the vaccinia virus is immunogenic itself, thus contributing to create an immune response. Second, when host's cells get infected, necrosis causes them to release pro-inflammatory signals and PSA which is taken from DCs to be processed and presented on their surface. The pro-inflammatory substances released also help to activate DCs enhancing the immune system's reaction. Furthermore, these vectors present the benefit of being able to deliver a great amount of target loads and are also relatively easy to produce and manipulate [162]. The vaccine creates an immune response measured by an increase of PSA specific T-cells. The toxicity is low, presenting little adverse effects. Compared to sipulecuel-T, it presents the advantage that the manufacturing process of PROSTVAC-VF is much less complicated, since it is not an individualized vaccine. The results of the ongoing phase III trial should be able to provide more information about the effects of PROSTVAC-VF on PC [146].

The results from the phase I/II trial with a DNA based vaccine encoding PAP and pro-inflammatory agents showed an antibody and T-cell response against PAP [147]. Also a deceleration of PSA rate rise could be observed. Even though it was a small group of patients, the data demonstrates the vaccine caused an immune response in the patients. A long-term follow-up of the patients included in the phase I/II trial or a larger trial with this vaccine could probably provide further information.

GVAX is a mixture of two allogeneic prostate tumor cell lines treated to secrete GM-CSF. They do not proliferate since they are previously treated with radiation. GVAX presents the advantage that the vaccine can be produced in large quantities, making the manufacturing costs lower and thus increasing the number of patients that can be treated. Because it contains whole tumor cells, the number of tumor antigens delivered (known and unknown prostate cancer tumor antigens) is considerable. This should provide a major advantage compared to the other vaccines, since, provided GVAX succeeded in creating an immune response, the variety of activated T- and B-cells would be very

large. Also, it is believed that with a larger variety of tumor antigens, the possibilities for the tumor to develop evasion mechanisms from the immune system are reduced [163]. However, since both studies testing the effect of GVAX were stopped prematurely, no definite statement can be made about the immunologic effectiveness of this treatment [141].

Immunotherapy for PC has been showing promising results and it seems that this approach should be further encouraged and analyzed in earlier disease stages instead of CRPC patients. However, the results of the present study showing a low number of cancer related deaths after a mean follow up of more than 5 years indicate that future studies using immunologic therapies in this stage of disease require a follow-up of at least 10 years. Alternatively, reliable surrogate parameters for survival improvement in patients treated with vaccination therapy have to be identified. As the rate of patients with radiologically detectable disease is lower in earlier stages of disease, the use of radiologic response is questionable. PSA has been assessed in many studies; however, to date there is a lack of data on correlation of PSA response and overall survival in patients treated with vaccination. Immunologic monitoring of patients in these trials provides a promising alternative but still remain a challenge, since there are no standardized procedures to follow immune cells' activities after treatment.

Future of immunobased therapies in recurrent prostate cancer

The improvement of overall survival in patients with prostate cancer by application of Sipuleucel-T has provided a proof of principle showing that immunobased therapies can have a significant benefit for patients with CRPC. Several other studies have supported the concept of prostate cancer as a tumor susceptible to modulations of the immune system. These observations have raised the hope that the application of immunotherapies may be even more promising in earlier stages of prostate cancer as the number of tumor cells the immune system has to cope with is lower. In fact, animal studies indeed show

that vaccination therapies show better results when the tumor burden is smaller [164]. However, to date no clear evidence exists that modulation of the immune system in the context of castration sensitive prostate cancer provides clinical benefit. The results of the present study should encourage further research on immunotherapies in the context of early disease relapse. Although the trial which formed the basis of the present study was not designed to detect a benefit on cancer-related outcome, the observed differences in time to recurrence should encourage the initiation on further trials addressing this issue. To investigate both recurrence-free and overall survival, these trials should include regular imaging, clear criteria for disease progression and a long term-follow-up of the patients. If immunotherapy based approaches should indeed demonstrate a significant benefit on oncologic outcome, the application of hormone deprivation therapy in recurrent prostate cancer would have to undergo complete re-evaluation and additional trials including different sequences of vaccination and hormone deprivation therapy would have to be performed.

Limitations of the study

The present study exhibits various major limitations. First, the clinical trial forming the basis of this study was not designed to evaluate the efficacy of multipeptide vaccination on cancer-specific outcome as its primary endpoint. The follow-up of patients varied broadly from patient to patient in both groups. Also, no regular imaging schedules were applied neither for patients included in the trial nor the control patients. As the patients' treating urologists were responsible for the follow-up, the differences observed between the two groups might be due to differences in follow-up schedules. The group of patients screened for the study was quite heterogeneous with a high proportion of patients having received additional cancer-specific therapies between prostatectomy and vaccination. Therefore, this cohort might not be representative for a typical cohort of patients with biochemical recurrence. The different therapies performed between prostatectomy and screening for the

study may have a major influence on the study outcome. To control for this bias, sub-analyses in patients without any cancer-specific therapy between vaccination and screening for the study were performed. The homogenicity of the vaccinated cohort was limited from the beginning since different adjuvants were applied during vaccination as determined by the study protocol to analyze the induction of the immune response. This however served to the purpose of assessing the impact of these adjuvants, so the heterogeneity of the group was taken into account. The follow-up of the study was limited preventing the power of the study for evaluating the effect of vaccination on survival.

Summary

PC is the most common cancer entity in men in Germany. The broad use of PSA screening has led to a significant increase of tumors diagnosed in a localized stage. Many of these patients undergo surgical therapy or radiation, although there is no clear evidence that this has a significant impact on the course of disease. Both surgery and radiotherapy are performed with a curative intention. However, a significant proportion of patients experiences biochemical disease recurrence. The optimal treatment of these patients with biochemical recurrence but no evidence of clinical recurrence is discussed controversially. A peptide vaccination against tumor specific surface antigens has shown efficacy in several tumor entities. Whether this approach might provide benefit for patients with biochemical relapse after prostatectomy is unclear. The aim of the present study was to evaluate the clinical outcome of patients taking part in a clinical trial evaluating the efficacy of a multipeptide vaccination for the treatment of patients with biochemical relapse. We analyzed data of 101 patients with PC who underwent RP and had a biochemical disease recurrence. 36 patients were treated with a multipeptide vaccination therapy and 65 served as a control group.

We observed that in patients treated with vaccination therapy, the time to clinical recurrence tended to be longer compared to non-vaccinated patients. Moreover, we observed that the first non-vaccination therapy could be delayed in the group of patients receiving vaccination therapy. We observed no impact of vaccination therapy on cancer-specific and overall survival. The time to initiation of hormone deprivation therapy was not delayed significantly by peptide vaccination.

The present study is the first evaluating a multipeptide vaccination for the treatment of biochemical relapse in patients with prostate cancer. In contrast to prior studies in other malignancies, the vaccination resulted in a significant delay of clinical recurrence whereas overall survival, an endpoint which is most frequently affected by immunization based therapies in cancer, was not

changed. These results implicate that immune therapy might have a short to mid-term effect on tumor dynamics in patients with biochemical relapse but does not significantly affects the long-term course of the disease. Although the mechanisms potentially leading to the delay of clinical recurrence are unclear, the results of the present study encourage further investigations on the use of immunotherapy in patients experiencing biochemical relapse after treatment with a curative intention.

Zusammenfassung

Das Prostatakarzinom ist die häufigste Krebserkrankung bei Männern in Deutschland. Die breite Verwendung des Serum PSA als Screening Methode hat zu einer signifikant erhöhten Inzidenz von Tumoren, die in einem lokalisierten Stadium entdeckt werden, geführt. Viele dieser Patienten werden chirurgisch oder mit Strahlentherapie behandelt, obwohl es keine klaren Hinweise darauf gibt, dass diese Therapieverfahren einen erheblichen Einfluss auf den Krankheitsverlauf haben. Sowohl radikale Prostatektomie als auch Bestrahlung werden mit kurativer Intention durchgeführt. Allerdings erlebt ein signifikanter Anteil der Patienten ein biochemisches Rezidiv im Verlauf der Erkrankung. Die optimale Behandlung von Patienten mit biochemischen Rezidiv ohne Hinweise auf ein klinisches Rezidiv wird kontrovers diskutiert. Eine peptidbasierte Impfung gegen tumorspezifische Oberflächenantigene hat für mehreren Tumorentitäten eine gute Wirksamkeit gezeigt. Ob dieser Ansatz einen Nutzen für Patienten mit einem biochemischen PSA Rezidiv hat, ist unklar. Das Ziel der vorliegenden Studie war die Untersuchung des Krankheitsverlaufs von Patienten, die an einer Multipeptidvakzinierungsstudie teilgenommen haben, um einen möglichen Vorteil dieser Therapie für Patienten mit biochemischem Rezidiv zu beurteilen. Wir analysierten Daten von 101 Patienten mit PC, die nach radikaler Prostatektomie einen PSA Rezidiv erlebt haben. 36 Patienten wurden mit einer Multipeptid-Vakzinierungstherapie behandelt und 65 dienten als Kontrollgruppe.

Das mediane Follow-up der Studie betrug 64 Monate. Die Zeit zum klinischen Wiederauftreten der Erkrankung war für die Patienten in der Vakzinierungsgruppe im Vergleich zu der Kontrollgruppe signifikant verlängert. Außerdem konnte beobachtet werden, dass die Zeit bis zur nächsten Prostatakarzinom spezifischen Therapie in der Gruppe der geimpften Patienten verzögert werden konnte. Die Zeit zur Einleitung einer antihormonellen Therapie wurde durch die Vakzinierung nicht signifikant verzögert. Die

Vakzinierungstherapie zeigte auch keinen signifikanten Einfluss auf das krebsspezifische Überleben oder auf das Gesamtüberleben.

Diese Studie ist die erste, die die Wirkung einer Multipeptidvakzinierung bei Patienten mit biochemischen Rezidiv analysierte. Im Gegensatz zu früheren Studien in anderen Tumorerkrankungen, führte die Impfung zu einer erheblichen Verzögerung des klinischen Rezidives, wohin Gesamtüberleben, ein Endpunkt, der am häufigsten zur Evaluierung von Immuntherapien bei Malignomen benutzt wird, nicht verändert wurde. Diese Ergebnisse deuten darauf hinaus, dass Immuntherapie eine kurze bis mittelfristige Wirkung auf die Tumordynamik bei Patienten mit biochemischen Rezidiv haben könnte. Ein Hinweis für einen Einfluss auf den langfristigen Verlauf des Prostatakarzinoms liegt bisher nicht vor. Obwohl die Mechanismen, die möglicherweise zur Verzögerung des klinischen Rezidiv führten, unklar sind, sollten die Ergebnisse dieser Studie das Interesse für Immuntherapien bei Patienten mit einem PSA Rezidiv wecken und die Forschung in diesem Bereich fördern.

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Statement of own contribution

I, Claudia María Avilés Escobar, hereby declare that this doctoral dissertation was written by myself only. I did not make use of any other means than the ones listed on this document. Any citation was correctly marked as such.

The idea to conduct this study came from the department of Urology and the department of Immunology at the University of Tuebingen. The persons involved in the development of this study were Prof. Dr. A. Stenzl, Prof. Dr. J. Bedke, Prof. Dr. C. Schwentner, Mr. J. Hennenlotter and Prof. Dr. H.G. Rammensee and several other staff members from the above mentioned university departments.

The vaccination trial was conducted at the urology clinic in Tuebingen under the supervision of Dr. Feyerabend, Prof. Dr. Stenzl and Prof. Dr. Bedke. The multipeptide vaccine was developed in cooperation with the department of immunology under the supervision of Prof. Dr. Rammensee. I did not take part in any clinical trial procedures.

I gathered all follow-up data of the trial patients and the patients who formed the control group by myself. I performed the analysis and interpretation of the data and results under the supervision of Mr. Hennenlotter and Prof. Dr. Bedke who gave me their input and helped me correct and improve the statistical analysis. The data was then matched with patient information provided by Prof. Dr. Bedke who perfomed the updated analysis of March 2015.

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