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Topic:

Can isoflavones influence prostate specific antigen serum levels in localized prostate cancer?

A systematic review

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for the
attainment of the academic
degree

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Table of contents

List of tables

Figures

List of abbreviation

ADT	Androgen deprivation therapy
AR	Androgen receptor
ASR	Age-standardised rate
BCR	Biochemically recurrent prostate cancer
BMI	Body mass index
BPH	Benign prostatic hyperplasia
BT	Brachytherapy
DER	Digital rectal exam
EQ-5D	EuroQol- 5 Dimension
ER	Estrogen receptor
GCP	Genistein combined polysaccharide
HDL, LDL	High-density lipoprotein and low-density lipoprotein
HIFU	High-intensity focused ultrasound
MRI	Magnetic resonance imaging
PCa	Prostate cancer
PSA	Prostate-specific antigen
QALY	Quality-adjusted life-year
RCT	Randomized controlled trial
RR	Relative risk
SHBG	Sex hormone-binding globulin
SR	Systematic Review
TRUS	Transrectal ultrasonography
TSH	Thyroid Stimulating Hormon

1. Introduction

1.1. Epidemiology

Prostate cancer is the most common diagnosed cancer in males in Germany. With 57,400 new cases in 2014, it accounted for nearly a quarter of all male carcinomas (23%). Despite a high 5-year survival rate of over 90%, prostate cancer was the second most common cause of cancer death, with 13,704 cases even before colon cancer. These accounted for 11.3% of all cancer-related causes of death [1]. In comparison, prostate cancer was the second most commonly diagnosed tumor worldwide, with 1.1 million documented cases in 2012 [2].

In this context, it should be noted that many deceased men may be found to have prostate carcinoma that was not prominent during their lifetime and would not have affected the patient's quality of life. The prevalence of these clinically insignificant prostate carcinomas increases with age and is as high as 60% in those over 80 years of age. The incidence of prostate cancer diagnoses, moreover, varies in different regions of the world, being highest in Western and Northern Europe (age-standardised rates [ASR] of 94.9 and 85 per 100,000) and other geographical areas of the western world like Australia/New Zealand and Northern America (ASR of 111.6 and 97.2 per 100,000). By contrast, the incidence in Eastern and South-Central Asia is low (ASR 10.5 and 4.5). The differences could be traced back to the systematic use of prostate specific antigen (PSA) tests and the aging population in the Western world. The variation in mortality rates is comparatively low in the different regions, while they tend to be higher in African-descended populations (Caribbean, 29 per 100,000 and Sub-Saharan Africa 19-24 per 100,000), rates are lowest in Asia (2.9 per 100,000 in South-Central Asia) [2; 3; 4].

Prostate cancer is essentially a tumor of older men, so it becomes a major health problem in countries with high and rising life expectancies. The population of men over 60 is expected to triple to 2 billion by 2050, leading to an unavoidable increase in PCa incidence in the future [5].

1.2. Localized prostate cancer

In about 90 percent of people with prostate cancer, the tumor is confined exclusively to the prostate (clinically localized tumor). Localized prostate cancer is by definition limited to the prostate only and has not spread throughout the body. The emphasis of this report is on this type of tumor, the clinically localized prostate cancer (T1-T3a), while focally advanced (T3b-T4), recurrent and metastatic prostate cancers are not covered [6].

At an early stage, the clinically localized PCa is less symptomatic; early urinary problems such as hematuria, a weak urine flow and associated burning, inability to urinate or frequent urination at night may occur. Based on this symptom constellation, a physical examination, a determination of the prostate specific antigen value (PSA) and a possible biopsy can be used to search exploratively for a carcinoma. After pathological confirmation of a carcinoma in a biopsy, the tumor grade is determined based on the Gleason score. Since most tumors typically have several histological patterns, the Gleason score adds together the two most common grade patterns in one tumor, with 5 different patterns to distinguish [7]. Accordingly, the Gleason score includes a scale of 2-10.

Increasingly, a new classification system called the ISUP Grade Group System is being used. This is a modification of the Gleason scoring system and is more precise in terms of tumor prognosis. The ISUP grade-group system uses five grades.

- Grade group 1 (Gleason score 6 or less): low risk; the cancer is slow growing and less aggressive
- Grade 2 (Gleason score 3+4 = 7): Moderately favorable; the cancer is moderately aggressive
- Grade 3 (Gleason score 4+3 = 7): Moderately unfavorable; cancer is moderately aggressive
- Grade 4 (Gleason score 8): High risk; the cancer is fast growing and aggressive
- Grade 5 (Gleason score 9 or 10): Highest risk; the cancer is fast-growing and aggressive [86]

Other diagnostic tools that determine the prognosis are PSA level, PSA kinetics (change in PSA level over time, PSA velocity and PSA doubling time), and digital rectal examination (DRE).

In addition, cancer staging is performed. The extent of tumor spread, including size and localization, is assessed. The staging system currently used is the TMN classification of the American Joint Committee on Cancer (AJCC). This includes the extent of the primary tumor (T stages), the spread to neighbouring lymph nodes (N stages) and the presence of distant metastases (M stages). The staging system is shown in Table 1-3.

Table 1: Extent of the primary tumor.*

T Category	T Criteria
Tx	Non-evaluable primary tumor
T0	No detection of a primary tumor
T1 • T1a • T1b • T1c	Clinically silent tumor that is not palpable. Tumour-related histological findings in 5% or less of the resected tissue. Casual histologic findings in more than 5% of tumor tissue resected Tumor detected by needle biopsy on one or both sides, yet not palpable.
T2 • T2a • T2b • T2c	The tumor is palpable and enclosed in the prostate. The tumor comprises half of one side or less. The tumor comprises more than half of one side, not both sides. The tumor comprises both sides
T3 • T3a • T3b	Extraprostatic tumor does not penetrate into adjacent structures. Extended prostate lengthening (on one or both sides) The tumor penetrates into the seminal vesicle(s)
T4	The tumor penetrates into neighbouring structures, apart from the seminal vesicles, such as the external sphincter, rectum, bladder and/or pelvic wall

*Modified after Buyyounouski MK et al. Prostate cancer - major changes in the American Joint Committee on Cancer eighth edition cancer staging manual. CA Cancer J Clin. 2017 May 6;67(3):245-253.

Table 2: **Regional lymph node involvement.***

N Category	N Criteria
Nx	Regional lymph nodes cannot be evaluated
N0	No regional lymph node involvement detectable
N1	Metastases are detectable in the regional lymph nodes.

***Modified after Buyyounouski MK et al. Prostate cancer - major changes in the American Joint Committee on Cancer eighth edition cancer staging manual. CA Cancer J Clin. 2017 May 6;67(3):245-253.

Table 3: **Distant metastases.***

M Category	M Criteria
M0	No formation of distant metastases.
M1 • M1a • M1b • M1c	Distant metastasis Nonregional lymph node(s) Bone(s) Other locations with or without bone involvement

*Modified after Buyyounouski MK et al. Prostate cancer - major changes in the American Joint Committee on Cancer eighth edition cancer staging manual. CA Cancer J Clin. 2017 May 6;67(3):245-253.

During staging, DRE, a transrectal ultrasound examination of the prostate (TRUS) or magnetic resonance imaging (MRI) can be performed, whereby the precision of the staging process is ultimately determined by the location and size of the tumor itself, the examiner's qualifications, and the interpretation of the imaging [8].

In order to anticipate the histopathological stage as well as the aggressiveness of a carcinoma, risk stratification systems were developed. These are combined observations of TMN classification, Gleason score and PSA values with the intention to get a holistic overview. A representative example can be found in the D'Amico classification. However, it is a three-stage classification with low-, intermediate-, and high-risk. It is intended to predict a possible tumor progression so that the probability of success of an early therapy can be estimated [8].

- Low risk: a Gleason score of 6 or less, a clinical stage of T1c or T2a and a PSA level of 10 ng/mL or less.
- Intermediate risk: a Gleason score of 7, in clinical stage of T2b and a PSA level of 10-20 ng/ml.
- High risk: a Gleason score of 8-10, in the clinical stage of T2c and a PSA score greater than 20 ng/ml.

Prostate carcinomas of stages I and II have often been described as "clinically localized" tumors, while "locally advanced" carcinomas have been specified as those that extended beyond the prostate capsule but did not reach the seminal vesicle. Since 2013, clinically localized carcinomas have been defined as clinical stages T1-T3a, NX, M0 or stage I-IIIa, according to the clinical guidelines of the National Comprehensive Cancer Network (NCCN). Locally advanced tumors have a very high risk of recurrence and are classified as stages T3b-T4. In general, it can be assumed that the use of other modified risk assessment schemes, such as the CAPRA (Cancer of the Prostate Risk

Assessment) score and the use of alternative biomarkers (e.g. actinin alpha 1, Derlin 1), will make diagnosis and therapy more targeted and efficient in the future [8].

Based on these schemes, treatment options are balanced against each other. The age and health status of the patient, life expectancy, probability of progression without therapy, costs and potential side effects (e.g. incontinence or sexual dysfunction) are taken into account in the decision-making process. After assessing the patient's general state of health, the following therapies may be considered for the treatment of localized prostate cancer:

- Observation or watchful waiting (largely passive follow-up with symptom management)
- Active monitoring (includes regular PSA value examinations and biopsies)
- Hormonal therapy (e.g. androgen deprivation therapy [ADT])
- Radical prostatectomy, including laparoscopic or robot-assisted prostatectomy (RALRP)
- External radiotherapy (EBRT), including conventional irradiation and other modulated radiation techniques
- Interstitial brachytherapy (BT), as internal radiotherapy
- Cryotherapy, in the context of temperature-induced cell destruction with freezing/thawing gases
- High Intensity Focused Ultrasound (HIFU) [8]

Even after definitive therapy, such as external or internal radiotherapy or radical prostatectomy, about 35% of the patients treated annually experience failure of primary therapy. Biochemical failure or a biochemical recurrence (BCR) manifests itself in a rise in serum PSA levels within 10 years of therapy [9].

Due to the toxicity and associated side effects of conventional therapy as well as considerable anxiety states of patients during the period of vigilant waiting or treatment intervals, non-hormonal treatment alternatives have gained in importance. The prevalence of herbal medicine use ranges from 1.2% to 24.5%. Due to this increase in importance, clinical studies have become increasingly relevant for demonstrating the efficacy of non-hormonal herbal methods (phytotherapy). Thus, several observational studies have already shown a reverse association between soy intake and prostate cancer risk [10; 11]. In particular, soy isoflavones, as well as green tea (Camelia sinensis), catechins, lycopene, curcumin from turmeric (Curcuma longa), sulforaphane, indole-3-carbinol from broccoli (Brassica oleracea) and many other plant substances have become the subject of scientific studies [12].

Before placing the main focus on soy isoflavones, the importance of prostate specific antigen (PSA) for prostate cancer must be discussed in detail.

1.3. Prostate-specific antigen

PSA (prostate-specific antigen) is an enzyme from the group of serine proteases with Kallikrein-like properties. It is predominantly produced by the epithelial cells of prostate tissue and is consequently an organ-specific marker.

According to the German guideline, the PSA value can be determined for the early detection of prostate carcinoma after informing about possible advantages and disadvantages [5; 87]. In particular, the significance of positive and negative test results, overdiagnosis and possible therapeutic consequences should be addressed [5; 13; 14; 15; 16; 17]. Thus, it is not a cancer-specific parameter, since prostatitis or benign prostatic hyperplasia also lead to an increase in PSA. Also, not every clinically significant PCa manifests itself by an elevated PSA level [5].

Therefore, a digital-rectal examination can be recommended in addition [87].

In the case of a controlled PSA level of ≥ 4 ng/ml at the initial screening consultation, or a result suspicious for carcinoma on digital-rectal examination, or in the case of an abnormal PSA increase, a prostate biopsy is subsequently recommended to confirm the diagnosis. Furthermore, the diagnosis can be supplemented by imaging techniques [87].

More important is the significance of the PSA level in the follow-up of diagnosed prostate cancer to assess disease progression [87].

1.4. Isoflavones

The epidemiological findings that the incidence of PCa in Asian countries is much lower than in Western countries have brought the lifestyle and eating habits of these people into focus. Studies have shown that Asian men generally consume more soy-based foods than Western men [10; 18]. The relevance of soybeans arises from the fact that several natural anti-carcinogens have been found in them, e.g: Protease inhibitors, different types of phytosterols and especially isoflavones [19; 20; 21]. Observations that the incidence of PCa of Asian people who emigrated to Western countries adapted to the local population after a few generations also increased the attention to isoflavones [22].

Isoflavones belong to the group of phytoestrogens and therefore have structural similarities with animal estrogen. Due to its low affinity to estrogen receptors, it has a certain estrogenic effect [23; 24; 25]. Daidzein, glycitin, equol, biochanin A and especially the well studied genistein are important isoflavones [26]. However, equol is formed from daidzein as a degradation product by the microbial intestinal flora [27]. Important sources for isoflavones are soy products such as beans and tofu but also kudzu root, American groundnuts, those of lignans are flaxseed, green tea and strawberries [28].

1.4.1. Isoflavones and PCa

Prostatic carcinogenesis as well as progression are probably influenced by androgens. Since PCa develops from androgen-dependent tissue, it also contains androgen receptors (ARs) [29]. Furthermore, the plasma androgen-to estrogen ratio seems to decrease with age, suggesting that estrogens may also be involved in tumor progression [30]. In vitro and in vivo studies have shown that estrogens play a role as potential agents in hormone-dependent malignancies such as PCa. Estrogens not only contribute to PCa development through estrogen receptor (ER)-induced interactions, but also influence carcinogenesis through epigenetic modifications, estrogen imprinting, hyperprolactinemia, direct genotoxicity, and inflammatory and immunological changes [31; [32].

Various epidemiological studies suggest that consumption of isoflavone containing foods reduces PCa incidence. Studies at the cellular level have shown that isoflavones act via various hormone-like and non-hormone-like mechanisms. In addition to the antagonisation of estrogen- and androgen-mediated signalling pathways, the inhibition of tyrosine kinases, the regulation of the cell cycle and apoptosis are also important [26]. Non-hormonal effects were observed in vitro in androgen-independent PC3 prostate cancer cell lines. Effects exerted by isoflavones to suppress cancer by pathways targeting cell cycle and apoptosis include G2/M arrest and p21 expression [26; 33]. Further modulating effects on cell proliferation, such as fibroblastic stromal cells, endothelial cells and immune cells, and inhibition of angiogenesis and tumor cell metastasis are discussed [34]. The most significant effects are achieved by binding the estrogen receptor β (ER-β). Human prostate stem cells/early progenitor cells mainly express the estrogen receptor isoforms ER-α and ER-β. ER-α and ER-β are competing systems. It is assumed that ER-β has an antiproliferative effect and can therefore counteract carcinoma development. The observation that the expression of ER-β in localized

malignant tumors is reduced in comparison to benign lesions also suggests a protective effect [35; 36]. In contrast, it is assumed that ER-α has a proliferative and accordingly a tumor progression enhancing effect [32]. Studies showed that due to its structural similarity to 17β-estradiol, genistein binds to ER-β with a higher affinity than ER-α. The chemopreventive potential is consequently achieved by the increased activation of the tumour suppressor ER-β [35; 36].

Other mechanisms, such as the defence against antioxidants, downregulating androgen receptor expression, inhibiting PSA secretion and regulating sex steroid hormone synthesis and secretion, are also investigated. In this respect, isoflavones have been shown to increase serum sex hormone binding globulin (SHBG) through increased hepatic synthesis, which reduces the bioavailability of testosterone [24; 31; 37; 38].

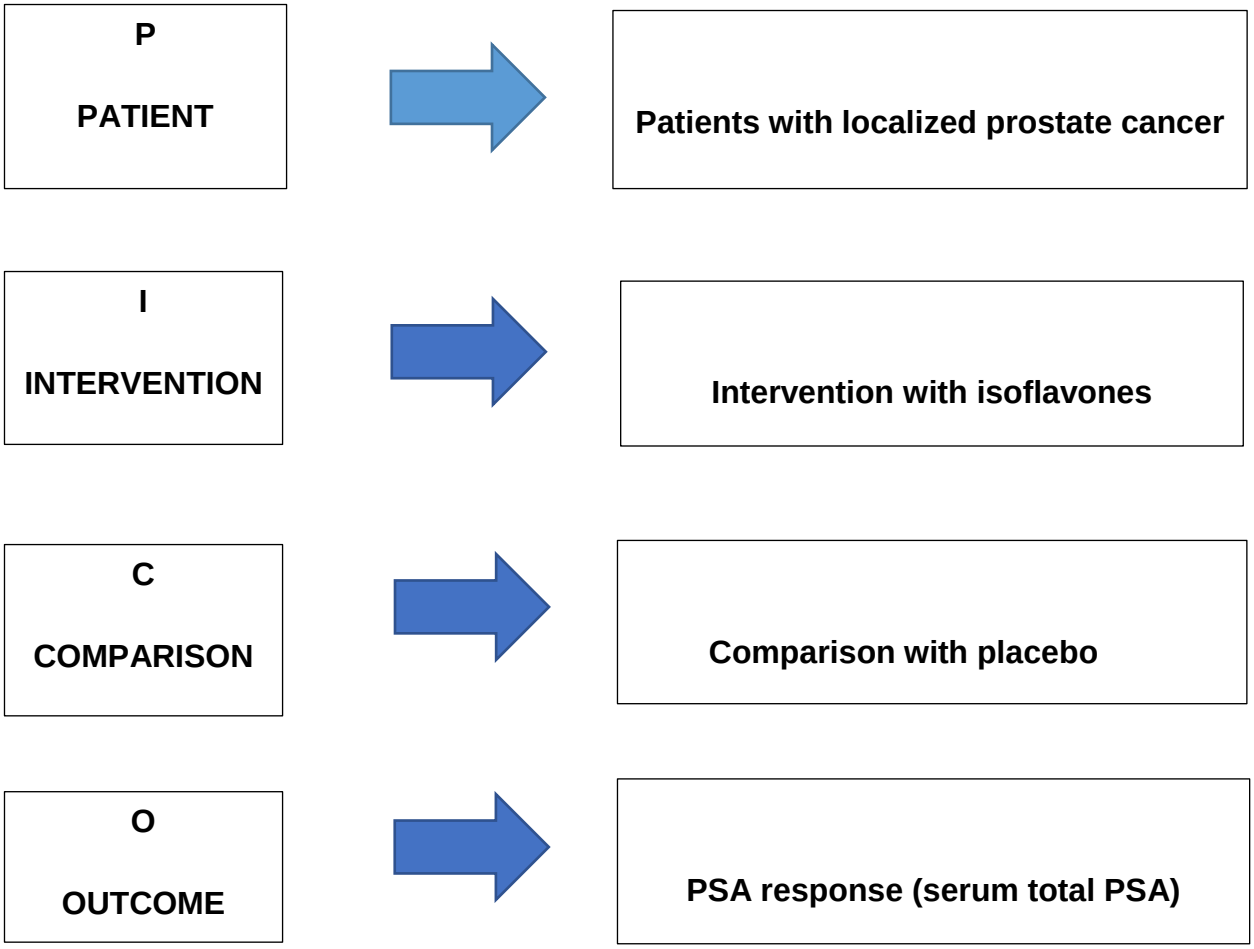
1.5. PICO scheme and objectives of this work

In scientific work, there is often a discrepancy between the desire for the greatest possible gain in knowledge and a limited data situation in the literature. The PICO (Patient, Intervention, Comparison and Outcome) scheme is a format that finds a compromise in between. Defining a question in terms of a specific patient problem is helpful to find clinically relevant evidence in the literature. Based on this concept, the primary objective of this review is to summarize the evidence for the use of isoflavones in localized prostate cancer with respect to PSA response (see Figure 1 below). In the context of chemoprevention, agents can be used on the one hand to reduce the risk of cancer and on the other hand to delay the development or recurrence of cancer. In this work, the effect on tumor progression, which is measured with PSA changes, is examined.

Despite numerous studies addressing the use of isoflavones as a treatment option or chemoprevention in prostate cancer [19; 26; 39; 40; 41; 42; 43; 44],

no systematic review or meta-analysis based on the results of randomized-controlled trials has been performed to date.

Figure 1: PICO scheme



2. Material and methods

The methodological quality was assessed using the recommendations from the Cochrane Handbook of systematic Reviews [45] and the PRISMA Reporting Guidelines [46]. The aim was to summarize the evidence available in the literature regarding our research question with the greatest possible objectivity. A measure of objectivity in this context is the reproducibility of the results. To meet these criteria, we followed standardized steps:

- The formulation of an appropriate review question using the PICO scheme (see Figure 1).
- A literature search based on a comprehensive search strategy (see Table 5) to identify relevant papers.
- Selection of the identified papers by means of formulated inclusion and exclusion criteria (see Table 4).
- Evaluation of the evidence with regard to methodological quality and relevance of content.
- Written summary and interpretation of the results in the format of a Cochrane Review.

2.1. Criteria for considering studies for this review

2.1.1. Types of studies

We considered randomized (RCTs) and quasi-randomized studies (if available) as well as systematic reviews (SRs) and meta-analysis. Other study designs such as cohort studies or case-control studies were not included. Full-text articles and abstracts were considered. No exclusions were made by publication date. We only included German and English publications.

2.1.2. Types of participants

2.1.2.1. Inclusion criteria

Men diagnosed with histologically confirmed, localized prostate cancer were included. We made no other exclusions like age or race.

2.1.2.2. Exclusion criteria

Studies that considered the effect of isoflavone intervention on focally advanced or metastatic tumors or on tumor-like disease such as benign prostatic hyperplasia (BPH) were excluded. Also, those studies that examined the effect on a non-uniformly composed group of patients with different disease geneses were not considered. Another reason for exclusion was therapeutic treatment taking place at the time of the study (radiotherapy or hormone therapy).

2.1.3. Types of interventions

We included any RCTs investigating the effect of isoflavones on men with localized prostate cancer compared to a placebo group. We differentiated all types of isoflavones, such as genistein or daidzein. Dietary supplements, which contained a number of different components or included unspecified phytoestrogens were excluded. Restrictions on dose, frequency, intensity or duration were not made.

2.1.4. Types of outcome measures

Measurement of outcomes assessed in this review will not be used as an eligibility criterion. The primary outcome for this review was serum PSA response. Studies that did not document the patients' PSA values or only to an insufficient extent were excluded. No restrictions were imposed on the time points of measurement. All pre-formulated inclusion and exclusion criteria are presented in the table below (Table 4).

Table 4: Summary of the formulated inclusion and exclusion criteria.

	Inclusion criteria	Exclusion criteria
Types of studies	• RCTs • SRs and meta-analysis	• Other study designs
Types of participants	• Patients with localized prostate cancer	• Focally advanced or metastatic tumors • BPH • Studies that only investigate the effect on tumor risk • Presence of multiple different prostate diseases • Tumor patients undergoing radiation or hormone therapy
Types of interventions	• Isoflavone intake (daidzein, genistein, glycitein, equol, also genistein combined polysaccharide (GCP))	• Phytoestrogens without further specification • Isoflavones combined with other phytotherapeutic substances • Isoflavones combined with drugs • Isoflavones combined with radiotherapy
Types of outcome measures	• Total serum PSA	• Incomplete presentation of PSA values • PSA velocity values only • Tissue PSA concentrations only • Urinary PSA measurements • PSA quotient • Evaluation based on transrectal sonography (TRUS) or digital rectal examination (DRE) only

2.2. Search methods for identification of studies

To gain an impression of the data situation regarding the influence of phytotherapeutic interventions on prostate carcinoma, an exploratory non-systematic literature search was first performed. In the second step, we extended the literature search after formulating a review question. This involved a combination of electronic and manual searches to identify appropriate studies.

2.2.1. Electronic searches

We searched the following databases: MEDLINE via PubMed from 1946 to 2019 (the search strategy is shown in Table 5) and the Cochrane Central Register of Controlled Trials 1900 to 2019. Last search was conducted on January 2019.

Table 5: Search strategy in MEDLINE, adapted for other databases (CENTRAL).

No.	Search
#1	Search ("prostatic neoplasms"[MeSH Terms] OR ("prostatic"[All Fields] AND "neoplasms"[All Fields]) OR "prostatic neoplasms"[All Fields] OR ("prostate"[All Fields] AND "cancer"[All Fields]) OR "prostate cancer"[All Fields])
#2	Search ("prostate"[MeSH Terms] OR "prostate"[All Fields] OR "prostatic"[All Fields]) AND ("adenocarcinoma"[MeSH Terms] OR "adenocarcinoma"[All Fields]) OR ("prostate"[MeSH Terms] OR "prostate"[All Fields] OR "prostatic"[All Fields]) AND ("carcinogenesis"[MeSH Terms] OR "carcinogenesis"[All Fields])
#3	Search (#1 OR #2)
#4	("phytotherapy"[MeSH Terms] OR "phytotherapy"[All Fields]) OR ("plant extracts"[MeSH Terms] OR ("plant"[All Fields] AND "extracts"[All Fields]) OR "plant extracts"[All Fields]) OR herbs[All Fields] OR ("dietary supplements"[MeSH Terms] OR ("dietary"[All Fields] AND "supplements"[All Fields]) OR "dietary supplements"[All Fields])
# 5	("isoflavones"[MeSH Terms] OR "isoflavones"[All Fields] OR "isoflavone"[All Fields]) OR ("soybeans"[MeSH Terms] OR "soybeans"[All Fields]) OR ("soybean proteins"[MeSH Terms] OR ("soybean"[All Fields] AND "proteins"[All Fields]) OR "soybean proteins"[All Fields]) OR ("soy foods"[MeSH Terms] OR ("soy"[All Fields] AND "foods"[All Fields]) OR "soy foods"[All Fields]) OR ("genistein"[MeSH Terms] OR "genistein"[All Fields]) OR ("genistein combined polysaccharide"[Supplementary Concept] OR "genistein combined polysaccharide"[All Fields]) OR ("daidzein"[Supplementary Concept] OR "daidzein"[All Fields]) OR ("phytoestrogens"[Pharmacological Action] OR "phytoestrogens"[MeSH Terms] OR "phytoestrogens"[All Fields]) OR ("flavonoids"[MeSH Terms] OR "flavonoids"[All Fields]) OR ("equol"[MeSH Terms] OR "equol"[All Fields]) OR ("glycitein"[Supplementary Concept] OR "glycitein"[All Fields]) OR ("dihydrodaidzein"[Supplementary Concept] OR "dihydrodaidzein"[All Fields])
#6	Search (#4 OR #5)
#7	Search (#3 AND #6)

The electronic searches were complemented by searching the World Health Organization International Clinical Trials Registry Platform Search Portal (WHO ICTRP) and ClinicalTrials.gov by using the term ‘prostate cancer AND phytotherapy’ (MeSH) to identify possible completed or ongoing trials.

2.2.2. Searching other resources

The reference lists of included studies were hand-searched for additional references. Conference proceedings of 5 journals *(The Journal of Urology, European Urology Supplements, Der Urologe, Annals of Hematology, Journal of Clinical Oncology)* were hand-searched as well from the year 2008 onwards.

2.3. Data collection and analysis

2.3.1. Selection of studies

Citavi 6.0 (Swiss Academic Software, Wädenswil, Switzerland) was used to manage the bibliographic references. The described search strategy was used to obtain titles and abstracts of studies that were of relevance to the review. In preparing the full publication, two review authors (PR and LS) independently screened these titles and abstracts to determine which studies should be assessed further. Two review authors (PR and LS) have rated all potentially-relevant records as full texts, mapped records to studies, and classified studies as included studies, excluded studies, studies awaiting classification, or ongoing studies in accordance with the criteria for each provided in the *Cochrane Handbook for Systematic Reviews of Interventions* [45]. Disagreements were resolved by consensus or by consulting a third party reviewer (TN).

2.3.2. Data extraction and management

For studies that met the inclusion criteria, the following information was extracted: Study dates and settings, participant details, grade of evidence, SIGN (Scottish Intercollegiate Guideline Network), definitions of relevant outcomes, method and timing of outcome measurement for this review as well as any relevant subgroups (see Table 10).

2.3.3. Measures of treatment effect

We extracted outcomes data relevant to this systematic review as needed for calculation of summary statistics and measures of variance. For dichotomous outcomes, we attempted to obtain numbers of events/measurements and totals for population of a 2 x 2 table, as well as summary statistics with corresponding measures of variance. For continuous outcomes, we attempted to obtain means and standard deviations or data necessary to calculate this information. For time-to-event outcomes, we attempted to obtain hazard ratios (HRs) with corresponding measures of variance or data necessary to calculate this information.

We resolved any disagreements by discussion, or, if required, by consultation with a third review author (TN). In addition, attempts were made to contact the authors of the included studies to obtain missing key data.

2.3.4. Assessment of risk of bias in included studies

We have attempted to assess the risk of bias by using the Cochrane risk of bias tool for RCTs and quasi-RCTs as well as systematic reviews and meta-analysis [45] (see Table 10). In this context, the following aspects were critically observed:

- Has the method for generating the allocation order been sufficiently described (Selection bias)? This serves to assess whether intervention and placebo groups are comparable (e.g., use of a computerized random number generator). [47; 48]
- Selection bias also relates to the participant recruitment process. In this context, the question arises whether there was biased participant recruitment into the intervention or placebo group? (Recruitment bias) [47; 48]
- Was there selection bias due to dissimilarity at baseline with respect to key prognostic indicators (e.g., demographic factors)? (Baseline imbalance) [47]
- Has the method of concealing the assignment order been adequately described? Could investigators and participants not predict allocation because, for example, drug containers with identical appearance were used? (Selection bias) [47]
- Bias due to knowledge of assigned interventions by participants and staff during the study. (Performance bias) [47; 48]
- Bias due to the outcome assessor's knowledge of the assigned interventions during the study. Are any steps described that were used to blind assessors from knowing which intervention a participant received? (Detection bias) [47; 48]
- Are all outcome data fully reported, including dropouts and exclusions, as well as the reasons for dropouts/exclusions from the study. (Attribution bias) [47]
- Is there an opportunity for selective reporting by the authors regarding the outcomes found? (Reporting bias) [47]

In this context, the studies were also assessed to determine whether they appeared to be free of other sources of bias that are not addressed elsewhere (e.g. study funding). Using the Cochrane Collaboration tool, studies were each

assigned a rating (high, low, or unclear) with respect to the five domains (selection, performance, attrition, reporting, and other). The results of this evaluation can be found in Table 10.

2.3.5. Assessment of study heterogeneity and data synthesis

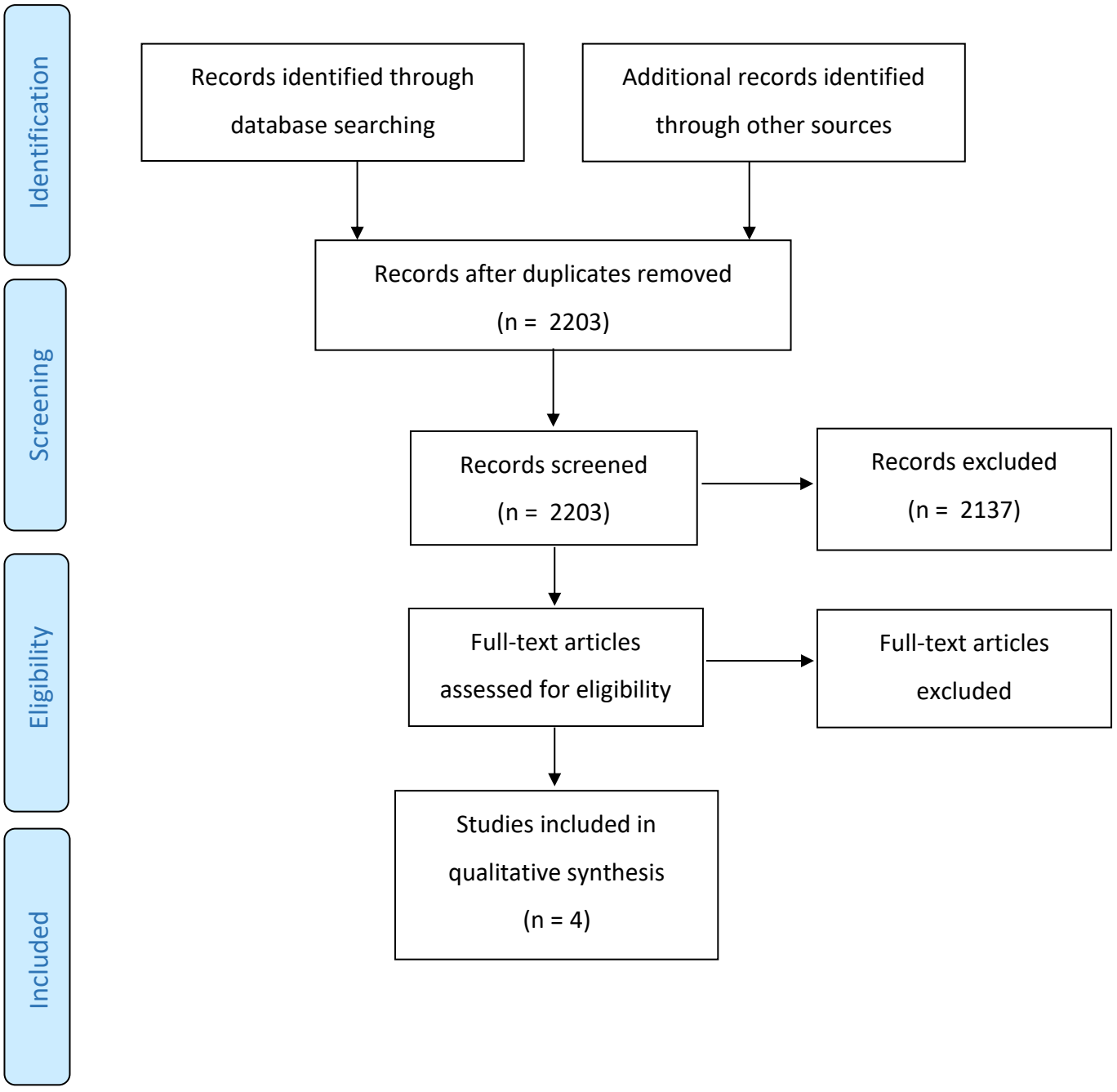
Due to the heterogeneity of the initial studies with regard to the interventions applied as well as the selected outcomes, we were ultimately unable to conduct a synthesis of results in the sense of a meta-analysis. The results were compiled descriptively in the form of a systematic review.

3. Results

3.1. Results of the search

The literature search for primary studies yielded 8494 results. The extraction of duplicates resulted in 2203 papers remaining. After reviewing the title and abstract, we excluded 2137 records of these. The remaining 66 full-text articles were screened for eligibility. After consideration regarding the inclusion and exclusion criteria presented in Table 4, we concluded that 4 studies were suitable for the qualitative synthesis. In total, the studies included 342 men with localized prostate cancer, of whom 298 completed the trials. [19; 26; 40; 49]. This process is shown graphically in the PRISMA diagram (see Figure 2 below).

Figure 2: **PRISMA flow chart**



3.2. Description of included studies

The following four studies were included:

- Kumar et al., 2004 [19]
- deVere White et al., 2010 [49]
- Lazarevic et al., 2011 [40]
- Hamilton-Reeves et al., 2013 [26]

Following our PICO scheme (see Figure 1), all studies had in common that they investigated the effect of soy isoflavones on PSA levels in patients with localized prostate cancer. In addition to the primary endpoint, numerous secondary endpoints, including hormone biomarkers, serum concentrations of each isoflavone, receptor status, or changes at the cellular level, were also examined. The heterogeneity of the studies was also reflected in different doses and application rates. To provide an overview, the individual RCTs are described in detail and illustrated in tabular form below.

3.2.1. B. Kumar et al.

The randomized controlled trial by B. Kumar et al. published in 2004 investigated the effect of isoflavone administration on patients in the early stages of prostate cancer [19].

Prostate cancer patients with a Gleason score of 6 or less between the ages of 50 and 80 years who met the criteria of the "watchful waiting" treatment strategy were enrolled. In total, out of 120 eligible patients seventy-six patients were recruited for the study, who were treated for 12 weeks either in the intervention arm with isoflavones or in the control arm with a placebo. During this time no other prostate cancer therapy was performed. Fifty-nine prostate cancer patients completed the trial. PSA and steroid hormone status were assessed both at the beginning and at the end of the intervention. The randomly assigned patients were divided into intervention group A (n=31) and control group B (n=28). While group A received an American standard diet supplemented with a soy protein drink (60 mg genistein), group B received the American standard diet with an isocaloric placebo [19].

The authors aimed to investigate the efficacy of a soy isoflavone-containing supplement in a group of early-stage prostate cancer patients. The focus was on changes in risk parameters associated with prostate cancer progression. These include, for example, the decrease in steroid hormones, serum levels of estrone, estradiol and sex hormone-binding globulin (SHBG) at baseline and after completion of the study [19].

No statistically relevant changes in mean levels of free serum estradiol or SHBG were observed. Although serum-free testosterone was reduced or constant in 61% of the subjects in the intervention group compared to 33% in the placebo group, the changes between the two groups were also not statistically significant [19].

Attention was also paid to baseline and final concentrations of total PSA and free PSA in serum. In the genistein-treated group 69% of the patients showed a decrease or no change in total serum PSA levels, while in the control group this was seen in 55% of the subjects. 19% of the subjects in the isoflavone group showed a significant reduction in total PSA over the entire period, compared to 0% in the placebo group. Despite the fact that several patients showed decreases in the values, these changes were not statistically significant for this study period. The table below (Table 6) illustrates the key points of the study [19].

Table 6: Illustration of the key data from the study by B. Kumar et al.

Kumar et al., 2004 **[19]**	
Patient population	- 76 patients with localized PCA (59 completed study) - Intervention group n=39 (31 have finished) - Placebo group n=37 (28 have finished)
Tumor stage	Patients in early and with localized stages of prostate cancer (Gleason score of 6 or less)
Duration	12 Weeks
Ongoing therapy	- Not involved in any other prostate cancer therapy/research protocol - Fulfilled the criteria of “watchful waiting“
Intervention substance	Soy supplement containing Genistein
Placebo substance	American standard diet supplemented with an isocaloric placebo without more precise specification
Dose and application rate	60mg genistein/d
Primary endpoints	Serum steroid hormone biomarkers (estrone, estradiol and sex hormone binding globulin (SHBG))

Secundary endpoints	Total and the free PSA
Results	No statistically significant changes in the primary and secondary endpoints.
Tolerability	Good tolerance with high compliance

3.2.2. deVere White et al.

deVere White et al. conducted a two-part study over a period of 12 months. In the first 6 months a double-blind randomized, placebo-controlled study was conducted. In the last 6 months an open label study was performed. In the following, we will focus on the first part of the study. Sixty-six men with histologically proven prostate cancer who were treated with the "Active Surveillance" strategy were included. The specific Gleason score was 10 or less. Another inclusion criterion was that the PSA concentration of the subjects had increased in two consecutive measurements. The serum PSA concentration was in the range of 0.7 to 22.6 ng/ml. No further prostate cancer therapies were performed either before or during the study period [49].

In the intervention arm 36 patients received 5g daily of a genistein-combined polysaccharide (GCP) containing several isoflavone components (450mg genistein, 300mg daidzein etc.). 30 patients from the control arm received 5 g inert cellulose daily for 6 months. Both the isoflavone supplementation and the placebo were administered in identical looking pods. During the implementation of the first 6 months of the study 13 participants stopped prematurely, resulting in a final number of 53 subjects. Of these, 28 were in the isoflavone group and 25 in the placebo group. The measurements were performed before study start, after 3 months and after 6 months. While the PSA value was measured as primary endpoint at all three points in time, the serum concentration of genistein, daidzein and equol were measured as secondary endpoints at baseline and after 6 months [49].

Of 24 volunteers from the intervention arm the genistein, daidzein and equol serum levels could be determined at the beginning and after 6 months. While 21 prostate cancer patients showed significant increases in serum genistein and daidzein, 3 men showed no or minimal increases. The measurements of equol, a metabolite of daidzein, showed no changes. From the control group,

the serum values of 22 of 25 participants could be evaluated. No significant changes in the values were found [49].

Although significant changes in isoflavone concentrations were measured in the intervention group**,** no correlation with PSA concentrations was found. The following overview (Table 7) illustrates the key aspects of the study [49].

Table 7: Illustration of the key data from the study by deVere White et al.

deVere White et al., 2010 [49]	
Patient population	- 66 patients with localized PCA (53 completed study) - Intervention group n=36 (28 have finished) - Placebo group n=30 (25 have finished)
Tumor stage	Patients with localized PCA (Gleason score of 10 or less)
Duration	Effectively 6 months
Ongoing therapy	"Active Surveillance" treatment
Intervention substance	Genistein-combined polysaccharide (GCP)
Placebo substance	Inert cellulose
Dose and application rate	- 5g GCP (containing 450mg genistein and 300mg daidzein)/d - 5g Inert cellulose/d

Primary endpoints	Serum PSA value
Secundary endpoints	Serum concentration of genistein, daidzein and equol
Results	Substantial increases in genistein concentrations without statistically significant PSA changes
Tolerability	Good tolerance with high compliance

3.2.3. Lazarevic et al.

Lazarevic et al. designed a double-blind, placebo-controlled, randomized study with two study arms. In a period of 3 to 6 weeks prior to performing a radical prostatectomy, 23 subjects in the intervention arm received 30 mg synthetic genistein per day, while 24 men in the control group received a placebo [40]. Various primary and secondary endpoints were formulated for the study (see Table 8). Changes in serum PSA and serum testosterone levels as well as PSA in prostate tissue were investigated as primary endpoints. Secondary endpoints included a postoperative examination of the prostate for any changes in Gleacon score compared to a preoperative biopsy. Macroscopic determinations of volume and extraprostatic expansion were also performed. Secondary endpoints included compliance during the study period, the safety of isoflavone administration based on the occurrence of adverse events, and genistein plasma concentration. In addition, serum cholesterol, HDL, LDL, triglycerides, lipase, amylase, TSH and INR values were determined as safety markers [40].

For the evaluation of the results the comparability of the patient characteristics was of great importance. The study showed a significant increase in total genistein plasma concentrations during treatment in the intervention arm (mean genistein value in the intervention arm = 79.1ng/ml and placebo group = 2.0 ng/ml). One exception was a patient from the genistein arm whose originally high genistein plasma value decreased significantly during the course of the study (209.9 ng/ml and 25.7 ng/ml, respectively). No accumulation of isoflavone could be detected in the postoperatively examined prostate tissue even though the resection was performed only a few days after the last intake. In addition, measurements of hormone parameters were also performed. There were no significant differences in the sex hormone variables (testosterone, luteinizing hormone (LH) and sex hormone-binding globulin) and in the thyroid parameters (free T3, free T4 and TSH) [40].

The measurement of blood lipids, however, showed a conspicuous difference. Total cholesterol values were reduced in the intervention group (p=0.0123), while HDL and LDL values showed no statistically relevant change [40].

The prostate preparation showed no significant change in Gleason score in either group. However, focal cancer was more frequently detected in the genistein group compared to the comparison group. No abnormalities were found in surgical margins as well. Interestingly, a decrease in PSA serum levels was observed in the intervention arm. In the genistein group there was a mean percentage decrease of 7.8%, while in the placebo group an increase of 4.4% was reported. The difference was borderline significant (P=0.051). P-values of less than 0.050 were found to be statistical significant [40].

In general, the study recorded few and only minor adverse events. Intake was well tolerated and no subject had to stop the study due to adverse events. The following overview (Table 8) summarizes the study [40].

Table 8: Illustration of the key data from the study by Lazarevic et al.

Lazarevic et al., 2011 **[40]**	
Patient population	- 47 patients with localized PCA - Intervention group n=23 - Placebo group n=24
Tumor stage	Patients with localized prostate cancer (histologically in clinical stage T1c or T2 and a Gleason Score of 6-8)
Duration	3-6 weeks prior to radical prostatectomy
Ongoing therapy	No further therapy
Intervention substance	Synthetic genistein
Placebo substance	Placebo substance not specified more precisely
Dose and application rate	30 mg synthetic genistein/daily
Primary endpoints	PSA in serum and prostate tissue and serum testosterone

Secundary endpoints	Modulations of Gleason score and tumor stage, safety and compliance of intake, genistein serum levels, as well as lipid and thyroid levels as safety markers
Results	No significant changes with respect to most endpoints. However, a significant reduction in total cholesterol levels was observed as well as a borderline significant PSA change.
Tolerability	Good tolerance with high compliance

3.2.4. Hamilton-Reeves et al.

Hamilton-Reeves et al. conducted a double-blind, randomized, placebo-controlled study involving 86 patients with localized prostate cancer. In the intervention arm, 42 subjects received a soy supplement daily (160mg/d). In the control arm 44 patients received a placebo. The study was conducted up to 6 weeks prior to a radical prostatectomy [26].

The primary endpoints of the study were changes in serum PSA, total cholesterol and various sex hormones (serum total testosterone, free testosterone, total estrogen and estradiol), with values determined at baseline, midline and at the time of radical prostatectomy. Furthermore, the expression of genes involved in the cell cycle and apoptosis was analyzed on a cellular level. At the beginning of the study, it was important that the personal data and characteristics of the subjects, as well as the initial values of PSA, cholesterol and hormones did not differ [26].

The results of this study were very similar to those of previous studies. No statistically significant differences in serum markers were found. The authors found that no relevant changes in PSA, total cholesterol or sex hormones occurred during the short-term intake of isoflavones of about 20 days compared to the placebo group. Isoflavone consumption was also well tolerated by the subjects. [26].

However, the analysis of gene expression revealed abnormalities. Using molecular biology assay systems, 12 genes involved in cell cycle control were identified to be downregulated in isoflavone-treated tumor tissue. For example, a reduced activity of the p53-dependent cyclin G1 and G2 factors as well as a downregulation of the CDC27 protein involved in mitosis was observed. In addition, a decreased activity of 9 apoptosis genes was observed in isoflavone treated tissue. Table 9 below summarizes the study [26].

Table 9: Illustration of the key data from the study by Hamilton-Reeves et al.

Hamilton-Reeves et al., 2013 [26]	
Patient population	- 86 patients - Intervention group n=42 - Placebo group n=44
Tumor stage	Patients with localized prostate cancer (clinical stage T1 or T2)
Duration	Up to 6 weeks
Ongoing therapy	No therapy
Intervention substance	Soy isoflavones of an average isoflavone distribution of 55% daidzein, 30% glycitein and 15% genistein
Placebo substance	No exact specification
Dose and application rate	160mg of a soy protein daily
Primary endpoints	PSA, total cholesterol and sex hormones
Secundary endpoints	Alignment of genes involved in cell cycle and apoptosis

Results	No significant changes regarding primary endpoints, Gene analysis showed downregulation of several genes
Tolerability	Good tolerance with high compliance

3.2.5. Comparison of the results of the interventions

In the following presentation, all studies are compared; Table 10 shows the main results of the four included RCTs. In addition, based on the study design and methodological quality of each study, the levels of evidence were assessed using the SIGN grading system [50].

Table 10: Overview and characterisation of all included studies (n=4), all studies are RCTs.

Study/ Reference	Study design, country	Evidence	Patient population	Intervention	Control	Endpoint to this review	Conclusion
Kumar et al., 2004 [19]	Double-blind, placebo-controlled RCT, USA	SIGN: 1+	76 patients with localized PCA 59 completed study	60 mg genistein/daily for 12 weeks (n=39; 31 completed)	Placebo (n= 37; 28 completed)	PSA response	No significant difference in PSA levels
deVere White et al., 2010 [49]	Double-blind, placebo-controlled RCT, USA	SIGN: 1-	66 patients with localized PCA in active surveillance program 53 completed study	450 mg genistein and 300mg daidzein/daily for 6 months (n=36; 28 completed)	Placebo (n=30; 25 completed)	PSA response	No significant difference in PSA levels (p=0.29)
Lazarevic et al., 2011 [40]	Double-blind, placebo-controlled RCT, Norway	SIGN: 1-	Localized PCA prior to radical prostatectomy 3-6 weeks (n=47)	30 mg synthetic genistein/daily 3 to 6 weeks prior radical prostatectomy (n=23)	Placebo (n=24)	PSA response	No significant difference in PSA levels (p=0.051)

Hamilton-Reeves et al., 2013 **[26]**	Double-blind, placebo-controlled RCT, USA	SIGN: 1+	Localized PCA prior to radical prostatectomy (n=86)	160mg isolavones/daily about 20 days (n=42)	Placebo (n=44)	PSA response	No significant difference in PSA levels

RCT = randomized controlled trial; PCA = prostate cancer; PSA = prostate specific antigen

3.2.6. Demographic characteristics of the study population

In general, it can be said that within each study the patients from the intervention group and the placebo group were relatively similar in terms of characteristics. There are slight differences between the different studies. For example, the patients in Kumar et al. and deVere White et al. are slightly older on average than in the others [19; 26; 40; 49]. In contrast, the patients of Hamilton-Reeves et al. show slightly higher BMI values [26]. In terms of ethnic composition, all studies were similar in that primarily caucasian people participated in the studies.

The studies also vary to some extent with regard to the Gleason scores of the patients. For example, Kumar et al. only recruited patients with a score of 6 or less [19]. While in the comparative studies, tumor patients with higher Gleason scores were also included in the evaluation. However, the mean Gleason score in deVere White et al. was 6 in both the intervention group and the placebo group [49]. In the study by Lazarevic et al. the mean score in the intervention group was 6.6 and in the placebo group 6.5 [40]. In Hamilton-Reeves et al., there were slightly higher scores in both groups with a mean Gleason score of 7 [26].

Patient characteristics are listed in detail in Table 11.

Significant heterogeneity is apparent with respect to the selected endpoints, study designs, and patient characteristics (Table 6-11).

Table 11: Illustration of patient demographics and characteristics.

	Kumar et al., 2004 [19]		deVere White et al., 2010 [49]		Lazarevic et al., 2011 [40]		Hamilton-Reeves et al., 2013 [26]	
	Intervention	**Placebo**	**Intervention**	**Placebo**	**Intervention**	**Placebo**	**Intervention**	**Placebo**
Mean Age	72,5	70,9	70,5	68,6	60,7	58,4	62	62
Race								
Caucasian	37	34	21	/	/	/	37	29
AA	0	1	3	/	/	/	3	9
Hispanics	2	1	0	/	/	/	1	2
Other	0	1	0	/	/	/	1	4
BMI	26,9	27,5	/	/	26,4	25,9	30	31
Clinical tumor stage								
T1	/	/	/	/	12	13	15	24
T2	/	/	/	/	11	4	27	19

Gleason Score*								
2-4	/	/	0	2	0	0	/	/
5-6	/	/	25	21	11	9	/	/
7	/	/	2	2	11	7	/	/
8-10	/	/	1	0	1	1	/	/

AA: African American; BMI: Body Mass Index; / = no detailed data available in the paper; * = see description in the text above

3.2.7. Occurrence of adverse side effects

In the study conducted by Kumar et al., 17 of the original 76 patients dropped out. Of these, 8 were from the intervention group and 9 from the placebo group. In the end, 59 cancer patients completed the study. Reasons for drop-out included gastrointestinal complaints such as diarrhea, constipation and flatulence (12) as well as other unspecified medical causes (1). General causes such as non-compliance, travel or intolerance to the texture were also documented (4). Excellent compliance of participation was observed among the people who completed the study. The paper contains only the documentation of adverse events of those patients who terminated the study prematurely. Adverse events that occurred within the population that completely terminated the study are not described in detail [19].

In the study conducted by deVere White et al., a total of 13 of the original 66 participants stopped prematurely. Of these, 8 were in the intervention group and 5 in the placebo group. Among the causes of discontinuation cited were mainly gastrointestinal complaints such as diarrhea (7), of which 5 cases occurred in the GCP group and 2 in the control group. Other causes were skin rash (1), transfer to other therapies (2), non-medical problems (2) and dissatisfaction with the many capsules to be taken (1). The remaining subjects tolerated the intake very well. A small group of persons in the isoflavone group, which completely terminated the study, showed loose stool. Although, as in the previous study, the complaints of the patients who completed the study are not described in detail [49].

In the study initiated by Lazarevic et al., adverse events occurred infrequently. There were 5 events in the intervention arm, of which 3 were gastrointestinal, 1 cardiovascular and 1 general. In the placebo group, 4 events were recorded, of which 2 were of gastrointestinal and 2 musculoskeletal origin. All events were mild, so no patient had to stop treatment prematurely. In addition, 2

laboratory chemical abnormalities were registered in the intervention group. In one case there was an increase in serum lipase and in another an increase in serum bilirubin, which had normalized after the end of treatment. The blood lipids did not show any abnormalities. In general, the intake was well tolerated [40].

In the study by Hamilton-Reeves et al. only mild events were documented. None of these side effects led to the termination of the study by the candidates. In total 4 adverse events were detected in the isoflavone branch. Of these, 2 were gastrointestinal and 2 were of general origin. What is meant by general is not described in detail in the paper. In the placebo branch there were 9 events. Of these 6 were gastrointestinal and 3 were of general nature. In general, isoflavone intake was considered safe and well tolerated [26].

In summary, good tolerability of isoflavone intake was documented in all studies. Adverse events were generally mild. Most frequently gastrointestinal complaints such as constipation, diarrhea and flatulence were documented. Problems of general nature were also frequently observed. For instance, tablet shape, texture and quantity caused problems for some individuals [19; 26; 40; 49].

3.3. Description of excluded studies

It can be seen from the PRISMA flowchart (see Figure 2) that numerous studies were excluded as part of the study selection process. In this context, the study by Bosland et al. [39] was particularly notable. This study followed a different study concept compared to the other included studies. A total of 177 men were selected who had undergone radical prostatectomy for clinically localized prostate cancer (T1c or T2) within 4 months prior to randomization. A soy-based supplement was administered daily versus placebo, and serum PSA was determined initially at 2-month intervals and at 3-month intervals beginning at year 2. The primary endpoints of the study were biochemical recurrence and time to tumor recurrence. Recurrence was defined as the development of a serum PSA level of ≥0.07 ng/mL within the first 2 years. The study found no significant differences between the two groups with respect to the formulated endpoints. The lack of effects led to premature discontinuation of the study. However, as no raw PSA data were included in this work, this study plays a minor role for the final discussion [39].

3.4. Risk of bias in included studies

Reporting of methodological quality parameters was incomplete in 2 of the studies. Overall, risk of bias was assessed as low and the quality of evidence was rated good. Table 12 shows the summary of risk of bias assessment using the Cochrane “risk of bias” assessment tool for each included study [45].

Table 12: Risk of bias summary of all included studies.

	Kumar et al. 2004	deVere White et al., 2010	Lazarevic et al., 2011	Hamilton-Reeves et al., 2013
Random sequence generation (Selection bias)	+	+	+	+
Recruitment bias	+	+	+	+
Baseline imbalance	+	+	+	+
Allocation concealment (Selection bias)	+	+	+	+
Blinding of participants and personal (Performance bias)	+	+	+	+
Blinding of outcome assessment (Detection bias)	+	-	+	+
Incomplete outcome data (Attribution bias)	+	+	+	+
Selective reporting (Reporting bias)	+	-	-	+
Other bias	?	+	?	+

+ = low risk of bias; - = high risk of bias; ? = unclear risk of bias

4. Discussion

4.1. Summary of main results

We conducted a systematic review of the role of isoflavones as a tertiary chemoprevention against biochemical progression in patients with localized prostate cancer. In total, we identified four relevant RCTs involving 298 treated men. The result of this synopsis was that none of the studies showed a significant effect on serum PSA levels, suggesting that isoflavone intake has no effect on biochemical progression.

4.2. Assessment of other results

Various primary and secondary endpoints were formulated in the included studies. In addition to PSA levels, the effect of isoflavone intervention on steroid hormone biomarkers, serum concentrations of genistein, daidzein and equol, lipid and thyroid levels, as well as on the activity of genes involved in cell cycle and apoptosis were also investigated [19; 26; 40; 49].

In general, the studies showed that the phytotherapeutic intervention resulted in a significant increase of isoflavone serum concentration in most of the patients. However, there were also patients in whom serum levels hardly increased. Thus, in the study of deVere White et al. there were 3 men in whom no or minimal increases occurred [49].

Many other parameters did not show statistically significant changes:

- No statistically significant changes in mean levels of free serum estradiol, SHBG, and testosterone [19; 26; 40].
- No statistically relevant changes in HDL, LDL, triglycerides, lipase, amylase, and INR values [40].

- No abnormalities in thyroid parameters (free T3, free T4 and TSH) [40].
- No significant changes in Gleason score [40].

A special feature in this context is total cholesterol, where inconsistent results were observed. In the study by Lazarevic et al. [40] total cholesterol levels were significantly reduced in the intervention group (p=0.0123), whereas in the study by Hamilton-Reeves et al. [26] short-term isoflavone intake did not result in relevant cholesterol changes. In the latter, however, several cell cycle-associated genes were identified in the tumor tissue that were downregulated.

In general, the four included RCTs showed that soy isoflavone intake was very well tolerated [19; 26; 40; 49] and was associated with only very mild side effects.

4.3. Agreements and disagreements with other studies or reviews

To assess the outcome of this review, this subsection considers it in context to the literature. As can be seen from the introduction, the data available to date regarding the efficacy of isoflavones is not clear. While many in vitro studies have found promising anti-carcinogenic effects at a cellular level [24; 26; 29; 30 ; 33; 34; 35; 36; 37; 38], again other clinical studies found no significant PSA-lowering effects [51; 52; 53; 54]. The result of this work is another piece of the puzzle that adds to the existing data and rather strengthens the thesis that isoflavone ingestion has no tumor growth limiting effect.

4.4. Limitations of the included studies

With regard to the discussion of limitations, a rough distinction can be made between two sections. On the one hand, the limitations of the own systematic review and on the other hand limitations of the included studies. Limitations within the studies that have an impact on the quality of the evidence are discussed separately under another subheading.

We can start with the assumptions made regarding the classification of interventions, primary outcomes, and patient groups. In this respect, one can be guided by our PICO scheme (Figure 1). In order to find a compromise between limited available data and most effective information gain, we defined the isoflavone substitution as a whole as intervention and did not distinguish between the individual representatives of this group. However, isoflavones include various substances, such as genistein, daidzein, as well as, among others, the daidzein metabolite equol [26]. Past studies suggest that the different components are subject to different pharmacokinetics and also have different potency [55]. In contrast to genistein, daidzein showed lower potency [56]. In the included studies, differently composed isoflavones were administered. For example, the study by Kumar et al. [19] used a pure genistein-containing preparation (see Table 6) while Hamilton-Reeves et al. [26] used a combination preparation with different isoflavone components (see Table 9). In the work of deVere White et al. [49], a genistein-combined polysaccharide (GCP) was used (see Table 7). It is likely that the efficacy of the isoflavone substitutions varied from study to study.

With regard to the bioavailability of the intervention compounds, patient-specific characteristics also play a role. An individual variation in the ER-β gene as well as polymorphisms in the CYP19 gene influence the effect of phytoestrogens [31]. A distinction is made between high and low metabolizers [55]. The different potency of different isoflavone components as well as

patient-specific differences make it difficult to compare the results of the studies as well as to establish causality between intervention and outcome.

In addition, it must be considered that isoflavones have an estrogen-like effect. Prostate carcinomas themselves can manifest in many ways. For instance, they can be castration-sensitive or castration-resistant [57]. Since none of the studies made these distinguishments within their patient populations, nor did they examine them, the impact of these factors on the final outcome is difficult to assess.

Another limitation that can be discussed in this context is that the tumor-inhibitory effect of isoflavones is dose-dependent. In older studies at a cellular level, effects were only detected at concentrations above 10 µmol/L [58; 59; 60]. To illustrate this, one can cite the results of the randomized trial by Gardner CD et al.. There, administration of 82 mg total isoflavones per day resulted in a total isoflavone serum level of 0.7 µmol/L and a tissue level of 2.3 µmol/L [61]. Also, in other previous studies, oral substitution of approximately 40-130 mg of isoflavones per day resulted in peak serum levels of approximately 2 µmol/L [55; 62; 63]. With this in mind, it is questionable whether sufficiently high serum/tissue levels were achieved in all participants in the included studies, as the amount of substitution varied widely from study to study. The amounts ingested ranged from a few tens to several hundred mg per day [19; 26; 40; 49].

Going back to our PICO scheme, another limiting factor that stands out is the preformulated primary endpoint. We chose serum PSA response as the primary endpoint for this review after exploratory literature search because it is a noninvasive endpoint that is easily accessible and comparable, but there are also problems with this endpoint. The PSA level is an organ-specific but not a cancer-specific biomarker [64]. It also has limited specificity because, on the one hand, there are PSA-negative prostate cancers and, on the other hand,

there are PSA increases that are due to tumor-independent diseases, such as BPH and inflammation [65; 66].

In addition to clinical parameters, long-term outcomes such as overall survival and quality of life also play an important role in oncology. Long study durations are required to evaluate survival parameters. In this context, the studies showed considerable deficits. In prostate cancer, a follow-up period of at least 10 to 15 years is needed to correctly answer the question of overall survival [67].The duration of the included studies was too short to comprehensively answer this question. As shown in Tables 6-9, the duration of the studies varied from 3 weeks to 2 years.

An important limitation is the completeness of patient demographics and characteristics. As shown in Table 11, this subtopic includes patient age, ethnicity, BMI, as well as tumor classification by clinical tumor stage and Gleason score. As can be seen from the tabulation, apart from age, there are significant gaps, which can be attributed to the lack of reporting by the authors in their papers. To demonstrate the importance of comparability of patient demographics, reference can be made to the influence of ethnic composition on outcome. Interestingly, one study [68] showed that isoflavone substitution had greater PSA-lowering efficacy in Caucasian men than in African-American men. To unmask such patient-characteristic influences, it is necessary that these characteristics are fully described.

In connection with inadequate reporting by the authors in their paper, the imprecise presentation of the side effects that occurred should also be mentioned. This issue is given particular attention under the subtopic "Quality and overall completeness of the evidence".

Finally, the structured discussion of limiting factors leads us to the main limitation in this review. The major difficulty of this work is based on the fact that a meta-analysis was not feasible due to the heterogeneity of the included

RCTs. The heterogeneity was related to the type of isoflavones administered and the dosage as well as the timing of application, as described above. Also, the inhomogeneity of the selected primary and secondary endpoints (see Table 6-9) played a role. Thus, with regard to the other endpoints of the studies, it is very difficult to make a statement about the influence on localized prostate cancer [19; 26; 40; 49].

4.5. Quality and overall completeness of the evidence

The relevance of the evidence to our question needs to be discussed in the context that it is important in clinical practice to be able to offer alternative treatment approaches to patients with prostate cancer. The question must be asked whether the identified studies are sufficient to address the objectives of the review. To make a generally valid statement, not only the quality of the evidence but also the completeness of the evidence is important.

On the one hand, the strength of evidence provided by an individual study depends on the ability of the study design to minimize the possibility of bias. On the other hand, the quality of evidence provided by a study also depends on how well the study was designed and conducted. Failure to address key aspects of the study methodology increases the risk of bias and thus reduces the reliability of the study [50]. Simplified, little bias in a paper leads to high quality and correspondingly vice versa, much bias to lower quality.

Since bias within a study has a significant impact on quality, discussion of such determinants is important. The quality assessment of the individual studies was performed separately using the "risk of bias" assessment tool [45] provided in the Cochrane Collaboration Handbook [69] (see Table 12).

Only the results of randomized controlled trials (RCTs) were used in the assessment. RCTs are considered the gold standard in scientific work as they

are less prone to bias. Nevertheless, various quality deficiencies of the included studies have been noticed. The reporting of methodological quality parameters in 2 of the studies is incomplete (see Table 12). In particular, the papers of deVere White et al. and Lazarevic et al. [40; 49] are affected. For example, both studies originally reported a number of measured variables or outcomes, but not all were reported (reporting bias) [69]. Selective reporting, in which mainly positive outcomes were published, can mislead the external appearance of these studies [70]. In addition, detection bias could not be ruled out in the study by deVere White et al.. The steps taken to exclude bias based on the assessor's knowledge of the assigned interventions are inadequately described.

The levels of evidence provided by each study were graded based on the study design and methodological quality using the SIGN grading system (see Table 10) [71]. The SIGN grading presented was done by considering the assessment of the studies in terms of the five domains (selection, performance, attrition, reporting, and other) (see Table 12) as well as using the SIGN methodology checklist for randomized controlled trials [71]. Thus, because of deficiencies described above, the studies by deVere White et al. [49] and Lazarevic et al. [40] were rated SIGN 1-. They are classified as RCTs with increased risk of bias. The RCTs by B. Kumar et al. [19] and Hamilton-Reeves et al. [26] are considered well-conducted studies with low risk of bias and were rated SIGN 1+.

For the methodological assessment, the SIGN method checklist also considers various aspects that map the completeness of the evidence. These include all relevant types of participants, interventions, and outcomes. All included studies met the criteria formulated in our PICO scheme (Figure 1). Therefore, indirectness, i.e., a discrepancy between the patient population, intervention, or outcomes of interest and the population, intervention, or outcomes studied in the research, can be excluded [72].

In summary, the quality of evidence of the individual studies is rated as good. Despite slight methodological limitations in two studies, the overall risk of bias can be rated as low.

4.6. Potential biases in the review process / Method critique

Consideration of dissemination bias is relevant to the discussion of the methodology of this systematic review. We used an extensive search strategy to screen two medical databases for relevant randomized controlled trials related to our research question. The tabular presentation of the search strategy can be seen in the "Material and methods" section of this review (Table 5). In addition to searching CENTRAL and MEDLINE via Pubmed, we also performed a hand search of several oncology-associated journals from the last 10 years to identify any overlooked studies. Accordingly, a low publication bias can be expected due to the extensive search. However, it should be noted that studies that have failed to demonstrate treatment success tend not to be published ("publication bias") [72]. The identification of such studies is also complicated by the fact that studies with negative results are cited less frequently ("citation bias") [72]. The results of this possibly missed study are therefore not included in the overall evaluation.

Another limiting factor concerning the methodology is that we restricted our search to German- and English-language studies. Since isoflavones in particular are of greater relevance as a component of soy products, especially in the East Asian region, it could be assumed that locally conducted studies were more likely to have been published in national language journals ("language bias") [72]. The timeliness of the search also plays a role. At the time of this review, no other RCT was registered at WHO ICTRP or

ClinicalTrials.gov. However, it should be considered that studies with negative results are published with a time lag ("time-lag bias") [72].

It should be noted that the studies included anyway did not show any effects of isoflavone intervention, so that the influence on the outcome of this work due to the above-mentioned biases are probably small.

The visualization of the dissemination bias by means of a funnel plot is not possible due to the low number of included studies. Funnel plots should only be used if at least 10 studies are included. With fewer studies, the power of the tests is too low to distinguish chance from true variance [72; 73].

Prior to writing the thesis, review methods were determined based on the Cochrane Collaboration Handbook [69]. Thus, without deviating from the protocol, a research question, a search strategy, inclusion and exclusion criteria, and an assessment of the risk of bias were established. Methological limitations can also be discussed with regard to the definition of inclusion and exclusion criteria. On the one hand, we limited our search to high-quality studies, such as randomized controlled trials and systematic reviews. On the other hand, we excluded numerous studies that had no or incompletely documented PSA values. For example, the review authors decided to exclude a particular study from the synthesis because it differed substantially from the other included studies in one aspect. This study is described in more detail under the subtopic "Description of excluded studies" [39].

Critically, we had to weigh up which priorities to set. In order to obtain unambiguous results, our criteria were strictly formulated. Also, the evaluation of the included studies was performed independently by the PhD student as well as by L. Schneidewind, MD, and T. Neumann, MD, without conflicts of interest regarding the investigated measures.

4.7. Outlook

In order to close the evidence gaps, standardization as well as optimization of study designs is required. A double-blind, randomized, placebo-controlled trial would be the most methodologically appropriate approach [74]. To increase the transferability of the results to patient care, potential sources of error and bias must be excluded. A discussion of what an optimal RCT might look like follows.

A major limitation of this work was the large heterogeneity of the included studies [19; 26; 40; 49]. For this reason, no meta-analytic evaluation could be associated with this review. To standardize studies, the isoflavone intervention should be based on a standardized supplement with uniform ratios of genistein, daidzein, and other naturally occurring isoflavones. Genistein appears to be the most potent isoflavone component [75]. It also represents the largest fraction in natural isoflavone-containing foods, such as soy [76; 77]. Accordingly, it would be reasonable to base such supplements largely on genistein as well.

Isoflavones have a dose-dependent effect [55; 58; 59; 60; 62; 63]. Regardless of which intervention is chosen, the difficulty lies in achieving sufficiently high active levels in serum and tissue under realistic conditions. On the one hand, it would be interesting to see whether the use of genistein-combined polysaccharides (GCP) increases bioavailability in a clinical setting. In previous cellular studies, GCP, being an isoflavone-rich extract, showed high bioavailability and low toxicity [78]. An alternative would be a long-term study to allow the phytotherapeutically active constituents to accumulate sufficiently in the tissues over time. A long-term study is also useful in view of the fact that prostate carcinomas grow very slowly [67]. Oncologically relevant aspects such as overall survival, which is of paramount importance in patient care, can also be determined in this way. In order to actually prove or disprove the

efficacy, the study must extend over several years. Isoflavones are considered to be a safe and well-tolerated therapeutic option, so such an approach is not associated with increased risk [19; 26; 40; 49].

In addition to the long-term recording of overall survival, quality of life also plays an important role. A useful addition in this context would be the regular use of health questionnaires such as the EQ-5D. The EQ-5D is a generic measure of health-related quality of life that captures overall health status in 5 dimensions (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression) [79]. Such methods can be used to provide a more fine-grained representation of health status, especially in older patients, and can be used to assess Quality Adjusted Life Years (QALY). This is a composite measure that represents both quantity and quality of life [79; 80].

An important limitation in the studies was the exclusive use of serum PSA levels as a progression marker. More useful would have been combined considerations of TMN classification, Gleason score, and PSA levels. With the help of such a risk stratification, important differential diagnostic causes of a PSA increase could be excluded on the one hand, and on the other hand, the probability of success of a therapy could be better estimated.

In this context, the regularly required biopsies are problematic. One option would be to recruit patients who undergo "active surveillance" monitoring. This is a method of monitoring localized prostate cancer that includes regular PSA testing as well as biopsies [81]. Thus, PSA values and biopsies taken as part of this therapeutic regimen could also be used for a parallel coupled study. This could simultaneously reduce the burden on the patient and increase efficiency.

The methodology and results of the studies performed based on these suggestions must finally be presented in a complete and evaluable manner.

On the basis of such long-term, methodically well planned and performed studies, clear statements can be made for patient care.

To conclude this section, other promising approaches can be discussed as an outlook for future research. Several approaches can be considered. Due to the versatile nature of isoflavones, they could be investigated as adjuncts in chemoprevention or established therapeutic regimens. Thus, genistein exhibits synergistic behavior with known anticancer drugs, such as adriamycin, docetaxel, and tamoxifen, suggesting a potential role in combination therapy [82]. Isoflavones also showed beneficial effects in combination with radiotherapy. The phytoestrogens sensitize tumor cells in the prostate to radiotherapy while showing antioxidant and anti-inflammatory effects that may potentially minimize the side effects of radiatio [83]. The use of isoflavones in combination with other phytotherapeutics, such as lycopenes and turmeric are already the subject of ongoing studies [84; 85].

The study by Lazarevic et al [40] showed a significant reduction in total cholesterol levels within the intervention group ($p=0.0123$). Accordingly, the question can be derived to what extent long-term isoflavone administration has an influence on serum triglycerides, HDL and LDL levels.

Due to its multifaceted nature, the topic offers many open research areas. In particular, the study of isoflavones with respect to lipid and cholesterol metabolism would be one of the most promising perspectives.

5. Authors' conclusions

Overall, the included studies are characterized by consistency of results. Isoflavones do not appear to have an impact on PSA levels in localized prostate carcinoma, yet the comprehensive assessment reveals insufficient precision. Even if the quality of the individual studies is good, they are too heterogeneous among themselves for a joint summary. Accordingly, it is difficult to draw a definitive conclusion. The impact of isoflavones on overall survival in localized prostate cancer remains unclear.

5.1. Implications for practice

At this time, isoflavone-containing chemoprevention will not replace conventional treatment options or obviate the need for radical prostate resections. Nevertheless, there are indications that isoflavones may be beneficial in patient care. For example, they may have positive effects on lipid and cholesterol metabolism [40]. Isoflavones represent a safe therapeutic option with few side effects, where further interdisciplinary research is needed. However, evidence-based statements are not yet possible here.

5.2. Implications for future research

The review answers basic questions derived from the PICO scheme (Figure 1) empirically, but raises relevant questions that could serve as a starting point for further research.

In particular, study designs need to be more standardized to address limitations arising from heterogeneity. In order to fill gaps in the completeness and applicability of previous studies and also to be able to perform a statistical evaluation in the sense of a meta-analysis, the optimizations mentioned in the discussion under "Outlook" may be helpful.

As the evidence base develops, it will also be beneficial to assess the efficacy of isoflavones on the symptomatology of tumor patients and the associated overall survival as well as quality of life.

I hope that the review of this topic has provided new approaches for further research.

6. List of References

[1] Robert Koch Institut (Hrsg.): Krebs in Deutschland 2013/2014. 11. Ausgabe, Berlin 2017. (14.11.2018)

[2] https://www.prostata.de/wissen/prostatakarzinom/haeufigkeit-des-prostatakarzinoms (14.11.2018)

[3] Mottet, N. et al.: Guidelines on prostate cancer. European Association of Urology (EAU) 2018

[4] Ferlay, J., et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer, 2015. 136: E359

[5] Alberts AR, Schoots IG, Roobol MJ. Prostate-specific antigen-based prostate cancer screening: Past and future. United Nations DoEaSA, Population Division. World population prospects: the 2008 revision, 2009.Int J Urol. 2015 Jun;22(6):524-32

[6] Wilt TJ, Ullman KE, Linskens EJ, MacDonald R, Brasure M, Ester E, Nelson VA, Saha J, Sultan S, Dahm P. Therapies for Clinically Localized Prostate Cancer: A Comparative Effectiveness Review. J Urol. 2021 Apr;205(4):967-976.

[7] Jennifer Gordetsky and Jonathan Epstein. Grading of prostatic adenocarcinoma: current state and prognostic implications. Diagn Pathol. 2016; 11: 25

[8] Sun F, Oyesanmi O, Fontanarosa J, Reston J, Guzzo T, Schoelles K. *Therapies for Clinically Localized Prostate Cancer: Update of a 2008 Systematic Review*. Comparative Effectiveness Review No. 146. (Prepared by the ECRI Institute–Penn Medicine Evidence-based Practice Center under Contract No. 290-2007-10063.)

[9] Fakhrejahani F, Madan RA, Dahut WL. Management Options for Biochemically Recurrent Prostate Cancer.Curr Treat Options Oncol. 2017 May;18(5):26

[10] Yan L, Spitznagel EL: Soy consumption and prostate cancer risk in men: a revisit of a meta-analysis. Am J Clin Nutr 2009; 89: 1155-63

[11] Hwang YW, KIM SY, Jee SH et al.: Soy food consumption and risk of prostate cancer: a meta-analysis of observational studies. Nutr Cancer 2009; 61: 598-606

[12] van Die MD, Bone KM, Emery J, Williams SG, Pirotta MV, Paller CJ. Phytotherapeutic interventions in the management of biochemically recurrent prostate cancer: a systematic review of randomised trials. BJU Int. 2016 Apr;117 Suppl 4:17-34

[13] Sadi MV. PSA screening for prostate cancer. Rev Assoc Med Bras (1992). 2017 Aug;63(8):722-725

[14] http://www.urologenportal.de/pressebereich/pressemitteilungen/presse-aktuell/presse-archiv/pressemitteilungen-aus-dem-jahr-2017/neubewertung-des-psa-tests-urologen-plaedieren-fuer-baseline-psa-nach-informierter-patienten-entscheidung-03052017.html (29.03.2019)

[15] Loeb S, Carter HB, Berndt SI, et al. Complications after prostate biopsy: data from SEER-Medicare. J Urol 2011;186:1830

[16] Loeb S, van den Heuvel S, Zhu X, et al. Infectious complications and hospital admissions after prostate biopsy in a European randomized trial. Eur Urol 2012;61:1110

[17] Ryu H, Song SH, Lee SE, Song KH, Lee S. A prospective randomized trial of povidone-iodine suppository before transrectal ultrasonography-guided prostate biopsy. Medicine (Baltimore). 2019 Mar;98(12):e14854

[18] Adlercreutz CH, Goldin BR, Gorbach SL, Höckerstedt KA, Watanabe S, Hämäläinen EK, Markkanen MH, Mäkelä TH, Wähälä KT, Adlercreutz T. Soybean phytoestrogen intake and cancer risk. J Nutr. 1995 Mar;125(3 Suppl):757S-770S

[19] Kumar NB, Cantor A, Allen K et al.: The specific role of isoflavones in reducing prostate cancer risk. The Prostate 2004; 59: 141-7

[20] Rosner W: The functions of corticosteroid binding globulin and sex hormone binding globulin: recent advances. Endocr Rev 1990; 11: 80-91

[21] Messina M, Barnes S: The role of soy products in reducing risk of cancer. J Natl Cancer Inst 1991; 83: 541-6

[22] Shimizu H, Ross RK, Bernstein L, Yatani R, Henderson BE, Mack TM. Cancers of the prostate and breast among Japanese and white immigrants in Los Angeles County. Br J Cancer. 1991 Jun;63(6):963-6

[23] Setchell KDR, Borriello SP, Hulme P et al.: Nonsteroidal estrogens or dietary origin: Possible roles in hormone depended disease. Am J Clin Nutr 1984; 40: 569-78

[24] Adlercreutz H, Höckerstedt K, Bannwart C et al.: Effect of dietary components, including lignans and phytoestrogens, on enterohepatic circulation and liver metabolism of estrogens and on sex hormone binding globulin (SHBG). J Steroid Biochem 1987; 27: 1135-44

[25] Pugeat M, Crave JC, Tourniaire J et al.: Clinical utility of sex-hormone-binding globulin measurement. Horm Res 1996; 45: 148-55

[26] Hamilton-Reeves JM, Banerjee S, Banerjee SK et al.: Short-term soy isoflavone intervention in patients with localized prostate cancer: a randomized, double-blind, placebo-controlled trial. Plos one 2013; 8: e68331

[27]Muthyala RS, Ju YH, Sheng S, Williams LD, Doerge DR, Katzenellenbogen BS, Helferich WG, Katzenellenbogen JA. Equol, a natural estrogenic metabolite from soy isoflavones: convenient preparation and resolution of R- and S-equols and their differing binding and biological activity through estrogenreceptors alpha and beta. Bioorg Med Chem. 2004 Mar 15;12(6):1559-67

[28] Reger MK, Zollinger TW, Liu Z, Jones JF, Zhang J.Dietary intake of isoflavones and coumestrol and the risk of prostate cancer in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. Int J Cancer. 2018 Feb 15;142(4):719-728

[29] Takayama K, Horie-Inoue K, Katayama S, Suzuki T, Tsutsumi S, Ikeda K, Urano T, Fujimura T, Takagi K, Takahashi S, Homma Y, Ouchi Y, Aburatani H, Hayashizaki Y, Inoue S. Androgen-responsive long noncoding RNA CTBP1-AS promotes prostate cancer. EMBO J. 2013 Jun 12;32(12):1665-80

[30] Carruba G. Estrogens and mechanisms of prostate cancer progression. Ann N Y Acad Sci. 2006 Nov;1089:201-17

[31] Hong-Yi Zhang, Jie Cui, Ye Zhang, Zhen-Long Wang, Tie Chong, and Zi-Ming Wang. Isoflavones and Prostate Cancer: A Review of Some Critical IssuesChin Med J (Engl). 2016 Feb 5; 129(3): 341–347

[32] Dobbs RW, Malhotra NR, Greenwald DT, Wang AY, Prins GS, Abern MR. Estrogens and prostate cancer. Prostate Cancer Prostatic Dis. 2019 May;22(2):185-194

[33] Handayani R, Rice L, Cui Y: Soy isoflavones alter expression of genes associated with cancer progression, including interleukin-8, in androgen-independent PC-3 human prostate cancer cells. J Nutr 2006; 136: 75-82

[34] Mahmoud AM, Yang W, Bosland MC. Soy isoflavones and prostate cancer: a review of molecular mechanisms. J Steroid Biochem Mol Biol. 2014 Mar;140:116-32

[35] Sylvain Lecomte, Florence Demay, François Ferrière, and Farzad Pakdel. Phytochemicals Targeting Estrogen Receptors: Beneficial Rather Than Adverse Effects? Int J Mol Sci. 2017 Jul; 18(7): 1381

[36] Mahmoud AM, Al-Alem U, Ali MM, Bosland MC. Genistein increases estrogen receptor beta expression in prostate cancer via reducing its promoter methylation. J Steroid Biochem Mol Biol. 2015 Aug;152:62-75

[37] Adlercreutz H, Fotsis T, Bannwart Z et al.: Urinary estrogen profile determination in young Finnish vegetarian and omnivorous women. J Steroid Biochem 1986; 24: 289-96

[38] Rose DP: Dietary fiber, phytoestrogens and breast cancer. Nutrition 1992; 8: 47-50

[39] Bosland MC, Kato I, Zeleniuch-Jacquotte A et al.: Effect of soy protein isolate supplementation on biochemical recurrence of prostate cancer after radical prostatectomy: a randomized trial. JAMA 2013; 310: 170-8

[40] Lazarevic B, Boezelijn G, Diep LM et al.: Efficacy and syfety of short-term genistein intervention in patients with localized prostate cancer prior to radical prostatectomy: a randomized, placebo-controlled, double-blind Phase 2 clinical trial. Nutr Cancer 2011; 63: 889-98

[41] Ahmad IU, Forman JD, Sarkar FH et al.: Soy Isoflavones in conjunction with radiation therapy in patients with prostate cancer. Nutr Cancer 2010; 62: doi:10.1080/01635581.2010.509839

[42] Ahn-Jarvis JH, Clinton SK, Grainger EM et al.: Isoflavone pharmacokinetics and metabolism after consumption of a standardized soy

and soy-almond bread in men with asymptomatic prostate cancer. Cancer Prev Res 2015; 8: 1045-54

[43] Kumar NB, Kang L, Pow-Sang J et al.: Results of a randomized Phase 1 dose-finding trial of several doses of isoflavones in men with localized prostate cancer: administration prior to radical prostatectomy. J Soc Integr Oncol 2010; 8: 3-13

[44] Dalais FS, Meliala A, Wattanapenpaiboon N et al.: Effects of a diet rich in phytoestrogens on prostate-spefific antigen and sex hormones in men diagnosed with prostate cancer. Urology 2004; 64: 510-5

[45] Higgins JPT, Green S: Cochrane Handbook for Systematic Reviews of Interventions: Cochrane Book Series 2008. The Cochrane Collaboration. ISBN 978-0-470-69951-5

[46] Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC et al.: The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. J Clin Epidemiol 2009; 62: e1-34

[47] Higgins Julian P T, Altman Douglas G, Gøtzsche Peter C, Jüni Peter, Moher David, Oxman Andrew D et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials BMJ 2011; 343

[48] Smith, J., & Noble, H. (2014). Bias in research. Evidence-Based Nursing, 17(4), 100-101

[49] deVere White RW, Tsodokov A, Stapp EC et al.: Effects of a high dose, aglycone rich soy extract on prostate-specific antigen and serum isoflavone concentrations in men with localized prostate cancer. Nutr Cancer 2010; 62: 1036-43

[50] Robin Harbour, Juliet Miller. A new system for grading recommendations in evidence based guidelines. BMJ. 2001 Aug 11; 323(7308): 334–336

[51] Hamilton-Reeves JM, Rebello SA, Thomas W, Slaton JW, Kurzer MS. Soy protein isolate increases urinary estrogens and the ratio of 2:16alpha-hydroxyestrone in men at high risk of prostate cancer. J Nutr. 2007 Oct;137(10):2258-63

[52] Ullmann U, Oberwittle H, Grossmann M, Riegger C: Repeated oral once daily intake of increasing doses of the novel synthetic genistein product Bonistein in healthy volunteers. Planta Med 71, 891–896, 2005

[53] Hong-Yi Zhang, Jie Cui, Ye Zhang, Zhen-Long Wang, Tie Chong, Zi-Ming Wang. Isoflavones and Prostate Cancer: A Review of Some Critical Issues. Chin Med J (Engl). 2016 Feb 5;129(3):341-7

[54] M Diana van Die , Kerry M Bone, Scott G Williams, Marie V Pirotta. Soy and soy isoflavones in prostate cancer: a systematic review and meta-analysis of randomized controlled trials. BJU Int. 2014 May;113(5b):E119-30

[55] Watanabe S, Yamaguchi M, Sobue T, Takahashi T, Miura T, et al.: Pharmacokinetics of soybean isoflavones in plasma, urine and feces of men after ingestion of 60 g baked soybean powder (kinako). J Nutr 128, 1710–1715, 1998

[56] Verma SP, Goldin BR. Effect of soy-derived isoflavonoids on the induced growth of MCF-7 cells by estrogenic environmental chemicals. Nutr Cancer. 1998;30(3):232–239

[57] Ulka Vaishampayan , Maha Hussain, Mousumi Banerjee, Soley Seren, Fazlul H Sarkar, Joseph Fontana, Jeffrey D Forman, Michael L Cher, Isaac Powell, J Edson Pontes, Omer Kucuk. Lycopene and soy isoflavones in the treatment of prostate cancer. Nutr Cancer. 2007;59(1):1-7

[58] Hedlund TE, Maroni PD, Ferucci PG, Dayton R, Barnes S, Jones K, Moore R, Ogden LG, Wahala K, Sackett HM, Gray KJ. Long-term dietary habits affect soy isoflavone metabolism and accumulation in prostatic fluid in caucasian men. J Nutr. 2005;135(6):1400–1406

[59] Zava DT, Duwe G. Estrogenic and antiproliferative properties of genistein and other flavonoids in human breast cancer cells in vitro. Nutr Cancer. 1997;27(1):31–40

[60] Swami S, Krishnan AV, Peehl DM, Feldman D. Genistein potentiates the growth inhibitory effects of 1,25-dihydroxyvitamin D3 in DU145 human prostate cancer cells: role of the direct inhibition of CYP24 enzyme activity. Mol Cell Endocrinol. 2005;241(1-2):49–61

[61] Gardner CD, Oelrich B, Liu JP, Feldman D, Franke AA, Brooks JD: Prostatic soy isofla-vone concentrations exceed serum levels after dietary supplementation. Prostate 2009;69: 719-726

[62] Heald CL, Ritchie MR, Bolton-Smith C, Morton MS, Alexander FE. Phyto-oestrogens and risk of prostate cancer in Scottish men. Br J Nutr. 2007;98(2):388–396

[63] Izumi T, Piskula MK, Osawa S, Obata A, Tobe K, Saito M, Kataoka S, Kubota Y, Kikuchi M. Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. J Nutr. 2000;130(7):1695–1699

[64] Matteo Ferro, Carlo Buonerba, Daniela Terracciano, Giuseppe Lucarelli, Vincenzo Cosimato, Danilo Bottero, Victor M Deliu 1, Pasquale Ditonno, Sisto Perdonà. Biomarkers in localized prostate cancer. Future Oncol. 2016 Feb;12(3):399-411

[65] Yii RSL, Lim J, Sothilingam S et al.: Predictive factors of prostate cancer diagnosis with PSA 4.0 – 10.0 ng/ml in a multi-ethnic Asian population, Malaysia. Asian J Surg 2019

[66] Maryam Esfahani , Negar Ataei, Mojtaba Panjehpour. Biomarkers for evaluation of prostate cancer prognosis. Asian Pac J Cancer Prev. 2015;16(7):2601-11

[67] European Association of Urology: Guideline on prostate cancer 2019: https://uroweb.org/guideline/prostatecancer [Last Access: 27 April 2019]

[68] Nagi B Kumar , Julio Pow-Sang , Philippe Spiess , Shohreh Dickinson , Michael J Schell. A phase II randomized clinical trial using aglycone isoflavones to treat patients with localized prostate cancer in the pre-surgical period prior to radical prostatectomy. Oncotarget. 2020 Apr 7;11(14):1218-1234

[69] Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). *Cochrane Handbook for Systematic Reviews of Interventions* version 6.1 (updated September 2020). Cochrane, 2020. Available from www.training.cochrane.org/handbook

[70] Cochrane Deutschland, Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften - Institut für Medizinisches Wissensmanagement. „Bewertung des Biasrisikos (Risiko systematischer Fehler) in klinischen Studien: ein Manual für die Leitlinienerstellung“. 1. Auflage 2016. Verfügbar: Cochrane Deutschland: http://www.cochrane.de/de/rob-manual; AWMF: http://www.awmf.org/leitlinien/awmf-regelwerk/ll-entwicklung.html

[71] Scottish Intercollegiate Guidelines Network SIGN 50. A guideline developer's handbook. Edinburgh: SIGN, 2008

[72] Cochrane Deutschland, Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften - Institut für Medizinisches Wissensmanagement. „Bewertung von systematischen Übersichtsarbeiten: ein Manual für die Leitlinienerstellung“. 1. Auflage 2017

[73] Higgins J, Green S. Cochrane Handbook of Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. Secondary Cochrane Handbook of Systematic Reviews of Interventions Version 5.1.0 [updated March 2011] 2011. http://handbook.cochrane.org/, Zugriff: 07.03.2020

[74] Bhide A, Shah PS, Acharya G. A simplified guide to randomized controlled trials. Acta Obstet Gynecol Scand. 2018 Apr;97(4):380-387

[75] Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology. 1998 Oct;139(10):4252-63

[76] Varinska L, Gal P, Mojzisova G, Mirossay L, Mojzis J. Soy and breast cancer: focus on angiogenesis. Int J Mol Sci. 2015 May 22;16(5):11728-49

[77] Hsieh, C.Y.; Santell, R.C.; Haslam, S.Z.; Helferich, W.G. Estrogenic effects of genistein on the growth of estrogen receptor-positive human breast cancer (MCF-7) cells *in vitro* and *in vivo*. *Cancer Res.* 1998, *58*, 3833–3838

[78] Batra N, Sam A, Woldemariam T, Talbott G, de Vere White RW, Ghosh PM, Gaikwad NW, Kotchoni SO, Vinall RL. Genistein Combined Polysaccharide (GCP) Can Inhibit Intracrine Androgen Synthesis in Prostate Cancer Cells. Biomedicines. 2020 Aug 11;8(8):282

[79] Bhadhuri A, Kind P, Salari P, et al. Measurement properties of EQ-5D-3L and EQ-5D-5L in recording self-reported health status in older patients with substantial multimorbidity and polypharmacy. *Health Qual Life Outcomes*. 2020;18(1):317

[80] Rabin R, de Charro F. EQ-5D: a measure of health status from the EuroQol Group. Ann Med. 2001 Jul;33(5):337-43

[81] Thomsen FB, Jakobsen H, Langkilde NC, Borre M, Jakobsen EB, Frey A, Lund L, Lunden D, Dahl C, Helgstrand JT, Brasso K. Active Surveillance for Localized Prostate Cancer: Nationwide Observational Study. J Urol. 2019 Mar;201(3):520-527

[82] Carmela Spagnuolo , Gian Luigi Russo , Ilkay Erdogan Orhan , Solomon Habtemariam , Maria Daglia , Antoni Sureda , Seyed Fazel Nabavi , Kasi Pandima Devi , Monica Rosa Loizzo , Rosa Tundis , Seyed Mohammad Nabavi. Genistein and cancer: current status, challenges, and future directions. Adv Nutr. 2015 Jul 15;6(4):408-19

[83] Iftekhar U Ahmad , Jeffrey D Forman, Fazlul H Sarkar, Gilda G Hillman, Elisabeth Heath, Ulka Vaishampayan, Michael L Cher, Fundagul Andic, Peter J Rossi, Omer Kucuk. Soy isoflavones in conjunction with radiation therapy in patients with prostate cancer. Nutr Cancer. 2010;62(7):996-1000

[84] Mahdi Mirahmadi , Shayan Azimi-Hashemi , Ehsan Saburi , Hossein Kamali , Mandana Pishbin , Farzin Hadizadeh. Potential inhibitory effect of lycopene on prostate cancer. Biomed Pharmacother. 2020 Sep;129:110459

[85] Maria G Grammatikopoulou, Konstantinos Gkiouras, Stefanos T Papageorgiou, Ioannis Myrogiannis, Ioannis Mykoniatis, Theodora Papamitsou, Dimitrios P Bogdanos , Dimitrios G Goulis. Dietary Factors and Supplements Influencing Prostate Specific-Antigen (PSA) Concentrations in Men with Prostate Cancer and Increased Cancer Risk: An Evidence Analysis Review Based on Randomized Controlled Trials. Nutrients. 2020 Sep 29;12(10):2985

[86] www.prostate.org.au (Last access: 29.08.2021)

[87] Leitlinienprogramm Onkologie (Deutsche Krebsgesellschaft, Deutsche Krebshilfe, AWMF): S3-Leitlinie Prostatakarzinom, Kurzversion 6.1, Juli 2021, AWMF Registernummer: 043/022OL, https://www.leitlinienprogrammonkologie.de/leitlinien/prostatakarzinom/ (Retrieved on: 05.09.2021)

7. Appendix

7.1. Original publications

Parts of this work were used for the following publications:

- P1002 - Can isoflavones influence prostate specific antigen serum levels in localized prostate cancer? A systematic review

  Ratha P., Neumann T., Schmidt C.A., Schneidewind L.

  Universitätsmedizin Greifswald, Klinik für Innere Medizin C, Hämatologie, Onkologie, Greifswald, Germany
  Universitätsmedizin Rostock, Urologische Klinik und Poliklinik, Rostock, Germany

  This publication was presented as a congress contribution in the form of a poster at the annual meeting of the German, Austrian and Swiss Society of Hematology and Medical Oncology in Berlin, Germany, Oct. 11, 2019 - Oct. 14, 2019.

- Can Isoflavones Influence Prostate Specific Antigen Serum Levels in Localized Prostate Cancer? A Systematic Review.

  Ratha P, Neumann T, Schmidt CA, Schneidewind L.

  Nutr Cancer. 2021;73(3):361-368.

8. Statutory declaration

I hereby declare that I have written this dissertation independently and have not used any auxiliary materials other than those indicated.

I declare that I have not yet unsuccessfully completed a doctoral examination and that I have not been deprived of a doctoral degree that I have already obtained.

Date Signature
09.03.2023

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10. Curriculum vitae

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Study:

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