Future directions in the prevention of prostate cancer

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Abstract | The high global incidence of prostate cancer has led to a focus on chemoprevention strategies to reduce the public health impact of the disease. Early studies indicating that selenium and vitamin E might protect against prostate cancer encouraged large-scale studies that produced mixed clinical results. Next-generation prostate cancer prevention trials validated the impact of 5α-reductase inhibitors in hormone-responsive prostate cancer, and these results were confirmed in follow-up studies. Other interventions on the horizon, involving both dietary and pharmacological agents, hold some promise but require further investigation to validate their efficacy. In this Review, we discuss the clinical and preclinical evidence for dietary and pharmacological prevention of prostate cancer and give an overview of future opportunities for chemoprevention.

Introduction
Prostate cancer is the most common cancer in American men, affecting one in six during his lifetime.1 The disease is ubiquitous, present in a growing fraction of men as they age; as life expectancy increases, this will become responsible for raising the number of cancer deaths in men.2,3 Unfortunately, patients are generally asymptomatic until their disease becomes metastatic. Although a range of new agents have been developed for advanced-stage prostate cancer, treatment is expensive, is associated with a host of adverse effects and most men with metastatic prostate cancer will ultimately die of their disease.

In response to this challenge, serum prostate-specific antigen (PSA) testing became highly prevalent in the late 1980s. Current US guidelines regarding PSA screening vary, but the 2013 American Urological Association Guideline recommends screening between ages 55 years and 69 years, as this seems to be the age during which individuals gain the greatest benefit from screening.4 Although prostate cancer mortality has certainly fallen after PSA screening was introduced because cancers were being detected at an earlier stage, the unintended consequence has been a high rate of overtreatment of indolent disease. As treatment is expensive—and frequently has a significant impact on a man’s urinary, sexual and gastrointestinal quality of life5—screening was deemed inappropriate by the US Preventive Services Task Force in 2012.6

Consequently, chemoprevention has been increasingly emphasized as an approach to mitigate the prostate cancer burden and the issues surrounding the overtreatment of indolent disease. Chemoprevention is defined as the use of drugs, vitamins or other agents to try to reduce the risk of—or delay the development or recurrence of—cancer.7 In prostate cancer, chemopreventive strategies have initially focused on sensitivity to androgens, although interest has grown in attempting to find inhibitors of chronic inflammation as this process is involved in tumour growth, angiogenesis and chemoresistance (Figure 1). In this Review, we discuss the clinical and preclinical data available for a range of chemoprevention options in prostate cancer, including the outcomes for studies assessing both dietary and pharmacological agents. We also give an overview of future opportunities for chemoprevention in this disease.

Lessons from phase III trials
Selenium and vitamin E supplementation
Substantial preclinical and epidemiological evidence has pointed to the potential of two agents—selenium and α-tocopherol (vitamin E)—that might reduce the risk of prostate cancer.8–10 Thought to exert protective effects by virtue of their anti-inflammatory activities, promising results were observed from secondary analyses of randomized clinical trials.11–13 In the Nutritional Prevention of Cancer Trial, sponsored by the National Cancer Institute (NCI), 1,312 patients who had had skin cancer were randomly assigned to receive 200 μg of elemental selenium per day in the form of high-selenium yeast.9 The primary end point of this study was a subsequent skin cancer and, although no reduction in skin cancer incidence was noted, a 63% reduction in subsequent prostate cancer was noted in those patients receiving selenium. In the Alpha Tocopherol Beta Carotene study11 (with a primary end point of lung cancer incidence), again sponsored by the NCI and conducted in Finland among smokers, new prostate cancer incidence was reduced by 32% and mortality by 41% in patients

Competing interests
The authors declare no competing interests.

receiving vitamin E. On the basis of these compelling data, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) trial was initiated by the NCI in 2001. In this study, 35,534 healthy men over the age of 50 years and from all races were randomly assigned to receive selenium (200 μg/day of l-selenomethionine), vitamin E (400 IU daily), both agents (at the same doses used for each single-agent arm) or placebo in a 2 x 2 study design. Initial findings of a preplanned interim analysis (median follow-up of 5.5 years) showed that daily selenium and vitamin E supplements, either taken alone or together, did not prevent prostate cancer. These findings led to cessation of administration of both agents, but monitoring of the participants continued. In a follow-up analysis that included 54,464 additional person-years of continued observation and found 521 more prostate cancers, this group reported—on the basis of a recommendation from the independent data and safety monitoring committee—a significant increase in the risk of prostate cancer of 17% (odds ratio [OR] 1.17; 99% CI 1.04–1.36) in the vitamin E-only group compared with those not receiving vitamin E. The results from this study suggest that neither selenium nor vitamin E likely have an impact on reducing prostate cancer incidence in the US population. Some unanswered questions remained at the end of the SELECT study. Could the lack of effect from selenium be attributed to the fact that only a small number of men might be deficient in this nutrient? Why was there no significant increase in risk of prostate cancer seen in the combination (vitamin E plus selenium) group? What effect would a lower dose of vitamin E have? Could the type of selenium used in the trial (selenomethionine) have blunted the effect of the agent? Certainly, other doses of vitamin E could have a potentially different effect, but given that the dose of 400 IU was carcinogenic, it is unlikely that a large study would be conducted to examine the effect of a lower dose. Additional confirmation of the lack of efficacy of selenium was provided by a randomized trial that enrolled 619 men with high-grade prostatic intraepithelial neoplasia, 423 of whom were randomly assigned to receive either selenium or placebo. In this study, selenium at a dose of 200 μg/day as selenomethionine had no effect on the subsequent risk of prostate cancer.

5α-reductase inhibitors
Prostate cancer is unequivocally an androgen-modulated disease. Circulating testosterone that enters prostatic cells is converted to dihydrotestosterone (DHT) by 5α-reductase. Although both testosterone and DHT can bind to the androgen receptor, ultimately resulting in nuclear transcription, DHT has a greater affinity and a slower rate of dissociation from the androgen receptor than testosterone, making 5α-reductase a target for prevention and treatment of prostate-related diseases. Testosterone at high concentrations interacts with the human androgen receptor in a similar way to DHT. Although the role of androgens in prostate carcinogenesis is less clear than their role in progression and treatment, a host of observations in the early 1990s suggested that modulation of the androgen axis could affect prostate cancer risk. With the development of finasteride, the first 5α-reductase inhibitor (5-ARI) for the treatment of urinary symptoms associated with benign prostatic hypertrophy, the Prostate Cancer Prevention Trial (PCPT) was initiated to determine if the risk of prostate cancer could be reduced by this agent. A total of 18,882 men with serum PSA levels <3.0 ng/ml were randomly assigned to receive finasteride or placebo. Treatment was continued for 7 years, and all participants who were cancer-free at this point were recommended to undergo prostate biopsy. More than 1 year before the planned study completion date, the independent data and safety monitoring committee recommended early closure because of overwhelming evidence that the primary objective (prostate cancer prevalence) had been met. A relative risk (RR) reduction for prostate

- Chemoprevention has been increasingly explored to mitigate the global burden of prostate cancer and the overtreatment of indolent disease that has arisen in the prostate-specific antigen (PSA) screening era
- Preclinical and epidemiological evidence suggested that selenium and α-tocopherol (vitamin E) might reduce the risk of prostate cancer
- A large trial found vitamin E to significantly increase the risk of prostate cancer
- The strongest evidence supports the use of 5α-reductase inhibitors for prostate cancer prevention, with recent data showing that the risk reduction with these agents is 30%

Figure 1 | Prostate cancer progression. Accumulated DNA damage, oxidative damage, genetic polymorphisms and chronic inflammation all contribute to disease progression. These events also provide opportunities for possible intervention with chemopreventive agents. Abbreviations: COX-2, cyclooxygenase 2; IL, interleukin; miR, microRNA; mTOR, mammalian target of rapamycin; PIN, prostatic intraepithelial neoplasia; PSA, prostate-specific antigen.
cancer of 24.8% with finasteride was observed compared with those in the placebo group (95% CI 18.6–30.6%, \( P < 0.001 \); 803 of 4,368 participants in the finasteride group versus 1,147 of 4,692 participants in the placebo group). However, this seemingly positive result was marred by an increased number of participants who developed high-grade (Gleason 7–10) tumours in the finasteride group: 280 of 757 (37%) versus 237 of 1,068 (22%) in the placebo group (\( P < 0.001 \)). Since that time, post hoc analyses have demonstrated that the increase in high-grade cancer was attributable, at least in part, to improved cancer detection in patients treated with finasteride through improved sensitivity of serum PSA, digital rectal examination and prostate biopsy for prostate cancer detection; in the cases of PSA and biopsy, sensitivity for detection of high-grade cancer was also improved with finasteride. Follow-up analysis of men in the PCPT, the longest randomized trial of this medication to date, also revealed a significant reduction in the risk of prostate enlargement complications in those patients treated with finasteride. This observation suggests a dual prevention benefit among men who are developing symptoms of prostate enlargement: reduction in the risk of prostate cancer as well as in the risk of complications related to prostate enlargement.

The results of the long-term follow-up of the PCPT have recently been reported. With up to 20 years of follow-up data, prostate cancer was found in 989 of 9,423 (10.5%) men in the finasteride group, compared with 1,412 of 9,457 (14.9%) in the placebo group, which translates to a risk reduction of 30% (RR in finasteride group 0.70; 95% CI 0.65–0.76; \( P < 0.001 \)). Although an increased risk of Gleason 7–10 prostate cancer remained in the finasteride group (RR 1.17; 95% CI 1.00–1.37; \( P = 0.05 \)), no difference in survival between the finasteride and placebo-treated patients was apparent when stratified by grade. These data confirm that finasteride is associated with a significant reduction in the risk of prostate cancer and, despite a greater number of high-grade tumours diagnosed in the finasteride group, no excess deaths were observed in patients treated with this agent. This observation suggests that men who have opted for PSA testing might achieve similar survival outcomes, but with 30% fewer cancers diagnosed if finasteride is used.

There are two isoenzymes of 5α-reductase; finasteride inhibits the type 2 isoenzyme, which is the most important in the prostate. An even greater reduction in DHT levels can be achieved by dual inhibition of both isoenzymes with, for example, the inhibitor dutasteride. The Reduction by Dutasteride of Prostate Cancer Events (REDUCE) trial investigated the preventive impact of dutasteride in 8,231 men with a PSA level 2.5–10.0 ng/ml and a previous negative biopsy. The results were similar to the PCPT: a 22.8% (95% CI 15.2–29.8; \( P < 0.001 \)) reduction in risk of prostate cancer was observed in the dutasteride treatment arm. Gleason 7–10 tumours were slightly more common in the dutasteride group (220 out of 3,299 patients in the dutasteride arm versus 233 out of 3,407 patients in the placebo group, \( P = 0.81 \)).

Concern in this study was the increase in Gleason score 8–10 tumours in study years 3 and 4, rising from one tumour in the placebo group to 12 tumours in the dutasteride group (\( P = 0.003 \)). Ideally, long-term follow-up study of this population, as carried out for the finasteride update, would clarify whether this excess of 13 Gleason 8–10 tumours is biologically relevant when compared with the 1,517 total tumours detected over the course of the trial.

Taking into account that >200,000 men are diagnosed with prostate cancer in the USA annually, a one-quarter reduction in prostate cancer risk using a 5-ARI, an agent that also reduces the risk of prostate enlargement symptoms and complications (such as urinary retention and need for surgical treatment), would have a tremendous public health impact. Further analysis of the PCPT and REDUCE studies should shed light on whether the increase in detection of high-grade disease was an artefact, and an ongoing large case–control study might help us understand which men stand to benefit the most from 5-ARIs. In this study, genetic variants of SRD5A2 (the polymorphic gene that encodes 5α-reductase), among others, are being examined for their influence on prostate cancer risk and the impact of finasteride as a cancer prevention intervention.

**Other preventive opportunities**

**Inflammation blockade**

The role of acute and chronic inflammation in the genesis of prostate cancer remains controversial; however, accumulating evidence indicates anti-inflammatory blockade provides a major opportunity for chemoprevention in this disease (Figure 1). Irritation to the prostate has been postulated to come from three potential sources: infection, dietary stimulation of prostanoid secretion and hormonal changes. Increasingly, proliferative inflammatory atrophy (PIA) of the prostate, a condition characterized by elevated markers of inflammation, is being recognized as preceding prostatic intraepithelial neoplasia (PIN) and prostate cancer. With long-term stimulation, NF-κB induces the expression of PTGS2 (which encodes cyclooxygenase 2 [COX-2]) and generates many downstream effectors that tip the balance toward an inflammatory state. Elevated levels of cytokines and chemokines hasten the process, aided and abetted by suppressed vitamin D receptor levels—itself a master regulator of inflammation—and elevated mTOR signalling, resulting in a sustained stimulus to angiogenesis and chemoresistance. Both *in vitro* and *in vivo* models of prostate cancer have demonstrated the antiproliferative and anti-tumour potential of vitamin D3 both in chemopreventive and chemotherapeutic studies. Ongoing clinical trials investigating the effects of vitamin D3 supplementation in patients with low-risk and localized prostate cancer are being conducted.

A range of factors have been postulated to contribute to prostatic inflammation, including bacterial infection (*Escherichia coli* or *Propionibacter acnes*), and dietary factors, such as polysaturated fats—sources of arachidonic acid—that are involved in prostanoid
biosynthesis and inflammation. Inflammatory cells (leukocytes and T lymphocytes) are frequently detected in prostate tumours; for example, in the REDUCE trial, 80% of patients had evidence of inflammation in biopsies. In another study, men of African American origin were found to have more inflammation in prostate tumours than those not of African American descent. Indeed, archived prostate tissues subjected to microarray gene-expression analysis revealed that specimens from African American men had elevated expression of interleukin (IL)-1β, IL-6, IL-8 and chemokine receptor type 4 (CXCR4) in prostate tissues compared with prostate tissue from white American men. A meta-analysis of 11 prospective cohorts with 194,796 patients revealed a link between elevated levels of C-reactive protein (CRP), indicative of systemic inflammation, and increased risk of cancer in general, as well as lung cancer specifically; CRP level was possibly indicative of breast, colorectal and prostate cancer. Urine reflux has also been hypothesized to have a role in the expression of inflammation markers and elevated levels of cytokines, although whether this is a common risk factor remains unclear. Finally, animal studies have shown that inflammation is a critical early step in the progression to prostate cancer, and Mineault and Batra suggest that elevated levels of TGF-β, CXCR1, CXCR2, CXCL8, NF-κB and IL-17 are possible contributors to this process. A number of additional agents have shown promise as preventive agents for prostate cancer. Some of these agents will soon enter clinical testing, whereas some have not yet reached the level of evidence required for their use in the clinic as chemopreventive agents.

**Lycopene**

Considerable evidence suggests that a high dietary intake of fruit and vegetables is associated with a reduced risk of many cancers, including prostate cancer. In this arena, the chemopreventive activity of lycopene and soy isoflavones is being investigated; the earliest hypotheses proposed that the protective mechanism of these and similar agents is the result of their antioxidant properties. Antioxidants protect cells against free radicals that can damage DNA, and as free radical damage increases with age, lycopene (a carotenoid found in red fruit and vegetables) could protect against age-related cancers, such as prostate cancer. On the basis of published cohort studies and a meta-analysis of six high-quality case–control studies, the American Institute for Cancer Research concluded that “foods containing lycopene probably protect against prostate cancer.”

Despite promising retrospective studies, prospective studies have been less-supportive of the protective role of lycopene. In the largest of these studies, the PCPT, lycopene intake was not associated with a reduction in the risk of prostate cancer among 9,559 men who completed the trial. Additionally, no relationship was detected between lycopene serum concentrations and risk of prostate cancer. Similarly, in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, which included approximately 28,000 men, serum lycopene levels were not related to risk of subsequent prostate cancer after 1–8 years of follow-up (OR 1.14; 95% CI 0.82–1.58 for highest versus lowest quintile; P for trend of 0.28). To determine the clinical efficacy of lycopene, the physiologically effective dose must be delineated, more-potent analogues must be synthesized, combinatorial studies with enhancers must be conducted and administration regimens must be optimized.

Ongoing phase II clinical trials are examining the preventive effect of lycopene in tandem with other antioxidants to reduce the size of prostate cancers, prevent the recurrence of prostate cancer, or augment therapy, and completed clinical trials might provide more insight into the therapeutic potential of lycopene. However, at this juncture, a considerable amount of preparatory research is needed before lycopene can truly be considered a promising avenue in prostate cancer prevention.

**Soy foods**

Soy-based foods, such as tofu and miso, contain isoflavones that are phytoestrogens. The two most abundant isoflavones that have been extensively studied mechanically are genistein and daidzein. Evidence suggests that in countries where soy consumption is a culinary mainstay, such as most of Southeast Asia, the incidence of prostate cancer is lower than in North America. A meta-analysis of five cohort and eight case–control studies that investigated the relationship between soy food consumption and the risk of prostate cancer suggested that high consumption of some soy-based foods can significantly decrease the risk of prostate cancer. A number of small clinical studies also examined the relationship between the intake of soy foods and prostate cancer risk. In one such study, 100 Japanese men who had undergone prostate biopsy and were found to be cancer-free were randomly assigned to receive soy isolavones (40 mg; comprised of 66% daidzein, 24% glycitin and 10% genistein) and curcumin (100 mg) or placebo for 6 months. No difference was observed in PSA levels between the two treatment groups (initial PSA levels were 8.0 ± 6.7 ng/ml in the placebo group versus 10.5 ± 9.5 ng/ml in the treatment group; PSA levels post-trial were 7.1 ± 5.6 ng/ml in the placebo group versus 7.4 ± 4.6 ng/ml in the treatment group). In another study, 58 men at risk of prostate cancer (defined as men with preneoplastic lesions) or with low-grade prostate cancer received one of three types of soy protein (either soy protein isolate, alcohol extracted soy isolate or soy milk isolate) for 6 months. Fewer cases of prostate cancer were reported in men who had received soy protein (P = 0.01), although tissue biomarkers were not affected. Several studies have focused on modulation of PSA as a short-term indicator of the health benefits of soy. In a study of 47 patients who were scheduled for radical prostatectomy, patients were randomly assigned to receive placebo or genistein (30 mg daily) for 3–6 weeks before surgery. Testosterone levels decreased by 7.8% in patients receiving genistein, whereas serum PSA levels increased by 4.4% in patients who received placebo. In a randomized trial of vitamin E, selenium and soy protein in men.
**Table 1 | Preclinical evidence for preventive activity of curcumin in prostate cancer**

<table>
<thead>
<tr>
<th>Study</th>
<th>In vitro model</th>
<th>Findings</th>
<th>In vivo model</th>
<th>Findings</th>
<th>Putative mechanism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sundram et al. (2012)</td>
<td>LNCaP; LNCaP C4-2 and PC3</td>
<td>Inhibition of cell proliferation, colony formation and cell motility; Enhanced cell-cell aggregation in prostate cancer cells</td>
<td>LNCaP C4-2 xenograft in athymic nude mice</td>
<td>Inhibition of tumour growth correlated with enhanced membrane localization of β-catenin</td>
<td>Activation of PKD1, resulting in changes in β-catenin signalling and inhibition of β-catenin transcription activity; Reduced β-catenin levels in PCa cells; Decreased active cofilin levels</td>
</tr>
<tr>
<td>Shankar et al. (2008)</td>
<td>LNCaP</td>
<td>No in vitro-specific results</td>
<td>LNCaP xenograft in Balb/c nude mice</td>
<td>Inhibition of tumour growth, metastasis and angiogenesis; Induction of apoptosis; Upregulation of TRAIL receptors; Inhibition of activation of NF-κB and its gene products</td>
<td>Sensitization of LNCaP cells by inducing death receptors, upregulating proapoptotic proteins, inhibiting antiapoptotic Bcl-2 proteins and inducing expression of cell-cycle inhibitors</td>
</tr>
<tr>
<td>Sliusarz et al. (2010)</td>
<td>TRAMP-C2 and PC3</td>
<td>Inhibition of cell growth</td>
<td>C57BL6/J TRAMP</td>
<td>Reduction and delay in prostate cancer initiation</td>
<td>Inhibition of Hedgehog signalling pathway</td>
</tr>
<tr>
<td>Barve et al. (2008)</td>
<td>NA</td>
<td>NA</td>
<td>C57BL6 TRAMP</td>
<td>Decreased incidence of prostate tumour formation; Inhibition of PIN; Decreased cellular proliferation; Increased apoptotic index</td>
<td>Downregulation of AKT signalling pathway to decrease cell proliferation</td>
</tr>
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</table>

*Curcumin and TNF-related apoptosis-inducing ligand. +Curcumin and phenylethylisothiocyanate. Abbreviations: NA, not applicable; PIN, prostatic intraepithelial neoplasia; PKD1, poly cystic kidney disease 1 protein; TRAIL, TNF-related apoptosis-inducing ligand; TRAMP, transgenic adenocarcinoma of mouse prostate.*

Curcumin

Curcumin is the major polyphenol component of the Indian spice turmeric (*Curcuma longa*), and has been extensively studied for cancer prevention (Table 1). Its anti-inflammatory activity has been confirmed in a wide variety of tumour types, including prostate, head and neck and mammary tumours. One clinical study, in 85 patients with previous negative prostate biopsies, showed that a supplement combining soy and curcumin reduced serum PSA levels sharply in those with initial PSA readings of >10 ng/mL. 

In vitro studies have shown that curcumin interferes with a number of components of cell signalling pathways dysregulated in prostate cancer, including COX-2, NF-κB, Bcl-XL, AKT and receptor activator of NF-κB ligand (RANKL). These effects, as well as modulation of a number of chemokines and cytokines, suggest that inflammatory components in prostate cancer development or progression could be targeted with curcumin. Promising data also indicate that curcumin, by acting as a kinase inhibitor, could inhibit prostate cancer metastasis by targeting cytokines CXCL1 and CXCL2 through inhibition of NF-κB. Studies have also shown that curcumin might be effective in castration-resistant prostate cancers; one study has shown that curcumin potentiates the effect of paclitaxel therapy in androgen-independent DU145 cells by circumventing absorption problems when complexed in a polymer.

Despite numerous studies supporting the anticancer properties of curcumin, its clinical use has been restricted because of its low bioavailability and poor tissue absorption. Several approaches have been undertaken to overcome these limitations, including synthesis of structural analogues, adjuvant agents (for example, piperine which improves bioavailability by increasing the solubility of curcumin in blood) and development of delivery systems (for example, nanoparticles, nanoemulsions and liposomes).
Green tea and green tea polyphenols

Studies in animal models have shown that green tea prevents prostate cancer (Table 2) through a mechanism of action that includes targeting apoptosis by preventing the degradation of p53.76–85 A meta-analysis of 13 studies involving 3,608 men with prostate cancer among 111,499 individuals revealed a protective relationship between prostate cancer and the long-term intake of green tea, mostly in Asia where the intake of green tea is common.86

Polyphenon E is a mixture of green tea-based polyphenols that has been used in clinical studies of chemoprevention for breast, leukemia and prostate cancer. In an open-label, single-arm, two-stage phase II clinical trial, 26 men with positive prostate biopsies were given daily doses of polyphenon E (800 mg/day) for 3–6 weeks until undergoing radical prostatectomy. Polyphenon E administration lowered serum levels of hepatocyte growth factor (HGF), VEGF, insulin-like growth factor-binding protein 3 (IGF-BP3), insulin-like growth factor 1 (IGF-1) and PSA in these patients.79 In another study, 60 men with high-grade PIN were randomly assigned to receive green tea catechins (in pill form) or placebo for 1 year.85 Although a small trial, patients receiving the polyphenols had a significantly reduced progression of high-grade PIN to cancer compared with those who received placebo ($P < 0.001$). However, this rapid inhibition of high-grade PIN might be attributable to sampling error rather than the intervention, and a confirmatory trial with more patients is required before a definitive conclusion can be reached.

Metformin

Metformin is the most widely prescribed biguanide worldwide for control of blood sugar levels in patients with type 2 diabetes mellitus (T2DM).87–89 Metformin might be a promising agent worthy of rigorous clinical assessment for prostate cancer prevention given the collateral benefits attributed to the drug’s glucose control mechanism—such as reducing the risk of cardiovascular disease and polycystic ovary syndrome—and its low toxicity profile. Indeed, a number of clinical studies have suggested that metformin might also reduce the risk of prostate cancer (Table 3). Lehman et al.89 examined metformin, sulfonylurea and statin use in >5,000 men with T2DM in the Veterans Affairs (VA) Healthcare System, hypothesizing that these agents have synergistic protective effects in prostate cancer. Metformin was associated with a 31% reduction in the risk of prostate cancer, but only in men receiving statins; the risk of cancer was more than twofold less than observed in those not receiving any intervention ($P < 0.0001$). Importantly, only 10% of the cohort was taking both metformin and statins; thus, this finding must be validated in larger studies. Other clinical studies have produced mixed results; a similar protective effect on prostate cancer has been shown in some studies, but these results have been contradicted in two meta-analyses of metformin use in patients with T2DM.90–94 Metformin use does have a low risk of nausea, diarrhoea and loss of appetite, which might limit application in some settings. Finally, although few studies of metformin and prostate cancer have been conducted using animal models, studies of other tumour types—notably in models of oral, ovarian and pancreatic cancer—have shown a chemopreventive effect for metformin.95–101 Metformin has been found to inhibit the growth and progression of xenografted LnCaP prostate tumours in mice.102
NSAIDs

The epidemiological evidence linking aspirin and other NSAIDs to protect against prostate cancer is still equivocal (Table 4), but growing in favour of possible use.110–115 Studies have included cohort and case–control studies and secondary analyses of large-scale clinical trials. For example, one case–control study, comparing 9,007 men diagnosed with prostate cancer with men without prostate cancer, showed aspirin conferred a 10% reduction in risk of prostate cancer (OR 0.90, 95% CI 0.81–0.95); this benefit was associated with >10 years of NSAID use.110 A meta-analysis of studies reported in the mid-2000s showed a 39% reduction in the risk of prostate cancer with aspirin, but not with ibuprofen.108 Using data from the PLCO study, investigators found a reduction of approximately 10% (OR 0.91, 95% CI 0.84–0.99) in prostate cancer risk with aspirin use, whereas, again, ibuprofen use did not confer a benefit.109 Aspirin has been available much longer than other NSAIDs, such as ibuprofen and naproxen, and as time passes it is likely that similar benefits to aspirin will become evident for these NSAIDs.

Not all studies have demonstrated a benefit for NSAID use in prostate cancer chemoprevention. In the PCPT, use of NSAIDs increased the risk of benign prostate hyperplasia (OR 1.21, 95% CI 1.01–1.46).110 A Finnish study of 24,567 case–control pairs gathered between 1995–2002 from a national registry of recorded prescription drug use reported an increase in risk of overall and advanced-stage prostate cancer in men who used NSAIDs, but aspirin use was associated with a reduced risk of prostate cancer.111 By contrast, Rothwell et al.112 did not find a protective association of aspirin for prostate cancer risk (OR 0.94; 95% CI 0.82–1.08), but did observe potential protection for other tumour types. In this literature review of case–control and cohort studies published from 1950 to 2011, regular use of aspirin was associated with reduced risk of colorectal cancer (pooled OR 0.62, 95% CI 0.58–0.67, P < 0.0001, 17 studies), with little heterogeneity (P = 0.13). Similarly, consistent reductions were observed in risks of oesophageal, gastric, biliary and breast cancer. That the authors found inconsistent results for other tumour types might be explained by less-detailed recording of aspirin exposure, a lack of updating of exposure between initial recruitment and subsequent diagnosis of cancer and by a lack of adjustment for imbalances in baseline clinical characteristics.

Few animal studies investigating the relationship between NSAIDs and prostate cancer prevention have been conducted. Most studies have examined the effects of celecoxib—a selective COX-2 inhibitor—on PC3 or LnCaP xenografts, either alone or in combination with other treatments (such as androgen ablation or atorvastatin).113–115 In general, these interventions have reduced progression of prostate cancer.
Table 4 | Evidence for preventive activity of NSAIDs in prostate cancer

<table>
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<th>Intervention</th>
<th>Study design</th>
<th>Findings</th>
<th>Putative mechanism(s)</th>
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<td><strong>Preclinical</strong></td>
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<tr>
<td>Zhang et al. (2010)</td>
<td>SSA</td>
<td>In vitro LNCaP, LNCaP C4-2, TRAMP-C2; In vivo C57BL/6 TRAMP model</td>
<td>SSA suppressed growth of human and mouse prostate AR+ cancer cells; Dietary SSA attenuated prostatic growth, suppressed AR-dependent glandular epithelial lesion progression via repressed cellular proliferation</td>
<td>Suppression of cell growth by G1 arrest at low concentration (&lt;10 μmol/l); At higher concentrations (≥10 μmol/l), suppression associated with caspase-mediated apoptosis</td>
</tr>
<tr>
<td>Zheng et al. (2010)</td>
<td>Atorvastatin and celecoxib</td>
<td>In vitro LNCaP; In vivo androgen-independent LNCaP tumour xenograft in SCID mice</td>
<td>Atorvastatin or celecoxib inhibited growth and stimulated apoptosis; Combination was more effective than either agent alone; Mice treated with atorvastatin or celecoxib alone suppressed regrowth or LNCaP tumours after castration; Combined low-dose treatment exerted more-potent effect on growth inhibition and progression</td>
<td>Inhibition of cell proliferation and induction of apoptosis</td>
</tr>
<tr>
<td><strong>Clinical</strong></td>
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<td>Schenk et al. (2012)</td>
<td>NSAIDs (aspirin, ibuprofen, sulindac, meloxicam, nabumetone, celecoxib) aspirin and nonaspirin NSAIDs</td>
<td>Cohort study for risk of symptomatic BPH men without BPH at baseline (n = 4,735) using data from the placebo arm of the PCPT</td>
<td>No reduction in symptomatic BPH; NSAID use associated with increased BPH risk of 23%, with similar risks for aspirin (29%) and nonaspirin NSAID (34%); Modest association of NSAID use with increased risk of BPH, the result of confounding indications</td>
<td>Intervention on inflammatory pathways might reduce risk of BPH</td>
</tr>
<tr>
<td>Shebl et al. (2012)</td>
<td>Aspirin and ibuprofen</td>
<td>Analysis of patient-reported aspirin and ibuprofen use in relation to prostate cancer risk in men aged 55–74 years in the PLCO study (n = 29,450)</td>
<td>Daily aspirin use, but not ibuprofen use, associated with reduced prostate cancer risk</td>
<td>Inhibition of COX; Suppression of cell proliferation; Induction of apoptosis</td>
</tr>
<tr>
<td>Mahmud et al. (2011)</td>
<td>Aspirin, arylacetic acids, butyrylpyrazolidines, oxicams and propionates</td>
<td>Nested case–control study using data from the SPDP and cancer registry to examine the effects of dose and duration of NSAIDs in men (n = 9,007 aged ≥40 years)</td>
<td>Any use of propionates (ibuprofen, naproxen) associated with modest reduction in prostate cancer risk; No clear evidence of dose-response or duration-response relationships for any NSAID</td>
<td>Inhibition of cell proliferation, COX, and angiogenesis; Induction of apoptosis</td>
</tr>
<tr>
<td>Dhillon et al. (2011)</td>
<td>Aspirin</td>
<td>Prospective cohort study of health professionals (40–75 years old) for long-term aspirin use (n = 51,529)</td>
<td>Men taking &gt;two adult-strength aspirin tablets weekly had 10% lower risk of prostate cancer; For T3b, T4 and N1 disease, no significant associations with aspirin use; For M1 disease, men taking &gt;six adult-strength tablets weekly had similar reductions in risk hazard ratio (HR 0.72, 95% CI 0.54–0.96)</td>
<td>Inhibition of cell proliferation and COX; Induction of apoptosis</td>
</tr>
<tr>
<td>Salinas et al. (2010)</td>
<td>Aspirin, ibuprofen and acetaminophen</td>
<td>US population-based case–control study of men aged 35–74 years (n = 1,001)</td>
<td>Reduction in prostate cancer risk (21%) in aspirin users compared with nonusers; Long-term aspirin (&gt;5 years) and daily use of low-dose aspirin associated with decreased risk; Significant interaction with aspirin use for PTGS2 rs12042763 polymorphism; Prostate cancer risk not associated with ibuprofen or acetaminophen use</td>
<td>Inhibition of cell proliferation, metastasis and PTGS2 enzymes; Induction of apoptosis</td>
</tr>
<tr>
<td>Coogan et al. (2010)</td>
<td>Statins (simvastatin, atorvastatin, lovastatin, fluvastatin, pravastatin, rosuvastatin) and NSAIDs (undefined)</td>
<td>Case–control study in men aged 18–79 years (n = 1,367)</td>
<td>No protective effect of statin use alone or in combination with NSAIDs on risk of advanced prostate cancer</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: AR, androgen receptor; BPH, benign prostatic hyperplasia; COX, cyclooxygenase; NA, not applicable; PCPT, Prostate Cancer Prevention Trial; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; PSA, prostate-specific antigen; PTGS2, gene encoding prostaglandin-endoperoxide synthase (PTGS) 2; SCID, severe combined immunodeficiency; SPDP, Saskatchewan Prescription Drug Plan; SSA, sulindac sulfide amide; TRAMP, transgenic adenocarcinoma of mouse prostate.

Resveratrol

Pigments naturally found in fruits and vegetables also hold promise for prostate cancer prevention (Table 5). Once such pigment is resveratrol—a phytoalexin found abundantly in red grapes, raspberries, plums, blueberries and red wine.62,63,66,116–118 Similar to curcumin, several preclinical studies have shown that resveratrol inhibits prostate cancer metastasis or enhances sensitivity of otherwise radioresistant PC3 cells to radiation.119–122 Indeed, resveratrol significantly inhibits the incidence of high-grade PIN in mice, but has paradoxically been shown to reduce survival of mice with LAPC4 human prostate cancer xenografts; in LnCaP xenografts, survival was not untowardly affected.124–126 The implications of these different results in LnCaP and LAPC4 models are unclear. However, the
therapeutic efficacy of resveratrol is limited owing to its low bioavailability and extensive metabolic clearance.\textsuperscript{127,128} Different drug delivery systems, analogues and compound co-administration are being investigated to enhance the pharmacokinetic properties of resveratrol.\textsuperscript{129-132}

Phase I clinical trials on the effects of resveratrol in healthy volunteers and in patients with colorectal cancer and hepatic metastases demonstrated an acceptable safety profile.\textsuperscript{133,134} However, a phase II study of resveratrol (SRT501) in 24 patients with relapsed or refractory multiple myeloma was suspended because of a high rate of adverse effects, especially renal toxicity.\textsuperscript{135} Notably, patients with multiple myeloma generally exhibit renal impairment or insufficiency, which might explain the observed high nephrotoxicity in this trial.\textsuperscript{135-137} Renal toxicity might not be observed in a healthier population, or in populations without this susceptibility; indeed, studies of resveratrol in otherwise healthy men should be performed to confirm if renal toxicity is a specific adverse effect of resveratrol observed in patients with multiple myeloma.

### Conclusions

Of the many chemopreventive strategies that have been explored in prostate cancer, only 5-ARIs are supported by compelling clinical evidence. Although an increased risk of high-grade disease observed with 5-ARIs initially tempered enthusiasm for this approach, the long-term evidence of safety and a further reduction in risk should reinvigorate consideration of this class of agents for most men at risk of prostate cancer. Studies indicate that vitamin E should not be used as it is associated with an increased risk of cancer. These two examples go some way to illustrate the nuances (and importance) of large-scale clinical trials of prostate cancer prevention; no clinical recommendations should be made unless agents are tested in an appropriately powered

### Table 5 | Preclinical evidence for preventive activity of resveratrol in prostate cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Intervention</th>
<th>Study design</th>
<th>Findings</th>
<th>Putative mechanism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klink \textsuperscript{et al.} (2013)\textsuperscript{124}</td>
<td>Resveratrol alone</td>
<td>\textit{In vivo} LAPC4 xenograft and LNCaP xenograft in CB17SC SCID mice</td>
<td>Decreased survival in LAPC4 xenograft model; No change in survival in LNCaP xenograft model; Decrease in IGF-1 and increased in IGFBP-2 in both models</td>
<td>Upregulation of oncogenic pathways E2F3 and β-catenin in LAPC4 xenograft model but not in LNCaP xenograft model\textsuperscript{*}</td>
</tr>
<tr>
<td>Ganapathy \textsuperscript{et al.} (2010)\textsuperscript{121}</td>
<td>Resveratrol, TRAIL or both</td>
<td>\textit{In vivo} PC3 xenograft in Balb/c nude mice</td>
<td>Inhibited growth of PC3 xenografts; Combination was more potent than either compound alone; Resveratrol alone upregulated TRAIL-R1, TRAIL-R2, Bax and p27; Resveratrol alone inhibited Bcl-2 and phosphorylation of FOXO3; Combination inhibited angiogenesis and markers of metastasis</td>
<td>Resveratrol potentially enhances apoptosis-inducing activity of TRAIL by activating FOXO3 and its target genes</td>
</tr>
<tr>
<td>Brizuela \textsuperscript{et al.} (2010)\textsuperscript{143}</td>
<td>Resveratrol alone</td>
<td>\textit{In vitro} PC3, C4-2B; \textit{In vivo} heterotopic PC3 xenograft in NMRI nude mice (PC3/SPHK1 and PC3/GFP)</td>
<td>Impeded cell growth in vitro and in vivo</td>
<td>Inhibition of SPHK1/SIP pathway</td>
</tr>
<tr>
<td>Slusarz \textsuperscript{et al.} (2010)\textsuperscript{126}</td>
<td>Resveratrol alone</td>
<td>\textit{In vitro} TRAMP-C2 and PC3; \textit{In vivo} C57BL6/J TRAMP model</td>
<td>Inhibited growth in human and mouse prostate cancer cell lines; Reduced or delayed prostate cancer in TRAMP mice</td>
<td>Inhibition of Hedgehog signalling pathway</td>
</tr>
<tr>
<td>Harper \textsuperscript{et al.} (2009)\textsuperscript{144}</td>
<td>Resveratrol, genistein or both</td>
<td>\textit{In vivo} SV40 large T-antigen-targeted probasin promoter rat model</td>
<td>Genistein and resveratrol treatment alone or in high-dose combinations suppressed prostate cancer development; Treatment with both polyphenols decreased cell proliferation and IGF-1 protein expression; Genistein alone induced apoptosis and decreased NCoA-3 in ventral prostate</td>
<td>Inhibition of cell proliferation, IGF-1 expression and upregulation of AR by resveratrol; Induction of apoptosis and inhibition of NCoA-3 signalling by genistein</td>
</tr>
<tr>
<td>Narayanan \textsuperscript{et al.} (2009)\textsuperscript{129}</td>
<td>Resveratrol and curcumin (with or without liposomal encapsulation)</td>
<td>\textit{In vitro} PTEN-CaP8; \textit{In vivo} prostate-specific PTEN knockout mouse model</td>
<td>Combination of liposomal forms of curcumin and resveratrol decreased prostatic adenocarcinoma in vivo, inhibited cell growth and induced apoptosis in vitro</td>
<td>Downregulation of targets (p-AKT, cyclin D1, mTOR and AR) that are activated by PTEN loss</td>
</tr>
<tr>
<td>Wang \textsuperscript{et al.} (2008)\textsuperscript{126}</td>
<td>Resveratrol alone</td>
<td>\textit{In vitro} LNCAP; \textit{In vivo} LNCaP xenografts in athymic Balb/c and AnNCr-nu/nu nude mice</td>
<td>Inhibited cell growth; Delayed initial development of LNCaP tumours; Resveratrol exposure promoted angiogenesis and inhibition of apoptosis in LNCaP xenograft model</td>
<td>Modulation of steroid hormone-dependent pathways (androgen-mediated and oestrogen-mediated events)\textsuperscript{3}</td>
</tr>
<tr>
<td>Seeni \textsuperscript{et al.} (2008)\textsuperscript{122}</td>
<td>Resveratrol alone</td>
<td>\textit{In vivo} TRAP model</td>
<td>Suppression of prostate cancer growth and induction of apoptosis without toxicity</td>
<td>Downregulation of AR expression; Suppression of the androgen response to glandular kallikrein 11\textsuperscript{3}</td>
</tr>
</tbody>
</table>

\textsuperscript{*}Preliminary data suggest that resveratrol might be harmful; caution is advised when using resveratrol in human patients until further studies are conducted. \textsuperscript{3}In vivo exposure is a concern due to increased angiogenesis and inhibited apoptosis. \textsuperscript{11}Provides mechanistic basis for resveratrol chemopreventive efficacy against prostate cancer. Abbreviations: AR, androgen receptor; IGF-1, insulin-like growth factor 1; IGFBP-2, insulin-like growth factor binding protein 2; mTOR, mammalian target of rapamycin; NA, not applicable; NCoA-3, Nuclear receptor coactivator 3 (also known as steroid receptor coactivator protein 3); PSA, prostate-specific antigen; SCID, severe combined immunodeficiency; TRAIL, TNF-related apoptosis-inducing ligand; TRAMP, transgenic adenocarcinoma of mouse prostate; TRAP, transgenic rat for adenocarcinoma of prostate.
and designed randomized trial. Despite the practical difficulties of conducting large trials with thousands of participants, preclinical and clinical data (from small studies) strongly support the exploration of various agents to reduce the risk of prostate cancer. A challenge for the future will be the translation of preclinical data into clinically useful strategies, which will require very large sample sizes on a par with those of the PCPT and SELECT studies.


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Author contributions
All the authors researched the data for the article, made a substantial contribution to the discussion of its content, wrote the manuscript and edited it prior to submission.