Molecular targets for cancer chemoprevention

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Abstract | Vaccines targeting infections with hepatitis B virus, a risk factor for hepatocellular cancer, and human papillomavirus, a risk factor for cervical cancer, are considered major clinical cancer chemoprevention successes. Molecularly targeted agents can prevent breast cancer (raloxifene and tamoxifen), colorectal adenomas (celecoxib), and prostate cancer (finasteride). Nevertheless, the broad translation of chemoprevention to the clinic is not yet a reality. Continuing research of molecular targets promises to expand the reach of chemoprevention and to personalize it as well.

Carcinogenesis
The process of molecular, physiological and histological changes by which normal cells are transformed into cancerous cells.

Intraepithelial neoplasia
(IEN). Lesions confined to the most superficial layer of an organ cavity and/or surface (that is, the epithelial layer). IENs are frequently precursors to invasive cancer.

Epigenetic alterations
Stable gene expression pattern changes that are transmitted from parent to daughter cells, largely irreversible, and different from genetic expression patterns. Epigenetic phenomena are important in determining cellular physiology and pathology. Examples of epigenetic changes are DNA methylation and acetylation.

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Originally proposed by Sporn *et al.* in 1976, the classical definition of cancer chemoprevention is the use of natural, synthetic or biological chemical agents to reverse, suppress or prevent either the initial phase of carcinogenesis or the progression of neoplastic cells to cancer[1]. This definition encompasses a major aspect of clinical cancer chemoprevention: the use of pharmacological interventions to treat or reduce the risk of intraepithelial neoplasia (IEN)[2,3] (BOX 1). The key concept underlying chemoprevention is that carcinogenesis is multistep (that is, it results from accumulated genetic and epigenetic alterations), multipath (that is, multiple functional pathways are involved, such as self-sufficiency in growth signals, insensitivity to anti-growth signals, apoptosis evasion, limitless replicative potential, tissue invasion and metastasis and sustained angiogenesis), and multifocal (both multiclonal, that is, 'field cancerization' occurs, and clonal, that is, cloning expansion leading to intraepithelial spread occurs)[4].

Identifying molecular mechanisms involved in carcinogenesis provides strong rationales for developing strategies for cancer treatment and prevention. The relatively few molecularly targeted drugs that have been evaluated in cancer prevention trials include cyclooxygenase 2 (COX2) inhibitors for colon cancer prevention[5–7], selective oestrogen receptor modulators (SERMs) for breast cancer prevention[8,9], and 5α-reductase inhibitors for prostate cancer prevention[10]. These approaches have provided the proof of principle for feasible and effective molecularly targeted cancer chemoprevention, albeit associated with substantial obstacles that hinder their widespread application. After summarizing the biology of cancer risk and proof-of-principle prevention trials, this Review addresses some of the ways that molecularly targeted agents could help overcome the substantial challenges that are involved in developing chemoprevention studies. Molecular targets that are relevant to risk assessment and patient selection, drug development and outcome evaluation will streamline chemoprevention research. In turn, these molecular targets will lead to effective, safe and personalized cancer prevention strategies for widespread clinical use.

Biology and cancer risk

Host–environment interactions and tissue injury are crucial factors of cancer risk. Genetic susceptibility of the host (for example, lung cancer susceptibility due to a single nucleotide polymorphism variation at 15q24/15q25.1 loci[11–13]) and environmental factors (for example, tobacco and microbial infections) interact to influence carcinogenesis. Persistent tissue injury[14,15] in the form of genetic and epigenetic changes such as mutations, loss of heterozygosity (LOH), methylation and global transcriptome changes (for example, in inflammation pathways) can lead to aberrant pathway activation, cellular dysfunction and, consequently, premalignant epithelial changes. These changes can occur in clonal patches, or patches of clonally (and subclonally) related cells. Clonal patches of 40,000–360,000 cells have been mapped in the lung[16], and the size and number of subclones in a clonal patch are associated with cancer risk in patients with Barrett's oesophagus[17].

Traditional cancer risk assessments are based on clinical and demographic criteria that can be bundled in models such as the Gail model[18] — which has identified women with a 5-year breast cancer risk of 1.67% or greater for inclusion in large-scale breast cancer prevention trials[8,9] — or on recent models of lung cancer risk developed by Spitz and colleagues[19,20]. Generally, the cancer risk computed from these models is far lower than that associated with a diagnosis of advanced (dysplastic)

Box 1 | **Intraepithelial neoplasia: an end point for clinical trials**

Intraepithelial neoplasia (IEN) is a premalignant lesion occurring in most epithelial tissues as moderate-to-severe dysplasia. It is frequently a precursor to invasive cancer and occurs at a relatively late stage in the pathway leading from normal tissue to cancer. Many IENs (for example, grades 2 and 3 cervical IEN, breast ductal carcinoma *in situ* and colorectal adenomas in patients with familial adenomatous polyposis) are recognized as diseases requiring treatment in their own right, independent of cancer prevention objectives. Chemoprevention drugs customarily must reduce cancer incidence in a clinical trial before being considered for standard of care; many investigators would prefer reduced cancer mortality to prove a drug's worth. The cancer end point and its reduction make trials long, large and costly. For example, the sample size of the Breast Cancer Prevention Trial (BCPT) was 13,388 women[8], and of the Selenium and Vitamin E [prostate] Cancer Prevention Trial (SELECT) was 35,534 men[125]. Establishing IEN as a surrogate end point for cancer chemoprevention trials could reduce the number of subjects (by thousands), the time (by a decade or more) and untold costs from the logistics of chemoprevention trials. For example, a drug-registration trial of celecoxib to prevent colorectal adenomas in patients with familial adenomatous polyposis required only 77 patients and 6 months to complete, with an undoubted concomitant reduction in cost[79]. In 2002, the American Association for Cancer Research recommended the development of chemoprevention strategies that are focused on carcinogenesis, not necessarily invasive cancer, as a measure of clinical benefit. This recommendation specified the prevention and regression of clinical/histopathological IEN[2]. A 2006 update of these recommendations highlighted the importance of molecular IEN (that is, molecular alterations detected early in the target histopathologic IEN) as a potential surrogate marker for invasive cancer and an end point for chemoprevention studies[3].

premalignant lesions. For example, a high 5-year risk of breast cancer begins at 1.67% according to the Gail model, whereas a diagnosis of breast ductal carcinoma *in situ* is associated with a 5-year risk of 13% (following resection and radiation)[21]. In the United States, patients with breast ductal carcinoma *in situ* are routinely considered for chemoprevention, but women with a high risk according to the Gail model are not. Although reliable in predicting risk at the population level, the Gail model has only modest accuracy in predicting risk at the individual level[22]. Promising new models of breast cancer risk that incorporate clinical, demographic and histopathological parameters are being developed[23]. The development, evaluation and application of numerous other cancer risk prediction models have been reviewed by Freedman and colleagues[24].

Molecular data seem to improve the accuracy of already-established risk factors in models that assess risk in healthy individuals and in patients with premalignant lesions. For example, Tlsty and co-workers demonstrated that expression of biomarkers indicating an abrogated response to cellular stress predicts a worse outcome for patients with breast ductal carcinoma *in situ*[25]. A panel of methylation markers in sputum marked a high lung cancer risk in chronic smokers[26]. Recently published lung cancer risk models that integrated genomic (somatic gene expression arrays and host DNA repair capacity) and clinical features were more accurate than clinical models alone[19,27]. In Barrett's oesophagus (a well-established but modest predictor of absolute cancer risk), a model incorporating a chromosome instability panel of LOH and DNA content profiles could distinguish between a high cancer risk (79% in 6 years) and a low cancer risk (0% in over 6 years) population[28]. Patients with oral leukoplakia who have LOH at specific loci in chromosomes 3p and/or 9p have a substantially increased risk of developing oral cancer (versus oral leukoplakia without such LOH)[29,30]. In particular, LOH at these chromosomes was also predictive of development of secondary oral malignancy in patients with a previously treated oral cancer[31]. A specific cyclin D1 genotype (adenine/guanine single nucleotide polymorphism at position 870 of exon 4 of *CCND1*, the cyclin D1 gene)[32] is also associated with a high cancer risk in patients with dysplastic oral and/or laryngeal lesions. A recent study of laryngeal dysplasia confirmed and extended this finding, showing that high cyclin D1 expression further increased the cancer risk of patients with the high-risk *CCDN1* genotype[128].

Many of these molecular risk factors improve the accuracy of traditional risk factors based on clinical, demographic and histopathological criteria and actively participate in the carcinogenic process. These molecular factors could therefore be targeted for chemoprevention. For instance, although there are no direct inhibitors of cyclin D1 in clinical use, several currently available drugs inhibit targets (for example, epidermal growth factor receptor (EGFR) and mammalian target of rapamycin (mTOR)) that are upstream of cyclin D1 and could ultimately downregulate it. One such agent, erlotinib (Tarceva; Genentech/OSI Pharmaceuticals), is being tested in a Phase III trial for prevention of oral cancer (FIG. 1). Drugs targeting methylation are another attractive chemopreventive approach, particularly in people with epigenetic changes (as mentioned above for lung cancer risk). Two demethylating drugs, azacitidine (Vidaza; Celgene) and decitabine (Dacogen; Eisai), are currently in routine clinical use for treating myelodysplastic syndrome.

Completed molecularly targeted trials

The feasibility of molecularly targeted chemoprevention has been demonstrated in people at risk of head and neck, breast, prostate and colorectal cancer. The seminal clinical trials that demonstrated this feasibility — which are summarized below in this section — have not, however, led to a broad acceptance of cancer chemoprevention in routine clinical practice. Broad acceptance will rest on three important issues associated with chemoprevention: agents with favorable side-effect profiles; high-risk populations to maximize the impact of chemoprevention; and translational advances that improve our understanding of molecular drug mechanisms and lead to more effective and less toxic strategies.

Vaccines and cancer prevention. Successful targeted chemoprevention strategies include vaccines against hepatitis B (to prevent hepatocellular carcinoma)[33] and human papillomavirus (HPV) (to prevent cancers of the cervix, vulva and vagina)[34–36] (BOX 2). These vaccines are considered to be molecularly targeted because they generate immune responses against specific proteins; that is, the L1 HPV viral capsid protein and the hepatitis B surface antigen. As these vaccines illustrate, molecular targeting through immunization against infectious agents related to neoplasia is a successful way to prevent or treat early steps of host cell damage that can otherwise

Angiogenesis
The growth of new blood vessels.

Cyclooxygenase 2
(COX2). The enzyme that is responsible for the synthesis of prostanoids (that is, prostaglandins, prostacyclin and thromboxane) from the precursor arachidonic acid, and that thus has a major role in inflammation. COX2 is generally upregulated during inflammation, whereas its counterpart, COX1, is constitutively expressed in most normal tissues.

Selective oestrogen receptor modulators
(SERMs). A class of drugs that can function as agonists or antagonists of the oestrogen receptor, depending on the tissue type.

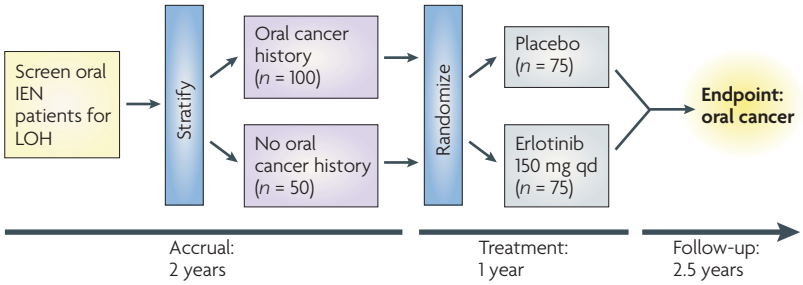


Figure 1 | **Schematic for the Erlotinib Prevention of Oral Cancer trial.** Erlotinib Prevention of Oral Cancer (EPOC) is an ongoing novel trial at M. D. Anderson Cancer Center, USA, and several partner centres in the United States. This trial is being conducted in patients with oral intraepithelial neoplasia (IEN) who are at a very high risk of oral cancer (up to 65% in 3 years) based on loss of heterozygosity (LOH) profiles (primarily LOH at 3p14 and/or 9p21) in their IEN lesions. This trial reflects the convergence between cancer prevention and therapy: it will test a molecularly targeted agent used in standard cancer therapy (lung and pancreatic cancer), and its population will include patients with or without a previous history of oral cancer. Data supporting the use of epidermal growth factor receptor (EGFR) targeting with erlotinib in this setting include increased EGFR expression in oral IEN and cancer and in association with a poor prognosis, EGFR tyrosine kinase inhibitor growth suppression in oral cancer xenografts and IEN cells, activity of EGFR signalling suppression (and its effects on downstream molecules such as cyclin D1) in preclinical head and neck cancer, and lung cancer prevention models, and EGFR targeting results in head and neck cancer therapy. EPOC is the first trial to directly develop personalized medicine for cancer prevention based on molecular markers. LOH profiling is feasible in the clinic and allows EPOC to test a highly promising molecularly targeted agent (erlotinib) in a population with the greatest need for prevention, to limit the number of people exposed to potential drug adverse effects (by excluding those with lower-risk LOH profiles) and to reduce the overall size, duration and cost of a trial needed to define cancer preventive efficacy. For example, and by loose comparison, EPOC will be conducted in a total of 150 patients (randomized to receive erlotinib or placebo for 1 year), whereas the Breast Cancer Prevention Trial was conducted in 13,388 women at a 5-year breast cancer risk of >1.66% (eligibility level) and the Prostate Cancer Prevention Trial was conducted in 18,882 randomized men at a 7-year prostate cancer risk of 24.4% (placebo group rate). EPOC is the first cancer endpoint (definitive) trial in the setting of oral cancer prevention; it is also evaluating clinical and molecular intermediate end points that may correlate with cancer risk. qd, daily.

lead to cancer. HPV vaccines have not been shown to accelerate HPV clearance and so are unlikely to prevent cancer in patients already infected with the virus[37].

A promising avenue of chemoprevention research is targeting infectious agents related to other tumour types. For example, targeting *Helicobacter pylori* to prevent gastric cancer, targeting HPV to prevent oropharyngeal cancer, and targeting herpes virus 8 to prevent Kaposi's sarcoma. Most solid tumours, however, have yet to be clearly associated with an infectious agent. Nonetheless, it is clear that the link between infection and cancer development is just beginning to be understood. For example, recent discoveries include a previously unknown infectious agent called Merkel cell polyomavirus and its link to Merkel cell cancer[38]. However, trials of traditional drugs, for example, antibiotics, to prevent infection-related cancer have been problematic so far[39–41].

Another promising molecular-based immunization strategy is prophylactic vaccines based on tumour antigens[42]. Successful examples of this approach include a vaccine based on the prostate stem-cell antigen; prolonged disease-free survival was demonstrated in mice genetically predisposed to prostate cancer and genetically engineered to overexpress the prostate stem-cell antigen[43]. The importance of the immune system in preneoplastic conditions is further supported by the ability of T cells isolated from the bone marrow of patients with monoclonal gammopathy of undetermined significance (the preneoplastic counterpart of multiple myeloma) to recognize autologous tumour-cell-loaded denditric cells[44,45].

Retinoids and molecularly targeted prevention. The translational study of retinoids (the natural and synthetic analogues of vitamin A) set the benchmark for molecularly targeted research in cancer chemoprevention. Beginning in the early 1980s, Hong and colleagues conducted a series of randomized chemoprevention trials[46–50] evaluating the retinoid 13-*cis*-retinoic acid in patients at risk of head and neck cancer. In one trial, 13-*cis*-retinoic acid reduced the number of second primary tumours in patients with resected early stage head and neck cancer[47,48], thereby providing the first proof of principle of cancer chemoprevention. This research group conducted intensive correlative studies of the biology of carcinogenesis and retinoid actions and mechanisms in the head and neck that significantly advanced the understanding of molecular markers of drug activity[51–53] and sensitivity[32] and cancer risk[54]. The methods of preclinical and clinical study (for example, tissue sample collection and analysis) and the overall translational approach of this programme helped to shape the methodological direction for novel molecularly targeted research[55]. It also aided in focusing on personalized risk assessment and evidenced-based selection of chemoprevention strategies for clinical trials.

SERMs and breast cancer prevention. The first agent approved specifically for the indication of cancer risk reduction by the US Food and Drug Administration (FDA) was tamoxifen. Approved in 1998, this molecularly targeted SERM agent was shown to reduce the risk of invasive and non-invasive breast cancer in the Breast Cancer Prevention Trial (BCPT)[8], one of four randomized trials that tested tamoxifen for breast cancer prevention[56–59]. In the BCPT, 13,388 women at higher-than-average risk of breast cancer were randomized to receive tamoxifen (20 mg per day) or placebo for 5 years. At a median follow-up of 55 months, tamoxifen reduced the risk of invasive breast cancer by 49% and non-invasive breast cancer by 50%. The protective effect was limited to oestrogen receptor (ESR)-positive tumours. In addition, there was a reduced risk of fractures of the hip, radius and spine. However, the incidence of endometrial cancer was significantly increased in the group receiving tamoxifen (risk ratio of 2.53), as was the incidence of thrombotic events. In a meta-analysis of the BCPT and the three other tamoxifen randomized prevention trials, the combined reduction in breast cancer incidence with tamoxifen was 38% (95% confidence interval, 28–46, $p<0.0001$)[60]. There was no effect for ESR-negative breast cancers, but the incidence of ESR-positive breast cancer was reduced by 48% (95% confidence interval, 36–58,

Molecular targets
Molecules involved in the development of intraepithelial neoplasia and/or cancer that can (or potentially can) be targeted by pharmacological agents designed to prevent or treat cancer. Examples include tyrosine kinase receptors (including epidermal growth factor, insulin-like growth factor 1, platelet-derived growth factor), other receptors (for example, oestrogen), enzymes (for example, cyclooxygenase 2 and 5α-reductase) and other proteins (for example, vascular endothelial growth factor).

Box 2 | **Human papillomavirus vaccines against cervical cancer**

Randomized clinical trials in women aged 16–26 years and not previously infected with human papillomavirus (HPV) produced striking results, leading to the approval of an HPV vaccine for reducing the risk of cervical cancer, adenocarcinoma *in situ* and high-grade intraepithelial neoplasia (IEN) and vulvar and vaginal IEN in girls and women aged 9–26 years. The recent placebo-controlled Females United to Unilaterally Reduce Endo/Ectocervical Disease (FUTURE) Phase III trial found that a quadrivalent vaccine against HPV6, 11, 16 and 18 reduced the risk of the primary composite end point (cervical IEN grades 2 and 3, adenocarcinoma *in situ* and cancer related to HPV16 or 18) by 98% in women aged 15–26 years with no virologic evidence of HPV16 or 18. The vaccine also reduced this end point by 44% in an intent-to-treat analysis that included women with previous HPV infection[35]. Another recent Phase III trial tested a bivalent (HPV16 and 18) vaccine in 18,644 girls and women aged 15–25 years[36]. The primary end point, grade 2 cervical IEN associated with HPV16 or 18, was reduced by 90% in women with no evidence of prior HPV infection. A Phase III trial of a quadrivalent (HPV6, 11, 16 and 18) vaccine was 100% effective at 7 months (the protocol-specified analysis period) in preventing the co-primary end points genital warts, vulvar or vaginal lesions (IEN or cancer) and cervical lesions (IEN, adenocarcinoma *in situ* or cancer) in 5,455 females aged 16–24 years who had no virologic evidence of HPV infection[34]. An intent-to-treat analysis including disease unrelated to these HPV types and women with prevalent HPV infection showed that vaginal and vulvar lesions were reduced by 34% and cervical lesions by 20%.

$p<0.0001$). Rates of endometrial cancer were increased in all tamoxifen prevention trials (consensus relative risk of 2.4), particularly in women aged 50 years or older, as were the rates of venous thromboembolic events (relative risk of 1.9).

Long-term follow-up studies suggest that the beneficial effects of tamoxifen persist for at least 10 years. After the 5-year treatment period, the incidence of most side effects of tamoxifen, including thrombotic events and endometrial cancer, were not higher than in the placebo group[61].

Despite the proven efficacy of tamoxifen as a cancer preventive drug and the endorsement of its use by governmental agencies and medical societies, only a small percentage of eligible high-risk women are being offered and agree to take tamoxifen for primary breast cancer prevention[62,63]. Resistance to accepting tamoxifen illustrates the difficulty of implementing pharmacological preventive strategies in a healthy population, especially when potential serious risks are associated with the intervention.

The Study of Tamoxifen and Raloxifene (STAR) tested the hypotheses that the SERM raloxifene (Evista; Eli Lilly) would be equivalent or better for breast cancer prevention and less toxic than tamoxifen. This hypothesis was derived largely from data obtained from an osteoporosis trial indicating the potential of raloxifene in reducing breast cancer risk and toxicity[9]. The STAR randomized 19,747 post-menopausal women who had an increased risk of breast cancer to receive tamoxifen (20 mg per day) or raloxifene (60 mg per day) for 5 years. The rates of invasive breast cancer did not differ between the treatment groups, but there were fewer cases of uterine cancer in the raloxifene ($n = 23$) than in the tamoxifen ($n = 36$) group (risk ratio, 0.62; 95% confidence interval, 0.35–1.08). There was also a statistically significant reduction in the risk of thromboembolic events and cataracts in women assigned to raloxifene. Tamoxifen-treated women had a lower incidence of non-invasive breast cancer. Following the STAR, the FDA approved raloxifene for reducing the risk of invasive breast cancer in post-menopausal women with osteoporosis and in post-menopausal women at a high risk for invasive breast cancer.

Finasteride and prostate cancer prevention. The Prostate Cancer Prevention Trial (PCPT) evaluating the 5α-reductase inhibitor finasteride (Proscar; Merck) was launched in 1993. The PCPT was based on the hypothesis that inhibiting the conversion of the androgen testosterone to the more potent dihydrotestosterone (via 5α-reductase) with finasteride would prevent androgen-driven cancer development[10]. In this study, 18,882 healthy men aged 55 years or older with a normal digital rectal examination and a serum prostate serum antigen level of less than 3 ng per ml were randomized to receive finasteride (5 mg per day) or placebo for up to 7 years, and were followed annually for detection of cancer. After 7 years, an end-of-study biopsy was offered to, but not mandated for, all subjects not diagnosed with prostate cancer.

The key finding of this trial was a statistically significant reduction in the prevalence of prostate cancer in the finasteride-treated cohort (18.4% versus 24.4% in the placebo group); however, high-grade prostate cancer (Gleason grade 7–10) was more prevalent in the finasteride group (6.4% versus 5.1% in the placebo group)[64]. Finasteride also significantly reduced high-grade prostate IEN[65]. Sexual side effects were more common in finasteride-treated men, whereas urinary symptoms were more common in men receiving placebo. Several issues were raised after the presentation of these results[64]. First, there was a higher than anticipated incidence of prostate cancer in both groups, which could be due to the detection of subclinical prostate cancer in end-of-study biopsies. Second, prevention of localized, low-grade prostate cancer, as occurred in the PCPT, might be clinically irrelevant. Third, it was unclear whether the increased incidence of high-grade prostate cancer in the finasteride-treated men was a true phenomenon or the artificial effect of a detection bias.

To address the issue of clinically inconsequential prostate cancer, a recently published analysis demonstrated that a substantial proportion of the cancers that were prevented by finasteride met previously established criteria for clinically significant cancer[66]. The issue of high-grade disease was clarified by subsequent histopathological analyses of biopsies and prostatectomy specimens[67], and by statistical modelling to account for biases introduced by biopsy in diagnosing prostate cancer in men treated with finasteride[68]. The histopathological analyses showed that increased high-grade cancers were due to the effects of finasteride on prostate volume and selective inhibition of low-grade cancer. That is, finasteride significantly shrank the prostate in the PCPT, and biopsies may have over-detected high-grade disease in the smaller prostates of finasteride-treated men[67]. Two

Personalized cancer prevention
The selection of preventive medicine for a patient who is at high risk of cancer, is most likely to benefit from the selected agent or agent combination, and least likely to experience adverse effects of the intervention.

Single nucleotide polymorphism
Variation of a single nucleotide in the DNA sequence, leading to a difference between paired chromosomes in an individual.

Loss of heterozygosity
(LOH). Loss of the normal function of one allele of a gene, when the other allele is already inactivated. In carcinogenesis, LOH frequently occurs in tumour suppressor genes, leading to an interruption of the function of that gene and an increased risk for cancer development.

Phase III trial
Randomized, controlled clinical study, generally conducted on large groups of patients, designed to definitively evaluate the efficacy of a given therapeutic strategy.

distinct statistical methodologies independently found that the increased incidence of high-grade prostate cancer may have been an artefact because finasteride increases biopsy sensitivity for prostate cancer[68] and/or caused higher Gleason misclassification rates in the finasteride group than in the placebo group[69]. These updated analyses further support the use of finasteride for prostate cancer chemoprevention[70].

Mathematical models of the potential impact of finasteride on prostate cancer prevention in the general population have also been published[71,72], and one demonstrates a putative positive impact of finasteride on survival[71]. Nonetheless, there are intensive ongoing investigations into the biology of the PCPT that are designed to clearly define high and low risk of prostate cancer, possibly the risk of high-grade prostate cancer, and the pharmacoecogenetics (environmental, metabolic and genetic factors) of finasteride for prostate cancer prevention. These projects are evaluating the influence of androgen metabolism, dietary factors, the insulin-like growth factor axis, inflammation and inflammatory factors, and DNA repair and oxidative damage on prostate cancer risk and the effects of finasteride on this risk. Results of these analyses are expected to produce comprehensive models of cancer risk and the pharmaco-ecogenetics of finasteride, which promise to advance prostate cancer prevention by selecting men at the highest cancer risk and who are most likely to benefit (and least likely to have serious adverse effects) from finasteride. These models also promise to provide a paradigm for such modelling in other sites and for other drugs. The ability to identify the people who are in greatest need of and who are the most likely to benefit from an intervention will reduce the risks of chemoprevention that were illustrated by the BCPT and the PCPT[72].

Selective COX2 inhibitors and colorectal cancer. Many trials have demonstrated that COX inhibition with non-steroidal anti-inflammatory drugs (NSAIDs) can prevent and treat colorectal polyps in different settings. For example, sulindac reduced the number of polyps in patients with the rare genetic disorder familial adenomatous polyposis (FAP)[73]. Four prospective, randomized, placebo-controlled trials have demonstrated that aspirin significantly prevents adenomatous polyps in patients with a previous history of adenomas[74–76] or colorectal cancer[77]. Two randomized trials in preventing cardiovascular events (British Doctors Aspirin Trials and UK-Transient Ischemic Attack Aspirin Trial) demonstrated that aspirin significantly reduced the long-term risk of colorectal cancer by 26%[78].

In 2000, Steinbach *et al.* reported the results of a randomized trial of the selective COX2 inhibitor celecoxib (Celebrex; Pfizer) versus placebo in 77 patients with FAP. Celecoxib significantly decreased the burden of colorectal polyps and was approved by the FDA (as an adjunct to usual care) for reducing polyp numbers in patients with FAP[79]. Following this successful trial, three studies were launched to assess the efficacy of coxibs (compared with placebo) in the prevention of sporadic adenomas in patients with a prior history of colorectal polyps: the Adenomatous Polyp Prevention on Vioxx (APPROVe) trial used rofecoxib, and the Adenoma Prevention with Celecoxib (APC) and Prevention of Colorectal Sporadic Adenomatous Polyps (PreSAP) trials used various doses of celecoxib. Interim rates of cardiovascular events were unexpectedly higher in patients receiving rofecoxib for more than 18 months in the APPROVe study, and in patients receiving celecoxib in the APC but not the PreSAP study[80,81]. However, all three studies were stopped early by their data-monitoring committees because of these safety issues.

In the APC trial (2,035 randomized patients), celecoxib (400 mg twice daily and 200 mg twice daily for 3 years) significantly reduced the incidence of adenomas compared with placebo (3-year adenoma incidence of 37.5%, 43% and 60%, respectively; $p<0.001$)[6]. However, treatment also significantly increased the rates of serious cardiovascular adverse events in a dose-dependent manner compared with placebo (total incidences of 3.4% (400 mg), 2.6% (200 mg) and 1% (placebo)); higher risk was associated with a previous history of cardiovascular disease. The incidence of advanced adenomas was reduced by more than 50% in the celecoxib groups (3-year incidence of 17% with placebo versus 8% and 6% in the 200 mg and 400 mg dose groups, respectively; $p<0.001$)[6]. Furthermore, the highest-risk patients (having three adenoma risk factors at baseline: more than three adenomas, aged 60 years or older, and at least one parent with a history of colorectal cancer) seemed to benefit the most. The relative adenoma risk reduction in this cohort was 51% with celecoxib 200 mg twice daily and 73% with celecoxib 400 mg twice daily. An extension study of the APC trial showed no rebound effect after treatment discontinuation — which was recommended by the data and safety monitoring board (because of increased cardiovascular events) after only 86.6% of patients had completed 3 years of intervention. The highest-risk patients also had persisting relative risk reductions of 14% for all adenomas and 41% for advanced adenomas at 5 years[82], potentially illustrating a higher impact of celecoxib in the advanced, more clinically relevant form of the disease. Furthermore, the extension study suggested that the severe adverse events (for example, cardiovascular toxicity) subsided 2 years after treatment discontinuation. In the PreSAP trial (1,561 randomized patients), celecoxib (400 mg once daily) lowered the incidence of adenomas (33.6% versus 49.3% in the placebo group; $p<0.001$)[5] and did not increase the risk of cardiovascular events (defined as a composite end point of myocardial infarction, stroke, congestive heart failure or death from cardiovascular causes). In the APPROVe study (2,587 randomized patients), adenoma recurrence was also less frequent in the rofecoxib group than in the placebo group (41% versus 55%; $p<0.0001$). Rofecoxib also reduced the risk of advanced adenomas ($p<0.01$)[7].

Viewed exclusively from the perspective of cancer prevention, selective COX2 inhibitors are an elegant example of the successful translation of basic science research into clinical practice, corroborating the role of COX2 in carcinogenesis and confirming the utility

Familial adenomatous polyposis
A rare inherited disease associated with a mutation in the *APC* gene that is clinically characterized by numerous polyps in the colon. If untreated, transformation of the polyps into colon cancer is inevitable.

of its inhibition for arresting tumour development in humans. Viewed more broadly, however, these inhibitors are blemished by the unexpected cardiovascular side effects observed in the randomized trials. These adverse effects might eliminate the clinical utility of COX2-specific inhibitors for cancer prevention in the general population, in part because serial colonoscopies and polypectomy might be safer and more effective for preventing colorectal cancer[83]. These results add to the findings of the completed molecularly targeted prevention trials[9,10,84], highlighting the complicated problem of drug toxicity despite beneficial effects in healthy populations. This problem underscores the importance of tailoring chemopreventive agents to individual patients — or personalizing preventive medicine — based on features that predict sensitivity or resistance to the preventive effect of a drug (and sensitivity to its potential adverse effects), level of cancer risk, and the availability of other pharmacological and non-pharmacological alternatives for cancer prevention. Personalized chemoprevention strategies comprise a triad of molecular-, patient- and population-targeted approaches that will maximize the chances of a patient to avoid cancer.

Emerging data on COX2 inhibitors for colorectal cancer prevention have shed new light on the issues and concerns discussed above. Regarding safety, an elegant and careful cross-trial analysis by Solomon *et al.* computed toxicity data from randomized Phase III studies of celecoxib for non-arthritic conditions[85]. By stratifying patients into low, moderate and high baseline cardiovascular risk (using previously defined Framingham criteria), the authors demonstrated that celecoxib did not cause increased adverse cardiovascular events in the low-risk group, which was also the finding of a secondary analysis of the APC trial data. Of note, 16% of the APC trial participants were in the low cardiovascular risk category. The new safety and efficacy data based on simple clinical characteristics open a window of opportunity for COX2 inhibitors in patients with a low risk for cardiovascular events and a high risk for adenomas at baseline. Furthermore, 5-year efficacy data of the APC trial suggest that COX2 inhibitors in this population would significantly reduce high-risk adenomas. This finding could translate into decreased colorectal cancer risk in the long term; for example, the effects of aspirin in preventing colorectal cancer appeared after a latency of 10 years from the intervention and were greatest 10–14 years after randomization in the British Doctors Aspirin Trial and UK-Transient Ischemic Attack Aspirin Trial[78].

Incorporating biomarker data into colorectal adenoma and cancer risk models may further improve the identification of optimum candidates for future coxib cancer-prevention trials. One step in this direction is the finding from the Nurses' Health Study and the Health Professionals Follow-up Study that aspirin use was associated with a decreased risk of colorectal cancers that overexpressed COX2, but not of those with low or no expression of COX2 (REF. 86). This finding suggests that COX2 inhibition specifically interferes with malignant cells (or their precursors) that are addicted to this activated pathway, thereby opening a window of opportunity for identifying a population that is likely to respond to COX2 inhibitors. This hypothesis would be further corroborated by a demonstration that premalignant lesions that overexpress COX2 lead to cancers in which COX2 is overexpressed, and that NSAIDs have greater activity in adenomas that overexpress COX2 than in adenomas with weak or no expression of COX2 (REF. 87).

Challenges of chemoprevention

The identification of new potential molecular targets and the development of agents aimed at these targets within cancer have already had a significant impact on advanced cancer therapy and provide a wealth of opportunities for chemoprevention. Nevertheless, there are a number of challenges that stand in the way of successfully translating these advances into effective molecularly targeted chemoprevention strategies (BOX 3).

Clinical development of chemopreventive strategies. A growing body of evidence indicates that certain pathways involved in advanced disease are also involved in early tumorigenesis and may therefore be rational targets for chemoprevention as well as for therapy. Some targeted drugs have migrated successfully from therapy to prevention, but none has yet moved from prevention to therapy. This developmental pattern does not result from scientific issues but largely from practical issues such as the lack of early clinical trials in prevention, the abundance of therapy trials at every level, and the acceptance of higher risks of toxicity in therapy than in prevention trials. For example, although tamoxifen developed first in therapy, it is effective across the spectrum of breast cancer therapy and prevention settings, and could have developed first in cancer prevention. As discussed in detail elsewhere[88,89], 'convergent' trials that are designed to include patients with both preneoplastic lesions and early stage cancers may facilitate the identification of promising agents for chemoprevention and therapy at early stages of clinical evaluation.

On the other hand, some molecularly targeted strategies might be differentially effective in prevention and therapeutic settings and so should follow distinct pathways of clinical development, as illustrated by angiogenesis inhibitors. The acquisition of a blood supply, or the angiogenic switch, is a vital step for the growth of an early neoplastic lesion beyond the size of 1–2 mm^3, and also has a role in the metastatic spread and growth of advanced tumours[90]. Therefore, angiogenesis could theoretically be a useful target for chemoprevention and adjuvant therapy against advanced disease. Consistent with this possibility, angiogenesis inhibitors affect multiple steps in the process of carcinogenesis in preclinical models[91]. Some angiogenesis inhibitors, however, seemed to be more effective in preventing the angiogenic switch, whereas others were more effective against advanced tumours. This dichotomy illustrates that the activity of a drug in treatment and prevention may not be correlated and needs to be investigated on a case-by-case basis. Agents with demonstrated activity in the setting of advanced disease and with an acceptable toxicity profile

are natural candidates for chemoprevention, but even if an agent lacks significant activity in advanced disease it may still have potential for chemoprevention, depending on the preclinical data.

Another strategy for identifying promising targets for chemoprevention is to apply knowledge gained from the study of hereditary cancer risk syndromes to the understanding of the pathophysiological process of sporadic and familial carcinogenesis, and then develop molecular-based interventions against the early steps of tumorigenesis. This strategy is illustrated by the study of hypoxia-inducible factor (HIF) in the development of renal cell carcinoma. Activation of the HIF pathway is known to promote a wide range of pro-tumorigenic processes. These include angiogenesis via vascular endothelial growth factor (VEGF) and other factors, cellular motility and invasiveness, increased glucose transport, and resistance to apoptosis[92]. Although initially thought to be primarily regulated by hypoxia, HIF was recently found to have several non-hypoxic regulators, including receptor tyrosine kinases such as EGFR[93], the phosphoinositide 3-kinase (PI3K)–AKT–mTOR pathway, and metabolic pathways including the tricarboxylic acid (Krebs) cycle (reviewed elsewhere[94,95]) (FIG. 2). Germline mutations in the *VHL* gene — the underlying cause of von Hippel–Lindau (VHL) disease — lead to a markedly elevated risk of developing renal cell carcinoma, haemangioblastomas of the central nervous system and other tumour types. The VHL protein encoded by this gene is part of a protein complex that targets HIFs for degradation[95]. Sporadic mutations in *VHL* also occur in sporadic clear-cell renal cell carcinoma.

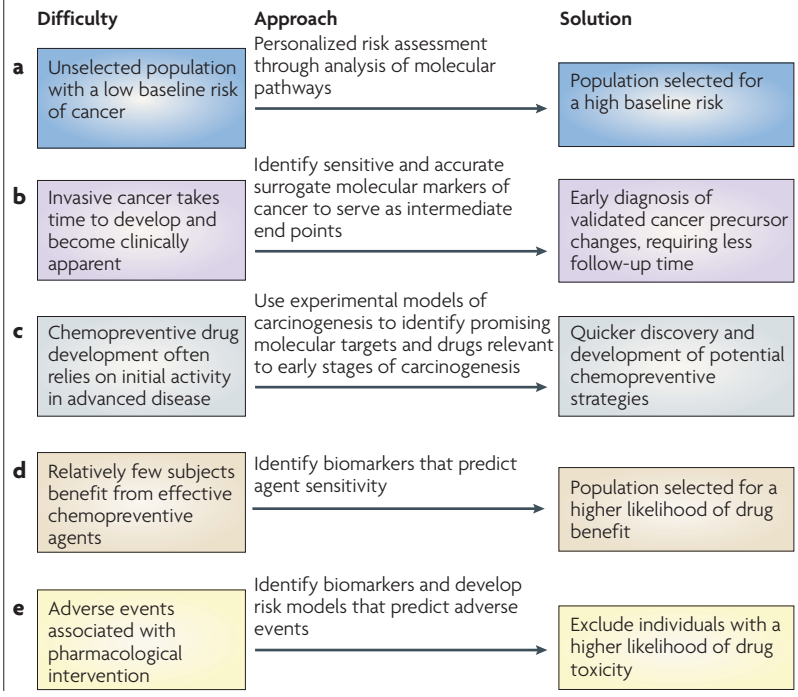
A second syndrome, hereditary leiomyomatosis and renal cell carcinoma, results from mutations in the fumarate hydratase (*FH*) gene[96]. FH is a mitochondrial protein involved in the tricarboxylic acid cycle. Although the mechanism(s) by which mutations in *FH* promote tumorigenesis is still under investigation, it seems that loss of FH function increases HIF expression by causing a build up of intracellular fumarate, which inhibits the enzymes that hydroxylate HIFαs (HIF hydroxylases, also known as EGLNs) and targets them for VHL-mediated degradation.

A third hereditary syndrome, tuberous sclerosis complex, is caused by mutations in the tuberous sclerosis complex genes, resulting in elevated HIF levels via the mTOR pathway[97,98]. Several other syndromes also lead to elevated levels of HIFs and downstream HIF-regulated gene products, causing a 'pseudohypoxic' state[94,97]. That multiple inherited cancer syndromes involve pathways that converge on HIFs suggests that HIFs probably have a role in sporadic cancers as well. Several drugs targeting HIFs are currently in clinical development.

The ideas described above, and others, such as translating celecoxib activity from patients with the rare FAP syndrome to patients with sporadic adenomas, illustrate that understanding the biology of inherited cancer syndromes can provide important leads for identifying molecular pathways and targets for chemoprevention. Because the risk of specific malignancies is usually markedly elevated in hereditary syndromes (compared with the general population) and because the molecular drivers of malignancy are often known, these syndromes provide a potential proof of concept for chemoprevention with agents that have an acceptable benefit-to-toxicity ratio. Furthermore, the pathways involved in these hereditary cancers can be dysregulated in sporadic cancers as well, raising the possibility that the work with hereditary syndromes could also be applicable to a broader cancer population.

Box 3 | **Chemoprevention trials: overcoming the obstacles**

Many molecularly targeted drugs that are in development for advanced cancer therapy have potential activity and tolerability for cancer chemoprevention. Few clinical trials, however, have been conducted or are under way to establish the role of these agents in prevention. Perhaps the key barrier to clinical trials of molecularly targeted chemoprevention is the low incidence of cancer in any given healthy, unselected population. This low incidence poses a low expected event rate that requires a large sample size in order to achieve statistical significance for a meaningful clinical outcome (versus control) or treatment effect. Large sample sizes, however, can be especially problematic for chemoprevention trials, in which recruiting patients is already challenged by potential toxicity and other obstacles (for example, tissue sampling) in relatively healthy people. Molecularly targeted approaches integrated with translational research provide an opportunity to potentially increase event rates and/or treatment effects in prevention trials, as outlined in the chart below. Recent studies have incorporated principles depicted in the chart, although they did not always integrate molecular data that could further improve trial design and conduct. The Erlotinib Prevention of Oral Cancer study selects high-risk patients based on loss of heterozygosity in specific loci of chromosomes 3p and/or 9p in intraepithelial neoplasia (difficulty 1; see part **a** of the figure). Based on preclinical models, Meyskens *et al.* identified a combination therapy (sulindac plus difluoromethylornithine) with minimal activity in colorectal cancer but dramatic activity in preventing adenomas (difficulty 3; **c**)[119]. The Adenoma Prevention with Celecoxib trial identified patients who were most likely to benefit from celecoxib (difficulty 4; **d**)[6,82]. The ornithine decarboxylase G316A genotype was predictive of aspirin efficacy in three colorectal adenoma chemoprevention trials (difficulty 4; **d**)[126,127]. Solomon *et al.* developed a risk model to predict adverse events associated with cyclooxygenase 2 inhibitors (difficulty 5; **e**)[85].



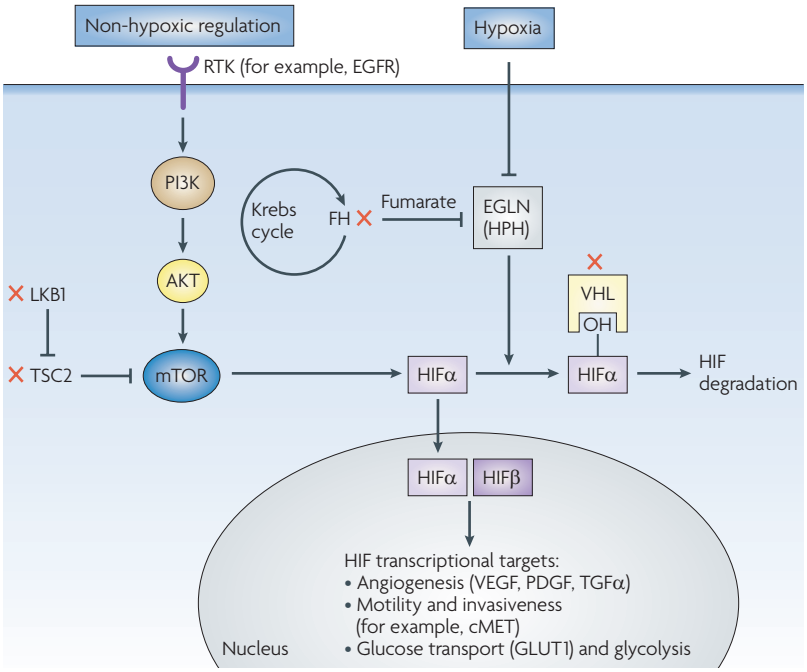


Figure 2 | **Regulation of the hypoxia-inducible factor pathway by hypoxic and non-hypoxic mechanisms and its role in inherited cancer syndromes.** Hypoxia increases levels of hypoxia-inducible factors (HIFs) by decreasing the activity of HIF prolyl hydoxylases (HPHs; also known as EGLNs). These enzymes hydroxylate HIFα, leading to its association with the von Hippel–Lindau (VHL) protein, which targets it for ubiquitylation and degradation. HIFs can also be regulated in a hypoxia-independent manner by mutations in the mitochondrial fumarate hydratase (*FH*) gene, which cause an increase in fumarate that inhibits HPH, or by activation of the phosphoinositide 3- kinase (PI3K)–AKT–mammalian target of rapamycin (mTOR) pathway, which can occur by activation of cell-surface receptor tyrosine kinases (RTKs) or through alterations in LKB1 (also known as STK11) or tuberous sclerosis complex (TSC) 1/2. Red crosses indicate proteins from genes that, when mutated, are known to cause hereditary cancer syndromes. These genes and syndromes include *VHL* (VHL disease), *FH* (hereditary leiomyomatosis papillary renal cell carcinoma), *LKB1* (Peutz–Jeghers syndrome) and *TSC2* (tuberous sclerosis). EGFR, epidermal growth factor receptor; GLUT1, glucose transporter 1 (also known as SLC2A1); OH, hydroxyl group; PDGF, platelet-derived growth factor; TGFα, transforming growth factor α; VEGF, vascular endothelial growth factor.

Scale of definitive chemoprevention trials. The goal of definitive chemoprevention trials is to reduce the occurrence or risk of a new cancer. Because the annual incidence of cancer, even for higher risk patients, is generally low (for example, the 10-year lung cancer risk of smokers is 0.8–15.0%[99]), definitive chemoprevention trials typically require a large number of patients and many years of follow-up before the number of cancer events is sufficient to detect a meaningful difference between the study groups. Consequently, few agents can be definitively evaluated for reducing cancer risk, and by the time the definitive studies are completed, potentially improved drugs have usually emerged. The scale of these trials can be reduced by increasing the anticipated treatment effect of agents, therefore increasing the power to detect the difference between treatment and placebo in a smaller population. In addition, identification of truly high-risk patients (for example, with a 3-year risk of greater than 50%) could also reduce sample size and trial duration by increasing the event rate in the placebo group. The ability to select eligible high-risk patients, in part based on their pharmaco-ecogenetic profile (comprising genetic, environmental and metabolic factors), and predicting the likelihood of drug sensitivity (and unlikelihood of serious adverse events) would also drive down the scale of a trial by increasing the estimated benefit in the treatment group.

Another major approach under investigation for reducing definitive trial scale is surrogate end-point biomarkers (SEBs), or biomarkers that correlate with the cancer end point. Although many potential SEBs have been examined in early-phase clinical trials to provide evidence of agent activity prior to more definitive testing, none have been validated or confirmed to correlate with the definitive cancer end point. For example, the short-term clinical response rate of a potential SEB in oral IEN was only marginally associated with long-term cancer outcome in the largest randomized trial to date in patients with oral IEN[129]. A prespecified substudy of the APC trial found no significant modulation of another potential SEB, aberrant crypt foci, by celecoxib. The presence or number of nondysplastic abberant crypt foci did not correlate with a higher risk of synchronous advanced or recurrent adenomas[100]. Validated SEBs are clearly needed before they can be substituted for the cancer end point in definitive trials. A better understanding of the molecular processes of carcinogenesis and their interaction with drug targets may lead to the discovery of potential SEBs that pan out in validation trials.

Optimizing the risk/benefit ratio. Although the benefits of an agent outweigh its risks for patients with advanced cancer, the reverse may be true in the setting of chemoprevention[72]. This principle was illustrated by the BCPT and COX2 inhibitor trials, which achieved their risk-reduction objectives but also revealed serious adverse effects. The risk of endometrial cancer has largely curtailed public acceptance of tamoxifen despite its FDA approval for reducing breast cancer risk in the BCPT. Cardiovascular toxicity has eliminated consideration of rofecoxib and celecoxib for colorectal cancer prevention despite their established ability to reduce colorectal adenomas in the APPROVe, preSAP and APC trials. Risk aversion in the setting of chemoprevention can be due to the substantial possibility that a person will not develop cancer even in the absence of the intervention. Adverse effects in chemoprevention can be higher because the drugs are usually taken for longer (versus therapy), which could increase the chance of unexpected late toxicities. Furthermore, the tolerance for potential adverse drug events is lower in the chemoprevention setting, in which most individuals are relatively healthy and cancer-free.

Enhancing the likelihood of benefit, and minimizing the risk of adverse events, will be crucial for realizing the potential role of molecularly targeted agents in prevention. These objectives are achievable in part by improving the identification of high-risk patients and of people who are more likely to benefit (and less likely to

Surrogate end point
A measure of the effect of a treatment (for example, an increase in the expression levels of a certain protein) that may correlate with a real clinical end point (for example, cancer development).

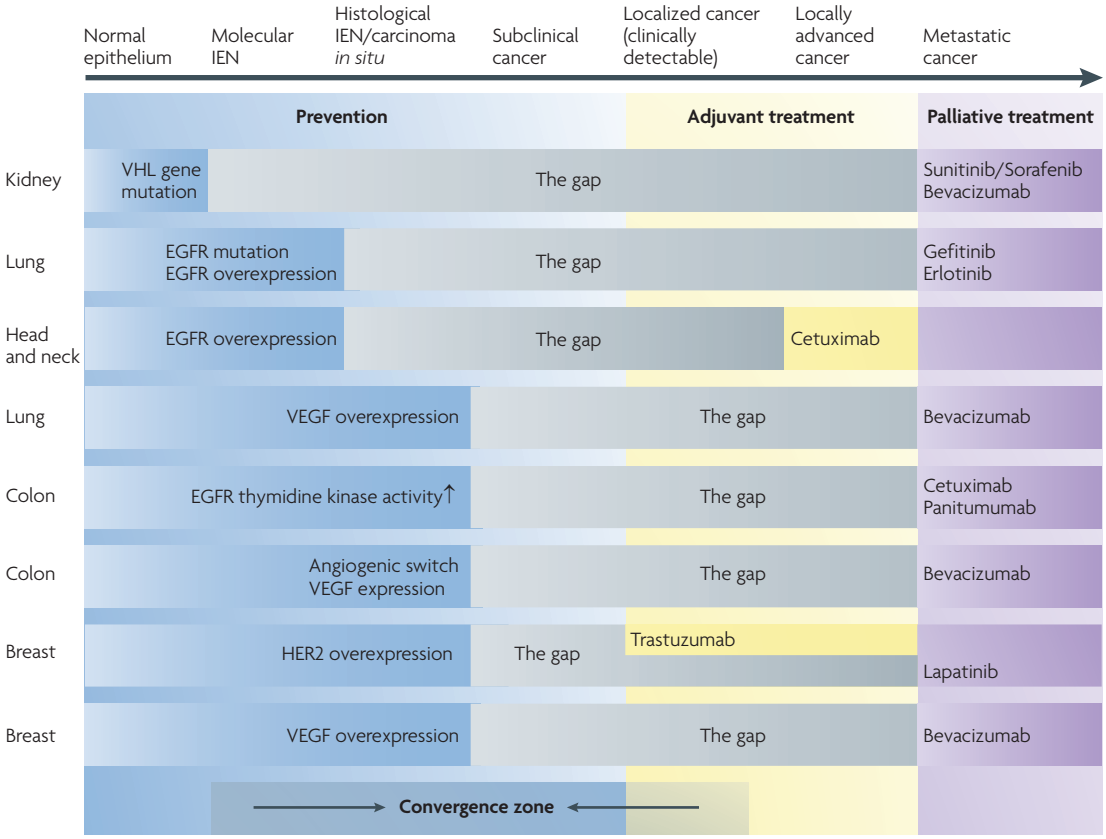


Figure 3 | **Bridging the gap between cancer treatment and prevention.** Molecular alterations (indicated in blue) occur early on during the process of carcinogenesis. However, agents targeted to these molecules are often incorporated into cancer treatment at a relatively late stage. The delay in pharmacologically addressing these molecular alterations is herein referred to as 'the gap' and represents a window of opportunity for the development of molecularly targeted strategies for the prevention and/or early treatment of cancer. The convergence zone illustrates lesions at different stages of development that might be grouped in clinical trials designed to efficiently asses the utility of selected targeted agents for prevention. EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; IEN, intraepithelial neoplasia; VEGF, vascular endothelial growth factor; VHL, von Hippel–Lindau.

experience toxicity) from a given agent, thus personalizing prevention based on validated clinical and (in the future) molecular features.

Increasing the efficacy of preventive agents. As outlined above, patient selection is a vital component of successful chemoprevention. Nonetheless, identifying high-risk patients is futile without effective chemopreventive agents. Drug development strategies that are focused on the following three types of agents are likely to improve the outcomes of chemoprevention programmes in the near future: novel targeted agents with proven activity in advanced disease, agents with strong preventive properties in preclinical models regardless of activity in advanced disease, and combined agents.

In recent decades, specific molecules involved in signal transduction and angiogenic pathways have emerged as targets of adjuvant or palliative therapy for solid malignancies of the head and neck, breast, lung, pancreas, kidney, liver and colon, and gastrointestinal stromal tumours. Growing evidence shows that these key molecular pathways are disrupted early on in the processes of carcinogenesis, suggesting that agents targeting these pathways may be active against premalignant disease as well (FIG. 3). The molecular origins of lung cancer[101] — for example, EGFR mutations in lung adenocarcinoma development — are an example of how molecular targets in advanced cancer treatment might be relevant to the whole range of carcinogenesis: from molecular IEN to carcinoma *in situ* to invasive disease. *EGFR* mutations in exons 18, 19 and 20, and L858R, have malignant transformation potential in fibroblasts and lung epithelial cells[102]. In animal models, exon 19 deletion or L858R mutation in lung tissues leads to atypical alveolar hyperplasia, followed by bronchoalveolar carcinoma and then adenocarcinoma[103]. In humans, EGFR tyrosine kinase domain mutations can occur early, or in histologically normal respiratory epithelium adjacent to tumours[104,105]. EGFR mutations have also been detected in pre-invasive disease, and precede EGFR amplification[104]. Even a germline EGFR mutation (T790M) has been identified and linked to lung adenocarcinoma development[106].

Several targeted agents have been tested for preventive activity in preclinical studies. Promising new prevention targets that have a strong preclinical rationale and are

Box 4 | **Active drugs reduce the daunting scale of chemoprevention trials**

The development of molecularly targeted drugs with improved clinical activity allows for estimating a higher magnitude of effect of the intervention. This reduces the sample size of the trial while retaining power to show a meaningful clinical benefit. Although cross-comparisons of trials are generally not advisable, the table below illustrates the point by depicting the 'number needed to treat' to prevent one patient from developing adenoma within the placebo-controlled trials using aspirin, selective cyclooxygenase 2 inhibitors or difluoromethylornithine (DFMO) plus sulindac. The DFMO plus sulindac combination is the most active regimen studied in this setting to date; the low number needed to treat implies that the difference between the placebo and the active treatment groups is large, clinically meaningful and readily demonstrable in a trial with a small sample size.

Trial (size)	Intervention	Number needed to treat to prevent adenoma(s) in one patient*	Relative risk of all adenomas (95% CI)*	Relative risk of advanced adenomas (95% CI)*
Baron *et al.*[75] (1,121)‡	Aspirin (81 mg daily)	11	0.81 (0.69–0.96)	0.59 (0.58–0.92)
APPROVe[7] (2,587)	Rofecoxib (25 mg daily)	8	0.76 (0.69–0.83)	0.70 (0.58–0.86)
PreSAP[5] (1,738)	Celecoxib (400 mg daily)	6	0.64 (0.56–0.75)	0.49 (0.33–0.73)
APC[6] (2,035)	Celecoxib (200 mg twice daily)	6	0.67 (0.59–0.77)	0.43 (0.31–0.61)
APC[6] (2,035)	Celecoxib (400 mg twice daily)	4	0.55 (0.48–0.64)	0.34 (0.24–0.50)
Meyskens *et al.*[119] (375)	DFMO (500 mg) plus sulindac (150 mg daily)	3	0.30 (0.18–0.49)	0.085 (0.011–0.65)

*Based on adenomas diagnosed within 3 years of follow-up. ‡This is the largest trial of aspirin specifically designed for colorectal adenoma prevention; it is representative of three other trials[74,76,77] revealing a number needed to treat of 9–16 to prevent adenoma(s) in one patient during the follow-up period. APC, Adenoma Prevention with Celecoxib; APPROVe, Adenomatous Polyp Prevention on Vioxx; CI, confidence interval; PreSAP, Prevention of colorectal Sporadic Adenomatous Polyps.

likely to be evaluated in the clinic include the insulin-like growth factor axis and the PI3K–AKT–mTOR axis pathway. Targeting insulin-like growth factor axis components and downstream pathways inhibits survival of premalignant and malignant bronchial epithelial and vascular endothelial cells, and decreases tumour growth and angiogenesis[107]. The AKT pathway is activated in bronchial premalignancy (both in the proximal airway and in the alveolar epithelium) in smokers and in patients with lung cancer[108]; deguelin and myo-inositol have preventive activities in lung carcinogenesis, in part by suppressing the PI3K–AKT pathway[109,110]. The mTOR inhibitor temsirolimus (Torisel; Wyeth) prevented the progression of atypical alveolar hyperplasia — the premalignant form of some types of adenocarcinomas[111] — in an elegant experiment in the K-Ras lung cancer mouse model. Inhibition of mTOR also reversed AKT-dependent prostatic IEN in a transgenic mouse model[112]. Other molecularly targeted agents, such as small-molecule receptor tyrosine kinase inhibitors, with promise in high cancer risk settings include VEGF pathway inhibitors, dual inhibitors of VEGF receptor and EGFR, and dual inhibitors of EGFR and human epidermal growth factor receptor 2 (HER2; also known as ERBB2). Indeed, the EGFR- and HER2-targeted small molecule lapatinib (Tyverb; GlaxoSmithKline) has been recently shown to prevent ESR-negative breast cancers in mice by suppressing the development of premalignant lesions[113].

As with cytotoxic chemotherapy in the setting of advanced disease, combining targeted agents is likely to enhance their activity against established cancers. Results of the first clinical trials of molecular-targeted doublets show that some combinations (for example, erlotinib plus bevacizumab for non-small cell lung cancer[114], and lapatinib plus trastuzumab for breast cancer[115]) have promising clinical activity. This strategy will naturally migrate to prevention settings once the efficacy and tolerability of combinations are better characterized.

Provocative preclinical work conducted over many years[116] has suggested the promise of combination chemoprevention[117,118]. This concept was recently validated by the first successful randomized Phase III trial of combination chemoprevention[119]. Patients with resected colorectal adenomas (375 patients recruited) were randomized to receive daily oral difluoromethylornithine (an ornithine decarboxylase irreversible inhibitor) plus sulindac (an NSAID) or placebo for 36 months. The 3-year adenoma recurrence rates were 12% for the combination group versus 41% for the placebo group ($p<0.001$). Notably, advanced adenoma rates were 0.7% in the combination group versus 8.5% in the placebo group ($p<0.001$), a greater than 90% reduction in relative risk. The combination was well tolerated, with no statistically significant increase in serious adverse events. This study is a landmark advance for cancer chemoprevention as it tested doses that were reduced from those previously shown for both agents to be ineffective against advanced colorectal cancer. The drugs for the combination were chosen on the basis of

extensive animal testing[120–122], and the dose de-escalation strategy was based on earlier clinical work with careful pharmacodynamic assessments of polyamine levels in the target tissue[123,124]. Comparing favourably with results of the previous COX2 inhibitor studies[5–7], results with this combination illustrate the desirability of targeting a high magnitude of effect in order to design smaller, yet definitive, studies with the potential to change clinical practice (BOX 4).

Concluding remarks

Cancer chemoprevention continues to evolve with the rapid integration of molecular approaches into its research and clinical practice. Early translational trials in the oral premalignancy model set the stage for modern trial design and conduct. Striking recent randomized trial results and FDA approvals will probably lead to relatively wide public use of an HPV vaccine to prevent cervical neoplasia and of raloxifene to prevent invasive breast cancer. Large randomized prevention trials in the breast, prostate and colon–rectum, however, have shown that major adverse events can prevent widespread public acceptance of active chemoprevention agents. This acceptance should be enhanced by future trials that can select patients with a truly high cancer risk, identify patients that are most likely to benefit from and least likely to experience serious adverse effects of the intervention, test single or combined agents with large anticipated treatment effects, and that can be completed in a relatively short time. Future chemoprevention research will include molecularly targeted approaches for patient selection, drug development and outcome evaluation. Paralleling personalized approaches in advanced cancer therapy, the evolution of molecularly targeted, personalized approaches will streamline chemoprevention research and facilitate the development of rational, effective and safe preventive drugs with the ability to intervene early in carcinogenesis.

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Competing interests statement

The authors declare competing financial interests: see web version for details.

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