The chemopreventive agent development research program in the Division of Cancer Prevention of the US National Cancer Institute: An overview

James A. Crowell

Division of Cancer Prevention, National Cancer Institute, DHHS, Bethesda, MD 20892, USA

Received 25 April 2005; accepted 25 April 2005
Available online 6 July 2005

Abstract

Chemoprevention is an innovative area of cancer research that focuses on the development of pharmacological, biological, and nutritional interventions to prevent, reverse, or delay carcinogenesis. Over the past two decades the Division of Cancer Prevention of the US National Cancer Institute has organized a research and development program to provide resources and infrastructure to the research community for the clinical evaluation of potential cancer preventive agents. This program now encompasses preclinical agent and molecular target identification, in vitro and in vivo screening, efficacy and intermediate endpoint testing, pharmacology and toxicology assessments, and finally chemical synthesis and manufacturing leading to Investigational New Drug applications and clinical studies. In this review, examples of agents currently in development, preclinical testing models, and phase 1 and 2 clinical studies are described. Continued commitment to cancer prevention will significantly reduce the economic and medical burden of cancer.

Published by Elsevier Ltd.

Keywords: Cancer prevention; Chemoprevention; Carcinogenesis; Drug development

1. Introduction

Cancer is the result of a multistep process in which the cumulative effect of successive genetic and molecular alterations leads to a gradual transition, typically over decades, from normal to increasing grades of dysplasia that culminate in an invasive and metastatic phenotype. The sequential accumulation of genetic and molecular alterations over time provides opportunities for the development of clinical interventions aimed both at preventing cancer initiation and at treating premalignant lesions.

Chemoprevention is an innovative area of cancer research that focuses on the prevention of cancer through pharmacologic, biologic, and nutritional interventions. As originally described, this involves the primary prevention of initiation and the secondary prevention, delay, or reversal of promotion and progression [1,2]. Several agents have demonstrated cancer preventive risk reduction in large phase 3 clinical trials in individuals with an increased risk of cancer [3–5] and other large trials are ongoing [6,7]. The extension of these successes in individuals at high risk for cancer, such as having an inherited predisposition to cancer e.g. familial adenomatous polyposis (FAP), to individuals at lower risk e.g. having a prior colonic adenoma, requires a risk/benefit assessment that may lead to the use of different agents, regimens, or to dietary recommendations intended to affect the same molecular targets. The degree of benefit that leads to compliance, the safety of the intervention, and the cost become increasingly important considerations in moving from adjuvant and secondary prevention to primary prevention and represent some of the challenges of chemopreventive agent development.
The organization of a research and development program to provide resources and infrastructure to the research community for the clinical development of chemopreventive agents was initiated within the Division of Cancer Prevention (DCP) of the US National Cancer Institute (NCI) approximately two decades ago. This effort now encompasses all phases of agent development from identification of lead agents and preclinical efficacy and toxicity evaluations to translational research (e.g., identification and validation of biomarkers of carcinogenesis) and phase 1 and 2 clinical studies. Technical support resources for regulatory affairs, drug supply and formulation, protocol development and management, and research information have also been established. Large phase 3 studies are conducted through the Cancer Cooperative Groups and the Community Clinical Oncology Program. The subject of this overview is the early agent development effort that comprises agent and molecular target identification, in vitro mechanistic and efficacy screens, in vivo preclinical efficacy and intermediate endpoint testing, preclinical pharmacology and toxicology testing, and chemical synthesis and formulation leading to clinical phase 1 safety and pharmacokinetic studies and phase 2 biomarker modulation studies.

2. Agent and molecular target identification

The identification of potential chemopreventive agents is conducted by the systematic review of the epidemiological and experimental carcinogenesis literature; collaborations with the pharmaceutical, nutritional, and biotechnology industries; and the Rapid Access to Preventive Intervention Development (RAPID) Program. Research involving non-steroidal anti-inflammatory drugs (NSAIDs) illustrates this discovery process.

Epidemiological observations suggesting that NSAIDs might be effective in reducing colorectal cancer were made almost 20 years ago. Preclinical efficacy testing of NSAIDs in rodent models of colon, bladder, and skin carcinogenesis showed that: (a) NSAIDs including piroxicam, sulindac, ibuprofen, and indomethacin are highly effective in blocking colon carcinogenesis in rats and mice; and (b) NSAIDs can be introduced late in the process of carcinogenesis and still be highly effective in preventing the development of cancer. However, most of the traditional NSAIDs have risks that must be weighed against their benefits, the most prominent being gastrointestinal bleeding and renal dysfunction due to cyclooxygenase (COX) inhibition and lack of protective prostaglandins. Several strategies have been suggested for improving the therapeutic index of NSAIDs. One approach evaluated is to lower the dose and combine the NSAID with another agent that is also effective but by an independent mechanism of action. In this regard, NSAIDs combined with z-difluoromethylornithine (DFMO), an ornithine decarboxylase (ODC) inhibitor, were found to have synergistic efficacy [8]. The discovery about 14 years ago of 2 COX forms (constitutive COX-1 and inducible COX-2) provided another opportunity for improving the therapeutic index of NSAIDs. Higher levels of COX-2 occur in inflammation and are widely associated with cancer progression and tumour angiogenesis. For example, COX-2 levels are increased in bladder, skin (basal cell, squamous cell, and melanoma), endometrial, breast, non-small cell lung (NSCLC), pancreatic, and esophageal cancers. This suggested that COX-2 specific inhibitors might be effective chemopreventive agents and that the toxicity associated with COX inhibitors might be reduced. Based on the mechanism of action and in vitro efficacy studies, DCP entered into collaboration with the pharmaceutical manufacturer of the selective COX-2 inhibitor celecoxib (now marketed as Celebrex®) to undertake initially in vivo cancer prevention studies.

One of the most common genetic mutations found in sporadic human colon cancer is in the adenomatous polyposis coli (Apc) gene. Mutations of this gene are also found in the germ line of individuals with the autosomal dominant cancer syndrome, familial adenomatous polyposis (FAP). These individuals typically develop hundreds of intestinal polyps and colon cancer at an early age. The Min (multiple intestinal neoplasia) mouse is one animal model of this syndrome. The Min mouse carries a germline mutation in the Apc gene and develops multiple intestinal adenomatous polyps. Exposure of Min mice to celecoxib prior to the development of polyps prevents adenoma formation. Treatment later in carcinogenesis, in mice bearing polyps, reduces the number of these adenomatous polyps [9]. The results of the late intervention in Min mice helped to justify the successful DCP-sponsored trial in patients with FAP, who have pre-existing polyps, that led to FDA labeling approval [4]. Celecoxib was also highly effective in preventing azoxymethane (AOM)-induced rodent colorectal cancers when administered either throughout the experiment or later in carcinogenesis [10]. The latter experimental design has particular relevance to patients who have had an adenoma(s) and are at increased risk for additional adenomas and colorectal cancer. Based on these preclinical data and on the clinical data in patients with FAP, a clinical trial to study the effects of celecoxib in individuals with prior sporadic colon adenomas was initiated and is nearing completion. Celecoxib was also evaluated in a number of other rodent tumour models: tongue, esophagus, breast, lung, prostate, bladder, and skin. Celecoxib was found to reduce the incidence by 90% both of 4-hydroxybutyl (butyl) nitrosamine (OHBBN)-induced bladder cancer [11] and of UV-induced skin cancers in rodents when administered at the time of initiation or later during cancer progression [12].
Based on these preclinical studies and on supporting epidemiological data, a clinical trial is currently underway to evaluate the efficacy of celecoxib in the prevention of recurrent bladder cancers in patients with previous superficial bladder cancers. Other trials are also underway in patients with actinic keratoses, basal cell nevus syndrome (BCNS), Barrett’s esophagus, and breast, prostate, and head and neck cancers.

Continued investigations with COX-2 inhibitors not only have revealed the recent controversial data regarding cardiovascular safety [13,14], but also have stimulated research and agent development for non-COX-2 targets of COX-2 inhibitors [15] and have coincided with the development of other agents to modulate the arachidonic acid cascade. The COX and the lipooxygenase (LOX) cascades are in balance [16], and agents are in development for arthritis that are dual inhibitors of these cascades, such as licofelone [17]. Altered LOX signaling has been implicated in cancer [18] and dual function inhibitors may be effective in cancer prevention. Administration of LOX substrates such as z-linolenic acid, docosahexanoic acid, and eicosapentaenoic acid in combination with NSAIDs may also be efficacious in cancer prevention [19]. Additionally, aspirin can acetylate the active site of COX-2 and convert it to produce 15R-hydroxyicosatetraenoic acid, which may be transformed by 5-LOX to the anti-inflammatory 15-epi-lipoxin A4 [20]. The development of AKT pathway modulators, secondarily via 3-phosphoinositide dependent kinase (PI3K) inhibition by COX-2 inhibitors [15], and independently as molecular targets for cancer prevention, represent newer, potential chemopreventive molecular targets associated with inflammatory oxidant stress.

Modification of NSAIDs by attaching nitric oxide (NO) has led to the development of a novel class of drugs with decreased gastrointestinal toxicity [21]. NO affects a vast number of physiological processes [22] and alterations in NO synthase are potential molecular targets for cancer prevention [23]. These NO-NSAIDs consist of a known NSAID linked via a chemical spacer to a NO-releasing group. The rationale for their development as pharmaceuticals is based on the observation that NO exhibits many of the same qualities as prostaglandins in the gastric mucosa. By stimulating vasodilation and mucous secretion, NO should compensate for the reduction in gastric prostaglandin release produced by NSAIDs and result in decreased gastrointestinal toxicity. NCX-4016 is a NO-releasing aspirin derivative that maintains the COX and platelet inhibitory activity of aspirin and exerts a number of NO-mediated actions. It is virtually devoid of gastric and duodenal toxicity both in animals and humans while maintaining, and in some cases increasing, pharmacological actions including anti-inflammatory, analgesic and anti-thrombotic activities. NCX-4016 is being investigated for its potential cancer chemopreventive action in humans based on several observations. In published animal studies, the drug inhibited the formation of carcinogen-induced pre-neoplastic colonic aberrant crypt foci (ACF) in rats [24]. Effects were superior to aspirin. Preliminary results from a NCI, DCP-sponsored study indicate that NCX-4016 inhibited AOM-induced colon tumour incidence and multiplicity even though it had no significant effect on ACF formation. The closely related NO-aspirin-releasing compound NCX-4040 (a positional isomer of NCX-4016) reduced the growth of a number of human cancer cells (pancreatic, prostate, colon, and tongue) in vitro at low micromolar levels [25]. The agent was more potent than both unmodified aspirin and NO-NSAIDs containing sulindac, ibuprofen or indomethacin. NO-releasing NSAIDs also strongly inhibited the expression of inducible NO synthase in colon cancer cell lines. NO synthase has been linked to promotion of carcinogenesis [26].

Interestingly, NO and angiotensin II (ANG II) are biological antagonists in sensing oxygen and in responding to oxygen stress [27]. ANG II is formed from ANG I by angiotensin-converting enzyme (ACE) in the rennin-angiotensin system. The effects of ANG II are mediated directly through receptors and indirectly through the release of other factors affecting other signalling cascades. Some of the pathophysiological effects of ANG II are mediated through nuclear factor κB (NFκB), which participates in inflammatory responses and has been implicated in cancer [28]. Long term use of several angiotensin converting enzyme (ACE) inhibitors for the treatment of hypertension was shown to reduce the risk of cancer in a retrospective cohort study [29]. To evaluate further the interaction of NO and the ANG II pathways, combinations of ACE inhibitors and NO-ASA are now being evaluated in preclinical cancer prevention models.

One means of upregulating COX-2 in cancer is through the AKT pathway and its effect on NFκB [30,31]. Phosphorylation of IκB kinase (IKK) by AKT activates it to phosphorylate the inhibitor of κB (IκB) which is then degraded, releases NFκB from inhibition, and allows NFκB to translocate to the nucleus and transcribe anti-apoptotic genes [32]. Further upstream, proteins in the receptor tyrosine kinase/PI3K/PTEN/AKT sequence are often altered in cancer and other diseases and are amenable to drug targeting (as noted above AKT is a non-COX-2 target of celecoxib). The AKT pathway affects cell proliferation, cell survival and resistance to apoptosis, cell metabolism and growth, angiogenesis, and cell migration [33–35]. Aberrant PI3K/AKT signaling has been described in multiple types of human cancers [33–37] including, for example, non-small cell lung cancer (NSCLC), prostate and pancreatic cancers, head and neck squamous cell carcinoma, melanoma, gastrointestinal cancers, and female breast and...
reproductive tract cancers, among others. AKT activation is an early response to carcinogen exposure and has been suggested to have a permissive role in the development of tobacco-related cancers [38,39]. Studies in transgenic mice that overexpress constitutively active AKT and in PTEN heterozygous knockout mice show that AKT contributes to tumour formation in these mice [40].

The DCP and others have developed several compounds to modulate targets in the AKT signaling cascade. Curcumin was initially identified as a potential anti-oxidant and anti-inflammatory agent that could modulate both the COX and the LOX pathways [41–44]. Recently, inhibition of IKKs by curcumin to prevent inactivation of IkB and release of active NFκB has been described [30]. Curcumin is the major yellow pigment extracted from turmeric, the powdered root of the herb Curcuma longa. In India and Southeast Asia, it is used extensively in food and also as a treatment (as turmeric, 1–5% curcumin) for inflammation, skin wounds and tumours. In Germany and Central America, a formulation is marketed for treatment of biliary disorders. In the USA, turmeric is classified as ‘generally recognized as safe’ (GRAS). Food-grade curcumin actually contains three similar compounds belonging to the curcuminoid family: curcumin (69–77%), demethoxycurcumin (∼17%), and bisdemethoxycurcumin (3–6%). The individual curcuminoids present in food-grade curcumin have similar activities, although differences in potency have been demonstrated in some systems. Other described effects that could contribute to chemopreventive activity include inhibition of mutagenesis, clastogenesis, carcinogen-DNA adduct formation, and inhibition of angiogenesis, cell proliferation and growth. Additional activities include induction of apoptosis, as well as modulation of immune responses, oncogene expression, and the activities of cytochrome P450s and phase II metabolic enzymes. Finally, curcumin may have hormonal and anti-viral effects. A pharmaceutical grade curcumin is currently in clinical studies sponsored by DCP and elsewhere [45–47].

SR13668 was developed at SRI International, Menlo Park, California USA using computational modeling based on the cancer chemopreventive activity of the naturally occurring agent indole-3-carbinol (I-3-C). SR13668 exhibits potent oral anti-tumour activity in xenograft models of estrogen-dependent and independent breast cancer, prostate cancer, and drug-resistant ovarian cancer [48]. In vivo inhibition of pAKT in tumours occurred by 24 h and increases in apoptosis, decreases in proliferation, and accumulation in G0/G1 were observed. SR13668 significantly inhibits pAKT (Ser473) in vitro in a dose- and time-dependent manner and inhibits blood vessel growth. No effects on blood glucose were seen in mice after 14 days of treatment; and specificity for AKT was demonstrated by lack of inhibitory effects on a broad selection of kinase targets. SR16338 is currently under development for cancer prevention in the RAPID program, described further below.

Deguelin, under development by researchers at MD Anderson Cancer Center, Houston, Texas USA, is a rooted derived from the bark of the African plant Mundulea sericea Willd. (Leguminosae) [49]. Deguelin inhibits the growth of NSCLC cell lines, malignant and premalignant human bronchial epithelial (HBE) cell lines, and squamous HBE, but does not affect the growth of normal HBE cells. These effects are associated with induction of apoptosis and increased expression of apoptosis associated proteins including BAX, P53, and P21. In some cells, BCL-2 levels are also decreased [50,51]. In premalignant HBE cells, deguelin inhibits PI3K activity and reduces levels of pAKT, but does not affect mitogen activated protein kinase (MAPK) signaling. That deguelin-induced apoptosis is mediated by inhibition of the PI3K/AKT pathway is suggested by the observation that overexpression of a constitutively active AKT blocks deguelin-induced growth arrest and apoptosis [50]. In squamous HBE cells, a model of bronchial metaplasia, deguelin inhibits COX-2, but not COX-1 expression [51]. Other investigators have reported reduction in ACF formation in the AOM-induced rodent model [52] and reductions in cancer incidence and multiplicity in rodent models of colon, mammary, and skin cancers [53,54]. Very little data is available to evaluate the potential toxicity of deguelin in humans. Deguelin can inhibit NADH:ubiquinone oxidoreductase [55], which couples the oxidation of NADH and the reduction of ubiquinone to the generation of a protein gradient which is in then used to synthesize ATP.

Another potential chemopreventive molecular target in the PI3K/AKT sequence is the serine–threonine kinase mammalian target of rapamycin (mTOR) [56]. mTOR is a critical nutrient-sensor checkpoint protein which integrates signals from amino acids, energy, hormones, and growth factors [57,58] to control cell cycle progression, cell size, cell migration, and survival [59,60]. mTOR controls the phosphorylation of at least two regulators of protein synthesis and cell growth: ribosomal p70S6 kinase (S6K1) and the eukaryotic initiation factor 4E (eIF-4E) binding protein 1 (4E-BP1). S6K1 phosphorylates ribosomal protein S6 which upregulates translation of selective mRNAs containing a 5′-terminal oligopyrimidine sequence (5′-TOP). These 5′-TOP mRNAs code for essential components of the protein synthetic machinery including ribosomal proteins and elongation factors [61,62]. The other major downstream effector of mTOR is 4E-BP1; mTOR phosphorylates and inactivates 4E-BP1, decreasing its affinity for eIF-4E. eIF-4E participates in the transfer of mRNA to the 40S ribosomal subunit and is rate limiting
in the translation of cap-dependent mRNAs [61,62]. Although mTOR mutations have not been reported in human cancers [63], upstream elements in the PI3K/PTEN/AKT sequence have (e.g. [64,65]). In the autosomal dominant cancer syndrome, tuberous sclerosis (TS), which is characterized by renal tumours, the tumour suppressor complex TSC2 that negatively regulates mTOR is mutated [66,67]. TSC2 is phosphorylated and inhibited by AKT; thus AKT appears to stimulate mTOR signaling by inhibiting TSC2 [68]. The macrolide antibiotic and immunosuppressant rapamycin is being explored for chemoprevention using in vitro systems. The toxicity of rapamycin precludes consideration as a chemopreventive except possibly when used topically, to minimize systemic exposure, for skin cancer. Interestingly, in the autosomal dominant basal cell nevus syndrome (BCNS) the hedgehog pathway is deregulated and rapamycin inhibits induction of transformed foci in vitro by GLI, a transcription factor that functions in the hedgehog pathway [69]. DCP is supporting an in vitro and in vivo study in BCNS patients using rapamycin topically applied to the phenotypically normal skin of BCNS patients.

Epidemiological evidence has also associated a number of dietary components (e.g. polyphenolics, isoflavones, and lycopene) with reduced cancer incidences and has identified additional potential molecular targets of these agents. Consumption of tea has been associated with reduced incidences of cancers of the breast, cervix, colon and rectum, gall bladder, liver, lung, nasopharynx, pancreas, prostate, stomach, ovary and uterus [70,71]. In DCP sponsored and published preclinical studies using green tea and tea derived polyphenols and mixtures, chemopreventive efficacy has been demonstrated in mouse forestomach, stomach, intestine, mammary gland, liver, lung, lymph node (metastases), prostate and skin; in rat mammary gland, lung, esophagus, stomach, small intestine and colon; and in hamster buccal pouch. Modulation of intermediate biomarkers has also been demonstrated in several organs, including skin, prostate, stomach, colon and liver [72,73]. Several mechanisms may be responsible for the anti-carcinogenic properties of green tea polyphenols, particularly epigallocatechin gallate (EGCG). Inhibition of epidermal growth factor receptor (EGFR) signaling by EGCG could affect numerous downstream processes [74]. Other reported activities include anti-oxidant and anti-mutagenic activities (inhibition of carcinogen-DNA binding and adduct formation), modulation of cytochromes P450 activity and gene expression, and induction of phase II enzyme activity (glutathione (GSH) peroxidase, catalase, and NAD(P)H:quinone oxidoreductase) as well as inhibition of LOX, COX, type I 5α-reductase, and enhancement of gap junction intercellular communication and apoptosis. DCP has systematically developed the polyphenol mixture Polyphenon E in collaboration with industry. A key question has been whether or not the plasma concentrations of EGCG and polyphenols that have shown efficacy in preclinical studies could be achieved in vivo in humans. In clinical phase 1 studies substantially higher plasma levels of polyphenols and their conjugates have been achieved when administered to fasting subjects [75,76]. Phase 2 biomarker studies are now underway in breast, bladder, lung, cervix, prostate, and other settings. The combination of Polyphenon E and other agent(s) is also an attractive approach to enhance efficacy [77].

The isoflavones genistein, daidzein, and glycine occur naturally in soybeans and are in soy food products. Epidemiological studies have associated isoflavone consumption with reduced risks of breast and prostate cancers [78–80]. Individuals consuming a soy-enriched Asian diet have plasma and urinary genistein concentrations that are up to 100- and 30-fold, respectively, higher than individuals consuming a Western diet [81,82]. Genistein, as well as daidzein and its metabolite equol, are estrogen receptor (ER) agonists. Their affinity for ERα is 100- and 1000-fold weaker, respectively, than estradiol but only 3- and 60-fold less potent than estradiol in binding to ERβ [83]. The isoflavones are also anti-oxidants [84], can inhibit tyrosine kinases [85] and angiogenesis [86], and can induce apoptosis [87,88] and cell differentiation [89]. Studies in animals indicate that the isoflavones may prevent breast and prostate cancers [90–93]. DCP developed an isoflavone formulation for clinical studies and completed clinical phase1 pharmacokinetic and safety testing [94–98]. Plasma concentrations that have shown efficacy in preclinical testing were reached in these studies. The formulation is currently being evaluated in phase 2 studies in breast and bladder for modulation of tyrosine kinase phosphorylation and other pathways.

Lycopene, the red pigment found in tomato and tomato products, has shown an inverse association for the risk of prostate, lung and stomach cancers in epidemiologic studies [99,100]. A large percentage of the carotenoids identified in human plasma and tissues is accounted for by lycopene and it is not converted to vitamin A. It is uniquely concentrated in testis, adrenals and prostate, with lower but significant amounts in liver and kidneys. Although all carotenoids have potent anti-oxidant activity, lycopene is the most efficient singlet oxygen quencher and can also trap peroxynitrate [101]. These activities decrease lipid, protein and DNA damage. Lycopene also affects intercellular communication via gap junctions, the immune response by decreasing interleukin (IL)-6 levels, carcinogen-metabolizing enzyme expression, and insulin growth factor (IGF)-1-signalled cell proliferation. Lycopene has chemopreventive efficacy in animal models of lung, colon and breast cancer. Lycopene also inhibits the growth of human prostate cancer cells in vitro and concentrates in human
prostatic adenocarcinoma tissue implanted in nude mice. Recently, lycopene was shown to interfere with androgen signalling and metabolism and with hormone-dependent gene expression in a rat prostate tumour implant model [102,103]. These effects of lycopene correlated with increased necrosis of the tumour implant. In a published pilot investigation in 21 men with localized prostate cancer, a defined lycopene supplement administered for 1 month prior to prostatectomy decreased proliferation and induced apoptosis and differentiation in prostatic tissue [104]. DCP is sponsoring clinical phase 1 pharmacokinetic and safety studies and phase 2 biomarker modulation studies in collaboration with industry.

Other compounds, such as indole-3-carbinol (I-3-C) and resveratrol, have been identified for development based on publications in the experimental carcinogenesis literature. I-3-C, a compound formed in Brassica vegetables, such as broccoli, cabbage, and Brussels sprouts, by the action of myrosinase on glucobrassicin, was reported in 1990 to be a potential breast chemopreventive agent affecting estrogen metabolism in humans [105]. Subsequently, modulation of estrogen stimulated proliferation by I-3-C in cells infected with human papillomavirus (HPV) led to its evaluation in the treatment of recurrent respiratory papillomatosis [106–108] and in cervical intraepithelial neoplasia [109]. Additionally, in animal models of cancer, I-3-C has been reported to prevent breast [110], endometrial [111], cervical [112], and lung cancers [113]. However, the potential for I-3-C to promote as well as to block carcinogenesis, due to induction of cytochromes P450, has made consideration of its use for cancer prevention controversial [114]. I-3-C is also highly sensitive to aqueous acidic conditions and forms numerous oligomeric products, making it challenging to maintain it as a pharmaceutical [115]. Diindolyl methane (DIM) is a biologically active oligomer of I-3-C and is the only readily measurable analyte in plasma after oral administration of I-3-C [116]. DIM is a stable compound and has shown efficacy in a preclinical breast cancer prevention model [117]. Interestingly, DIM has also been reported to bind as an antagonist to the androgen receptor and to have efficacy in vivo in mice injected with prostate tumour cells [118]. DCP developed I-3-C for clinical studies and completed clinical phase 1 pharmacokinetic and safety testing. DIM is currently being evaluated in phase 1 studies in collaboration with industry. The compound SR13668, described above, was designed based on efficacy results with I-3-C and its oligomeric products; SR13668 is being developed by the DCP RAPID program and should enter clinical testing within 1 year.

The initial publication by Jang in 1997 [119] attributing chemopreventive activity to resveratrol (3,5,4′-trihydroxy stilbene), a compound found in grapes, mulberries, and peanuts, led to increased research and publications related to resveratrol. Favorable cancer preventive attributes include, for example, anti-oxidant, anti-inflammatory, anti-proliferative, anti-mutagen, and pro-apoptotic activities [120–125]. Mechanisms of action relevant to cancer prevention include inhibition of NFκB and AP-1 [126,127] and modulation of COX, LOX, NO synthase, and protein kinases [128–130]. Additionally, potentially cardioprotective properties have been ascribed to resveratrol [131], and it has shown efficacy as a topically applied anti-viral against herpes simplex [132]. Resveratrol is also a phytoestrogen and has some structural similarity to diethylstilbestrol (DES) [133]. However, resveratrol has a higher affinity for the ERα than α and transcriptionally activates ERβ at low concentrations [134]. Resveratrol is being developed by DCP in collaboration with industry and is currently in clinical phase 1 studies.

Since the natural products resveratrol and genistein are phytoestrogens, the optimal risk/benefit setting for their use in cancer prevention has yet to be determined (e.g. postmenopausal breast cancer, prostate or colon cancer). Interestingly, these compounds have become structural scaffolds for the development of ERβ agonists [135]. ERα and ERβ are distinct gene products with non-overlapping functions [136], and ERβ ligands have important chemopreventive properties [137]. ERβ is abundantly expressed in prostate and intestinal epithelium, the urogenital tract, lung, ovarian follicles, certain brain regions, and muscle, while ERα expression in these tissues is generally low or undetectable (reviewed in [138]). Tissues that exhibit high ERα content include the mammary gland, uterus, placenta, liver, central nervous system, cardiovascular system, and bone. In general, ERα is considered an activating factor for proliferation, whereas ERβ curtails the effects of ERα, serving an anti-proliferative function. ERβ has been identified in cancers of the breast [139], prostate [140], colorectum [141], ovary [142], esophagus [143], and others; however, its precise role in these cancers is not yet clearly defined. For example, evidence that ERβ may play a protective role against prostate tumour growth in humans includes multiple reports of diminished Erβ mRNA and/or protein expression during prostate cancer progression in comparison to levels found in normal prostastic epithelial tissues [140,144–147]. The loss of ERβ expression is further corroborated by studies showing that as prostate cancers evolve from low grade to high grade lesions, methylation of the ERβ promoter increases, leading to transcriptional inactivation and silencing of the ERβ gene [148–150]. Conversely, other investigators have failed to show a correlation between ERβ expression, histological grade and prostate cancer pathogenesis [151]. In fact, higher recurrence rates were reported in prostate cancers that retain ERβ expression [144]. Studies by Leav and colleagues revealed that ERβ protein levels are dramatically increased in prostate can-
cer metastases, particularly in bone and lymph node [152]. Other data suggests that ER β cx (a splice variant) may have prognostic value in prostate cancer, based on significantly greater levels of ERβ cx found in high-grade prostate tumours, compared with low grade tumours or benign tissues [153]. Given the disparities noted above, additional research is clearly needed to ascertain fully the function of ERβ in prostate cancer, and other organ sites, and to establish where ERβ agonists or antagonists would be more useful as chemopreventive agents. DCP is supporting clinical studies of resveratrol and isoflavones and additionally is using molecular modeling techniques to screen compounds in silico for their ability to interact with the estrogen receptors.

The RAPID program provides a foundation for the development of potential chemopreventive agents by making the resources of DCP available to academically based investigators. While DCP has historically provided investigational agents, toxicological, and regulatory support to investigators with grants and to institutions on an ad hoc basis, the RAPID program supports the formal expansion of this effort to compound synthesis and formulation under Good Manufacturing Practices regulations, preclinical efficacy and toxicity testing, and regulatory support through phase 1 clinical testing. The RAPID program supports academic investigators by providing those aspects of applied agent development that have historically not been supported through the research grant review process. The projects are supported as long as there is positive progress at defined agent development milestones. Applications are reviewed once yearly and information on the program can be found at http://www3.cancer.gov/prevention/rapid/index.html.

Agents that are under development within the RAPID program are listed on the website and those farthest along in development are described briefly here. Sulforaphane, its precursor glycone glucoraphanin, and 1,2-dithiole-3-thione are blocking agents, functionally similar to the anti-schistosomiasis drug oltipraz. Oltipraz has shown chemopreventive efficacy in numerous animal models and, in a clinical trial in China, it was effective in reducing the urinary concentration of aflatoxin adducts, which are associated with liver cancer. However, the side effect profiles of sun sensitivity and finger tip pain (possibly related to capillary vasospasm) are unacceptable for cancer prevention [154]. Sulforaphane and the other two agents have not shown significant toxicity, and will be evaluated based on the hypothesis that modulating the anti-oxidant response element or the transcription factor Nrf2 and the binding protein Keapl to induce particularly NAD(P)H:quinone oxidoreductase or GSH-S-transferase (GST) directly, or by unmasking hypermethylation and epigenetic silencing of GST, may be an effective chemopreventive strategy [64,155].

UAB30 is a retinoid compound that binds to the RXR receptor and has preclinical chemopreventive activity in the mammary gland, but unlike other retinoids it does not cause hypertriglyceridemia in vivo. This agent is being developed as a differentiation-enhancing agent that might provide additional benefit when combined with a selective estrogen response modifier in ER positive breast cancer prevention or with an EGFR1-4 antagonist in ER-progesterone receptor negative breast cancer prevention. SL-11217 is an anti-proliferative that inhibits polyamine formation through ODC. The predecessor drug in this class, DFMO, is in advanced clinical trials but has undesirable auditory side effects. Other examples in the RAPID program include the flavone compound tricin [156]; the selenium compound methylene sulfoxocysteine (MSC), which is the most proximal precursor of the presumed chemopreventive methylenebenzo and which, unlike selenomethionine, does not accumulate in striated muscle [157]; a non-hypercalcemic vitamin D analog [158]; a stable and robust vaccine to HPV 16; and the AKT modulator SR13688 [48]. Thus, numerous compounds with potential chemopreventive activity affecting different pathways in carcinogenesis continue to be nominated by academic investigators for development and clinical testing. An Investigational New Drug (INDs) application to the FDA for phase 1 clinical testing of MSC was recently submitted and INDs for SR13688 and other RAPID compounds are nearing submission.

3. In vitro and in vivo screening

Potential chemopreventive agents are first screened in a battery of short term in vitro and in vivo assays that measure the inhibition or induction of biochemical, molecular, and histological processes thought to be involved in carcinogenesis. The series of assays are continually evolving based on our increasing knowledge of carcinogenesis. The strategy of using screening assays has a number of important advantages. Most screening assays can be conducted within a short time period at a relatively low cost per assay, which not only maximizes the number of agents and agent combinations that can be tested in a single year, but also allows for an initial assessment of biological activity profiles, mechanisms of action, and classification of compounds. In vitro assays provide some initial information regarding the concentrations that might need to be achieved in vivo, organ specificity, and toxicity of potential agents, given the recognition that pharmacokinetic and pharmacodynamic parameters are not assessed. These types of assays may assist both in identifying appropriate animal models for additional in vivo testing and in considering the rationale for further development based on the efficacy-toxicity ratio. Also, comparisons of structure–activity
data and toxicity profiles of candidate agents makes it possible to identify and select optimal agents for additional testing. Other important criteria such as agent cost, commercial availability, chemical properties, such as solubility [159,160], and genotoxicity data are also considered before a promising agent is finally elected for further preclinical investment.

Historically, the screening of chemopreventive compounds [1,2] was organized around the general activity categories of: (1) anti-initiating agents, such as anti-mutagens or blocking agents, including, for example, direct blocking agents, phase I and II enzyme modulators, and anti-oxidants; (2) anti-promotional agents, such as anti-proliferatives, anti-hormones, and anti-inflammatories; and (3) anti-progression agents, such as differentiation enhancing and pro-apoptotic agents. More recently, six essential alterations that cancer cells manifest have been described and these alterations can be used to refine the screening assay categories [161]: self-sufficiency in growth signals, insensitivity to growth-inhibiting signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. Mechanism based in vitro assays and molecular targets can be categorized under these activities, such as receptor transactivation and telomerase inhibition. The next step to in vitro screening assays may provide indications of additional mechanisms of action that can be confirmed by an iterative process with the in vitro assays. Since the alterations in developing cancer cells may occur simultaneously, many of the most promising chemopreventive agents display multiple cancer blocking activities, as discussed above. For example, COX-2 inhibitors with anti-inflammatory activity may also have non-COX-2 molecular targets affecting AKT signaling [162,163] and, by inhibiting PGE$_2$ production, may decrease aromatase activity and estrogen synthesis, affecting proliferation [164]. Genistein is an anti-oxidant but is also an ER modulator and a tyrosine kinase inhibitor possibly affecting focal adhesion and metastasis [165].

Many anti-mutagenic agents possess cancer blocking capabilities and are typically compounds which inhibit carcinogen uptake or binding to DNA, or are effective in preventing the formation or activation of carcinogens. Epidemiological and experimental carcinogenesis studies have found that as many as 90% of all epithelial cancers may be associated with mutagens, mitogens, and the interactions of genes with the environment [166]. In vitro assays which assess anti-mutagenic-related activities include the glutathione induction assay [167], measurement of GST [167], and NADPH:quinone oxidoreductase [168] induction in a human liver cell line. For example, anti-mutagenic agents may work by enhancing electrophile processing via GSH conjugation and tissue-specific oxidative detoxification via induction of phase II enzymes such as GST and NADPH:quinone oxidoreductase [169,170]. Examples of chemopreventive agents of this type include anti-oxidants (e.g. vitamins E and C, genistein, EGCG, resveratrol), compounds upregulating the anti-oxidant response element (dithiols, sulforaphane), and compounds modulating phase I and II enzyme expression (I-3-C, flavones, thiones).

Many sites on deregulated signalling pathways are also possible targets for chemoprevention intervention. For example, deregulated expression of EGFR and its ligands has been associated with the development of neoplasia in both animals and humans [171]. EGFR tyrosine kinase inhibitory activity can be assessed in vitro by measuring EGFR-mediated incorporation of $^{32}$P-ATP into various substrate peptides (e.g. [172,173]). Examples of agents altering EGFR and related HER2/neu signaling are EGCG and genistein as well as erlotinib and the dual EGFR/HER-2 antagonist lapatinib [174]. Another approach to hormonally mediated signalling has been to screen commercially available chemical libraries and selected agents using receptor mediated transactivation or reporter assays.

Other in vitro screening assays have included inhibition of ODC [175,176], a key enzyme in polyamine biosynthesis, which is elevated in some tumour tissues and is believed to play a significant role in cell proliferation and malignant transformation [177]; angiogenesis inhibition [178]; apoptosis induction [179,180]; retinoid RAR and RXR binding and reporter gene assays [181,182]; anti-oxidant potency [183]; and telomerase inhibition [184]. Imaging assays have also been developed using computer assisted image analysis of cellular and nuclear structural changes [185] as well as following protein expression using green fluorescent protein (GFP) tagging [186]. To evaluate some of the earliest changes in carcinogenesis, phenotypically normal human cells isolated from subjects with inherited predispositions to cancer due to mutations in tumour suppressor, oncogenes, and DNA repair genes [187] are being cultured and characterized by genomic and proteomic technologies. Renal epithelial cells isolated from subjects bearing germline mutations in the TSC and von Hippel Lindau genes showed altered expression profiles representing early molecular changes in tumourigenesis that may serve as targets for chemopreventive intervention [188]. In other screening assays, human cell lines from different types of melanoma, the bronchial epithelium, and colon polyps, as well as cells from transgenic mice, have been used to assess biochemical markers associated with transformation. Several of the most well established assays are described below.

The rat tracheal epithelial cell assay (RTE) measures the ability of candidate chemopreventive agents to block the benzo[a]pyrene (B[a]P)-induced transformation of primary RTE cells [189,190]. Compounds are considered positive if they reduce the formation of foci by 20% or
more without producing signs of cytotoxicity. Once test agent solubility and cytotoxicity are determined, tracheal epithelial cells are plated at a uniform density and are incubated with B[a]P in the presence of test compounds for 24 h. After 24 h, the cells are rinsed to remove the carcinogen and are subsequently incubated for an additional 30 days in the presence of test agent. Cells are stained and the number of neoplastic cells and morphologically transformed foci are determined. Inhibition is based on the decrease in foci number in comparison to cells grown with B[a]P alone. Computer assisted image analysis has been incorporated to standardize quantitative endpoints. Additionally, endpoints such as immunohistochemical staining for proliferation, apoptosis, keratins, and other markers are being included.

In the mouse mammary gland organ culture (MMOC) [191,192], which is similar in appearance to the alveolar nodules produced in mouse mammary glands in vivo [193], the inhibitory activity of test compounds on the development of carcinogen-induced hyperplastic alveolar nodules is evaluated. When grown in hormonally defined culture media, MMOC goes through the developmental stages of lobulo-alveolar morphogenesis, differentiation, involution, and glandular regression [194,195]. Following exposure to chemical carcinogens, these organ cultures undergo oncogenic epithelial transformation; transformation is induced by treating MMOC with the carcinogen 7,12-dimethylbenz[a]anthracene (DMBA). The protocol requires excision of thoracic mammary glands from estradiol- and progesterone-primed mice (BALB/c), which are then incubated for 10–12 days in culture media containing prolactin, insulin, aldosterone, and hydrocortisone. After 12 days, glandular involution is induced by insulin, and the culture is incubated for an additional 14 days. Accordingly, two types of protocols can be followed: a complete carcinogenesis protocol, which involves pretreating organ cultures with test compounds prior to DMBA exposure on day 3; or an initiation/promotion protocol in which cultured mammary glands are treated with test agents either prior to, during, or following exposure to DMBA and 12-O-tetradecanoylphorbol-13-acetate (TPA, culture days 9–24). Following incubation, cells are fixed, stained, and scored for transformation and cytotoxicity. Depending on the treatment schedule, this assay can distinguish between anti-initiation and anti-proliferative activities. This assay has been modified to incorporate quantitative image analysis, ERα and ERβ responses, gene array profiling, and other endpoints.

The ductal carcinoma in situ (DCIS) mammary carcinogenesis induction protocol provides valuable toxicity and efficacy data for identifying candidate chemopreventive agents prior to testing in established mammary carcinogenesis models. Accordingly, induction of mammary tumourigenesis is initiated in weanling female Sprague–Dawley rats by intraperitoneal injection of the carcinogen 1-methyl-1-nitrosourea (MNU) [196]. Test agents are generally administered in the diet 1 week after carcinogen administration and continued until termination 45–50 days later. Mammary glands are excised and processed for histopathological analysis and classification. Efficacy is measured as percent reduction in the number of DCIS lesions compared to carcinogen controls.

The rat and mouse colon ACF assay is a short-term model that identifies agents that may be effective in preventing colon cancer. ACF are putative early neoplastic lesions consisting of aggregates of single and multiple crypt cells that exhibit dysplasia and are thought to be the earliest detectable lesions of colon cancer [197,198]. Two different protocols have been developed: to identify compounds that inhibit initiation, and a second treatment schedule which evaluates potential chemopreventive agents during the post-initiation phase of colon carcinogenesis. Details of these regimens have been published previously [199]. In the initiation protocol, male F344 rats are given test agents in the diet 1 week prior to carcinogen (AOM) administration, and continuing throughout the 5-week study period. In the second regimen, rats are first treated with AOM, followed 4 weeks later by test agent, which is given for an additional 4-week period. Animals are sacrificed and ACF frequency determined by histological evaluation.

4. In vivo efficacy and intermediate endpoint testing

The next level of the chemopreventive drug development process is the use of animal efficacy models to establish organ specificity and to generate cancer incidence reduction data. These tests, with the toxicological safety tests, are used for ‘go–no go’ decisions regarding recommendations for clinical development. Numerous models are used to study inhibition of chemical carcinogenesis in mice, rats, and hamsters (Table 1). Important criteria considered in selecting an in vivo model for chemoprevention drug screening include: (1) short study duration and induction of carcinogenesis in less than 6 months; (2) target-specific experimental model evidenced by the production of cancer in the target organs comparable in such factors as histologic type and hormone dependence to that found in humans; and (3) evaluation of in vitro mechanistic activities, efficacy profiles, and relevant published data prior to the selection of models for a given chemopreventive agent. Typically, test agents are administered in the diet unless problems with stability are encountered. The highest dose level that does not cause >10% reduction or gain in body weight, or other gross major organ pathology, over a 6-week period is determined in a range-finding study,
if information about tolerable doses is not available in
the literature. It is recognized that the pharmacokinetic
parameters of dietary and oral administration, which is
most often used clinically, differ. However, the objective
of the efficacy studies is to obtain an indication of effi-
cacy at relatively high dose levels and not to identify
anticipated human dose ranges, which is done in the pre-
clinical toxicology studies. At least 2 dose levels are eval-
uated, typically 0.8 and 0.4 times the highest tolerated
dose from the range-finding study. Treatment schedules
include administration of test agents either before, con-
currently, or following carcinogen exposure. Efficacy is
based upon percent inhibition of tumour incidence or
multiplicity, or increased tumour latency compared to
carcinogen-treated controls. Representative models,
with emphasis on carcinogen-induced models, are dis-
cussed in the following sections.

4.1. Respiratory tract cancer models

Tumours of the head and neck are common and typ-
ically associated with tobacco smoke. Such tumours are
particularly important for clinical cancer prevention
studies because the local recurrence rate following surgical
intervention is high.

The primary lung cancer prevention model used in
the program is the strain A/J mouse model [200]. Strain
A/J mice spontaneously develop lung tumours as early
as 3–4 weeks, with lung tumour incidences approaching
100% at 24 months of age. Different chemical carcino-
gens (e.g. B[a]P, vinyl chloride) can be used to accelerate
carcinogenesis. The tumours resulting from B[a]P are
primarily alveolar adenomas, a tumour type that has in-
creased in incidence with the use of filtered, low tar cig-
arettes that are inhaled deeply. When the animals are
kept on study longer, some of the tumours induced by
vinyl chloride are squamous lesions of the bronchial
tree, more typical of unfiltered, large particulate ciga-
rettes. Squamous cell cancers represent the primary hu-
man cohort for phase 2 studies of chemoprevention in
lung cancer at this time. The strain A/J mouse tumours
do not have mutations in the P53 tumour suppressor
gene, which is altered in a high percentage of human tu-
mours of the upper aerodigestive tract. Recently, models
in mice with germline mutations in p53 or p16 have been
developed for invasive tumours in aerodigestive sites
[201]. These models will allow evaluation of agents in

Table 1
Examples of animal models of carcinogenesis used in DCP NCI studies to evaluate chemopreventive efficacy

<table>
<thead>
<tr>
<th>Target</th>
<th>Species (strain)</th>
<th>Carcinogen</th>
<th>Endpoints (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hamster (Syrian)</td>
<td>MNU</td>
<td>Tracheal adenosquamous carcinoma [203]</td>
</tr>
<tr>
<td></td>
<td>Hamster (Syrian)</td>
<td>DMBA</td>
<td>Squamous cell carcinoma of buccal cavity [204]</td>
</tr>
<tr>
<td></td>
<td>Rat (F344)</td>
<td>4-NQO</td>
<td>Squamous cell carcinoma of the tongue [205,206]</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>Rat (F344)</td>
<td>AOM</td>
<td>Aberrant crypt foci, adenoma, adenocarcinoma [8,207]</td>
</tr>
<tr>
<td></td>
<td>Mouse (Min)</td>
<td>Spontaneous</td>
<td>Small intestinal adenoma [208]</td>
</tr>
<tr>
<td></td>
<td>Mouse (Swiss-Webster; Min)</td>
<td>AOM then Dextran Sulfate; DS only</td>
<td>Ulcerative colitis and adenocarcinoma [211]</td>
</tr>
<tr>
<td></td>
<td>Rat (Sprague-Dawley)</td>
<td>Esophageal duodenal anastomosis</td>
<td>Barrett’s esophagus [212,213]</td>
</tr>
<tr>
<td>Mammary gland</td>
<td>Rat (Sprague-Dawley)</td>
<td>MNU, DMBA</td>
<td>Adenoma, adenocarcinoma, fibroadenoma [214–216]</td>
</tr>
<tr>
<td></td>
<td>Mouse [C3(1)TAG]</td>
<td>Spontaneous</td>
<td>Adenocarcinoma (mostly ER –) [217]</td>
</tr>
<tr>
<td></td>
<td>Mouse [C3(1)TAG]</td>
<td>Oncogene (e.g. neu)</td>
<td>Adenocarcinoma (ER+ or ER – after ovariectomy) [218]</td>
</tr>
<tr>
<td>Prostate gland</td>
<td>Rat (Wistar-Unilever)</td>
<td>MNU + hormones</td>
<td>Adenoma, adenocarcinoma [219]</td>
</tr>
<tr>
<td></td>
<td>Mouse [C3(1)TAG]</td>
<td>Spontaneous</td>
<td>Adenocarcinoma [217]</td>
</tr>
<tr>
<td></td>
<td>Mouse (TRAMP)</td>
<td>Spontaneous</td>
<td>Adenocarcinoma [220]</td>
</tr>
<tr>
<td>Bladder</td>
<td>Mouse (BDF) or Rat (F344)</td>
<td>OH-BBN</td>
<td>Transitional cell carcinoma [221]</td>
</tr>
<tr>
<td>Skin</td>
<td>Mouse (CD1 or SENCAR)</td>
<td>TPA, DMBA</td>
<td>Papilloma, keratoacanthoma, carcinoma [222–224]</td>
</tr>
<tr>
<td></td>
<td>Mouse (SKH-1)</td>
<td>UV</td>
<td>As above [225]</td>
</tr>
<tr>
<td>Cervix</td>
<td>Mouse (K14-HPV16)</td>
<td>Estradiol</td>
<td>Cervical intraepithelial neoplasia and invasive carcinoma [226]</td>
</tr>
<tr>
<td>Ovary</td>
<td>Rat (Lewis)</td>
<td>DMBA</td>
<td>Epithelial carcinoma [227]</td>
</tr>
</tbody>
</table>

Abbreviations: AOM, azoxymethane; B[a]P, benzo[a]pyrene; DMBA, 7,12-dimethylbenz[a]anthracene; ER, estrogen receptor; MNU, 1-methyl-1-
nitrosourea; NNK, N-nitrosonornicotine; 4-NQO, 4-nitroquinoline-1-oxide; OH-BBN, 4-hydroxybutyl (butyl) nitrosamine; TPA, 12-O-tetra-
decanoylphorbol-13-acetate; TRAMP, Transgenic Adenocarcinoma Mouse Prostate; VC, vinyl chloride.
an adenocarcinoma/adenoma model that more closely mirrors the cancers currently seen in humans.

It is also highly efficient to deliver chemopreventive drugs directly to the target site in an unmetabolized, highly concentrated form. This local route of exposure has the added advantage of minimizing systemic toxicity to non-target organs. In the case of pulmonary tissue, one clear option for local drug delivery is inhalation of aerosols containing cancer preventive agents. Certainly, pulmonary epithelia exposure to carcinogens is overwhelmingly due to inhalation of particulates, aerosolized liquids, or gases. Strain A/J mice are induced with carcinogen, and the aerosolized chemopreventive agents are administered by the nose-only inhalation route beginning 1 week after the last carcinogen dose and continuing throughout the experiment (4 months). The aerosolized administration of a number of agents including the corticosteroids budesonide and dexamethasone has been shown to markedly inhibit B[a]P-induced adenoma formation in lungs of the mice [202].

Other models of tracheal, buccal mucosa, and tongue cancers are also evaluated. The carcinogen MNU when administered by intratracheal instillation will induce tracheal cancers in the Syrian Golden hamster. These can-becancers are histologically similar to adenosquamous cell carcinomas in the [203]. Chemopreventive test agents are given prior to carcinogen exposure. In the hamster buccal pouch model, the carcinogen (e.g. DMBA or a nitrosamine) is administered over a 15-week period, resulting in buccal pouch squamous cell carcinomas in approximately 40–50% of treated animals after 6 months [204]. Oral lesions in rats induced by 4-nitroquinoline-1-oxide (4-NQO) are similar to human oral lesions since many are ulcerated and endophytic tongue lesions [205]. These tumours are primarily squamous cell carcinomas [206]. Male F344 rats at 7–8 weeks of age are given NQO at 20 ppm in drinking water for 10 weeks. Test agents are typically administered in the diet from 1 day or 6–8 weeks after the completion of 4-NQO exposure, and the rats are followed for 26 weeks after the first day of carcinogen exposure. In another schedule the chemopreventive agent is administered from 1 week before the first carcinogen dose until the end of the study.

4.2. Mouse and rat gastrointestinal tract (colon, esophagus) models

Potential inhibitors of colon carcinogenesis can be assessed utilizing models in both rat and mouse species [8,207]. The primary colon cancer prevention model used by DCP is the AOM induction model. Two subcutaneous doses of AOM, administered 1 week apart, to F344 rats results in the formation of colon adenocarcinomas and adenomas in approximately 70% of treated animals by 40 weeks. Potential chemopreventive agents can be administered before, during, or following carcinogen treatment.

Transgenic and gene knockout mice that carry well-characterized genetic lesions predisposing to carcinogenesis are also appropriate models for chemoprevention testing. One of the best developed models is the Min mouse [208] and other strains possessing lesions in the APC gene [209]. As described above, the Min mouse carries an Apc mutation similar to that found in human FAP patients. However, the mice are predisposed to develop predominantly intestinal adenomas and carcinomas rather than colorectal polyps and carcinomas. By manipulating 2 or more carcinogenesis-associated genes, such as modifier genes in a single animal model, closer approximations of human carcinogenesis may be possible. For example, it is feasible to knock out p53 in an animal that already carries another tumour suppressor defect such as mutant Apc or p16. Studies of Min and Apc1638 mice, also carrying genes with defective DNA repair mechanisms, are ongoing.

Individuals with ulcerative colitis are at high risk for developing colorectal cancer. The efficacy of agents in inhibiting colitis-associated colorectal cancer can be studied in mouse models that mimic the human disease. In the Min/DSS mouse model of induced colitis, Min mice are treated with cycles of dextran sulfate sodium (DSS) which induce cycles of ulceration/necrosis/regeneration [210]. In a second model, Swiss Webster mice are treated with an intraperitoneal injection of AOM with cycles of DSS [211]. Animals receive chemopreventive agents in the diet from 1 week prior to carcinogen and throughout the experiment, excluding periods of DSS treatment; DSS is administered in the drinking water during weeks 1, 4, 7 and 10.

Adenocarcinoma of the esophagus is frequently preceded by Barrett’s esophagus, a metaplastic lesion that is associated with gastroesophageal reflux. Recently, a model for esophageal adenocarcinoma has been developed in rats to mirror the human disease [212]. Cancers are generated by performing an esophageal duodenal anastomosis [212,213] resulting in esophageal reflux followed by treatment with iron dextran (50 mg/kg-body weight, intaperitoneally) 2 weeks after surgery and once per month thereafter. Chemopreventive agents are then evaluated after wound healing.

4.3. Rat mammary gland models

Chemopreventive efficacy against mammary gland carcinogenesis is routinely assessed by either the MNU or DMBA induction models [214,215]. Female Sprague–Dawley rats are administered the carcinogen as a single dose at 50 days of age. In some instances, the carcinogen is administered to older animals (180 days)
which is more representative of the human adult target population [216]. Tumour incidences at 120 days post-carcinogen are similar, ranging from 80% to 100% in the DMBA protocol, and 75% to 95% in the MNU model; however, the types of tumours produced by the two carcinogens differ. DMBA-induced mammary tumours are predominantly adenomas and fibroadenomas, with some adenocarcinoma development, whereas MNU-induced mammary tumours are invasive adenocarcinomas. Chemopreventive activity is based on the percent reduction in tumour incidence or percent increase in tumour latency relative to carcinogen-treated controls. These models produce hormonally responsive tumours.

Most transgenic mouse models involve the overexpression or altered expression of one or more oncogenes; one of the most common being the transforming antigens of SV40 or polyoma. With the possible exception of HPV, transformation by oncogenic viruses is probably not involved in carcinogenesis in most human epithelia. However, the transforming proteins of SV40, HPV, and some of the transforming adenoviruses bind to and cause the dysfunction of two major tumour suppressor proteins (P53 and RB), which are involved in a wide range of human tumours. Green and colleagues [217] have developed the C3(1)TAG transgenic mouse line in which the SV 40 T antigen has been combined with a hormonally responsive promotor. The specific regulatory control element is derived from the rat prostatic steroid binding protein. The resulting animals express T antigens in a variety of tissues including prostate, mammary gland and salivary tissue. Female mice show nearly 100% mammary tumour incidence by 6 months and demonstrate multifocality at later time points. These adenocarcinomas are predominantly ER negative. Another approach under investigation, that produces ER+/− cancers, if ovariectomy is performed, is to administer by intraductal lavage a retrovirus with the inserted neu gene (EGFR2, HER2, erbB2) [218].

4.4. Prostate cancer models

The rodent prostate cancer induction model using the Wistar HsdCpd:wu strain has provided a reliable assay for the evaluation of potential prostate chemopreventive agents [219]. Male Wistar–Unilever (HsdCpb:wu) rats are pretreated with daily intragastric doses of cyproterone acetate (CA) for 21 days. Starting 1 day after the last CA treatment, animals receive daily subcutaneous injections of testosterone propionate (TP) for three consecutive days. A single dose of MNU is administered 60 h after the first TP injection. Crystalline testosterone pellets are implanted subcutaneously 2 weeks after MNU dosing and every 6 months thereafter. Chemopreventive agents are administered in the diet for 12 or 24 weeks beginning 32 or 44 weeks after MNU administration. The model produces adenomas and adenocarcinomas that do not metastasize. While reliable, the induction period is long and large amounts of chemopreventive agent are required.

In the Green SV 40 T antigen model, cited above under mammary models, prostate tumours develop in approximately 50% of male mice by 8 months of age. In addition, virtually 100% of these male mice display dysplastic lesions in the prostate by 3–4 months of age. Using this model, the efficacy of chemopreventive agents on the development of prostate intraepithelial neoplasia (PIN) lesion within the dorsolateral prostate is being studied. Another genetically engineered model is the Transgenic Adenocarcinoma Mouse Prostate (TRAMP) model which develops spontaneous metaplastic prostate carcinomas [220]. TRAMP mice express a probasin-Tag transgene and develop spontaneous multistage prostate carcinogenesis, which resembles human prostate cancer progression. Males express this transgene by 8 weeks of age and develop abnormal pathology in the dorsolateral prostate by 12 weeks of age. By 28 weeks 100% of the animals have acquired prostate cancer. These carcinomas progress through PIN forms and thus mimic the human carcinogenesis process. Additionally, these tumours metastasize to the lung, lymph nodes, liver and bone. Potential intermediate biomarkers of prostate carcinogenesis as well as tumour incidence reduction by chemopreventive agents have been evaluated in this model. Other genetically engineered models involving perturbations, for example, in the IGF receptor or tumour suppressor gene p27kip1 are being evaluated. Often, the practical difficulty in utilizing such genetically engineered models is that adequate numbers of animals cannot be simultaneously bred and genotyped in order to obtain adequate group sizes.

4.5. Bladder cancer models

Bladder tumours are typically induced by the carcinogen OH-BBN, which causes development of invasive transitional cell carcinomas (TCC) that are morphologically similar to a type of TCC found in humans [221]. The carcinogen is given intragastrically over a 8-week period to 50-day old BDF mice (C57BL/6 x DBS/2-F1) or F344 rats, resulting in a 40–50% bladder tumour incidence at 180 days post-treatment. Treatment schedules for agent administration vary but emphasis is on chemopreventive intervention later in carcinogenesis to model the clinical presentation of early stage and grade bladder cancer.

4.6. Mouse skin carcinogenesis

Agents effective in inhibiting skin carcinogenesis are identified in a two-stage skin carcinogenesis protocol utilizing DMBA and TPA, which are applied topically
to the back skin of SENCAR or CD-1 mice [222,223]. Both strains of mice are highly susceptible to skin tumour induction. Skin papillomas appear as early as 6 weeks post-carcinogen treatment, eventually progressing to squamous cell carcinomas by 18 weeks [224]. Test agents are generally administered in the diet, or in some experiments they are topically applied according to several predefined treatment regimens.

Ultraviolet (UV) radiation is a ubiquitous, continuous source of carcinogenic exposure in humans; skin cancers have been attributed to solar radiation in the UVA and UVB range of 290–400 nm. The SKH-1 mouse is an excellent model for the study of UV-induced skin carcinogenesis. While UV radiation can penetrate to the dermis, it is the epidermis that typically serves as the ultimate target for UV-induced carcinogenesis in humans and mice. The SKH-1 mouse is a hairless albino stock as a consequence of a retroviral insertion on the ‘haired’ locus of chromosome 14 [225]. Unlike nude (athymic) mice, SKH-1 mice possess normal immune function. In this stock, UV radiation produces tumours that are entirely epidermal in origin. Using this model, the ability of chemopreventive agents to regress established preinvasive (intraepithelial) neoplasia induced by UVB in SKH-1 hairless mice is studied. Female SKH-1 mice (age 7–8 weeks) are exposed daily to UVA at 8.5 J/cm²/day and UVB at 18 mJ/cm²/day 5x/wk. The UVB dose is increased over 10 weeks to 160 mJ/cm²/day and this dose is maintained at the end of the study at 20 weeks. Chemopreventive agents are administered from weeks 16 to 20. The lesions are papillomas that would progress to squamous cell cancers if the animals were maintained longer. In vitro model systems for basal cell and melanoma skin cancers are currently being evaluated.

4.7. Cervix and ovary models

HPV infection is associated particularly with cervical cancer. Viral proteins E6 and E7 bind to major tumour suppressor gene products (P53 and RB). The Keratin14-HPV16 transgenic mice spontaneously develop epidermal carcinogenesis that progresses through stages of hyperplasia, dysplasia, complex premalignant precursors, and invasive squamous cancers [226] and are currently being used to test efficacy of chemopreventive agents.

A rat ovarian tumour model employing DMBA-coated sutures is also being utilized [227]. In this model, roughly 50% of rats develop relatively undifferentiated ovarian cancers that appear to be of epithelial cell origin. Tumours are induced by implantation of carcinoma-impregnated sutures into the left ovary of rats. The domestic fowl has also been used as a model of human ovarian cancer; the most relevant feature of the chicken is its high incidence of spontaneous ovarian cancer, which ranges from 11% to 35% at 4–6 years of age [228]. Since chickens ovulate daily and no carcinogens are used, the possibility that the pathogenesis of both chicken and human ovarian cancers is related to ovulation-induced genetic damage of ovarian epithelial cells can be investigated. Also, chicken ovarian cancers have genetic alterations similar to those identified in human ovarian cancer. The chicken model is not practical, however, due to duration, costs, experimental group sizes to achieve power (2000 chickens), limited ability to control dose, and large amounts of test agent required.

4.8. Intermediate endpoints

Intermediate biomarkers for carcinogenesis are also investigated as part of the animal efficacy testing protocols. The development of molecular progression models over the last decade has identified intermediate biomarkers that might serve as surrogate end points for cancer incidence reduction in clinical trials. Additionally, new technologies have been implemented that offer opportunities to detect and monitor changes associated with carcinogenesis and intervention. Because of the shorter intervention times needed to demonstrate modulation of intermediate biomarker end points and the smaller cohorts required for both preclinical and clinical chemoprevention studies, the identification and development of intermediate biomarkers are important for timely and cost effective progress in cancer prevention. Most obviously, as an example of current projects in this area, genomic array technology has been used to survey broadly genes that might be modulated in samples from animal efficacy studies both as part of the carcinogenic process and in response to drug treatments. Additionally, focused arrays of a limited number of genes have been performed using gene array and RT-PCR in early stage cancers and after chemopreventive interventions across the animal efficacy models, examples include: COX-1 and COX-2, ODC, EGFR1 and EGFR2 (new), 5-lipoxygenase, ERα and ERβ, RXR and RAR isoforms, peroxisome proliferator activated receptor (PPAR) δ, vitamin D and androgen receptor, PI3K, c-kit, P16, P27, survivin, FHIT, cyclin D1, cyclin E, proliferating cell nuclear antigen (PCNA). Another current example is to develop further, using immunohistochemical and fluorescence activated cell (FAC) sorting technologies, the observation that ablation of proliferating mammary ductal epithelial cells results in an increased sensitivity to MNU, suggesting that mammary stem and early progenitor cells are more highly susceptible to carcinogenesis than the differentiated cells [229]. Expression of the Wnt-1 proto-oncogene in the mammary gland of transgenic mice leads to expansion of a population of epithelial cells expressing stem cell antigen Sca-1 [230] and more than 50% of the cells in mammary tumours arising in these mice are Sca-1 positive. In this investigation, using the DMBA mammary tumour model, a transgenic mouse expressing the green fluorescent protein...
protein (GFP) reporter gene under the control of the 14 kb genomic stem cell antigen (Sca-1/Ly-6A) cassette is being used [231,232]; Sca-1 directs expression of GFP to all adult stem cell populations, including those in the mammary gland [233]. The objective is to determine if chemopreventive agents have selective effects on specific progenitor cell populations, and hence have efficacy for selectively intervening in or influencing the formation of specific tumour lineages. Intermediate biomarkers, such as Sca-1 and cytokeratins, and FAC sorting are being used to identify progenitor and differentiated ductal epithelial, myoepithelial, and squamous cell lineages in the tumours. A final current example is the evaluation of the technique of interferometry-based light scattering spectrophotometry to quantify selectively in situ the nuclear morphometry of basal cell nuclei, excluding the suprabasal layers, in the rat model of esophageal carcinogenesis, as biomarkers for detecting and characterizing neoplastic change in response to chemopreventive interventions [234].

5. Preclinical pharmacology and toxicology

Potential chemopreventive agents must have a risk:benefit ratio that is appropriate for potential users. Individuals with dominantly heritable cancer syndromes, for example, may tolerate a different (higher) ratio than a broader population, such as individuals who have had a non-cancerous colon polyp removed. Safety is a paramount issue in the development of chemopreventive agents and is distinctly different from therapeutic drugs used in cancer treatment. As with all investigational new drugs, a standard battery of tests is required by the Food and Drug Administration (FDA), USA in order to consider a request for an Investigational New Drug (IND) Application. The studies considered by the FDA for chemopreventive agent development are generally the same as for other drugs [235], but might be considered analogous to those currently in developing agents for cardiovascular disease prevention.

The initial battery typically includes genotoxicity testing, toxicity evaluations in two species after at least 28 days of agent exposure, pharmacokinetic measurements, and reproductive performance studies. Genotoxicity testing is constituted by: (1) gene mutation studies in Salmonella typhimurium; (2) evaluation of genetic mutations in mammalian cells (either L5178Y mouse TK+/− lymphoma or Chinese hamster AS52 ovary cells); and (3) in vivo assessment of cytogenetic damage by either the mouse bone marrow micronucleus assay and/or mouse or rat chromosomal aberration tests. In those instances where a positive test for genotoxicity occurs, then either compound development is stopped or additional in vivo tests, such as the 6-month carcinogenicity study in P53+/− mice, are performed after consultation with the FDA. Assessments such as the COMET assay for DNA strand breaks or fluorescent in situ hybridization (FISH) for chromosomal aberrations have been incorporated into early clinical studies in some instances [96]. Toxicity evaluations in different species, typically rodent and dog, are used to estimate no-observed-adverse-effect (NOAEL) and maximum tolerated dose (MTD) levels which, in turn, are used for initial dose setting in humans. Absorption, distribution, metabolism, and elimination studies are valuable to establish the methodology for plasma drug level measurements that will be used later clinically and to define and connect the dose-response relationships of toxicity and efficacy from in vitro and prior in vivo testing. The data is also used to identify the most appropriate species for subsequent safety testing. The initial tests related to reproduction are teratology assessments. Additionally, in vitro studies using human hepatocytes are conducted to evaluate metabolic profiles of potential agents and potential drug interactions due to cytochromes P450 or phase II enzyme modulation. Such studies may also be conducted in vivo in rodents. Extensive additional safety and specialized studies to address immunological, neurological, or mechanistic toxicology are performed as needed and to support longer durations of clinical exposure as trials progress to phase 2 and 3 stages.

6. Chemistry and manufacturing

The cost of producing the active pharmaceutical ingredient (API) often becomes rate limiting. Even compounds with high potency, therefore requiring lower dosages, may not be feasible to scale-up if the synthetic pathway is complex, such as for some steroid, flavonoid, and vitamin D compounds. Tens of kilograms of a compound that has minimal toxicity may be required to complete toxicity studies in dogs. The early standardization of production processes is critical to protect early investments in expensive preclinical development studies and to assure that the studies will support clinical testing. Assurance of product identity, purity, shelf-life stability and acceptance criteria for batch-to-batch manufacturing and for residual solvents, microbial, pesticide, and metal contaminants are required to document acceptability and to develop the profile required for Good Manufacturing Practices compliance. Standardization of production, product identity, and acceptance criteria for complex mixtures derived from natural sources can be especially challenging. Among the applications to the RAPID program, this aspect of chemopreventive agent development is the most needed by academic investigators wishing to bring products to initial clinical testing.
7. Phase 1 and 2 clinical studies

Phase 1 studies of chemopreventive agents typically have safety as the primary endpoint and pharmacokinetics secondarily. INDs are usually opened with protocols for escalating, single dose safety and pharmacokinetic assessments in, for example, three groups of six normal volunteers or in higher risk subjects, depending upon the preclinical agent safety profile. Dose ranges are based on preclinical toxicity studies and are initially a fraction of the NOAEL in the most sensitive species. Stopping rules within grade 2 adverse events are incorporated into cancer prevention protocols. Some investigators choose to include placebo treatments within dose groups and to include pharmacodynamic assessments. The second phase 1 study is typically an escalating, repeat dose study in similar group sizes with the primary endpoint again of safety and the secondary endpoint of pharmacokinetics after repeated administration to assess potential changes in metabolism. The potential for drug–drug interaction is evaluated using substrates for P450 enzymes before and during treatment with chemopreventives. The effect of fasting on absorption may also be assessed. Phase 1 clinical studies sponsored by the DCP NCI and currently open to accrual are listed in Table 2. As noted above, several other agents under development in the RAPID program are nearly ready for phase 1 studies. Phase 1 studies currently also include proteomic and genomic evaluations in vitro using phenotypically normal cells isolated from individuals with inherited cancer predispositions [188] in order to identify potential molecular targets for drug development in the earliest stages of cancer.

Phase 2 studies may start at the MTD identified in phase 1 studies and are designed to identify the lowest dose effectively modulating intermediate endpoints of cancer and other pharmacodynamic chemopreventive agent effects. These randomized, placebo controlled, blinded study designs vary across organ sites. Typically, these protocols involve ≤200 subjects. In many instances, this is the entry level for chemopreventive evaluation of agents being provided by the pharmaceutical industry that have prior clinical evaluations, such as the COX-2 or EGFR inhibitors. The details of this program area are too many to review here. There are currently >40 active protocols and examples of agents in phase 2 testing are listed in Table 3.

Table 2
Phase 1 chemoprevention clinical studies sponsored by DCP NCI currently open to accrual

<table>
<thead>
<tr>
<th>Agent</th>
<th>Endpoints</th>
<th>Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCX4016 (nitroxyphenylaspirin)</td>
<td>Safety, pharmacokinetics, aberrant crypt foci modulation (ACF)</td>
<td>Subjects w/prior colon cancer or polyps</td>
</tr>
<tr>
<td>3,3’-Diindolylmethane (DIM)</td>
<td>Safety, pharmacokinetics, CYP 450 induction</td>
<td>Healthy volunteers</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>Modulation of aromatase activity in mammary tissue</td>
<td>Breast cancer patients</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Safety, pharmacokinetics, biomarker modulation in colon tissue</td>
<td>Healthy volunteers, colon cancer patients</td>
</tr>
<tr>
<td>Polyphenon E (epigallocatechin gallate and other green tea catechins)</td>
<td>Safety, pharmacokinetics, CYP 450 induction; modulation of EGFR phosphorylation cascade</td>
<td>Healthy volunteers; patients w/esophageal hyperplasia</td>
</tr>
<tr>
<td>Rapamycin (topical)</td>
<td>Safety, genomic/proteomic profiles in phenotypically normal skin</td>
<td>Basal cell nevus syndrome patients</td>
</tr>
<tr>
<td>Isoflavones (genistein, daidzein, glycitein)</td>
<td>Safety, pharmacokinetics, biomarker modulation in cervix and lymphocytes</td>
<td>Healthy volunteers, postmenopausal women, prostate cancer patients</td>
</tr>
<tr>
<td>Lycopene</td>
<td>Safety, pharmacokinetics, biomarker modulation in prostate tissue</td>
<td>Healthy volunteers, men undergoing prostate biopsy</td>
</tr>
<tr>
<td>Bowman-Birk Inhibitor Concentrate</td>
<td>Safety, pharmacokinetics, biomarker modulation in prostate tissue</td>
<td>Healthy volunteers, men undergoing prostate biopsy</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Safety, pharmacokinetics, ACF modulation</td>
<td>Smokers with prevalent ACF</td>
</tr>
<tr>
<td>Se-Methyl-Seleno-L-Cysteine</td>
<td>Safety, pharmacokinetics, biomarker modulation in prostate tissue</td>
<td>Healthy volunteers, men undergoing prostate biopsy</td>
</tr>
<tr>
<td>Folate</td>
<td>Safety, biomarker modulation in colon tissue and lymphocytes</td>
<td>Subjects at risk for colon cancer</td>
</tr>
</tbody>
</table>

Table 3
Examples of agents in phase 2 chemoprevention clinical studies sponsored by DCP NCI and currently open to accrual

<table>
<thead>
<tr>
<th>Agent</th>
<th>Endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celecoxib</td>
<td>Tamoxifen, raloxifene, doxercalciroferol</td>
</tr>
<tr>
<td>Isoflavone mixture G-2535</td>
<td>Toremifene</td>
</tr>
<tr>
<td>HSP E7 Vaccine</td>
<td>Atorvastatin, lovastatin</td>
</tr>
<tr>
<td>Rosiglitazone, pioglitazone</td>
<td>Zileuton</td>
</tr>
<tr>
<td>T4 Endonuclease V</td>
<td>Erlotinib</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Imiquimod</td>
</tr>
<tr>
<td>Aminolevulinic acid</td>
<td>S-Adenosylmethionine</td>
</tr>
<tr>
<td>S-Adenosylmethionine</td>
<td>Anetholetrihione</td>
</tr>
</tbody>
</table>
8. Summary and conclusions

Chemoprevention is an innovative area of cancer research that focuses on the prevention of cancer through pharmacologic, biologic, and nutritional interventions. Over the past two decades the DCP within the US NCI has organized a research and development program to provide resources and infrastructure to the research community for the clinical development of chemopreventive agents and this effort continues to identify and develop new opportunities in prevention. With our increasing understanding of the molecular processes involved in carcinogenesis, we will hopefully come intervention modalities to prevent, reverse, or delay invasive cancer. While the paradigm of developing effective tertiary interventions [236] and moving them into primary prevention settings [237] will continue to prove effective in high risk cohorts, the recommendation of interventions to the general population will require increasing emphasis on safety and cost [13,14,238,239]. New approaches in cancer chemoprevention will continue to mature and include the administration of multiple agents, with additive efficacy against different molecular targets or sets of targets within the carcinogenic process, and with low toxicity; the development of aerosol and topical products to limit systemic exposure; the development of vaccines against infectious, carcinogenic pathogens; the development of epigenetic approaches to prevention, such as reversing gene methylation silencing; and the evaluation of new technologies for quantitative imaging and early detection. Continued commitment to cancer prevention will significantly reduce the economic and medical burden of cancer.

Conflict of interest statement

None declared.

References


