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Review

Colorectal cancer chemoprevention: biochemical targets and clinical development of promising agents

R.A. Sharma [a,*], M.M. Manson [b], A. Gescher [b], W.P. Steward [a]

[a] *University Department of Oncology, Leicester Royal Infirmary, Leicester LE1 5WW, UK*
[b] *MRC Toxicology Unit, Lancaster Road, Leicester LE1 9HN, UK*

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Abstract

Colorectal cancer (CRC) remains a cause of significant mortality in developed countries despite extensive knowledge of its epidemiology and molecular basis. Since multiple molecular steps that collectively bring about this disease are known, its chemoprevention is a realistic proposition. Biochemical targets of CRC chemopreventive agents include carcinogen metabolising enzymes, arachidonic acid metabolism, the transcription factor nuclear factor-kappa beta (NF-κB), enzymes responsible for polyamine metabolism, and events associated with proliferation and apoptosis of preneoplastic cells. Aspirin, celecoxib, calcium and α-difluoromethylornithine are examples of drugs that have undergone clinical testing. Critical evaluation of these trials allows optimisation of methodologies for clinical advancement of novel chemopreventive agents. Cancer patients can be a suitable cohort of subjects for pilot studies of certain new agents. Such studies and larger trials in high-risk healthy individuals require the stringent use of carefully validated 'preneoplastic' biomarkers which are intrinsically related to defined stages of colorectal carcinogenesis and/or to mechanisms of action of the agent under investigation.

Keywords: Biomarker; Carcinogenesis; Clinical trial; Colorectal cancer; Chemoprevention

1. Introduction

The concept of chemoprevention is not new. For many decades, chemicals have been used to prevent dental caries, heart attacks and stroke. Similarly, many individuals modify their diets and lifestyles in an attempt to prevent or delay the onset of disease. Although the history of cancer chemoprevention is relatively brief, many studies have been reported and a few efficacious agents have been identified. The most obvious example is tamoxifen, which can now be considered an established agent in the prevention of breast cancer [1]. Furthermore the vitamin A analogues isotretinoin and retinol palmitate have been demonstrated to prevent second primary cancers in patients with malignancies of the lung and head/neck [2,3]. There is little doubt that the identification of novel chemopreventive agents with efficacy against the major human cancers could have an enormously beneficial impact on the public health of Western societies.

This review, which is targeted at a readership concerned primarily with treatment rather than prevention of cancer, will initially attempt to define the parameters which determine whether a chemoprevention study will be useful or not. Therewith, biochemical events associated with colorectal carcinogenesis will be described as potential targets for chemopreventive agents. The final aim is to summarise recent clinical chemoprevention trials of novel agents.

Colorectal cancer (CRC) is the second most common malignancy in developed countries, accounting for approximately 20–30 deaths per 100 000 standard population each year [4]. Although recent years have seen marginal improvements in mortality for white US citizens [4], the prognosis remains poor; similar to that described in 1932 by Dukes [5] and in 1954 by Astler and Coller [6]. The 5-year survival rate for a patient presenting with lymph node involvement (Dukes' C) is approximately 39% following surgery [7]. Although 80% of patients undergoing surgical resection have complete macroscopic clearance of their disease, 50%

* Corresponding author. Tel.: +44-116-258-7597; fax: +44-116-258-7599.
E-mail address: ras20@le.ac.uk (R.A. Sharma).

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suffer recurrence [8]. Moreover, approximately 25% of patients have advanced disease at presentation [4], when palliative management strategies remain the only option.

The epidemiology and molecular biology underlying CRC have been studied more thoroughly and are better understood than for most other neoplasias. Clinical and molecular evidence suggests that there are several somatic pathways to CRC, and that two of these pathways appear to parallel certain well-defined processes described for inherited forms of the disease [9]. The incidence of CRC has increased rapidly in the latter part of the twentieth century in many countries, including Italy, Japan and urban China [10]. Such changes have occurred within one generation and suggest an important role for environmental influences in the aetiology of this disease. Epidemiological studies have also pinpointed environmental factors, especially dietary, which appear to affect individuals' chances of developing CRC [11]. Diets low in vegetables and folate, and high in fat, red meat and alcohol appear to increase relative risk. Lack of exercise and cigarette smoking are also risk factors. However, the relative risk ratio for each individual factor appears small when compared with factors linked to other cancers, such as smoking which increases the risk of lung cancer almost 20-fold. Red meat intake, for example, may at most double the risk for CRC [11]. Prospective cohort studies have confirmed certain links, such as the protective role of high-dose folate [12], but have failed to substantiate others such as the benefits of dietary fibre [13]. Large prospective studies of dietary risk factors are ongoing, such as the European Prospective Investigation of Cancer, in which data from over 400 000 individuals are being evaluated [14].

Intense research efforts are aimed at integration of our knowledge of molecular pathways with that of the epidemiology of CRC. Integration may ultimately allow the generation of 'risk profiles' for individuals, based on knowledge of family history, lifestyle, dietary factors, genetic analysis and risk/preneoplastic biomarkers (see below). Such profiles may play a significant role in the selection of subjects for clinical chemoprevention trials. Improved genetic screening for inherited disease traits and an increasing number of pilot screening programmes for sporadic cancer in normal populations [15] are likely to identify increasing numbers of individuals with early stages of colon carcinogenesis. Thus the need for intervention strategies in high-risk individuals and those with preneoplastic lesions will undoubtedly increase.

Cancer chemoprevention can be defined as the inhibition, retardation or reversal of carcinogenic processes by chemical means [16]. Most clinical trials focus on the prevention of carcinogenesis at a premalignant stage or an early phase of malignancy, which is termed 'primary' or 'secondary' chemoprevention, depending on whether it involves normal or high-risk individuals. The treatment with chemopreventive agents of patients who have undergone successful therapy of a primary malignancy but are at increased risk of a second malignancy, is referred to as 'tertiary' chemoprevention [17]. It is notable that the original definition of the term chemoprevention also embraces inhibition of growth and delay of progression of cancers [18].

2. Features of clinical trials

Clinical trials of cancer chemotherapeutic agents have traditionally been divided into three phases; phase I studies to assess potential toxicity and pharmacokinetics; phase II studies to assess potential benefit; and phase III studies to compare the efficacy and toxicity of the new treatment with current therapeutic regimes. The clinical evaluation of agents for chemopreventive properties can also be separated into three phases, albeit with objectives which differ significantly from those in chemotherapy trials. Table 1 summarises salient features of the design of contemporary chemoprevention trials. Unique challenges posed by these studies include trial duration, agent safety and number of subjects enrolled. Although the initiation phase of carcinogenesis may last only hours or days, promotion and progression

Table 1
Basic generalised design for chemoprevention trials (modified from [19])

Phase	Number of subjects	Design	Primary endpoint
I	15–30	Small, short-term pilot study in cancer patients with rapid dose escalation if no toxicity observed	1. Pharmacokinetics 2. Preliminary data on preneoplastic biomarkers
IIa	30–60	Small, short-term dose de-escalation study	Sensitivity and response of preneoplastic biomarkers
IIb	500–1500	Larger, medium-term randomised study in high-risk individuals	Response in definitive preneoplastic biomarkers
III	Several thousand	Large, long-term randomised study with low- and high-risk levels	1. Response in definitive preneoplastic biomarkers 2. Cancer incidence and mortality

of tumours probably take years, if not decades. Potentially efficacious cancer chemopreventive agents are therefore likely to be administered regularly to healthy individuals who will gain no visible benefit for many years. It is, therefore, important that there be a minimal risk of toxicity. Any adverse effect must be carefully considered in the risk/benefit estimation for the subject population chosen. Moreover, the number of individuals in chemoprevention efficacy trials is generally substantially greater than the number of patients in most chemotherapeutic trials in order to allow observations to reach significance levels.

3. Choice of subjects

Thousands of years ago, Hippocrates and Galen cautioned against the treatment of hidden cancers, arguing that treatment more often than not hastened death [20]. The ethical dilemmas of treating healthy or 'at risk' individuals were highlighted recently by two major lung cancer intervention trials, the α-tocopherol, β-carotene study [21], and the β-carotene and retinol efficacy study [22]. The rationale for the studies was the finding that eating fruit and vegetables is associated with consistently elevated levels of β-carotene and a lower incidence of cancer. However, the results of the trials suggested that β-carotene not only failed to protect against lung cancer, in high-risk groups of smokers and/or workers occupationally exposed to asbestos, it even increased the risk of developing the disease. Several criticisms have been levelled at the study [23], and consideration of these criticisms may be useful in optimising the design of future CRC chemoprevention studies. Especially noteworthy among the criticisms are the lack of a rigorous scientifically based mechanistic rationale for the trial and the absence of adequate pharmacokinetic/dynamic information to decide proper dose and scheduling. The importance of detailed mechanistic studies, as a prelude to clinical evaluation, in order to minimise the occurrence of unexpected adverse effects was highlighted recently by the suggestion that β-carotene boosts the detrimental effects of cigarette smoke carcinogens by upregulating carcinogen-metabolising enzyme activities [24]. Furthermore, it is conceivable that the fruit or vegetable constituent which is responsible for chemopreventive efficacy is not β-carotene, but a different agent such as its isomer α-carotene [23].

It is also clearly desirable to obtain information on pharmacokinetics and potential toxicity in humans before administering chemopreventive agents over long periods of time to healthy volunteers. Some of the agents under consideration in cancer chemoprevention inhibit steps in the late stages of carcinogenesis and have chemotherapeutic activity in clinical trials. This notion is certainly true for tamoxifen in breast cancer prevention and treatment, and there is evidence in preclinical models and case reports to suggest similar activity for putative CRC chemopreventive agents such as curcumin, an active constituent of the spice turmeric [25,26]. Therefore it may be justifiable to conduct small pilot studies of certain chemopreventive agents in patients with cancer, with measurement of radiological/chemical tumour markers as well as preneoplastic biomarkers (see below) and pharmacokinetics.

4. Preneoplastic biomarkers

Biomarkers are increasingly used in programmes of screening, chemoprevention and chemotherapy, and there is some degree of confusion in the literature regarding their classification. 'Surrogate endpoint biomarkers' (SEBs) have been identified for several different stages in the carcinogenic process, and represent a means of monitoring disease progression without having to wait for true neoplasia and metaplasia to develop. SEBs can be termed 'preneoplastic biomarkers', and should be distinguished from 'risk biomarkers' and 'tumour markers'. In Table 2 these three basic marker types are defined and their uses outlined. Of these three types, preneoplastic biomarkers are the most useful for assessing the efficacy of cancer chemopreventive agents [27,28].

Carcinoma incidence is the ultimate endpoint of cancer chemopreventive intervention. However, if this acts

Table 2
Types of biomarkers (modified from [27])

Type of biomarker	Stage of disease	Description	Application
Risk biomarker	No disease detectable	Genetic predisposition, past medical history, lifestyle factors, exposure	Useful to molecular epidemiologists to assess risk of developing cancer
Preneoplastic biomarker	Initiation, promotion, progression	Biological alterations representing the early and intermediate stages of carcinogenesis, which may show alterations by chemopreventive agents	Identification of carcinogenesis and chemoprevention
Tumour markers	Established neoplasia, adhesion–migration	Elevated levels associated with particular cancers	Diagnosis and treatment

as the only endpoint, clinical trials are beset by practical difficulties, most notably the prolonged time periods necessary to obtain results. For example, the delay before preventive effects are seen may be 9 years for aspirin [29] and as long as 15 years for high-dose folate [12]. Therefore, sensitive and specific biomarkers are required that accurately reflect the development of malignancy, and may be differentially expressed during the multiple stages of carcinogenesis. In Table 3 some preneoplastic biomarkers of potential use in CRC chemoprevention studies are shown. The frequency of measurement of biomarkers depends on factors such as the accessibility of the biological material required and the potential risk involved in making the measurement. In cardiovascular chemoprevention for example, clinical measurement of arterial blood pressure and laboratory measurement of blood cholesterol are well established as valid predictive markers upon which intervention can be based. Fortunately, in the case of CRC the target organ is anatomically accessible with a relatively small risk from biopsy collection. But it could be desirable to correlate preneoplastic biomarker levels in the target organ with those in peripheral blood. An example of such a correlation has been provided recently in a pilot study [31] preceding a chemoprevention study of broccoli supplements [32]. Although glutathione S-transferase (GST) activity of colon mucosa correlated with that of blood lymphocytes in 29 subjects at increased risk of colorectal cancer, neither was raised significantly by the supplements. Apart from colonic biopsies and blood, patient samples that may be collected for preneoplastic biomarker measurement are urine, stool and colonic lavage fluid.

Preneoplastic biomarkers have not only to be identified, but also painstakingly validated. This task involves the judicious use of the most suitable animal models which reflect the multifactorial nature of human colorectal cancer [33]. Furthermore, the relationship between biomarker and risk of cancer has to be established in prospective studies in high-risk individuals. Such studies constitute a significant undertaking, but are necessary to bridge the gap between preclinical model systems and clinical trials.

5. Biochemical targets and chemopreventive mechanisms

5.1. Features of colorectal cancer

Most CRC arises within pre-existing adenomatous polyps or adenomas, which are common lesions, particularly in the elderly [34]. However, it is relatively rare for individual adenomas to progress to malignancy, despite the high rate of cell division [35], and it is, therefore, important to identify 'high-risk' polyps. In order to assess this risk, molecular biological criteria are increasingly used in addition to histological ones. A cell accumulates a combination of genetic defects, including activation of oncogenes and inactivation of tumour suppressor genes, to undergo full malignant transformation [36]. This concept, known as the multistep model of carcinogenesis, has been characterised in some detail for CRC (Fig. 1). Although in reality the progression of events may be less linear and less temporal than this simplified scheme suggests, the model has provided the basis for understanding the interaction between genetic predisposition and environmental factors as outlined in several contemporary reviews [8,9]. Irreversible mutations are known to occur in the initiation phase, followed by continued mutations, uncontrolled cellular proliferation and clonal expansion in the promotion phase. Progression involves genotypically altered cells developing the histological changes associated with CRC.

Identification of high-risk polyps should not only be based on histological criteria and assessment of genetic defects, but might also benefit from consideration of cell signalling pathways, downstream from isolated gene

Table 3
Examples of preneoplastic biomarkers identified for colorectal cancer (modified from [30])

Type of biomarker	Variable measured	Biomarker
Pathological	Histology	Adenoma, aberrant crypt foci
Cellular	Proliferation	BrDU, PCNA, Ki67, lectin labelling
	Differentiation	Lectin labelling
	Apoptosis	TUNEL assay
Biochemical	Arachidonate metabolism	Prostaglandins, COX-2, arachidonic acid, lipooxygenase, leucotrienes
	Polyamine metabolism	Polyamines, ornithine decarboxylase
	Detoxification enzymes	GST, DT-diaphorase
Molecular	DNA methylation, DNA adducts	Methyl groups, MTHFR, MDA–DNA adducts
	Cell cycle	Cyclin D1, TGFα
Genetic	Gene/product	K-ras, APC, DCC

BrDU, bromodeoxyuridine; PCNA, proliferating cell nuclear antigen; TUNEL, terminal deoxyribonucleotidyl transferase mediated nick-end labelling; COX-2, cyclooxygenase-2; GST, glutathione S-transferase; MTHFR, methylenetetrahydrofolate reductase; MDA, malondialdehyde; TGF-α, transforming growth factor-α. APC, adenomatous polyposis coli; DCC, deleted in colorectal cancer.

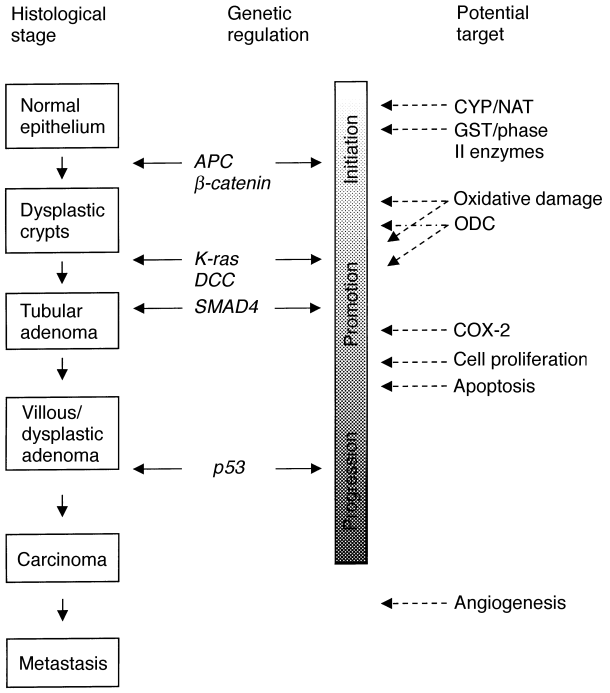

Fig. 1. Multistep model of carcinogenesis with targets for chemopreventive agents (modified from [36]). APC, adenomatous polyposis coli; COX-2, cyclooxygenase-2; CYP, cytochromes P450; *DCC*, deleted in colorectal cancer; GST, glutathione S-transferase; NAT, N-acetyltransferases; ODC, ornithine decarboxylase.

defects which give rise to an abnormal phenotype. It is now recognised that multiple, sequential mutations in genes critical to the control of cellular signal transduction, transcription and thus proliferation and apoptosis are important throughout carcinogenesis [37]. An example is provided by the *APC* gene which is commonly mutated in polyps and early cancer [38]. Loss of wild-type *APC* leads to transcriptional activation of *c-myc* expression through the β-catenin Tcf-4 complex [39], which may affect a number of downstream targets including the initiation factor eIF-4E [40]. Elucidation of the signalling pathway downstream of the wild-type *APC* gene has pinpointed a crucial role for β-catenin and E-cadherin, the expression of which is frequently abnormal in colorectal cancer [41]. Although *APC* function may be normal, mutations in *β-catenin* can result in the abnormalities of adhesion–migration and proliferative signalling found in neoplasia [42].

5.2. Chemopreventive agents

Historically, chemopreventive agents have been categorised as either 'blocking' or 'suppressing' according to the stage of the carcinogenic process with which they interfere [43]. Blocking agents exert their effect at the initiation stage, for example by altering carcinogen-metabolising activities in target tissues or by antioxidant effects. Suppressing agents act at more advanced stages in the carcinogenic process during promotion and/or progression, by inhibiting cell proliferation and/or inducing apoptosis. Although it is now known that single chemopreventive agents may exert effects at multiple stages (see below), this broad and simple classification remains useful.

It is not yet clear whether highly specific inhibitors, e.g. selective inhibitors of the enzyme cyclooxygenase-2 (COX-2), or agents with more 'pleiotropic' effects, e.g. naturally occurring polyphenols, offer the greatest potential in the chemoprevention of human cancers. In order to effectively counteract processes leading to a disease as multifactorial as cancer, a satisfactory chemopreventive agent may have to possess a variety of mechanistically distinct, but complementary, anticarcinogenic properties. Some important biochemical targets for cancer chemopreventive agents are outlined below.

5.3. Antioxidants and agents which affect xenobiotic metabolism

Reactive oxygen species, such as superoxide anions and hydroxyl radicals, are thought to be involved in carcinogenesis [44]. Consequently, mopping up activated oxygen species is a chemopreventive mechanism displayed by some blocking agents such as flavonoids, vitamin E and isothiocyanates. Phase I drug metabolising enzymes, particularly cytochromes P450, activate many carcinogens, and phase II enzymes, including GST, detoxify carcinogenic metabolites. The balance between carcinogen activation and detoxification is probably a critical arbiter of an individual's risk of developing cancer [45]. This balance is influenced by many blocking agents. One example is indole-3-carbinol which, despite inducing both cytochromes P450 and phase II enzymes, prevents aflatoxin B1-induced hepatocarcinogenesis in rodents [46]. Another example is the isothiocyanate sulphoraphane contained in broccoli, which not only induces xenobiotic conjugating enzymes [47], but also inhibits some cytochrome P450 isoenzymes, prominent among them CYP2E1 [48].

Preclinical and clinical studies have established an association between decreased GST enzyme activity and increased risk for CRC [31]. In subjects at risk of colorectal cancer, the putative chemopreventive agent oltipraz significantly increased GST expression in colon mucosa and in blood lymphocytes [49]. Similarly, administration of non-steroidal anti-inflammatory drugs (NSAIDs) and curcumin has been shown to increase levels of GST isoenzymes in the gastrointestinal tract in preclinical models [50,51].

5.4. Modulators of arachidonic acid metabolism

The arachidonic acid pathway and one of its key enzymes, COX, or prostaglandin H synthase, have

recently received much attention with respect to both the development and prevention of colon cancer. COX catalyses the production of prostaglandins and occurs as two isozymes, COX-1 and COX-2, the latter inducible by infection or inflammation. Several observations support the notion that COX-2 is important in the aetiology of cancer of the colon and several other tissues. A significant increase in COX-2 mRNA has been observed in the majority of colon carcinomas when compared with normal surrounding mucosa, as well as in some adenomas [52,53]. Increased COX-2 expression is often accompanied by elevated levels of eicosanoids including prostaglandins [54,55].

The NSAID, sulindac, which inhibits the expression of COX non-selectively, causes regression of adenomatous polyps in patients with familial adenomatous polyposis (FAP) [56]. Competitive inhibition of COX-2 activity by selective agents such as celecoxib has been shown to suppress the formation of polyps and neoplasias in rodents with carcinogen-induced colon cancer [57,58] and in the minimal intestinal neoplasia (MIN) mouse, a rodent model of the human *APC* gene defect [59]. When $APC^{\Delta 716}$ knockout mice which possess a phenotype similar to that of the MIN mouse, were crossed with COX-2 knockout mice, the resultant offspring were significantly protected from tumour development [60].

5.5. Inhibitors of NF-κB activation

Nuclear factor-kappa beta (NF-κB) is a transcription factor pivotal for the expression of many genes regulating proliferation, immunity, inflammatory response and cellular adhesion. It is sequestered in an inactive state in the cytoplasm by an inhibitory protein, IκB [61]. Upon stimulation of the cell by tumour promoters, cytokines or the products of oxidative stress, IκB is phosphorylated by upstream kinases and degraded to release NF-κB. NF-κB undergoes translocation to the nucleus where it initiates upregulation of genes containing suitable binding sites. Activation of NF-κB has been shown to inhibit apoptosis [62]. This finding suggests that its inhibition could downregulate genes involved in the promotion and progression of carcinogenesis by restoration of the sensitivity of cells towards apoptotic stimuli [63]. Both aspirin and its hydrolysis product salicylate [64], as well as curcumin [65], interfere with NF-κB activation by inhibiting the IκB kinase (IKK) complex involving a heterodimeric kinase IKK-α and -β.

5.6. Inhibitors of polyamine biosynthesis

Polyamines are short-chain aliphatic molecules required for normal cellular growth. Their concentration in tissues has been shown to correlate with cellular proliferation [66]. Polyamines appear to be involved in the activation of the proto-oncogenes *c-myc* and *c-fos* [67]. Proliferating tissues and tumours differ from normal non-proliferating tissues not only because they produce more polyamines, but also in that they contain individual polyamines at different concentrations. For example, N1-acetylspermidine is generally undetectable in normal mammalian tissues, but is present in high levels in human CRC [68]. The rate-limiting step in polyamine biosynthesis is the enzyme ornithine decarboxylase (ODC), which is constitutively overexpressed in colorectal dysplasia and neoplasia [69,70]. Levels of ODC and polyamines, especially putrescine, are significantly elevated in the colorectal mucosa of individuals with the *APC* germ line mutation before they develop polyposis [71]. ODC has been suggested to be critical for cell transformation [72], and it might thus act as a preneoplastic biomarker of colorectal carcinogenesis [73]. α-Difluoromethylornithine (DFMO) is an irreversible inhibitor of ODC that is currently under clinical evaluation (see below). Many diet-derived chemopreventive agents, such as genistein, curcumin, indole-3-carbinol and green tea polyphenols, have also been shown to inhibit ODC activity [74–77].

5.7. Modulators of cell proliferation and apoptosis

The evidence for antiproliferative and pro-apoptotic properties as important mechanistic determinants of chemopreventive activity is particularly robust in the case of the NSAIDs. Aspirin has been shown to retard proliferation of human colorectal tumour cells by inducing arrest in the G0/G1 phase of the cell cycle and programmed cell death [78]. Salicylate increased the susceptibility of cells at a late stage of neoplastic progression towards induction of apoptosis. Intriguingly, aspirin suppressed the mutator phenotype associated with hereditary non-polyposis CRC by genetic selection for a subset of cells that do not express microsatellite instability [79]. Other chemopreventive agents like curcumin and indole-3 carbinol have also been shown to induce cell cycle arrest and/or apoptosis in cancer cell lines [80,81].

In general, cancer chemopreventive agents have cytostatic rather than cytotoxic properties. A case in point is the specific COX-2 inhibitor SC-58125, which arrested the growth of COX-2-expressing human colon adenocarcinoma cells both *in vitro* and when implanted into nude mice, but it did not affect adenocarcinoma cells lacking COX-2 [82]. This degree of selective activity of a chemopreventive agent is reminiscent of the current emphasis in modern anticancer drug development on compounds which target cellular signalling events selectively [83]. Many of these 'antisignalling molecules', in contrast to their classical anticancer drug precursors, also cause cytostasis rather than cytotoxicity. Such similarities between chemopreventive and antisignalling

chemotherapeutic agents intimate a convergence in the philosophies underpinning new agent development in both areas. This notion is supported by the fact that many cellular signal transduction targets such as *ras*, the mitogen-activated protein kinase pathway and cell cycle regulating molecules, are equally attractive to both areas.

6. Clinical trials

Agents that have entered clinical chemoprevention trials have been chosen on the basis of epidemiological research and activity in preclinical models. The relevance of published clinical trials is discussed in the following paragraphs. Relevant ongoing trials of the same agents, posted on commonly accessed international cancer websites, are shown in Table 4 to allow the reader to monitor future progress.

6.1. Celecoxib

Celecoxib is a highly selective COX-2 inhibitor (see above), which was recently approved in the USA as an adjunct to standard care for patients with FAP based on the results of a double-blind, placebo-controlled, multicentre trial in 77 patients [84]. Of the two dose levels studied for six months, 400 mg of celecoxib twice daily was found to cause a significant reduction in the number of colorectal polyps on endoscopy. Further NCI-sponsored phase II/III trials in patients with sporadic polyps and hereditary non-polyposis coli are ongoing. Preclinical studies of such agents [82] and clinical experience of NSAIDs such as sulindac [56] would suggest that the chemopreventive effects of highly selective COX-2 inhibitors are likely to be transient, and that polyps may increase in size and number once treatment is curtailed. Long-term safety is being elucidated (see Table 4).

6.2. Aspirin

A number of retrospective epidemiological studies suggest a decreased incidence of colorectal cancer of up to 50% in regular aspirin users [85–87]. One case–control study of 5815 cases of CRC over a 14-year period has suggested that there may be a 9-year delay before any preventive effects are seen from daily aspirin use, and that the reduction in risk may be dose-depen-

Table 4
A selection of clinical trials in progress measuring preneoplastic colorectal biomarkers (obtained from commonly accessed cancer websites, June 2000)

Agent	Trial design[a]	Subject population (recruitment status)	Variables measured[b]	Principal investigator/institution
Celecoxib	Phase I/II	Patients with HNPCC (R)	Pharmacokinetics Histology Others N/D	National Cancer Institute (Bethesda, MD, USA)
Celecoxib	Phase III	Patients with polyps resected (R)	Histology	Bertagnolli M (Boston, MA, USA)
Aspirin	Phase III	Patients with sporadic polyps (R)	Histology	Baron J. (Dartmouth Uni., NH, USA)
DFMO + Sulindac	Phase IIb	Patients with polyps resected previously (N)	Histology Gene/product Apoptosis Arachidonate metabolism Polyamine metabolism	Meyskens F.L. (California Irvine University, CA, USA)
Sulindac versus curcumin/rutin/quercetin	Phase I/II	Normal volunteers (R)	N/D	Shiff S. (Rockefeller University, New York, USA)
Curcumin	Phase I	Patients with advanced CRC (R)	Pharmacokinetics DNA adducts Detoxification enzymes Arachidonate metabolism	Sharma R.A. (Leicester University, UK)
Calcium	Phase III	Patients with sporadic polyps (C)	Histology, others N/D	Baron J. (Dartmouth Uni., NH, USA)
Calcium/Fibre	Phase III	Patients with polyps (R)	Histology, others N/D	Biasco G. (Bologna, Italy)
Folate	Phase II	Patients with polyps resected (R)	Histology, DNA methylation	Mason J. (Boston, MA, USA)

R, recruiting; N/D, not defined; N, not yet recruiting; C, recruitment completed; DFMO, α-difluoromethyl ornithine; HNPCC, hereditary non-polyposis colorectal cancer; CRC, colorectal cancer.

[a] See Table 1.

[b] See Table 3.

dent [29]. However, the interpretation of retrospective studies is fraught with potential pitfalls, such as individual selection, compliance and confounding variables.

Unfortunately, prospective studies of the incidence of colorectal cancer among users of aspirin or other NSAIDs are still lacking. The only large study published so far has been the Physicians' Health Study [88]. This randomised trial assessed the effects of 325 mg of aspirin taken on alternate days in 22 071 male physicians in the USA. The trial was closed prematurely because of a significant benefit with respect to cardiovascular mortality in the aspirin group. Analysis of 12-year follow-up of the aspirin group has not shown any significant effect on CRC incidence [89]. Questions have been raised about the suitability of the subject population, the dose regime adopted, and the fact that the actual period of continual dosing lasted only five years and subsequent data were collected post-trial when follow-up of subjects may have been less stringent.

More recently, strategies to investigate the chemopreventive potential of aspirin have changed. In a pilot phase I de-escalating dose study involving 65 healthy human subjects taking the drug daily for two weeks, preneoplastic biomarkers were measured in colonic tissue in addition to plasma levels of aspirin and salicylic acid [90]. The results of this study suggest that the optimal dose for aspirin as a chemopreventive agent may be 81 mg/day, which is lower than the dosage previously considered necessary for efficacy. Colonic prostaglandin levels continued to be suppressed long after aspirin and salicylic acid had been cleared from the plasma. Subsequent phase II results in patients with colon adenomas have proved encouraging [91], and phase III studies of aspirin and other NSAIDs are ongoing.

6.3. Calcium

Calcium ingested orally forms salts with bile acids and fatty acids once it reaches the colon, and it may thus have a direct anticarcinogenic effect on colonic epithelial cells [92]. Although a few case–control epidemiological studies suggest an inverse association between calcium intake and incidence of colorectal cancer [93], the results are by no means consistent. Similarly, chemoprevention trials have studied histological endpoints in the colon, with mixed results. In certain studies, calcium supplementation decreased epithelial cell hyperproliferation [94,95] and reduced the incidence of metachronous colorectal adenomas [96]. However, two large studies failed to show an effect of calcium on colonic mucosal proliferation [97,98]. Although two recent studies of patients with a previous history of dysplasia or neoplasia have suggested that calcium supplementation may decrease adenoma recurrence [99,100], the optimal dosing regime and the role of confounding dietary constituents remain unclear.

6.4. DFMO

Another agent which has been administered to individuals at risk for CRC is DFMO. Unlike chemoprevention trials in which only histological endpoints were measured, contemporary chemoprevention trials of DFMO have focused on potential toxicity and the tissue levels of polyamines and ODC as preneoplastic biomarkers. In two studies, significant changes in biomarker levels were observed in the target organ, but reversible ototoxicity was experienced at high-dose levels [101,102]. Depending on the subject population being targeted, safety may prove to be the main obstacle in the development of DFMO as a cancer chemopreventive.

6.5. Selenium

Often the secondary analyses of results of negative chemoprevention trials provide surprising results, as exemplified by the selenium skin cancer study [103]. Selenium (200 μg/day) was administered as a constituent of brewer's yeast to 1312 patients with a history of skin cancers, living in low selenium-intake regions of America. Secondary subset analysis found statistically significant reductions in total cancer mortality, and specifically in the incidence of prostate, lung and colon malignancies.

6.6. Other potential agents

Agents which are currently under consideration for colorectal cancer chemoprevention trials are the dietary substances curcumin, isothiocyanates, vitamin E, vitamin D3, folate, perillyl alcohol and polyphenols from tea [104,105]. On account of their long history of use in food, these compounds are considered harmless, at least at the dose levels consumed in certain diets. Whether this assumption is reasonable for the compounds in their pure forms remains to be demonstrated. Non-dietary compounds under consideration include oestrogens, oltipraz and N-acetylcysteine.

7. Conclusions

With improved molecular and epidemiological knowledge of colorectal carcinogenesis and risk of developing CRC, effective chemoprevention of this common disease is a realistic proposition. Reactive oxygen species, metabolising enzymes, COX-2, NF-κB, polyamines, and key molecules in cell proliferation and apoptosis are potential targets for chemopreventive agents. Integration of knowledge of the molecular biology of CRC with known mechanisms of action of such agents should lead to the discovery of new efficacious

agents and optimisation of clinical trials. CRC chemoprevention trials of aspirin, celecoxib, calcium and DFMO have provided valuable insights into choice of subject cohort, dose levels and the use of biomarkers. The trial of β-carotene in lung cancer patients has highlighted the need for new approaches to subject selection and trial design, especially the desirability of incorporating preclinical and early clinical mechanistic data. There is a need for small pilot studies of putative agents in healthy subjects or cancer patients to obtain information on safety, pharmacokinetics and pharmacodynamics. On account of the prolonged time periods necessary for chemoprevention trials, optimisation will depend on the selection and validation of preneoplastic biomarkers. Adherence to these principles may facilitate the discovery and development of effective chemopreventive agents. The old adage 'to name is to know, a disease known is half cured' may not currently hold for the chemotherapy of CRC. Nevertheless, the optimised understanding of this disease may ultimately contribute to its prevention.

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