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An Update on Cancer Prevention Approaches

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Majority of human cancers are caused, mediated and modified by environmental and lifestyle factors; and the multi-factorial, multi-step and multi-path process of carcinogenesis involves a series of genetic and epigenetic events. In spite of tremendous advancement in understanding of the molecular basis of cancer and identification of several environmental carcinogens, avoidance of exposure to carcinogens and early detection and/or successful treatment for most cancers have met with limited success. Based on the susceptibility to modulations of the multi-step process of carcinogenesis by a multitude of environmental compounds, lifestyle changes and host factors, and the demonstrated success of prevention of certain infectious diseases and cardiovascular events, cancer preventive interventions are receiving increasing attention. Several cancer preventive interventions such as vaccination, chemoprevention, weight control and lifestyle changes have been implemented. The current review focuses on several approaches and agents that have been scrutinized by way of randomized clinical trials in humans for their cancer prevention potential. Successful chemopreventive agents include selective oestrogen receptor modulators and aromatase inhibitors for breast cancer, the 5-α-reductase inhibitors for prostate cancer, non-steroidal anti-inflammatory drugs (NSAIDs) for colorectal lesions and vaccines for viruses that are associated with cervical and liver cancers. Several experimentally proven chemopreventive agents have been observed to lack efficacy with and without toxicity. In spite of numerous chemoprevention trials, the number of successful agents is rather small. Identifying novel approaches and chemopreventives holds tremendous potential for reducing the burden of cancer.

INTRODUCTION

Cancer comprises a multiple of diseases in which the cells proliferate autonomously without control, and accumulated abnormal cells spread to other parts of the body by invasion and/or distant metastasis via the blood and lymphatic systems. Cancer continues to be one of the major physical, social and economic burdens and public health threats worldwide and accounts for over 12% of deaths globally. The studies in migrant populations, changes in cancer incidence with time within same country and identical twins indicate that environment and lifestyle factors are major players in the causation of human cancer. The etiology of all cancers can be categorized into two main groups i.e. hereditary and environmental. Of the total, 5–10% of cancers are associated with inherited genetic aberrations; other 90–95% of

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cases are caused by exogenous/endogenous environmental factors.

The International Agency for Research on Cancer (IARC) has classified human carcinogens and recently completed reviewing human cancer sites associated with more than 100 carcinogenic agents (Cogliano *et al*., 2011). In brief, the list is given below:

- chemicals and mixtures e.g. aflatoxins, 4-aminobiphenyl, benzene, benzidine, coal tar pitch, ethylene oxide, formaldehyde, 2-naphthylamine, tobacco-specific nitrosamines, shale oils, soot, sulfur mustard, vinyl chloride, etc.
- several occupations e.g. productions of aluminium, auramine, coke, isopropyl alcohol, magenta, painting, rubber production industry, welding, etc.
- metals e.g. arsenic, beryllium, cadmium, chromium, nickel, etc.
- dusts and fibres e.g. asbestos, dust from leather, silica, wood, etc.
- radiations e.g. all types of ionizing radiations, UV/solar radiations
- biological agents e.g. EBV, HBV, HIV1, highly oncogenic HPVs, HTLV1, Kaposi sarcoma herpes virus, parasites such as liver flukes and schistosoma and bacteria such as *Helicobacter pylori*, etc.
- personal habits e.g. smoking/smokeless tobacco use, alcoholic beverages, use of areca nut, betel quid with/without tobacco, indoor emissions from household combustion, consumption of salted fish, etc.
- pharmaceuticals e.g. several anti-cancer agents, immunosuppressants, hormonal preparations, etc. (Cogliano *et al.*, 2011).

Nevertheless, several common human cancers have not been associated with identified causative agents and the quest for causative factors continues.

Although dose and duration of exposure to exogenous/endogenous carcinogen(s) are some of the determining factors, these aspects are not sufficient to explain exposure-related outcome as majority of cancers result from complex interactions between environmental exposure(s) and genetic/acquired susceptibility or protective host factors. Response to carcinogen exposure may further be modulated by other risk-enhancing or protective factors such as diet, tobacco and alcohol, physical activity, obesity leading to associations with cancer.

Carcinogenesis

Carcinogenesis refers to chemical/physical/biological agent(s)-mediated etiologic pathway that leads to cancer. It is a complex, multi-factorial, multi-step, multi-path process characterized by at least three stages viz., initiation, promotion and progression (Fig.1). Initiation is an irreversible event which begins when the cells in normal tissues are exposed to carcinogen and the genomic DNA undergoes damage and subsequent fixation of the damage. In the promotion process, initiated

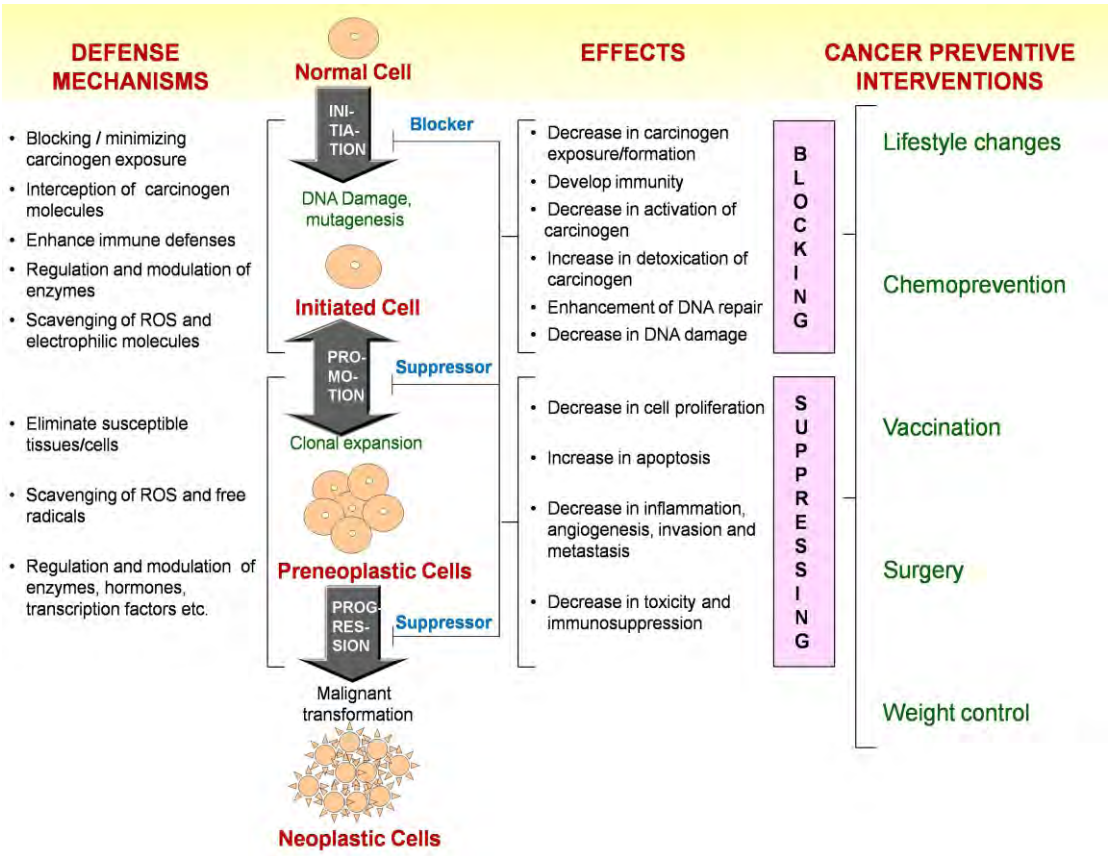

Figure 1: Schematic representation of the multi-step process of carcinogenesis, steps defining cancer preventive interventions and their effects, and observed defense mechanism(s).

cells expand to form an actively proliferating multi-cellular pre-malignant tumor cell population; while progression is an irreversible process producing a new clone of tumor cells with increased proliferative capacity, invasiveness and metastatic potential.

Among several models of carcinogenesis, Knudson (1971) proposed a 'two-hit' model requiring a mutation in both copies of a gene resulting in cancer. Expansion of this concept by Vogelstein and colleagues emphasized that cancer is ultimately a disease of damaged DNA comprising of a series of genetic mutations that lead to the transformation of normal cells to cancerous cells (Vogelstein and Kinzler, 2004). The genetic mutations include inactivation of tumor suppressor genes and/or activation of oncogenes. Further expansion of the concept by Hanahan and Weinberg (2011) proposed hallmark events at the cellular level that lead to cell transformation. The hallmarks of cancer include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis and activating invasion and metastasis. Underlying these hallmarks are genome

instability and inflammation, which fosters multiple hallmark functions leading to cell transformation and imparting the ability to invade and metastasize.

In spite of tremendous advancements in understanding the molecular basis of cancer, early detection and/or successful treatment approaches for most cancers have met with limited success. It is expected that the number of cancer-related deaths may double in the next 50 years and global cases of cancer will rise to 15 million new cases by 2020, when the world population reaches 7.5 billion. Generally, patients with metastatic cancer do not successfully respond to even the most advanced treatment methods, and often their lives are not saved. While in patients with less advanced cancer, treatment extracts tremendous social and economic devastation. Moreover, an increasing trend of chemo-/radio-resistance and recurrence of tumor results in limitations in the fight against cancer.

In this context healthy aging and disease prevention by preventive interventions is increasingly a subject of academic and public interest and research. The approach of cancer prevention by avoiding/minimizing exposure to proven carcinogens, and/or altering the metabolism of carcinogen(s), and/or pursuing lifestyle or dietary changes to modify effect of cancer causing factors or genetic predispositions, and/or medical interventions (chemoprevention), and/or prophylactic resection of high-risk organs in certain germline mutation carriers is considered to be an alternative, probably more realistic, cost-effective and a fundamental strategy. This is based on the experience that preventable illnesses make up ~80% of the burden of illnesses and 90% of all healthcare costs, and saves us from undergoing sufferings and discomfort.

The rationale for prevention approaches are based on:

- process of carcinogenesis involves a series of genetic and epigenetic events (multi-step, multi-path), and that many of these events are susceptible to modulation by variety of environmental compounds, lifestyle changes and host factors, and
- prevention approaches have been demonstrated to be successful in other environmental diseases such as certain infectious diseases (vaccines) and cardiovascular events (treating risk factors with stents, statins, lifestyle changes).

The concept of prevention is best defined in the context of levels traditionally called primary, secondary and tertiary prevention.

Primary Prevention

Primary prevention is an important means to improve public health, and it is by far the most cost-effective and sustainable intervention for reducing mortality and disability, by reducing the incidence of cancer globally. Primary

prevention of human cancer can be accomplished in two ways:

- avoiding the introduction of carcinogenic agents into the environment, and
- eliminating or drastically reducing exposure to carcinogenic agents that are already present in our environment

The first approach is theoretically possible but practically it has proved to be difficult. The second approach involves actions aimed at reducing or eliminating exposure to carcinogens, and/or enhancement of resistance to the effects of exposure to causative agents (e.g. vaccination). All these approaches are suitable for application in general public.

Elimination of carcinogen/avoidance or minimization of exposure can probably be achieved by improving technology, and/or replacement of agent by less toxic or non-chemical means. Avoidance or minimization of exposure can also be achieved by implementing control measures resulting in a decrease in exposure conditions such as concentration, duration, time, frequency and also reducing exposure to the number of persons. This can be facilitated by increasing awareness, reducing workplace limits, improving compliance with exposure limits by legislation, regulation and policies. This is likely to be achieved in occupational and environmental exposures. In addition, motivating individual members, institutions/organizations and society for changes, in attitudes and behaviors are also important in exposure control and risk reduction. Although possible, it has proved difficult, possibly due to addiction, especially with respect to societal exposures e.g. smokeless tobacco/tobacco smoke, alcohol, etc.

Although primary prevention of occupational carcinogen(s)-induced cancers appears to be simple and logical, reductions in cancer rates are not easily documentable in quantitative terms (Tomatis *et al.*, 1997) perhaps due to the following reasons:

- Few follow-up studies designed to determine whether cancer rates actually declined as a result of implementation of defined preventive measures.
- Most reports predicting decline in cancer risk are based on assumptions about exposure-response curves and not on actual observations on changes in risk after exposure reduction.
- The time that must elapse after intervention, before a reduction in cancer risk (that may vary depending on the dose and duration of exposure) can be observed, may be one of the reasons for the absence of data.
- The multi-factorial origin of most tumors makes it particularly difficult to measure the role and quantify the contribution of a single agent.
- Estimates of attributable risks are largely based on unverified assumptions and hence most assessments of the percentage

of cases that could be avoided by intervening on single factors are uncertain.

- The concept of prevention is further complicated because 'attributable risk' is taken as the proportion of all cases of the disease caused by individual exposure.
- Research funding may be more difficult to obtain for (long-term) studies to demonstrate the effectiveness of primary preventive actions.

In spite of convincing evidence for their causal association with cancer for more than 100 environmental carcinogenic agents, their elimination from the environment has proved to be difficult due to lifestyle factors and modern developments. Overall there is limited success in elimination of carcinogen, and/or avoidance of carcinogen exposure. In addition, specific causative agents have not been identified for several cancers, partly because of the limitations of available experimental/epidemiological studies, and hence primary prevention is not achievable for them. This approach has demonstrated some success and feasibility especially in preventing societal, occupational and environmental exposure-related, as well as some biological agent-induced cancers. Some of the examples are indicated as follows:

- The decreasing risk of cancer in British male doctors who quit smoking provides strong evidence that the elimination of exposure results in reduction of risk (Doll *et al.*, 1994). Decrease in lung cancer rates with exposure reduction in terms of number of cigarettes per day, or duration of smoking and time since stopping smoking (Lubin, 1984), and decreasing mortality from lung cancer in males from younger cohorts in the western world, have been linked to the decreasing proportion of smokers among the young (Coleman *et al.*, 1993). Lung cancer death rates in USA have mirrored smoking patterns i.e. increase in smoking followed by dramatic increase in lung cancer death rates and, more recently, decrease in smoking followed by decrease in lung cancer death rates in men (Jemal *et al.*, 2001).
- Banning production and use of carcinogenic aromatic amines has resulted in reduction of bladder cancer among dye workers after the elimination of exposure to aromatic amines (Swerdlow, 1990).
- A significant reduction in incidence and mortality for gastric cancer has been attributed to the elimination of environmental carcinogens, and/or the improvement in food preservation techniques (Hwang *et al.*, 1994).
- Initial observation based evidence suggest the success of vaccines against specific causative biological agents such as HBV, HPVs (Chang *et al.*, 1997; Frazer, 2004).
- Reduced melanoma after regular sunscreen use has been reported based on

observations from randomized follow up trial (Green *et al.*, 2011). This result has further been complimented by the effect of sunscreen on the response of melanocytes *in vivo* to ultraviolet radiation (Hacker *et al.*, 2013).

Creating awareness and implementing appropriate preventive measures may help us in decreasing the incidence of region-specific cancers such as Kangri Cancer (due to prolonged exposure to heat) in Kashmir, Sari and Dhoti Cancer (Chronic friction) in Maharashtra, and tongue/mouth cancer due to sharp tooth.

In traditional primary cancer prevention approaches, research on healthy lifestyles e.g. strategies to reduce unhealthy behaviors, and identification and elimination/avoidance of environmental risk factors receive major emphasis. While complementary cancer prevention activities may include,

- screening of populations for genetic risk factors and genetic counseling of individual with genetic risk
- research to develop new, more sensitive and specific biomarkers for early detection of cancer
- screening of populations for early detection of certain cancers

Screening is the early detection of disease, precursors to disease, or susceptibility to disease, in individuals who do not show any signs of disease.

Screening populations for genetic risk factors

Hereditary cancer is cancer risk that is inherited or passed on in a family. Hereditary cancer results from an abnormal alteration in a single gene and 5–10% of all cancers are considered to be hereditary, e.g. breast-ovarian cancer syndrome, familial adenomatous polyposis (FAP), familial melanoma, hereditary non-polyposis colorectal cancer (HNPCC), multiple endocrine neoplasia (MEN), Von Hippel Lindau (VHL) syndrome, xeroderma pigmentosum, and hereditary diffuse gastric cancer (HDGC), etc.

Compared to cancers arising in the general population, individuals with a major inherited predisposition to cancer are born with inherited (e.g. germline) mutations in genes involved in cancer causation. Since the heritable component of some cancer predisposition has been linked to mutations in specific genes, clinical interventions have been formulated for mutation carriers within affected families. Surgery represents the primary approach to cancer prevention for carriers of mutations in genes associated with high penetrance cancer syndromes, such as MEN, FAP, HNPCC and HDGC. Prophylactic resection of high-risk organs in certain germline mutation carriers although radical, may be recommended. However, standard prevention approach e.g. colostomy can reduce colorectal cancer risk in FAP patients who have adenomatous polyposis coli

mutations (Chau and Cunningham, 2002; Steinbach *et al.*, 2000) and bilateral mastectomy (Meijers-Heijboer *et al.*, 2001) and oophorectomy (Haber, 2002; Metcalfe, 2009) can reduce breast cancer risk as well as breast and ovarian cancer risk in BRCA1/BRCA2 mutation carriers. Several studies have provided evidence that genetic counseling and testing increased surveillance and led to risk-reducing operations, which resulted in a diagnosis of early-stage tumors in patients with BRCA1 and BRCA2 mutations (Scheuer *et al.*, 2002).

Several studies have employed alternative prevention approaches in carriers of gene mutations, e.g. tamoxifen in individuals with germline BRCA2 mutation. Tamoxifen reduced breast cancer incidence among healthy BRCA2 carriers by 62%, similar to the reduction in incidence of ER positive breast cancer among all women in the breast cancer prevention trial (King *et al.*, 2001). In contrast, tamoxifen use beginning at the age of 35 years or older did not reduce breast cancer incidence among healthy women with inherited BRCA1 mutations.

Screening for early detection of certain cancers

Screening for early detection of several cancers in asymptomatic individual to reduce mortality and morbidity has been employed. Beyond the potential of avoiding death, screening may reduce cancer morbidity since treatment of earlier stage cancers is often less aggressive than that for more advanced-stage cancers. These interventions are often directed to entire populations or large and easily identifiable groups within the population. Several simple and/or advanced methods/markers have been employed for early detection of breast cancer (e.g. Mammography, Breast self-examination), cervical cancer (Pap smear, VIA), colorectal cancer (Fecal occult blood testing, Sigmoidoscopy, Colonoscopy), prostate cancer (PSA, Digital rectal examination) and several other cancers. The approach of early detection of cancer has demonstrated success in reducing cancer mortality (Christopherson *et al.*, 1976; Mandel *et al.*, 1993; Shapiro *et al.*, 1982; Shastri *et al.*, 2014); however, screening use remains low in resource-poor countries. This approach may also facilitate enrollment of subjects for secondary or tertiary chemoprevention trial ultimately leading to a reduction in mortality and morbidity due to cancer.

Secondary Prevention

In secondary prevention targets are specific risks in closely defined high-risk subjects rather than general populations, and intervention is undertaken to prevent the consequences of carcinogen exposure, and preclinical disease.

Tertiary Prevention

In tertiary prevention, aim is to prevent or control the symptoms and morbidity due to cancer or cancer therapy, and prevent recurrence of pre-existing cancer or a subsequent different cancer (second primary).

Preventive interventions

Several preventive interventions such as chemoprevention, vaccination, weight control and lifestyle changes (avoiding/minimizing exposures, physical exercise, eating healthy) have been employed (Fig.1) and extensive experience and literature on various aspects of 'chemoprevention' and 'vaccination' have accrued.

Chemoprevention

Chemoprevention of cancer refers to intervention with natural or synthetic compounds to retard, block or reverse the process of carcinogenesis ultimately leading to prevention of pre-neoplastic and/or clinically detectable cancer and/or recurrence of cancer. It is a 'prescription' approach and forms an adjunct to other cancer control and prevention approaches.

Identification of environmental chemo-preventive agents

Considering the limited scope of the present update only a very brief summary of this aspect has been covered. Putative chemopreventive agents are subjected to rigorous evaluations employing a series of *in vitro* and *in vivo* experimental model systems (Table 1).

Studies employing *in vitro* and/or *in vivo* animal models have contributed significantly in identification of a number of environmental chemopreventive agents and helped in understanding the complexity of gene-environmental interactions (Patel *et al.*, 2007). After extensive evaluation of preclinical efficacy and safety of an agent, further evaluation in appropriate clinical trials is

Table 1: Experimental models and end points employed in identification of environmental chemopreventive agents

***In vitro* assays**	**End points**
Bacteria, mammalian cells, tissues, organ cultures, cancer cell lines, cell-free extracts or biochemical reactions	**Inhibition of carcinogen/mutagen-induced effects –** mutations, chromosomal aberrations, clastogenic effects, DNA adducts and free-radical formation, DNA strand breaks, levels and activity of metabolic and repair enzymes **Inhibition of cell proliferation –** colony growth in soft agar, transformed cell foci, alterations in response to a known stimuli **Enhancement of cell differentiation and apoptosis**
***In vivo* assays**	**End points**
Normal/genetically engineered rodents and other models	Decrease in incidence and/or multiplicity of carcinogen-induced or spontaneous tumors or inhibition of carcinogen-induced premalignant lesions or markers or pathways. Increase in tumor latency period

undertaken.

Clinical trials are prospective biomedical studies on human subjects to answer specific questions about interventions (drugs/ treatments/vaccines/devices or new ways of using known interventions) generating safety and efficacy data. Preventive interventions initially enroll volunteers and/or patients in pilot studies and subsequently conduct progressively larger scale comparative studies. Clinical trials can vary in size involving single or multiple research entities in one or more countries. The goals, enrollment of the study population, study protocols, acceptable level of toxicity, etc. in the design of chemoprevention trials are different when compared to therapeutic oncology drug treatment trials (Table 2).

Interventions demonstrating chemo-preventive efficacy

a) Earlier reports demonstrated that anti-estrogens play an important role in preventing breast cancer development (Jordan *et al*., 1980). Tamoxifen, a selective estrogen receptor modulating agent (SERM), was discovered as an anti-estrogen compound and has been used for over 30 years in patients with early stage breast cancer as adjuvant therapy to prevent breast cancer recurrence; and in those with metastatic breast cancer to slow down its growth (Fisher *et al*., 1989; 1998).

The breast cancer prevention trial (BCPT) and follow up study of tamoxifen against raloxifene (STAR), two randomized, double blind, placebo controlled trials, enrolled a total of more than 32,000 women (35 years or older) at risk of breast cancer (Fisher *et al*., 1998; Vogel *et al*., 2006) to study whether tamoxifen can reduce the risk of developing breast cancer. The BCPT trial demonstrated conclusively that 20 mg/day of tamoxifen reduced the incidence of invasive breast cancer by 45%; ductal carcinoma was reduced by 48% compared with women on placebo; at least a 1.66% risk of invasive breast cancer over 5 years. Women, who took tamoxifen, had a significant increase (2.4 fold) in the risk of developing endometrial carcinoma and an increase in venous thromboembolic events (Fisher *et al*., 1998). The STAR trial compared tamoxifen and raloxifene for preventing breast cancer in post-menopausal women. Both the agents exhibited ~ 50% reduction in breast cancers and raloxifene showed fewer adverse effects including uterine cancers, cases of thrombosis and hot flashes) (Vogel *et al*., 2006). These trials have demonstrated efficacy of either SERM for breast cancer prevention and also suggested potential for improved safety in the iterative generation of agents.

In the worldwide adjuvant tamoxifen:

Table 2: Design of prevention trials vs therapeutic treatment trials

	Prevention Trials	Therapeutic Treatment Trials
Goals	**Cancer prevention**	**Cancer treatment**
	decrease in incidence and mortality	increase in cure or remission rates
	prevent/ameliorate precancerous lesions/biomarkers that serve as surrogates of risk	decrease in mortality and morbidity
	prevent second primary tumor	improvement in survival and/or efficacy against an established surrogate end point
Study Population	**Subjects without cancer** (asymptomatic, ostensibly healthy subjects)	**Cancer patients** (diagnosis confirmed before therapy)
	general population	
	high-risk population	
	persons with precancerous lesions	
	cancer patients	
	small to large-scale	small to moderate
Toxicity of Agent	**Mild to moderate – acceptable**	**Moderate to severe – acceptable**
Study Protocol	**Design**	**Design**
	intervention vs placebo	therapy vs placebo
	intervention A vs intervention B Vs intervention AB vs placebo	therapy A vs therapy B
		therapy A vs therapy B vs therapy C
	pilot study usually required	pilot study rarely needed
	placebo run-in is useful	placebo run in is inappropriate
	study may require 5-10 years or more of intervention and follow up	study length may be short for aggressive cancers, longer for slow growing cancers/adjuvant studies
	adherence to protocol may be difficult to maintain (subject-dependent)	adherence to protocol easier to maintain (physician-dependent)
Basis	**Based on cellular and molecular insights**	**Based on symptoms and loss of normal function**

longer against shorter (ATLAS) trial, 12,894 women with early breast cancer who had completed 5 years of treatment with tamoxifen were randomly allocated to continue tamoxifen for 10 years or stop at 5 years (open control). Results demonstrated that women with ER +ve disease, continuing tamoxifen for 10 years rather than stopping at 5 years produced a further reduction in recurrence and mortality particularly after year 10 (Davies *et al*., 2013).

Following their successful implementation for the treatment of metastatic breast cancer, the third-generation aromatase inhibitors (anastrozole, letrozole, exemestane) have now become the standard adjuvant endocrine treatment for postmenopausal estrogen-receptor-positive breast cancers (Lonning and Eikesdal, 2013). In a randomized, placebo-controlled, double blind trial, 4560 women (median age 62.5 years, median Gail risk scores 2.3%) were assigned to either exemestane or placebo. At a median follow-up of 35 months, 11 invasive breast cancers were detected in those given exemestane, and 32 in those given placebos, with a 65% relative reduction in the annual incidence of invasive breast cancer, and no serious toxic effects (Goss *et al*., 2011).

b) The high-risk human papilloma virus (HPV) is the etiologic agent associated with cervical cancers in females, penile and anal cancers in males, and 5–10% oropharygeal cancers in both males and females (Dunne *et al*., 2012). A causative relationship between high-risk HPVs e.g. HPV16 and HPV18 infection and cervical cancer has been established (Boshart *et al*., 1984; Durst *et al*., 1983). Approximately 70% of cervical cancers are caused by HPV16 and HPV18. Quadrivalent and bivalent vaccines against high-risk HPV types HPV16 and HPV18, and/or HPV6 and HPV11 have shown 95–100% effectiveness at preventing the cervical cancer precursor lesion (cervical intraepithelial neoplasia grade 3 or greater) and 100% effective at preventing cervical adenocarcinoma *in situ*. When considering the entire cohort tested, including those with prior HPV infection, the level of protection conferred is highly variable with 12–46% protection from cervical intraepithelial neoplasia grade 3 or higher, and 28–83% protection from cervical adenocarcinoma *in situ* (Garland *et al.*, 2007; Group FIS, 2007; Lehtinen *et al.*, 2012). Two HPV vaccines i.e. Gardasil (quadrivalent vaccine against 4 HPV types – 6, 11, 16 and 18 from Merck) and Cervarix (bivalent vaccine against HPV types 16 and 18 from Glaxo SmithKline) have been approved by Food and Drug Administration, USA are globally in use.

Another cancer with the potential for vaccine-based prevention is hepatocellular carcinoma (HCC). In Taiwan when HBV vaccination was implemented the rates of childhood HCC decreased in children (Age 6–14 years) from 0.70 cases per 100,000 in 1981–1986 to 0.36 cases per 100,000 in 1990–1994 (Chang *et al.*, 1997). The impact of HBV vaccination on HCC occurrence will be realized after about 20 years of vaccination (Wong and Chan, 2012).

c) Multiple lines of evidence suggest non-steroidal anti-inflammatory drugs (NSAIDs) are active in colorectal adenoma and cancer prevention (Chan *et al.*, 2012; Giardiello *et al.*, 1993). Observational evidence for the association between NSAIDs and colorectal adenomas indicated that the relative risk of colorectal adenomas was 0.57 with regular use of any NSAID; and for non-aspirin NSAIDs the effect was somewhat smaller with a relative risk of 0.7. Colorectal adenomas are considered as precursor lesions to cancer. NSAIDs-mediated adenoma risk reduction of 22–53% puts NSAIDs at the forefront of agents of interest in colorectal cancer prevention (Gill and Sinicrope, 2005; Rostom *et al.*, 2007). At low levels of cardiovascular risk, the benefit of aspirin for colorectal adenoma prevention assumes increased importance in the balance against the complications of aspirin/NSAID use. After 5 years follow up in the Physician's Health Study with participants randomized to 325 mg of aspirin every other day versus placebo, the point estimate for colorectal polyps and *in situ* cancer was 0.88 (Gann *et al.*, 1993). In the Women's Health Study, 100 mg of aspirin every other day was compared with placebo and after 10 years, the relative risk of colon polyps was 0.97 (Cook *et al.*, 2005). Although the doses tested may be suboptimal for colorectal adenoma prevention, concern about adverse events with higher doses is justified. A recent, notable success was reported from evaluating a longer-term follow-up study of Colorectal Adenoma/Carcinoma Prevention Program (CAPP2), evaluating aspirin and resistant starch for the prevention of adenomas and carcinomas in Lynch syndrome patients. Long-term follow-up demonstrated a significant increase in time-to-first colorectal cancer occurrence in those who took aspirin for at least two years (Burn *et al.*, 2011). A recent review presenting analysis of available evidence from studies and clinical trials suggests that prophylactic aspirin use in a general population for a minimum of 5 years at 75–325 mg/day appears to have favorable benefit-harm profile. For average risk individuals aged 50–65 years taking

aspirin for 10 years, a relative reduction of between 7% (women) and 9% (men) in the number of cancer, myocardial infarction or stroke over a 15 year period and an overall 4% relative reduction in all deaths over a 20 year period was reported (Cuzick *et al*., 2014).

With the development of selective COX-2 inhibitors (coxibs), the option of blocking the inducible form of COX offered an attractive opportunity. Three randomized controlled trials of COX-2 inhibitors confirmed a significant (28%) reduction of adenoma risk (Arber *et al.*, 2006; Baron *et al.*, 2006; Bertagnolli *et al.*, 2006; Steinbach *et al.*, 2000). Adverse effects included increase (2.6 fold) in cardiovascular events for a celecoxib dose of 400 mg and 3.4 fold for a dose of 800 mg. In addition to the proof-of-principle established by the results, coxibs may find a role in individuals at high risk of colon cancer with low cardiovascular risk. This scenario should apply to young patients diagnosed with familial adenomatous polyposis (FAP). A subsequent study of children with FAP found that celecoxib at a dosage of 16 mg/kg per day for 3 months was well tolerated and reduced the number of colorectal polyps by 44% (Lynch *et al.*, 2010).

Another successful chemoprevention trial was reported a few years ago, using the combination of difluromethyl ornithine (DFMO, 500 mg/day) and sulindac (150 mg/day) to prevent sporadic colorectal adenomas (Meyskens *et al.*, 2008). Individuals receiving this intervention experienced a 70% reduction in colorectal adenomas in contrast to participants assigned to the placebo arm of the trial, where 41% developed adenomas over 3 years (Meyskens *et al*., 2008). This trial utilized low doses of both DFMO and sulindac, thus limiting the potential toxicities associated with the medications. This approach allows for the targeting of multiple aspects of a single pathway (Gerner and Meyskens, 2009).

d) Melanoma and non-melanoma skin cancers are among the most prevalent cancers in human. Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are non-melanoma skin cancers and actinic keratosis (AK) is a precancerous lesion that may progress to SCC if left untreated. Several methods of treating AK to prevent skin cancers are used based on demonstration of their efficacy in clinical trials. Actinic keratosis has been treated successfully by surgical removal of the lesion through cryosurgery or laser surgery, especially when the number of lesions is limited (Thai *et al.*, 2004). The other successful approach has been medical therapy wherein a chemical (e.g. chemical peeling) or medicated creams, gels and solutions are effective by

themselves or in combination with surgery (Berlin and Rigel, 2008; Jorizzo *et al.*, 2006). Medical therapy has advantages in being able to treat large areas with multiple lesions. Based on their success in several clinical trials five medications have been approved by FDA for the treatment of AK. These are topical 5-fluorouracil (5-FU) 0.5–5% (ointment or liquid) (Gupta *et al.*, 2005; Kurwa *et al.*, 1999; Loven *et al.*, 2002; Tutrone *et al.*, 2003a); topical imiquimod 2.5%, 3.75% and 5% (cream) (Gupta *et al.*, 2005; Hanke *et al.*, 2010; Stockfleth *et al.*, 2007; Ulrich *et al.*, 2006; 2007); topical diclofenac sodium 3% gel (Berlin and Rigel, 2008; Tutrone *et al.*, 2003b); photodynamic therapy (PDT) wherein light sensitizing compound such as delta-aminolevulinic acid is applied topically followed by exposure to light with appropriate wavelength that results in selective killing of dysplastic cells (Kaufmann *et al.*, 2008; Kurwa *et al.*, 1999); and topical ingenolmebutate, 0.015 or 0.05% gel (Lebwohl *et al.*, 2012; 2013; Rosen *et al.*, 2012). In four randomized, double-blind, and placebo-controlled studies, patients with AKs were randomized either to self-applied ingenolmebutate or placebo for 2–3 days. By 57th day, 42% of the patients with facial or scalp AKs who had received treatment experienced complete clearance of AKs compared with 3.7% in the placebo arm. Similarly, 34.1% of the participants with AKs on their trunks or extremities experienced complete clearance, whereas only 4.7% of those in the placebo arm had similar results (Lebwohl *et al.*, 2012). Amongst the agents employed ingenolmebutate appears to be more effective due to the shorter time of treatment.

e) In a community-based pragmatic trial of sunscreen (SPF 15+) to prevent skin cancer in Queensland, Australia; 1621 randomly selected residents of Nambour (Age 25–75) were randomly assigned to daily or discretionary sunscreen application to the head and arms for 4 years and participants were observed for 10 years. The reduction in invasive melanoma was substantial (n = 3 in active vs. n = 11 in the control group). Findings suggest the general preventability of melanoma after the regular application of broad-spectrum sunscreen (Green *et al.*, 2011).

f) The use of Bacilli-Calmette-Guerin (BCG) for treatment and prophylaxis of carcinoma *in situ* of the urinary bladder, and for prophylaxis of primary or recurrent stage Ta and/or T1 papillary tumors after transurethral resection (Sylvester, 2011), and use of valrubicin in BCG-refractory carcinoma *in situ* of the urinary bladder in patients for whom

immediate cystectomy would be associated with unacceptable morbidity or mortality have been reported (Steinberg *et al.*, 2000). Similarly, use of photofrin plus photodynamic therapy developed largely as adjuvant therapies for treatment of pre-invasive neoplastic lesion and for esophageal dysplasia have also been approved (Davila, 2011; Overholt *et al.*, 2005).

g) In a study involving the use of celecoxib for prevention of lung cancer in former smokers, emerging evidence shows that long-term use of celecoxib is associated with significant cardiovascular risk, and hence the study was suspended. Following reopening of the study, impressive response to celecoxib in a subset of patients was observed. As the study used small number of patients, it was difficult to generalize the results with confidence (Mao *et al.*, 2011). Based on this and other studies, celecoxib and other similar agents may need further evaluation as a chemopreventive in former smokers with high-risk lesions, and/or in individuals with high risk of developing colon cancer and low cardiovascular risk.

h) The androgen receptor blockers continue to be of interest for the prevention of prostate cancer. Finasteride that lowers the concentration of dihydrotestosterone by blocking 5α-reductase type 2, was tested in prostate cancer prevention trial (PCPT). In the phase III design, 18,882 men were randomized to receive finasteride versus placebo (Foley and Kirby, 2003). There was a reduction in prostate cancer prevalence of the order of 25% during a 7 year follow-up period, but apparently also an increase in high-grade tumors (Thompson *et al.*, 2003). Subsequent careful analysis showed that the excess high-grade tumors were probably due to biopsy artifacts (Logothetis and Schellhammer, 2008; Lucia *et al.*, 2007; Redman *et al.*, 2008). A similar reduction in prostate cancer has been reported with dutasteride – a dual 5α-reductase inhibitor (type 1 and 2) in a placebo-controlled trial, wherein 6729 men showed 23% reduction in the risk of prostate cancer at the end of 4 years with no apparent increase in high-grade cancer (Andriole *et al.*, 2010).

i) In a Physician Health Study, PHS-II, 14,641 male physicians were randomly assigned to receive either a daily multivitamin supplement or a placebo for a median of 11 years. Multivitamin supplements were associated with an 8% relative decrease in cancer incidence. The overall reduction in cancer risk was more pronounced in men diagnosed with cancer before the study began than those with no history of cancer. The study suggested that the small benefit of multivitamins in reducing overall cancer incidence largely stemmed from the prevention of second

primary cancer (Gaziano *et al.*, 2012).

Interventions demonstrating lack of chemopreventive efficacy and/or toxicity

a) Earlier studies (Hong *et al.*, 1986; 1990; Meyskens *et al.*, 1994) with high doses of retinoids for its effects on oral intraepithelial neoplasia (IEN) and cervical IEN (Hong *et al.*, 1986; Meyskens *et al.*, 1994) suggest a protective role by prevention of secondary head and neck malignancies (Hong *et al.*, 1990). Based on these initial observations of protective effects of β-carotene, the alpha tocopherol and β-carotene (ATBC) prevention study was undertaken. In this study > 29,000 Finnish male workers (age 50–60 years) were randomized to one of the four groups: α-tocopherol (vitamin E), β-carotene (a precursor of vitamin A), both α-tocopherol and β-carotene, or placebo with a 5–8 year follow-up. Men who took β-carotene alone or in combination with vitamin E had an 18% increased incidence of lung cancer and an 8% increase in overall mortality; whereas vitamin E alone had no effect (ATBC Prevention Group, 1994).

   The CARET trial that was a double-blind, placebo-controlled study enrolled > 18000 male and female smokers, former smokers and asbestos-exposed workers to study the effects of β-carotene and retinyl palmitate (vitamin E) or placebo on lung cancer and cardiovascular disease. The trial was stopped early after an interim analysis showed a 28% increase in lung cancer incidence and a 17% increase in overall mortality in the treatment group (Omenn *et al.*, 1996). Subsequent trials employing lower/less toxic doses of retinoids evaluating its effect on cancer and other endpoints have been negative (Decensi *et al.*, 2000; Lippman *et al.*, 2001). Thus retinoids have not been successful in reducing the cancer risk.

b) In selenium and vitamin E cancer prevention trial (SELECT), > 35,000 men (Caucasians aged > 55 years; African Americans aged > 50 years) from US, Peurto Rico and Canada were randomized to treatment with vitamin E and selenium together or placebo. The study was terminated earlier due to lack of efficacy in interim analysis (Klein *et al.*, 2003). Continued follow-up of study participants showed a 17% increase in prostate cancer risk in healthy men receiving vitamin E alone (Klein *et al.*, 2011).

c) The results of the Physicians' Health Study II demonstrated that supplementation with vitamin E and/or vitamin C had no benefit compared with placebo in preventing either prostate cancer or total cancer incidences (Gaziano *et al.*, 2009).

d) The results of the Women's Antioxidant Cardiovascular Study indicated that,

compared with placebo, supplementation with vitamin C, vitamin E or β-carotene was not efficacious in reducing the total cancer incidence (Lin *et al.*, 2009). In the same study, daily supplements containing folic acid, vitamin B6 and vitamin B12 as compared to placebo was not efficacious in reducing the overall risk of developing cancer (Zhang *et al.*, 2008). An exploratory analysis of pooled data from two Norwegian randomized controlled trials showed an increase in both cancer incidence and cancer death in patients treated with folic acid and vitamin B12 versus those receiving placebo or vitamin B6 alone (Ebbing *et al.*, 2009).

e) Evidence on the efficacy of vitamin D supplements (400–1100 IU daily) with or without calcium in preventing cancer incidence is available as a secondary endpoint from randomized controlled trials. All the trials showed lack of efficacy (Avenell *et al.*, 2012; Chung *et al.*, 2011).

A recent search of 'clinicaltrials.gov' showed a list of 280 on-going/completed chemoprevention trials. The majority of the chemopreventive agents fail to achieve endpoint results in randomized clinical trial settings due to lack of efficacy and/or unexpected toxicity. Most of the agents demonstrating preventive effects in experimental models have failed to exhibit chemopreventive effects in clinical trials. These failures can broadly be attributed to lack of (a) ability to replicate the conditions of human exposure (route, dose, sequence and frequency, duration etc.) and other host factors in animal models; and (b) knowledge about the mechanism(s) of action and toxicity of the agent on normal physiological processes in different organ systems (Patel *et al.*, 2007).

In spite of numerous chemoprevention trials, the number of successful or approved agents is rather small (Table 3). This is partly due to the challenges and barriers including (a) choice of cohorts – including difficulties in identifying and recruiting participants, which influences the outcome of the trial by affecting time lines, statistical power and adherence; (b) agent(s) with powerful efficacy in preclinical studies, selection of optimal doses, route, duration and frequency and toxicities; and (c) endpoint including the long latency period to cancer endpoints, selection of biomarkers as surrogates and accessibility to the target organ(s). The difficulties lead to very high costs, extended follow up periods and complexity in assessment of risk-benefit of the cancer risk reducing drugs (Table 3).

There is dire need to (a) improve existing experimental models or develop new experimental models/approaches to achieve better replication of human host factors and/or exposure conditions; (b) generate adequate information about the mechanism(s) of action of observed chemopreventive efficacy and/or toxicity; (c) study, understand and if feasible exploit the role of diet, calorie content and

Table 3: Agents for treating precancerous lesions and/or reducing cancer risk

Target Lesion/Organ	Treatment Agent	Target
Actinic keratosis	5-fluorouracil	DNA synthesis?
	Diclofenac sodium	Synthesis of prostaglandins?
	Imiquimod	Toll-like receptors 7-8, NFkβ?
	Ingenolmebutate	Mitochondria, Neutrophils?
	5-aminolevulinic acid + Photodynamic therapy	Damage to cellular machinery by ROS?
Bladder dysplasia	Bacillus Calmette-Guerin	Cellular immune machinery?
	Valrubicin	Not established
Breast cancer	Tamoxifen	Estrogen receptors
	Raloxifen	Estrogen receptors
	Exemestane	Aromatase enzyme (CYP450, CYPC19)
Cervical IEN and cancer vulvovaginal, anal IEN and cancer	HPV vaccines	
	Gardasil	HPV types 6, 11, 16, 18
	Cervarix	HPV types 16, 18
Colorectal polyps, adenomas and cancer	Aspirin	Cyclooxygenases
	Celecoxib	Cyclooxygenase-2
	Rofecoxib	Cyclooxygenase-2
	Sulindac	Ras pathway
	DFMO + Sulindac	Polyamine biosynthesis; Ras pathways
Esophageal dysplasia Preinvasive neoplastic lesion	Photofrin + PDT	Procaspase-3?
Hepatocellular carcinoma (HCC)	HBV vaccine	HBV
	Engerix-B	HBV-DNA
	Recombivax HB	HBV-surface antigen
Prostate	Finasteride	5-α-Reductase-type 1
	Dutasteride	5-α-Reductase-type 1+type 2
Skin (Melanoma)	Sunscreen (SPF 15+)	Blocking UVB (92%)

DMFO = Difluoromethylornithine, PDT=Photodynamic therapy, IEN=Intraepithelial neoplasia

physical activity (singly and in combination) on the standard agent mediated responses in humans. This is based on the extensive experimental and epidemiological observations on the protective effects of caloric restriction (Hursting *et al.*, 2010; 2013), diets rich in fruits and vegetables and moderate physical activity (World Cancer Research Fund/American Institute for Cancer Research, WCRF/AICR, 2007). Evidence on the protective effects of 'aspirin', 'statins' and 'metformin' in epidemiological studies further

suggest the role of carcinogen-mediated perturbation of common physiological pathways in cancer causation. Evidence generated may help in improving the planning, execution, monitoring and interpretation of outcomes from clinical trials. While consolidating on several of these aspects, efforts to search and exploit novel concepts such as 'synthetic lethality' (Davis and Wu, 2012) that has the potential to address both the issue of toxicity and efficacy in chemoprevention may also be undertaken.

This concept takes the advantage of the specific genetic changes in the precancerous cells to target them for destruction without harming the normal cells. Briefly, utilizing synthetic lethality approaches, chemo-preventive agents are delivered systemically, causing apoptosis selectively in premalignant cells without harming the normal cells, thus substantially decreasing toxicity. Furthermore, treatments are given for a short period of time, followed by intervals of drug-off periods. This method has been demonstrated to be effective in a mouse model of familial adenomatous polyposis (FAP) (Zhang *et al.*, 2010) as well as a mouse model of K-ras driven lung cancer (Huang *et al.*, 2011). Thus, targeting driver mutations with short-term Intermittent Therapy to Eliminate Premalignancy (SITEP) holds great promise for the future of chemoprevention.

Overall, clinical evaluation based on mechanistic studies, gene-environment interactions and complementary nutritional studies constitute the major thrust areas in chemoprevention research.

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CONFLICT OF INTEREST

The author claims no conflict of interest.

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