

**The challenges for cancer prevention**

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The Challenges for Cancer Chemoprevention

Abstract

The incidence of cancer is rising in parallel with an ageing population thus increasing the strain on both treatment options and budgets for healthcare providers worldwide. New cancer therapies are being developed but at what cost? The new treatments are expensive and poor survival rates still exist for some cancers. What is needed now is to prevent or at least limit the disease occurring in the first place. This review evaluates the current situation and the progress in upcoming strategies as well as suggesting some areas for further research within the increasingly important field of cancer chemoprevention. The key principles of cancer chemoprevention are discussed and areas for improvement highlighted. Despite significant progress, chemoprevention has not been widely adopted. Cancer chemoprevention has many challenges to face but this only emphasises the size of the task. These hurdles include a lack of awareness of the benefits, a lack of interest and a lack of investment in taking prevention forward. Despite the huge potential importance of cancer prevention and clinical success stories such as the well-publicised HPV vaccine, the challenges remain significant. With cancer and its treatment being a global issue, the opportunities offered by chemoprevention must be re-evaluated and uptake of chemoprevention actively encouraged. If chemoprevention is to be adopted successfully, a holistic approach is required. This approach will involve multidisciplinary teams of healthcare providers and scientists with the big challenge particularly for medicinal chemists being to design and synthesise the ideal chemopreventative agent.

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Introduction

Cancer treatment and its cost

Cancer is a major health problem with 14.1 million new cases and 8.2 million cancer deaths worldwide in 2012¹. Treatments for cancer have improved significantly over the last two decades with more effective drugs with better safety profiles and precise molecular targeting of the treatment options. Despite these improvements unwanted side effects are still a major problem as are the poor survival rates for certain cancers such as lung cancer¹. The worldwide the incidence of cancer continues to rise as, unfortunately, does the cost of new treatments. Put simply, newer cancer treatments are expensive. For example, the tyrosine kinase inhibitor, Ponatinib, used for treatment of chronic myeloid leukaemia costs approximately \$140,000 (USD) per patient per year². The average course of cancer treatment with a non-hormonal drug cost 34% per GDP capita in 1995-1999, 53% in 2000-2004 and 67% in 2005-2009³. This trend is accelerating and is limiting the number of treatment options approved by the National Institute for Health and Care Excellence (NICE) in the UK. An alternative approach would be to prevent cancers developing. If the occurrence of cancer could be prevented or reduced some of these expensive treatment options could be avoided. It would, therefore, be of huge benefit to healthcare providers worldwide if this increasing incidence could be halted, decreased or more importantly prevented.

Currently, the majority of anticancer drugs are antiproliferative, preventing cell growth and division but generally having little effect on invasion and metastases. There are more than four hundred different drugs used in therapy and all of these have unwanted side effects to a greater or lesser extent. Despite many individual and combinatorial anticancer treatments offering potent therapy, these intense treatments are all limited by long term and short

term side effects of the drug treatment. A number of drugs which are targeted more selectively to specific cancers have been developed recently which has improved outcomes for patients by decreasing the side effects. Combining these with the concept of stratified medicine has also resulted in benefits. Stratified medicine aims to select patients so that those who will benefit from drug A will be given drug A while those who will benefit from drug B will be prescribed drug B. This constitutes the “5 Rights”:

- the right drug
- the right disease
- the right patient
- the right time
- the right dose

While these strategies are advantageous, the alternative to treating a cancer once it has developed is to prevent it occurring i.e. chemoprevention. The idea of chemoprevention is highly desirable not only from the point of view of the patient but also from the healthcare providers worldwide who are under financial pressure to provide therapy.

The first chemopreventative agent approved by the FDA was tamoxifen in 1998⁴. However, the concept of chemoprevention has not been adopted widely or welcomed. This review will discuss progress within this field since the discovery of tamoxifen’s preventive effects in breast cancer. It will also highlight the potential for chemoprevention and encourage its adoption in the future.

What is chemoprevention?

It is the use of pharmacological agents (synthetic or natural) to prevent the development of disease (in this case cancer) in an individual. Chemoprevention is typically described under the categories of primary, secondary and tertiary chemoprevention (Table 1).

Table 1: Classification of chemoprevention

Type of Chemoprevention	Definition
Primary Chemoprevention	Preventing high risk cohorts of the population developing precancerous markers and cancer
Secondary Chemoprevention	Preventing the development of precancerous markers/lesions into cancer
Tertiary Chemoprevention	Preventing the recurrence of cancer

Table 1: A brief description of each individual classification of chemoprevention

One good example of chemoprevention in use currently is the use of low dose aspirin in the prevention of myocardial infarction in patients with cardiovascular disease⁵. This is an example of secondary prevention where patients have already experienced a cardiovascular event and aspirin is used to prevent another potentially more serious occurrence.

A chemopreventative agent needs to have certain characteristics as it is intended to be taken for extended periods of time or for life. This lays down a challenge to medicinal chemists to design the ideal agent with the following characteristics:

- low toxicity
- little or no side effects
- effective in low dose
- easy to administer (take)
- readily available
- cost effective

Many agents, both natural and pharmaceutical, have been investigated for their ability to match these desired criteria for a successful chemopreventative against cancers.

Current Chemopreventive Strategies

Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are some of the most widely used drugs worldwide with 30 million people taking an NSAID daily and over 111 million NSAID prescriptions issued in the USA annually^{6,7}. Most NSAIDs have 3 characteristic properties: they are anti-inflammatory, anti-pyretic and analgesic. NSAIDs exhibit their effects by the inhibition on the synthesis of prostaglandins by blocking the enzyme, cyclo-oxygenase (COX)⁸. There are two common forms of COX known as COX1 and COX2. COX1 is constitutive and produces prostaglandins that are required for normal cellular functions whilst COX2 is associated with the synthesis of prostaglandins involved in the inflammatory response. A link between inflammation and cancer has been shown thus NSAIDs might be expected to influence cancer development and/or progression. As yet it is not clear whether inflammation is causal or simply an association but the linkage is powerful. COX2 dependent prostaglandin E2 (PGE2) synthesis is suggested to be important for many hallmarks of cancer such as mutagenesis, angiogenesis and immunosuppression (reviewed extensively by Harris, 2007). For example, COX2 upregulation alone in animal models has been shown to cause the transformation of normal cells. Many cancer associated environmental factors such as smoking tobacco are highly associated with COX2 upregulation and these known effects have been well studied⁹. Additionally, COX2 over expression has also been found to increase intracellular telomerase levels which are also associated with tumourigenesis, proliferation and cellular immortality¹⁰. This growing body of evidence suggests that if COX2 was inhibited effectively then cancer could be prevented. This can, theoretically, be achieved by long-term, regular use of NSAIDs and this is supported by a large meta-analysis. The meta-analysis shows that regular use of over the counter NSAIDs, reduces the risk of certain cancers significantly (figure 1).

Figure 1: Reduction of cancer risk with taking regular NSAIDs

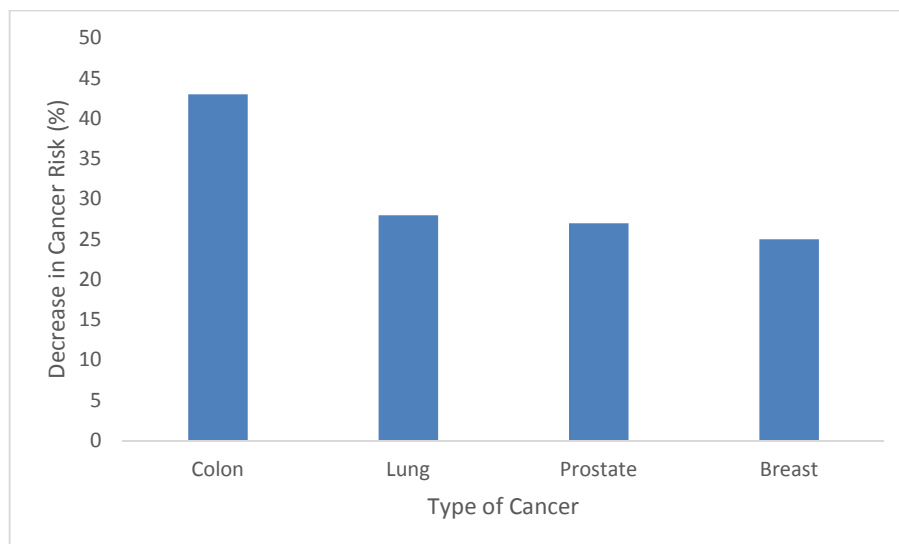


Figure 1: Regular NSAID use was defined as 325mg aspirin or 200mg ibuprofen more than twice a week. Meta-analysis is based on the following number of studies: Colon – 32, Breast – 33, Lung – 18 and Prostate - 17 (Redrawn from Harris, 2009).

This is further supported by the fact that daily intake of aspirin (which has greater COX1 selectivity) reduced the composite risk of these 4 malignancies by 54%, ibuprofen (equally inhibitory for COX1 and COX2) by 60% and celecoxib (COX2 selective) by 70%¹¹. The latter figure indicates that COX2 has potential to be a target for chemoprevention. As these 4 malignancies total more than half of the cancer deaths in UK and USA, this emphasises the need for chemoprevention against colon, breast, prostate and lung cancer.

NSAIDs show clear efficacy as chemopreventive agents and one of the most investigated examples is aspirin. Meta-analysis of 5 large UK trials has shown that daily aspirin halves the risk of metastatic adenocarcinoma of the colorectum and reduces the risk of distant metastasis by 30-40% when compared to a no aspirin control group¹². NSAIDs have also shown potential with regards to tertiary chemoprevention. Aspirin has been shown to significantly reduce recurrent colorectal carcinomas in patients who have previously had colorectal cancer^{13,14}. A recent study of over 15,000 patients has also shown that NSAIDs can significantly reduce hepatocellular carcinoma recurrence¹⁵. Importantly, aspirin also matches key criteria of an ideal chemopreventive agent. For example, they are taken orally which allows simple dosing regimen and are mass produced globally therefore they are

readily available/accessible. Equally as important, they are cost effective being only a fraction of pence per tablet.

Despite these many advantages, NSAIDs have the major disadvantage of having some significant side effects associated with long-term use which may limit their utility as chemopreventative agents. The gastrointestinal side effects of aspirin are well known. A meta-analysis of 24 trials of almost 66,000 subjects showed that 2.5 % of long term aspirin users (minimum 1 year use) vs 1.4 % of placebo group suffered from a GI haemorrhage which was considered significant¹⁶. Inhibition of COX1 is linked to the gastric side effects commonly found with NSAID therapy and this is supported by users of COX2 inhibitors showing significantly less risk of upper gastrointestinal bleeding when compared to users of non-specific COX inhibitors¹⁷.

Another example of potential long-term side effects was discovered in the high profile case of Vioxx (Rofecoxib), a selective COX-2 inhibitor. Vioxx was approved initially as a treatment option for acute pain and osteoarthritis in 1999¹⁸. However, during a chemoprevention based study it was noticed that the incidence of myocardial infarctions and ischemic cerebrovascular events was higher in the Rofecoxib group when compared to placebo. The number of serious thrombotic events was 77/1287 in the Rofecoxib group compared to 44/1299 in the placebo group. When this trend was confirmed by a meta-analysis the drug was withdrawn from the market¹⁹. Fortunately, these cardiovascular based side effects were deemed to be specific to Vioxx as a meta-analysis of 72 studies showed no evidence that dosing with the COX2 specific NSAID, celecoxib, increased CVD risk¹¹.

The use of NSAIDs in cancer prevention thus becomes a risk benefit challenge: a significant decrease in cancer development versus a potential GI bleed. As a result of this dichotomy NSAIDs are unlikely to be used universally for cancer prevention until a safer NSAID is designed but there may be role of their use in cases where there is a high risk of developing cancer. Despite this limitation associated with COX1 inhibition, the COX2 specific inhibitor celecoxib has been shown to be more effective with regards to reducing cancer risk and reducing GI bleed risk when compared to non-specific NSAIDs. With growing evidence of a positive risk - benefit ratio to prevent a wide range of cancers, NSAIDs may yet be used

clinically in the future in cancer chemoprevention. With aspirin being off patent, well studied, cheap and with clinical benefits that are only becoming clearer, one wonders why it has not been used routinely in a clinical setting for cancer chemopreventive purposes. Despite GI bleeding offering a disadvantage, major handicap to the use of aspirin or aspirin derivatives in prevention is the lack of interest and investment from pharma with their preferred option still to fund cancer therapy rather than prevention due, in the main, to the potential financial gains for the companies involved.

Specific breast cancer chemopreventative agents

Oestrogen plays an integral role in human physiology. This includes vasomotor control, increasing 'good' cholesterol, maintaining bone density and controlling the synthesis of key proteins involved in growth and development. When this hormonal signal is deregulated increased growth i.e. cancer occurs^{20,21}. This led to the original hypothesis that cancer initiation and promotion could be inhibited if the concentration of oestrogen could be controlled. With the role of oestrogen in breast cancer induction being well established, both selective oestrogen receptor modulators (SERMs) and aromatase inhibitors have been used clinically for effective breast cancer chemoprevention.

Selective oestrogen receptor modulators (SERMs)

The class of compounds called selective oestrogen receptor modulators (SERMs) compete and act upon the oestrogen receptor and exhibit effects dependent on the tissue. For example, the SERM, Tamoxifen, is an antagonist of the oestrogen receptor (ER) in breast tissue yet an agonist in bone and uterine tissue. Despite originally being designed as a contraceptive, Tamoxifen is now used as a chemotherapeutic option for early and advanced oestrogen receptor positive (ER+) breast cancer. Tamoxifen has also shown efficacy as a breast cancer chemopreventative agent and has been approved for this purpose by the FDA since 1998. The first large US cohort study of 13388 subjects published in 1998 showed Tamoxifen reduced the occurrence of ER+ invasive breast tumours by 69% and 49% in high risk women²². Tamoxifen has also been shown to be a useful tertiary chemopreventive agent with treatment of intraductal breast cancer with tamoxifen, lumpectomy and radiation therapy had 8.2% breast cancer events 5 years post treatment compared to 13.4% with lumpectomy and radiation therapy alone²³. Despite being FDA approved and being

deemed safe, there are side effects that limit its use and patient adherence. Some side effects noted were beneficial such as a 45% reduction of hip fractures and 26% reduction of spinal fractures in the original trial due to tamoxifen acting as an ER agonist in bones with osteoporosis and reduced bone density affecting many postmenopausal woman globally, this is a major benefit of Tamoxifen and SERMs. However, some rare, yet severe, side effects such as thromboembolic disease by 2-5 fold are increased in postmenopausal women taking Tamoxifen²². The incidence of uterine cancer is also increased due to Tamoxifen being an ER agonist in uterine tissue. Despite having a better safety profile in premenopausal women, Tamoxifen still plays an important role in modern day chemoprevention for both pre and postmenopausal women.

With a small increase in the incidence of uterine cancer and thromboembolic events in postmenopausal women who were administered Tamoxifen, other SERMs have been investigated in an attempt to improve upon the safety profile of tamoxifen. One example is Raloxifene. The initial idea of using Raloxifene as a chemopreventative agent came from the data of the 1999 MORE trial which was testing its use in women with osteoporosis in order to reduce fractures. Its secondary endpoint was invasive breast cancer and it was concluded that Raloxifene reduced the risk of ER+ breast cancer by 72% over 4 years in postmenopausal women²⁴. A study published in 2009 called **Study of Tamoxifen And Raloxifene (STAR)** compared the chemopreventative profiles of 60 mg/day raloxifene vs 20 mg/day tamoxifen in high risk postmenopausal women. With 19,747 participants over the 5 year project, it was shown that Raloxifene was almost equal to tamoxifen with regards to reducing risk of invasive breast cancer (incident of breast cancer per 1000: was 4.30 in the tamoxifen group and 4.41 in the Raloxifene group). Despite the similar efficacy of the two agents, Raloxifene reduced significantly the risk of thromboembolic events and uterine malignancy when compared to Tamoxifen²⁵. The reduction in uterine malignancy is likely due to the fact Raloxifene is an ER antagonist in the uterus whereas tamoxifen is a partial agonist. Despite being approved by the FDA and showing good efficacy, there is still room for improvement with common side effects such as hot flushes, gallstones and thromboembolic events. Third generation SERMs have also been investigated such as Arzoxifene and Lasofoxifene. Despite both these drugs reducing invasive breast cancer in postmenopausal women by 56% and 83%, showing good tolerability and reducing the

effects of osteoporosis, both SERMs increase the incidence of thromboembolic events^{26,27}. Lasofoxifene is, however, deemed to be a promising agent for the future not only due to its significant efficacy in breast cancer prevention but also because it showed benefits for vertebral fractures, cardiac events and stroke with no increase in endometrial cancer that is associated with the other SERMs.

Aromatase inhibitors

SERMs are not the only pharmacological agents that can effectively alter the proliferative and carcinogenic properties of oestrogen in postmenopausal women. With aromatase being a key enzyme responsible for the biosynthesis of oestrogen in these women, aromatase inhibitors such as Exemestane and Anastrozole have shown efficacy as chemopreventative agents. It was shown that Exemestane was an effective chemopreventive agent against breast cancer in postmenopausal women who were classed as average to high risk of breast cancer development. The 35 month study in 4560 women concluded that daily Exemestane (25 mg/day) treatment reduced breast cancer risk by 65% when compared with a placebo treatment. Adverse events were higher in the Exemestane group (88% compared to 83% in placebo) which was considered statistically different. Despite this difference, the side effects were considered minor and there was no association with major side effects such as cardiovascular events or skeletal factors. It was concluded that only minimal quality of life differences could be seen and this tended to be a heightening of menopausal symptoms and mild bone density loss²⁸. This good safety profile is paramount for chemoprevention and is improved by the excellent efficacy of a drug. The patent on this drug has expired thus the agent will be available generically and so it will meet one of the key criteria – low cost.

Another aromatase inhibitor, Anastrozole, has just completed an international, double-blind, randomised placebo-controlled trial for breast cancer prevention in high risk postmenopausal women. In a cohort of nearly 4000 women, it was shown that Anastrozole halved the risk of breast cancer diagnosis from 4% to 2% when compared to placebo over a 5 year period. This significant efficacy has been shown before across the pharmaceutical agents mentioned but what makes Anastrozole more attractive is its superior safety profile. Both placebo group and Anastrozole showed 89% adverse events across the 5 year period

with none attributable to the treatment group being serious. Side effects deemed significantly increased in the treatment group included minor side effects such as dry eyes, vasomotor symptoms and musculoskeletal adverse events ²⁹.

It can be seen that there is now a wealth of tried and tested options for breast cancer chemoprevention. With safety being paramount for the success of chemoprevention, aromatase inhibitors may begin to be more widely adopted compared to the current FDA approved SERMs such as tamoxifen. Just to reiterate, tamoxifen is currently the chemopreventative agent of choice for premenopausal high risk women and is the only FDA approved option for this cohort of women. As previously shown with NSAIDs, chemoprevention has a delicate and complex balance between risk and benefit.

Specific skin cancer chemopreventative agents

Chemoprevention of skin cancer would be of great benefit due to its high prevalence with one in five Americans developing skin cancer over their lifetime ³⁰. Chemopreventative agents for skin cancer aim to treat precancerous skin called actinic keratosis i.e. secondary chemoprevention. Actinic keratosis are caused by damage from ultraviolet light and approximately 10% of these lesions develop into squamous cell carcinomas. Fortunately, skin is readily accessible compared to other targets for chemoprevention thus allowing a much more direct pharmaceutical approach i.e. topical therapies rather than oral dosing. This offers the major advantage of limiting “off target” or side effects. Current FDA approved examples include Fluorouracil cream, Imiquimod cream, sodium diclofenac gel and ingenol mebutate gel.

Fluorouracil cream

As thymidine is a necessary nucleotide for DNA synthesis and growing cells, Fluorouracil cream offers a chemoprevention possibility. Fluorouracil acts as a natural nucleotide undergoing ribosylation and phosphorylation effectively acting as a pyrimidine (thymidine) analogue. By binding and inhibiting thymidylate synthetase which is necessary to convert deoxyuridine nucleotides to thymidine nucleotides, fluorouracil inhibits DNA synthesis³¹.

One study showed that a 4 week treatment (0.5%) on facial actinic keratosis achieved total clearance of the actinic keratosis lesions in 43% of patients. Despite being deemed effective there are side effects including crusting oozing, burning, pain and erythema which can be severe³².

Imiquimod cream

Imiquimod is classed as an agonist of toll like receptor 7 (TLR 7) which activates cell mediated immunity through specific T cell activation. This is achieved by inducing interferon and cytokines through the innate immune system. Apoptosis of the actinic keratosis cells is increased by activating an immune response. A phase 3 trial showed Imiquimod to be effective as a topical treatment option for superficial basal cell carcinoma which was followed soon after by FDA approval³³. Initially approved for genital warts in 1997 and subsequently approved for basal cell carcinoma FDA approved Imiquimod 5% cream for the treatment of actinic keratosis in 2004. The efficacy and safety of Imiquimod 5% cream treatment for actinic keratosis was evaluated in a multicentre trial. It was concluded that imiquimod reduced target lesion count by 80.2% and had a complete clearance rate of 36.4%. The treatment was deemed well tolerated but there was a high dropout rate of 28.1% and common side effects did include local skin reactions which were evaluated as severe³⁴. Imiquimod cream 3.75% (Zyclara) approved in 2010 by the FDA and has offered slightly lower side effect incidence and a similar efficacy over a twice a week dosing over 16 week period.

Sodium diclofenac gel

Despite sodium diclofenac being classed as a COX-2 specific NSAID (mechanism and benefits previously discussed), the exact mechanism of actinic keratosis treatment is not fully understood but has been shown to be effective. A trial of 96 people showed sodium

diclofenac 3% gel application twice a day for 3 months on to actinic keratosis showed a 100% clearance rate in 50% of the subjects. Pruritus, dry skin and application site reactions were some of the more common side effects (Side effects: 64% placebo vs 79% treatment) but were minor and no major adverse effects could be seen. The trial concluded the gel to be efficient and well tolerated ³⁵.

When important chemopreventative factors are weighed against each other including cost, effectiveness and tolerability, diclofenac 3% tends to be the treatment of choice ³⁶.

However, the fact that Imiquimod becomes a generic drug in 2015 should be considered as this will lead to a significant price reduction.

Ingenol mebutate gel (Picato)

Recently FDA and EMA approved in 2012, Picato (0.015% ingenol mebutate gel) which offers another exciting chemopreventative treatment option for actinic keratosis. It was approved after a successful phase 3 trial showed Picato to completely clear actinic keratosis in 42.2% of patients vs. 3.7% in the placebo group. The most predominant advantage lies in the fact that this treatment takes 3 days rather than months which is an important factor for better compliance. Adverse events were similar to those observed with the other treatments reviewed but were deemed very mild. These include redness, scaling and crusting and their minor nature will also encourage compliance. The fact these side effects occur after the 3 day treatment rather than during the treatment period supported a low dropout rate. The trial concluded with a study completion rate of an excellent 98% of the initial patients ³⁷. With good efficacy and tolerability, it is likely that Picato will have a major role in preventing the development of actinic keratosis into squamous cell carcinoma in the present and future.

Despite the therapeutic benefit of Picato being clear, the mechanism of action remains unknown. Recent evidence has suggested ingenol mebutate gel works through a 2 step process. The 1st step consisting of rapid lesion necrosis which occurs in the 1-2 hour period after application. The 2nd step is a lesion specific immune response which occurs in the days after application. More specifically, this phase consists of neutrophil mediated antibody dependent cellular cytotoxicity meaning B cells produce antibodies against specific antigens upon dysplastic epidermal cells and neutrophil receptors. Specific binding activates the

'killing mechanism' of the activated neutrophils which releases cytotoxic molecules such as reactive oxygen species to destroy the dysplastic epidermal cells³⁸.

In conclusion, for secondary chemoprevention of actinic keratosis, there are effective and relatively safe agents which are improving steadily especially in terms of tolerability. Despite chemoprevention becoming an effective reality with regards to actinic keratosis treatment, protection from UV light to limit their prevalence should be actively encouraged.

Chemoprevention of cancers associated with infectious agents

In 2008, there were 12.7 million new cancer cases and infectious agents including bacteria, viruses and parasites were deemed to be the causative factor in 2 million of these (16.1%)¹. Certain infectious agents offer strong risk factors for specific cancers e.g. human papilloma virus is attributed to 100% of all cervix uteri carcinomas. 660,000 cases of cancer worldwide are attributed to *H. Pylori* which include 86% of all gastric non Hodgkin lymphoma and 90% of all non-cardia gastric cancers^{39,40}. The pharmacological eradication or vaccination against these specific causative agents would allow a large proportion of selected cancers to be prevented.

Human papilloma virus (HPV)

610,000 cases of cancer worldwide are attributed to the human papillomavirus including 70% of all vaginal carcinomas, 70% of all penile carcinomas, 88% of all anal carcinomas, and 100% of all cervix uteri carcinomas. HPV has also been shown to be a causative factor in oropharyngeal cancer with 56% of cases being attributed to HPV in USA. The specificity of this virus is highly desirable as vaccination offers a very effective method of chemoprevention. Despite there being over 140 different identified strains of HPV, not all are associated with increased cancer risk. Epidemiological studies have identified 18 different high risk HPVs with HPV 16 being most predominant which is found in 61% of cervical cancer cases^{39,40}.

Due to HPV-16 being the highest risk strain, a HPV 16 L1 virus like particle vaccine was designed and was tested in a trial of 2392 women aged 16-23 across 16 US centres. After

nearly a year and a half, the results showed the vaccine to have 0 incidence of persistent HPV 16 infection per 100 women years compared to 3.8 per 100 women years of the placebo group. There were 9 cases of HPV 16 related cervical intraepithelial neoplasia which were all in the placebo group with adverse events being non-significantly different in the vaccine and placebo groups ⁴¹.

Another similar trial of 1113 women aged 15-25 in North America and Brazil stated that HPV 16/18 vaccination could prevent 70% of cervical cancers worldwide as HPV 16 and HPV 18 are responsible for 60% and 10% of cervical cancers respectively. The double blind trial used a bivalent HPV 16/18 L1 virus like particle vaccine and was shown to be 93.9% efficient against the cytological abnormalities associated with HPV 16/18 infection. It was also shown to be 100% efficient against persistent infection of HPV 16/18 and treatment was deemed to be well tolerated, highly immunogenic and safe ⁴².

In 2007, Australia introduced a free school based vaccination system using the quadrivalent HPV vaccine (6,11,16,18) called Gardasil. By 2012, a coverage rate of 69% of 18-19 year old women and 70-72% of 16-17 year old females was achieved. It will take decades to establish if Gardasil has prevented cervical cancer but HPV is a known cause of genital warts which are a more accessible measurement over a shorter time frame. It was found that there has been a significant decrease of 61% of genital wart incidence in 15-27 year olds since the vaccination programme begun in 2007 ⁴³. This shows preliminary efficacy over a national cohort of women. Since the FDA approval of the HPV vaccination against HPV 6, 11, 16 and 18 with Gardasil in 2006, 2 other vaccinations have been approved including Cervarix in 2009 which protects against the HPV strain 16 and 18 and Gardasil 9 which was approved in 2014 which protects against 9 HPV strains (6, 11, 16, 18, 31, 33, 45, 52 and 58).

Despite an increase in the number of FDA approved vaccinations against HPV, it should be mentioned that there are side effects. 92.4% of the total 25,176 adverse events were considered not serious. Despite an increase in pulmonary emboli and syncope, 90% of these subjects already had associated risk factors for blood clots thus the vaccine has not been deemed responsible. There has also been 83 associated fatalities including 2 unexplained variants of amyotrophic lateral sclerosis that resulted in the death of 2 young females but there were no pattern to the fatalities. It should be stated that these number of events are

small as between June 2006 and March 2014 there were 67,000,000 vaccinations administered nationally in America ⁴⁴.

Over one hundred countries have accepted these vaccination programmes and are beginning to extend these to males aged 9-26. This is due to many factors including the association of HPV with genital warts, penis tumours, oropharyngeal cancer and anal cancers being discovered thus vaccination offering a lot more than just cervical cancer prevention ⁴⁵.

Helicobacter Pylori

The link between *Helicobacter pylori* and its association as a risk factor for cancer has been known for over 2 decades. The initial findings suggested that the risk of gastric cancer increased 6 fold in subjects with a *H. Pylori* infection when compared to no infection ⁴⁶. The first study of *H. Pylori* eradication showed that no overall reduction of cancer or precancerous physiology was seen but it did conclude that *H. Pylori* eradication reduced the number of gastric cancer incidence when compared to placebo thus deeming it a causative factor of initiating gastric cancer. However, it does not appear to play a role in the actual tumour progression ⁴⁷. This highlights that if *H. Pylori* was to be eradicated, it could prevent initiation of most gastric cancers. A 15 year follow up study showed that *H. Pylori* eradication with Omeprazole and Amoxicillin reduced gastric cancer incidence by 39% ⁴⁸.

Unfortunately, as resistance is acquired, antibiotics are beginning to show reduced efficacy in *H. Pylori* eradication. In addition, antibiotics are ineffective in 20% of cases and relapse rates range from 15-30%. A true solution may lie in a vaccine based therapy but this approach has been fraught with difficulties. However, preliminary results from a phase 3 trial of an oral recombinant *H. Pylori* vaccine in 4464 Chinese children are exciting. The study showed the children aged 6-15 were protected against *H. Pylori* with 75% efficacy. The protection did drop to 65% but overall the protection was sustained for up to 3 years. What is equally important here is the lack of side effects. The placebo group saw more major and minor adverse events than the vaccinated group resulting in an excellent safety profile for this vaccine ⁴⁹.

The Future of Chemoprevention

A number of examples of the successful use of chemopreventative agents that do offer protection against certain cancers in high risk patients now exist providing “proof of concept” for cancer chemoprevention. However, the concept has not been adopted as widely as might have been expected with the major exception of the HPV vaccine. One possible reason for this is that chemopreventative trials tend to be long term studies and therefore expensive. A great deal of effort has gone into trials with nutraceuticals and many of these have proven to be ineffective or indeed harmful. For example, the β -Carotene And Retinol Efficacy Trial (CARET) aimed to reduce lung cancer and cardiovascular disease using a combination of β -Carotene and retinol (vitamin A). After an average of 4 years of supplementation, the study showed across 18314 smokers and workers exposed to asbestos that the supplementation actually appeared to increase lung cancer incidence and death rates whilst also increasing the risk of death from cardiovascular disease⁵⁰. Disappointing results such as this example have delayed investment in chemoprevention studies. In this review we have focused on pharmacological intervention. With new chemotherapeutic agents becoming increasingly more expensive to develop for limited benefit, the time is ripe for an alternative approach to cancer treatment and chemoprevention is now a realistic and much needed alternative.

The major problem with the adoption of chemopreventative agents is low uptake as highlighted by the study by Owens *et al.* in 2011. The American study identified that 15.2% of 5718 women who were considered high risk for developing breast cancer. Of this 15.2%, only a small fraction opted for screening, genetic testing or chemoprevention. In fact, of the 15.2% cohort of high risk women, only 50% appeared at the health center despite being referred for follow up consultation and a mere 2.0% were actually administered a chemoprevention agent⁵¹. With a growing library of chemopreventative agents available to high risk women, these numbers are too low. Despite some success to date, to be truly successful and a reality, chemoprevention must improve. Possible principles of current and future improvements include:

- Improving the perception of patients and healthcare providers.
- Improving the identification of high risk patients
- Improving the current system and reevaluating trial design
- Improving the chemopreventative agents

Improving the perception of patients and healthcare providers.

Worryingly, a recent meta-analysis of breast cancer chemoprevention decisions has shown little correlation between breast cancer risk and uptake. However, increased uptake does correlate consistently with patients with perceived vulnerability to breast cancer⁵². Despite being at high risk, individuals are usually healthy and the benefits of taking a drug with side effects, albeit minor, are difficult to see thus leading to a reluctance to risk current chemopreventative agents. Uncertainty in making the right choice is also a major issue within patient perception. Using SERMs as an example, although menopausal symptoms may seem like a minor side effect, SERMs are used for a 5 year period and these side effects may overshadow the potential benefit which is already difficult to determine. This uncertainty is also exacerbated by the fact that despite taking a chemopreventative agent, there is no guarantee that the cancer will be avoided or that cancer would not occur without chemoprevention. In spite of the complexity within the risk/reward choices involved with chemoprevention, the decision should be made with as much information and knowledge as possible. This clarity can only be achieved with the effective identification of patients within the various risk categories (high, intermediate, low). With some healthcare providers having doubts over current risk modelling, the appropriate and effective stratification of patients would be a positive step for chemoprevention.

Improving the identification of high risk patients

As mentioned, there are concerns over the accuracy and benefit of risk assessment software. One currently used example is the GAIL model which is used to calculate breast cancer risk. Its limitations are its simplicity but it does take into account key factors such as age, family and reproductive history, breast disease and race. This free software gives a

simple basis for risk assessment. It is far from a complete analysis but is the most used model in America and has been used widely and accepted across many trials⁵³. There are other options such as the IBIS model (Tyrer-Cuzick model) which is considered more useful for individuals with a stronger family history of the disease. It allows a calculation of invasive breast cancer risk and the chance of carrying a BRCA1 or BRCA2 gene. It includes a more complex insight into the more distant family history which takes into account the familial ovarian and breast cancer incidence as well as the age of diagnosis⁵⁴. Unfortunately, only 18% of physicians use any form of risk assessment software for breast cancer risk⁵⁵.

More novel systems are being developed to improve the identification of high risk patients. For example, a recently published study from Denmark has identified an efficient method of predictive screening of cancer risk in individuals by a biocontour. A biocontour can be defined as a mathematical pattern of phenotypic and biologic analysis. It is created through combining metabolic analysis of plasma and lifestyle information by analysing 27 individual variables, it can forecast diagnosis of breast cancer in patients several years ahead. It has shown early signs of validation through predicting a breast cancer diagnosis within 2-5 years. Excitingly, it shows early specificity and sensitivity of over 80% accuracy⁵⁶. Another recent study, has shown a strong link between blood telomere length (BTL) and cancer. By measuring the (BTL) of 792 participants between 1999 and 2012, it was shown that there was accelerated BTL shortening of participants who developed cancer. What was more striking was the fact that the BTL shortening in these patients halted 3-4 years before diagnosis⁵⁷.

These 2 exciting studies help identify people predicted to develop cancer years before a diagnosis. These individuals would be classed as very high risk and intervention would be appropriate and likely effective in this cohort.

Other patients who may be more interested in taking a chemopreventative agent would be those who suffer from syndromes/diseases which increases their risk of specific cancers. For example, due to germline mutations within DNA mismatch repair genes, Lynch syndrome is associated with a life time risk of 52%-82% for colorectal cancer⁵⁸. Since aspirin has shown significant benefit in reducing the development of colonic cancer then individuals with

Lynch syndrome deemed at high risk would be more likely to adopt the chemoprevention option ⁵⁹.

Improvements in the system and trial design

Chemoprevention trials are costly thus identifying markers of risk and surrogate markers of chemopreventative success are critical to moving this field forward. Using cancer incidence as the primary end point is a major obstacle for chemoprevention trials as it may take decades to obtain data. New drugs are protected by patents which last for 20 years. With the lengthy incidence based endpoints of chemoprevention trials, pharma are unwilling to invest in these studies. Early biomarkers need to be identified. This would also help bridge the difficult transitions between *in vitro* to *in vivo* and into man. Development of predictive markers to gauge the benefit and toxicity of treatment will be paramount for the future of stratifying chemoprevention studies.

Developing better chemopreventive agents

One simple way to improve chemoprevention in general would be to simply improve the quality and safety profile of the agents. Their potential can be improved through a number of different and novel approaches discussed below. These approaches include improving bioavailability of agents, improving target accessibility, combinatorial approaches and a medicinal chemistry approach for improving agents through molecular redesign.

Improving bioavailability

One class of agents that would benefit from this would be natural chemoprevention agents. Many of these agents meet key criteria such as tolerability, readily available globally, cheap and alter key signalling pathways involved in carcinogenesis (reviewed extensively by Saunders and Wallace, 2010) ⁶⁰. One recent example would be using the established benefits of green tea with regards to cancer prevention ⁶¹. The 10 year study showing that a combination of drinking green tea and supplementation can delay cancer onset in males by

3.2 years and females by 7.3 years. The tertiary prevention arm of the study showed that green tea can also reduce the recurrence of colorectal adenomas in polypectomy patients from 31% to 15%⁶². However, despite numerous advantages of these dietary and natural agents, many have poor bioavailability which limits their efficacy. One good example would be curcumin which offers chemopreventative potential by targeting and modulating various cell signalling molecules involved within cancer. These include modulation of pro inflammatory cytokines, apoptotic proteins, growth promoters, telomerase reverse transcriptase and reactive oxygen species⁶⁰. The main reason for the use of curcumin not being translated into clinical success is its limited bioavailability. Ways of improving bioavailability are being developed. For example, theracurmin is a nanoparticle formulation that aims to increase bioavailability and water solubility of curcumin and a phase 1 study showed a 210 mg dose of theracurmin has shown peak plasma concentration of 275 ng/ml⁶³. This is a marked improvement from previous curcumin bioavailability data. A previous phase 1/2 study for an 8 g/day dose of curcumin achieved peak plasma concentration of 134 ng/ml hence showing that this nanoparticle formulation can significantly increase the bioavailability of curcumin⁶⁴. Using a delivery system to improve efficacy could also benefit other natural or pharmacological chemopreventative agents. For example, oral dosing of Raloxifene has a poor median bioavailability of only 2%⁶⁵. Efforts have been made to improve this and a solid nanoparticle formulation of Raloxifene has been shown to increase peak plasma concentrations by over 3 fold when compared to free drug control⁶⁶. This is another excellent example of how current or potential agents with limited bioavailability can be improved upon with the use of “state of the art” drug formulations.

A further example of improving bioavailability would be dry frozen berries. As approximately 90% of berries are water, dry frozen berries offer a much stronger concentration of inflammatory modulating and anti-oxidant nutrients such as essential minerals, flavonoids and vitamins. These include, but are not limited to, ellagic acid, anthocyanins, folic acid, ascorbic acid and selenium thus giving dry frozen berries large potential as a multi-mechanistic chemopreventative agent⁶⁷. Following promising safety and animal data, a phase 2 trial gave 38 patients a 60 g/day dose of freeze dried strawberry powder for 6 months to prevent dysplastic lesions developing into oesophageal squamous cell carcinoma.

The histological grade of the premalignant lesion was significantly reduced in 80.6% of the patient group and was well tolerated with no serious side effects. Treatment also showed a reduction in expression of key markers such as COX-2 by 62.9%, iNOS by 79.5%, NFkB-p65 by 62.6% and Ki-67 (marker of proliferation) by 37.9%⁶⁸. Recently, freeze dried black raspberries have been shown to be effective in small clinical trials with regards to preventing the progression of premalignant lesions into oral and colon cancer^{69,70}. What is particularly exciting from these trials is not just the efficacy of various dry frozen berries but the excellent safety profile, accessibility and cost of this chemopreventative agents. All of these are essential criteria for long term cancer prevention.

Improving target accessibility

Another issue that needs to be addressed with chemoprevention is that it is limited by its lack of accessibility to some cancers. There are many chemoprevention strategies for skin cancer is due to the easy access for the chemopreventative agent. If agent delivery was improved and developed, it would only increase the bioavailability of the agent specifically to the site of interest thus increasing efficacy whilst limiting off target effects. For example, one recent study has used a demethylation agent called azacytidine delivered by aerosol to treat premalignant lesions of the lungs in mice. Demethylation in the context of DNA refers to the chemical process of removing a covalently bonded methyl group (CH₃) from the major groove of DNA thus demethylation agents can remove methyl groups that previously inhibited transcription of genes of medicinal interest⁷¹. With 80% of aerosol azacytidine droplets measuring between 0.1-5 microns, it allows lower and more complete bronchial deposition. This therapy significantly reduced the methylation of 9 significant tumour suppressor genes related to lung cancer with no detectable toxicity. Pharmacokinetic studies showed that aerosol delivery was significantly more successful at deposition compared to intravenous delivery (therapeutic index is 100 fold greater)⁷². Not only does this preclinical data show the potential of Azacytidine and its medicinal chemistry approach, it shows that a more specific delivery allows a better safety profile and a concentrated effect of the desired therapy in a targeted manner. Tissue specific chemoprevention would add to the potential of chemopreventative agents. With lung cancer being the most

common cause of death from cancer worldwide, its prevention is paramount regardless of its difficulty to access ¹.

Combinatorial strategies

A straightforward means of improving chemopreventative strategies would be combination treatments i.e. targeting multiple pathways to improve efficacy. A good example of this would be the work of Meyskens and Gerner who combined a NSAID with an inhibitor of polyamine biosynthesis. In a study of 375 patients with a history of resected adenomas, a dual dose of 500 mg of the polyamine synthesis inhibitor, α -difluoromethylornithine (DFMO), and 150 mg of the NSAID, sulindac, for a 3 year period was compared against a matched placebo. The combination reduced recurrent adenomas by 70% and advanced adenomas by 92% ⁷³. As the doses of each drug were low, this limited the side effects which is a prerequisite for long term chemopreventative adherence. The agents were deemed to be synergistic allowing lower doses of each agent to be administered without compromising their efficacy. The trial was stopped early as the results were so significant.

Combinatorial strategies do not necessarily have to mean a pharmacological combination. The proposal of adding chemopreventative treatment to post-cryosurgery with regards to actinic keratosis treatment appears to be effective. A phase 4 multi-centre study of 714 patients showed that full lesion clearance was seen in 32% of patients with cryosurgery alone but this was increased to 64% of patients when diclofenac sodium 3% was used post cryosurgery ³⁶. This again emphasises that the concept of chemoprevention is likely to be more successful with a combinatorial approach rather than an individual monotherapy.

Chemical modification

Another strategy that could improve the art of chemoprevention would be the enhancement of chemopreventive agents through molecular modification. The idea of enhancing chemopreventive potential is desirable. These approaches include improving

bioavailability of agents, improving target accessibility, combinatorial approaches and a medicinal chemistry approach for improving agents through molecular redesign.

One example would be hydroxylated analogues of resveratrol. Resveratrol has shown great potential as a chemopreventive agent due to having proapoptotic, anti-proliferation, and anti-inflammation mechanisms with potent antioxidant properties⁷⁴ but *in vitro* studies have shown that the chemical modification of resveratrol may enhance its chemopreventive profile. Hydroxylated analogues of the 3, 4', 5-trihydroxytrans-stilbene (resveratrol) structure were shown to have up to 6600 fold higher anti-radical activity when compared to the original resveratrol molecule. With regards to cytotoxicity against human leukaemic cells, hydroxystilbenes with orthohydroxyl groups showed 3 fold higher cytotoxicity when compared to 3, 4', 5-trihydroxytrans-stilbene (resveratrol)⁷⁵.

This advantage of chemical redesign is not limited to increasing activity nor hydroxylation of original natural molecules. Another example would be the methylation of curcumin. It was shown that 5 μmol of dimethoxycurcumin reduced proliferation in HCT116 human colon cancer cells 4 fold more effectively than the same dose of natural curcumin. The same dose also showed dimethoxycurcumin to induce apoptosis more effectively with 65% of cells becoming apoptotic whereas natural curcumin only achieved 16% in the HCT116 colon cancer cells. This study also concluded that dimethoxycurcumin was more stable within an *in vivo* mouse model when compared to curcumin with its well published low bioavailability⁷⁶. The concept of editing or building an ideal molecule with a better molecular profile is a concept that could help improve chemopreventive agents of the future.

A recent study designed and synthesised a sulindac based molecule which incorporated curcumin and evaluated its anti-inflammatory properties. This was achieved by chemically inducing inflammation and promoting tumourigenesis with TPA in mice's ear skin. When compared to curcumin, sulindac and a combination of the two, this novel compound was more significantly effective at reducing the weight of the ear, thickness of the ear, histological score and more significantly reducing key inflammatory and cancer associated markers such as IL-1 β , IL-6, TNF- α and COX-2⁷⁷. This novel structure which was rationally

designed on the basis of combining sulindac and curcumin shows early but great promise as a chemopreventive agent.

Another recent study compared several aspirin analogues to aspirin with regards to NF- κ B signalling, anti-proliferative activity and apoptosis within in vitro and in vivo colon cancer mice models. The analogues fumaryl diaspirin and diaspirin were both shown to demonstrate anti-tumour and proliferative activity beyond that of aspirin. They also showed a level of specificity in vitro and no obvious side effects could be seen within the mice over a 10 day period. This not only showed these 2 analogues to be of novel therapeutic value for cancer prevention/therapy but also helped identify a structure function relationship within aspirin with regards to anti-tumour effects. Through analysis of the analogues, it was shown that increased anti-tumour activity of the analogues was associated with 2 suitably spaced salicylate moieties separated by a range of approximately 8–10Å^{78, and 79}. Identifying molecular structure relationships that are associated to a drugs activity or side effects could be exploited by medicinal chemists to improve upon current chemopreventive options. The idea of modifying a molecule to increase activity or eliminate a side effect would be very beneficial to the likes of 'near perfect' agents such as aspirin which meets a large majority of chemopreventive criteria but it's use is limited due to the likes of GI bleeding.

A final example would be the isolation of COX inhibitor stellatin from *Dysophylla stellate* followed by chemical synthesis of derivatives to establish a structure activity relationship for COX2 inhibition. 18 derivatives of stellatin were evaluated, 7 of which showed better COX2 inhibition than stellatin. It was also revealed that a chromone scaffold with a double bond between positions 2 and 3, a carbonyl group at position 4 and a hydroxyl group at position 5 are key features for COX2 inhibition. The derivative with the most potential appeared to be compound 17 (5,6,7-trihydroxy-2-methylchromone). It showed the highest COX2 inhibition and potent free radical scavenging activity whilst also reducing inflammation better than stellatin and the NSAID indomethacin in a TPA induce mouse ear model^{80, 81, 82}. Not only has this research identified a potential anti-inflammatory derivative which may have therapeutic value. It has begun to establish a structure activity relationship for COX2 inhibition.

Increasing a chemopreventive agent's specificity to inhibiting a cancer and inflammation associated target such as COX-2 can only be of benefit to improving the library of chemopreventive options for the future.

These medicinal chemistry approaches can clearly play a major role within improving and redesigning the library of molecules for chemopreventive use as these *in vitro* and *in vivo* examples show. The characterisation of structure activity relationships and chemical redesign of molecular scaffolds that inhibit cancer associated pathways is a positive and potentially productive way forward for this topical area of research.

Conclusion

The incidence of cancer is rising in conjunction with an aging population which will lead to higher healthcare and treatment costs. An alternative to treating cancer once it has developed is rapidly becoming a necessity rather than a luxury. Chemoprevention is one solution and so investment is needed in better education of the general public, identifying surrogate biomarkers of disease and developing better and safer agents. Much progress has been made since the initial proof of concept of chemoprevention with the global adoption of HPV vaccines and there are increasing numbers of FDA approved chemopreventative options. This is still a field still in its infancy in relation to cancer treatment and one that can and must evolve quickly. This review of the current state of play of cancer chemoprevention highlights that a multidisciplinary approach from healthcare providers and scientists including medicinal chemists is essential for the clinical success needed in the future if we are to combat the relentless increase in cancer incidence across the world.

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