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Review

Cancer chemoprevention: Much has been done, but there is still much to do. State of the art and possible new approaches

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ABSTRACT

Over the past three decades great efforts have been made in search of cancer chemoprevention strategies. The increase in knowledge of the long process from normal to cancer cell has enabled interventions in terms of lifestyle modifications, natural compounds or drugs to block or reverse the process. Great successes have been achieved, especially for breast and colorectal cancer. However, these strategies have yet to find clinical application on a large scale.

In this article we identify the achievements, the pitfalls and the next steps to be taken to improve the efficacy and applicability of chemoprevention strategies. Among the crucial key points to be implemented are educational activities for physicians to appropriately disseminate the aim and indeed the culture of chemoprevention. It is essential to improve the risk-benefit balance, seeking the minimal active doses, intermittent schedules, a better characterization of the risk categories via a more personalized intervention based on individual characteristics, and ensure the containment of costs of public and private health prevention programs.

1. Introduction

The leading chemoprevention strategies in medicine are those employed in cardiovascular disease. This approach began around the 1960s, but became established standard care only many years later. In this field it has finally been demonstrated that the modulation of a biomarker such as blood hypertension has a definite impact on the true endpoint risk of major cardiovascular events (PAGE, 1952; Wong et al., 1989).

Cardiovascular mortality has greatly declined and most of this is due to chemoprevention. Since cardiovascular deaths have been reduced, cancer has become a predominant cause of mortality in the United States and the main industrialized countries.

In the late 1970s chemoprevention began to be considered as a potential strategy to lower cancer incidence and ultimately cancer-related death (Sporn, 1976). It soon became clear that the need existed to establish biomarkers to screen the huge variety of potential preventive agents (Kelloff et al., 1994). However, the task is more difficult than it may appear, and so far no validated biomarkers or surrogate biomarkers have been completely established (Baker and Kramer, 2013).

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To avoid large and costly phase III trials, the focus has recently been directed towards phase II studies, which provide the possibility to screen a large number of potential agents within a reasonable time-frame and within budgetary constraints (Meyskens and Szabo, 2005).

Surrogate endpoints are intermediate events along the carcinogenesis pathway, and their modulation should be reflected in the modification of the ultimate outcome of cancer incidence. While the relationship between hypertension (surrogate endpoint) and cardiovascular disease (primary endpoint) has been proven and gained acceptance, no such correlation has yet been proven in oncology, with the exception (albeit still with some reservations) of the adenoma-carcinoma pathway. There are two major concerns before one can arrive at a conclusive decision based on endpoint biomarkers: 1) the drug dose exerts an effect on the biomarkers but with limited clinical effect and/or is associated with side effects that outweigh the clinical benefit; 2) the drug tested may have prevention activity but via a different pathway in which the selected biomarkers are not modulated by the chosen drug, while the drug may still have a preventive effect nonetheless.

Another question centers on how epidemiological evidence can be reliably employed to set up prevention studies. There is a clear discrepancy between observational and randomized studies, which has led to clinical study results being painfully detrimental. In the lung prevention trial with beta carotene, the supplementation showed an increased risk of lung cancer in smokers. No effect of fiber and/or low fat intake in colorectal adenoma prevention has been confirmed (Beresford et al., 2006). In the case of prostate cancer, the promising (even if indirect) result in the ATBC Study (Albanes et al., 1996), showed null results when tested as primary endpoint in the SELECT study (Lippman et al., 2009). Several factors can be taken into consideration to explain such discrepancies: patient selection, differences in therapeutic regimen (compliance, intention-to-treat analysis), control of confounding factors (diet, lifestyle, co-medications), follow-up length, and outcome measurement. RCTs have typically been considered the only valid design to evaluate therapeutic efficacy. However, both study designs are susceptible to bias: overall observational studies might overestimate the outcome, while on the other hand, RCTs, being highly selective in inclusion and exclusion criteria and clinical procedures, might not give a true reflection of the general population. Furthermore, RCTs are very costly and it may be very difficult to apply phase III RTCs to drugs that fall outside the interests of the pharmaceutical companies (Alberts et al., 2000; Schatzkin et al., 2000; Sorensen et al., 2006).

Three major avenues have been pursued to balance efficacy, side effects and adherence to the program: minimal dose efficacy, the intermittent schedule approach, and “poly-chemoprevention” intervention.

For example, we have extensively studied low-dose tamoxifen for breast cancer prevention (see the following section), while in colorectal cancer prevention it has been proposed that low-dose aspirin may have a similar efficacy to higher doses (100 mg vs 325 mg/d) (Baron et al., 2003).

The combination of two or more agents with a view to targeting different pathways simultaneously has also been explored, since pathway redundancy is a common problem that has to be considered in order to avoid drug resistance. In colorectal cancer several compounds have been tested in association with NSAIDs (Mohammed et al., 2013).

Studies with an intermittent approach should also be implemented; short intermittent therapy could periodically reduce or eliminate premalignant cells inhibiting or delaying carcinogenesis while reducing toxicity (Wu and Lippman, 2011). In addition, this strategy may reduce drug resistance.

In this article we briefly review – mainly by agent category rather than by target organ – the major goals achieved in the main chemoprevention areas and the strategies taken to overcome their weaknesses. In addition we suggest several novel agents and biomarkers which warrant further investigation with the aim of providing new approaches for more effective, real-world cancer prevention.

2. Selective estrogen receptor modulators (SERMs)

A recent meta-analysis by Cuzick (Cuzick et al., 2013) of nine randomized trials comparing Selective Estrogen Receptor Modulators (SERMs) with placebo or another drug in women without breast cancer showed a 38% reduction in breast cancer incidence overall, with 42 women needing to be treated to prevent one case of breast cancer, over a 10-year follow-up period. The largest risk reduction was observed in the first 5 years. There was a significant increase in the incidence of thromboembolic disease with all SERMs odds ratio (OR) 1.73 (95% CI 1.47–2.05).

2.1. *Tamoxifen*

Tamoxifen is the most studied drug in breast cancer prevention so far, with four major phase III trials: The Royal Marsden, NSABP P-1, the Italian study, and IBIS I. Results of these trials have been published with subsequent long-term follow-up. The NSABP P-1 study (Fisher et al., 2005) was the largest, and the striking positive results led to it being closed ahead of time and to the FDA approving tamoxifen as treatment to lower breast cancer risk. In this study tamoxifen reduced the risk of invasive breast cancer by 49% ($P < .00001$). The decreased risk occurred in women aged 49 years or younger (44%), 50–59 years (51%), and 60 years or older (55%); risk was also reduced in women with a history of lobular carcinoma in situ (56%) or atypical hyperplasia (86%) and in those with any category of predicted 5-year risk. Tamoxifen reduced the risk of non-invasive breast cancer by 50% ($P < .002$) and the occurrence of ER-positive tumors by 69%, but no difference in the occurrence of ER-negative tumors was seen. After 7 years of follow-up (Fisher et al., 2005), the effects were similar to those seen in the initial report. Tamoxifen was associated with a higher rate of endometrial cancer relative risk (RR) 2.53 (95% CI 1.35–4.97), pulmonary embolism and deep vein thrombosis. These increased risks occurred predominantly in women aged 50 years or older. All endometrial cancers in the tamoxifen group were stage I (localized disease) and no endometrial cancer deaths occurred in this group. No increase in other tumors was observed in the tamoxifen group. Tamoxifen led to a 32% reduction in osteoporotic fractures (RR 0.68,

95% CI 0.51–0.92). The net benefit achieved with tamoxifen varied according to age, race, and level of breast cancer risk.

The 20-year follow-up of the Royal Marsden trial (Powles et al., 2007) analysis reported an overall 22% non-significant risk reduction, but which became statistically significantly in the post treatment period (23 in the tamoxifen arm and 47 in the placebo arm; hazard ratio (HR 0.48, 95% CI 0.29–0.79; $p = 0.004$).

Similarly in the Italian Tamoxifen Prevention Study after 11 years of follow-up, an RR of 0.84, 95% CI 0.60–1.17 was shown. Notably a post hoc analysis showed an RR of 0.24, 95% CI 0.10–0.59 in the subgroup of women at higher risk for BC.

Finally, the IBIS-I study (Cuzick et al., 2007), after a median follow-up of 96 months, showed an RR of 0.73 (CI 0.58–0.91, $p = 0.004$). The positive effect of tamoxifen was constant for the entire follow-up period, and no reduction of benefit was observed for up to 10 years after randomization. Conversely, side effects in the tamoxifen group were much lower after completion of the active treatment period than during active treatment. Deep vein thrombosis and pulmonary embolism were statistically significantly higher in the tamoxifen arm than in the placebo arm during active treatment (RR 2.26, 95% CI 1.36–3.87) but not after tamoxifen was stopped (RR 1.14, 95% CI 0.52–2.53).

2.2. *Raloxifene*

The first trials to show an effect on breast cancer incidence were the MORE trial (Cauley et al., 2001), which showed a decrease of all breast cancers by 62% and of invasive breast cancers by 72% compared with placebo (RR 0.28, 95%; CI 0.17–0.46; and the CORE trial (Martino et al., 2004), which was a follow-up of the previous one. Over the eight years of both trials, the incidence of invasive breast cancer and ER-positive invasive breast cancer was reduced by 66% (HR 0.34, 95% CI 0.22–0.50) and 76% (HR 0.24, 95% CI 0.15–0.40) in the raloxifene group compared with the placebo group, respectively.

The RUTH trial was specifically designed to evaluate the effect of raloxifene treatment on the incidence of coronary events and breast cancer (Barrett-Connor et al., 2006), and raloxifene compared to placebo reduced the risk of invasive breast cancer (HR 0.56, 95% CI 0.38–0.83).

Overall, raloxifene was associated with an increased risk of cardiovascular disease but no statistically significant increase in the incidence of endometrial cancer.

Based on these results, the NSABP-2 Study (STAR) (Vogel et al., 2006) was launched, a face to face comparison of raloxifene with tamoxifen. The first report showed a comparable risk reduction of breast cancer by approximately 50%, with tamoxifen being more effective on non-invasive breast cancer than raloxifene. Fewer, though not statistically significantly fewer, endometrial cancers were observed under raloxifene (RR 0.62, 95% CI 0.35–1.08). Thromboembolic events occurred less often in the raloxifene group (RR 0.70, 95% CI 0.54–0.91).

An updated analysis of the STAR trial with an 81-month median follow-up (Vogel et al., 2010) showed that raloxifene was less effective than tamoxifen for the prevention of invasive breast cancer, retaining only 76% of its long-term effect compared to tamoxifen. The preventive effect of raloxifene was not as persistent as that of tamoxifen. On the other hand, raloxifene had the advantage of fewer side effects (less uterine cancer and venous thromboembolism compared to tamoxifen). Thus, tamoxifen and raloxifene became two valid options to take into account by physicians and patients when deciding whether to reduce breast cancer risk pharmacologically, and also received approval for use in breast cancer risk reduction by the Food and Drug Administration (FDA) in the USA.

However, their risk-benefit was subsequently often considered far less than ideal and the two drugs are not currently utilized on a large scale by healthy women for chemoprevention.

In 2013 the National Institute for Health and Care Excellence (NICE) in the UK issued an updated set of clinical guidelines including tamoxifen and raloxifene for the prevention of breast cancer in healthy women at increased risk. It thus became the first European body to recommend chemoprevention of breast cancer in high-risk women. Notably, the guidelines indicate raloxifene as an alternative that may be more helpful to older postmenopausal women. (NICE CG164: Familial Breast Cancer, June 2013 http://guidance.nice.org.uk/CG164). Despite these positive findings, however, the public's attitude toward breast cancer chemoprevention remains ambivalent, and the toxicities associated with tamoxifen, particularly endometrial cancer and thromboembolic events, still hamper the drug's uptake by high-risk women who should benefit from its preventive effects.

Among the strategies to overcome the side effects of tamoxifen, we have intensively investigated alternative low doses of tamoxifen.

In a dose-finding presurgical trial we observed an equivalent KI-67 reduction from the standard dose (20 mg/d) to 1 mg per day, after 4 weeks of treatment (Decensi et al., 2003).

In a phase II trial in premenopausal women at higher risk for breast cancer (either based on the Gail model or due to a previous diagnosis of an intraepithelial neoplasia), we have shown that 5 mg daily of tamoxifen significantly lowered IGF-I and mammographic density by 12% and 20%, respectively, and after a median follow-up of 5.5 years, tamoxifen showed a numerical reduction in annual odds of breast tumor events (Decensi et al., 2009).

In a phase III study in postmenopausal healthy HRT users, we also tested the preventive effect of tamoxifen at 5 mg per day. After 6.2 ± 1.9 years follow-up, a risk ratio of 0.80 (95% CI 0.44–1.46) was observed. Tamoxifen showed a significant trend in luminal-A tumors (RR, 0.32; 95% CI 0.12–0.86). No differences in serious adverse events were observed compared to placebo (Decensi et al., 2013).

An ongoing phase III study is testing the 5 mg/d dose in patients with intraepithelial neoplasia.

All these findings suggest possible beneficial clinical preventive effects by low-dose tamoxifen regimens and they are strongly supported by observational studies (Guerrieri-Gonzaga et al., 2013).

Another alternative approach is topical administration of active tamoxifen metabolites directly onto the breast, i.e. directly onto the cancer prevention site itself. By avoiding systemic administration it is expected to reduce the distribution of drug to tissues susceptible to tamoxifen-induced toxicity.

Clinical trials of topical tamoxifen or with the active metabolite 4-OH-tamoxifen are still ongoing, whereas pharmacokinetic studies have already shown that appropriate formulations of drug successfully penetrate the skin to reach breast tissue, where a preventive effect is sought (Lazzeroni et al., 2012).

3. Aromatase inhibitors

Aromatase inhibitors (AIs) work by inhibiting the action of the enzyme aromatase, which converts androgens into estrogens by a process called "aromatization."

Two large clinical trials have compared aromatase inhibitors with placebo in primary breast cancer prevention settings.

The Mammary Protocol 3 (MAP.3) trial (Goss et al., 2011) was a randomized, placebo-controlled, double-blind trial of exemestane 25 mg administered to postmenopausal women 35 years or older with at least one of the following risk factors: 60 years or older; Gail 5-year risk score greater than 1.66%; prior atypical ductal or lobular hyperplasia or LCIS; or DCIS with mastectomy. A total of 4560 women (median age 62.5 years, median Gail risk score 2.3%) were randomly assigned to receive either exemestane or placebo. At a median follow-up of 35 months, 11 invasive breast cancers were detected in those administered exemestane and in 32 of those administered placebo, with a 65% relative reduction in the annual incidence of invasive breast cancer (0.19% vs 0.55%; HR 0.35, 95% CI 0.18–0.70; $p = 0.002$). The annual incidence of invasive plus non-invasive (DCIS) breast cancers was 0.35% on exemestane and 0.77% on placebo (HR 0.47, 95% CI 0.27–0.79; $p = 0.004$). Adverse events occurred in 88% of the exemestane group and 85% of the placebo group ($p = 0.003$), with no significant differences between the two groups in terms of skeletal fractures, cardiovascular events, other cancers, or treatment-related deaths. Minimal quality-of-life differences were observed.

The International Breast Cancer Intervention Study-II (Cuzick, 2008) (IBIS-II) is currently testing anastrozole for its ability to reduce the risk of invasive breast cancer in postmenopausal women at increased risk of disease. The overall IBIS-II design comprises two independent strata, one testing anastrozole in comparison with tamoxifen in women with ductal carcinoma in situ (DCIS) and the other testing this AI versus placebo in postmenopausal women at increased risk of breast cancer. The eligibility criteria for this "high-risk" portion of IBIS-II are similar to those used in IBIS-I, including risk based on family history, history of benign breast biopsies, lobular carcinoma in situ (LCIS) and/or atypical hyperplasia, and nulliparity. All women (aged 40–70 years) are postmenopausal. Exclusion criteria include a dual-energy X-ray absorptiometry (DXA) T score of 4 or lower or more than 2 fragility fractures. The IBIS-II trial stopped accrual in December 2012, with a total of 6844 women enrolled (3864 to Prevention and 2980 to DCIS). In the IBIS-II Prevention trial 1920 women were randomly assigned to receive anastrozole 1 mg daily and 1944 were assigned to placebo. After a median follow-up of 5 years, 2% of women in the anastrozole group and 4% in the placebo group developed breast cancer HR 0.47 (95% CI 0.32, 0.68; $p < 0.0001$). The predicted cumulative incidence of all breast cancers after 7 years was 5.6% in the placebo group and 2.8% in the anastrozole group (Cuzick et al., 2014). Musculoskeletal adverse events were common in the anastrozole group, but mostly of moderate severity. Vasomotor symptoms were commonly seen in both groups but the incidence was higher in women on anastrozole. No increases in fractures, myocardial infarction, or cardiac failure were seen between the groups.

In summary, similarly to tamoxifen and despite their strong preventive effect, AIs may not be well accepted by high-risk women due to undesirable symptoms, bone density loss and, last but not least, possible impact on QoL, significantly compromising their motivation and adherence to a preventive treatment. Therefore, a significant step forward to promoting BC prevention in postmenopausal women is to ameliorate QoL by finding the AI minimal active dose and an intermittent schedule. A lighter schedule, compared to the standard regimen, may be much more appealing to a healthy at-risk woman.

To this end – following the track of our group's long-term experience on window-of-opportunity (WOP) trials – we are currently working on the design of a pre-surgical phase IIb, randomized, double-blind, multi-centric study on postmenopausal patients with histologically confirmed ER-positive primary BC (Stage 0-II) to be assigned to receive exemestane 25 mg/day, or 25 mg/three times a week (M-W-F schedule), or 25 mg once a week (weekly schedule) for 5 weeks before primary surgery.

4. Non-steroidal anti inflammatory drugs (NSAIDs) and aspirin

It has been postulated that inflammation can play an important role in carcinogenesis (Coussens and Werb, 2002). The clearest evidences supporting this association are inflammatory bowel diseases (IBDs), that have been associated with an increased colorectal cancer risk (Itzkowitz and Yio, 2004). Consequently NSAIDs treatment, and especially aspirin, shows a chemopreventive activity.

4.1. *Colon cancer*

The well established adenoma-carcinoma pathway together with the data regarding the increased cancer risk in subjects with IBDs have, for a long time, stimulated great interest in validating cancer prevention strategies. Starting from epidemiological studies up to RCTs, there is now overwhelming evidence of a reduction in CRC incidence and mortality from the regular use of aspirin or other NSAIDs (Flossmann and Rothwell, 2007). Based on the intermediate biomarker, i.e. adenoma incidence, both aspirin and celecoxib have shown a strongly statistically significant reduction in adenoma incidence compared to placebo (Arber et al., 2006; Baron et al., 2003; Bertagnolli et al., 2006; Sandler et al., 2003).

To show a clear benefit on cancer incidence and mortality, aspirin has to be administered for a relatively long period and the effect can appear after treatment discontinuation. Two high-dose aspirin trials showed an overall 37% reduction in

CRC incidence (Flossmann and Rothwell, 2007). Other studies with lower aspirin dose (75–300 mg/day) found a smaller but significant reduction in CRC incidence (Rothwell et al., 2010). Another very important result was shown with the long-term follow up of the Colorectal Adenoma/Carcinoma Prevention Programme 2 (CAPP2), where carriers of Lynch syndrome were randomized to aspirin 600 mg/d versus placebo. The treated group showed a 63% reduction ($p = 0.008$) in incidence among those completing two years of treatment, although results for the intention-to-treat were not significant (Burn et al., 2011). A CAPP3 study is set to be launched soon.

4.2. *Breast cancer*

Case–control studies on aspirin indicate an 18% reduction in breast cancer incidence, and an 8% reduction was seen in cohort studies. Results were similar for mortality, 5% reduction in case–control studies and 14% in cohort studies, even though not statistically significant (Chan et al., 2007; Ratnasinghe et al., 2004; Thun et al., 1993). In addition to the prospective studies, randomized trial data have demonstrated an effect of aspirin on cancer recurrence.

Altogether, the effect of aspirin on breast cancer incidence is probably too small to justify its use as a single preventive agent. However, taking into account the risk reduction seen with other cancers (e.g., gastrointestinal cancers), as well as the beneficial cardiovascular effects, this drug may form part of a broad pharmacological intervention approach (Wald et al., 2011).

4.3. *Lung cancer*

Aspirin reduced lung cancer-specific mortality by 29% (95%, CI 11–42) in the 20-year period after one trial commenced: no trend with dose (above 75 mg/day) was observed, but the effect on all cancers was more evident in adenocarcinomas and was present in both smokers and nonsmokers (Rothwell et al., 2011). A suggestion of a protective effect of low-dose aspirin was seen in the Women's Health Study (Cook et al., 2005). A hospital-based case–control study found that lung cancer risk was significantly lower in aspirin users compared to non-users and prolonged duration of use was associated with reduced lung cancer risk. Risk reductions were observed in both sexes, but a significant dose–response relationship was only seen among male participants (Moysich et al., 2002).

4.4. *Aspirin and other cancers*

Mortality benefit from aspirin can also be seen for esophageal, gastric, prostate cancer. It begins five years after the commencement of treatment and can last for up to ten years after the cessation. Overall the major benefit can be seen for adenocarcinomas, including also pancreatic and ovarian cancer. Even though there is a certain lack of consistency in the published literature, a recent case–control study reported an overall risk reduction of pancreatic cancer with regular use of aspirin (OR, 0.52; 95% CI 0.39–0.69), and – interestingly concordant with the other cancer types – no dose effect has been observed (Streicher et al., 2014). Pooled data from 12 population-based case–control studies of ovarian cancer showed an OR of 0.91 (95% CI 0.84–0.99). Fewer studies reported the dosage and these showed that low-dose aspirin (<100 mg/d) had an OR of 0.66 (95% CI = 0.53–0.83) (Trabert et al., 2014).

5. Metformin

Epidemiological studies have suggested that metformin can reduce cancer risk and mortality in diabetic subjects. A recent meta-analysis (Gandini et al., 2014b) on 47 independent studies and 65,540 cancer cases in patients with diabetes, with a particular focus on confounders and biases, including body mass index (BMI), study type, and time-related biases, showed that metformin reduced the overall cancer incidence by 31%, although between-study heterogeneity was considerable ($I^2 = 88\%$). Cancer mortality was reduced by 34% (SRR, 0.66; 95% CI, 0.54–0.81).

Specifically for CRC, a borderline significant risk reduction was seen (12 studies, SRR, 0.80, 95% CI, 0.64–1.00). The SRRs from subgroups of studies adjusted for BMI and with prospective designs did not suggest a significant reduction in cancer risk. At this stage it is difficult to dissect the potential role of metformin on colon risk. Certainly in subjects with a metabolic imbalance this drug may help to improve this condition that can be considered a cancer risk factor.

Several early phase breast cancer clinical trials testing the effects of metformin on tissue biomarkers tried to determine whether these observations apply to non-diabetic populations. Each of these studies measured proliferation by Ki-67 staining in breast tumors and they reported reductions of up to 5% in cell proliferation following treatment with metformin in women with early stage breast cancer. The study of Bonanni and colleagues (Bonanni et al., 2012) reported a different drug effect depending on insulin resistance, with a non-significant mean proportional decrease in Ki-67 of 10.5% in women with a homeostasis model assessment (HOMA) index (fasting blood glucose [mmol/L] X insulin [mU/L]/22.5) (Bonora et al., 1998) of more than 2.8 and a non-significant increase of 11.1% with a HOMA index of less than or equal to 2.8. A different effect of metformin according to HOMA index was also noted in luminal B tumors.

Several other clinical studies are under way, aiming to address metformin and cancer issues (https://clinicaltrials.gov/ct2/results?term=metformin+and+breast+cancer&recr=Open&no_unk=Y). In particular the NCIC MA.32 is an ongoing, full-scale clinical trial that will enroll 3582 patients with early stage and resected breast cancer receiving standard breast cancer therapy. Subjects will be randomized to receive metformin 850 mg (or placebo) twice daily for 5 years (Goodwin et al., 2011).

6. Vaccines

Immunoprevention is a strategy to lower cancer risk by manipulating the immune system. This can target pathogens associated with cancer pathogenesis or can directly target cancer "specific" antigens. This strategy can use vaccines, antibodies, or immune modulators. The goal is to modulate the

so-called "immune surveillance", the complex balance between suppression, tolerance, activation and cytotoxicity. The "tumor surveillance" theory was developed in the 1950s, hypothesizing that the immune system protects against cancers by killing abnormal cells before they fully develop into cancer cells. However the concept that infections, inflammation and their related reactions are related to cancer is longstanding (Finn, 2014; Herberman et al., 2006).

Tumors that have a viral component in their etiology can be more "easily" targeted with vaccines directed against the viral peptide. Two major achievements have been made so far with vaccine programs. The first is the reduced incidence of hepatocellular carcinoma (HCC) due to the hepatitis B virus (Chang, 2014). The second is HPV vaccination against cervical cancer and the other HPV-related diseases (Bosch, 2011). Four phase III trials have been conducted: FUTURE I and FUTURE II (both with Gardasil®), PATRICIA (PApilloma TRIal against Cancer In young Adults) and the Costa Rica HPV Vaccine Trial (with Cervarix®) Both vaccines showed a highly significant efficacy against CIN in women who were immunologically naïve to the corresponding virus type at the time of vaccination. Vaccines also reduced other related diseases including vulvar, vaginal and anal intraepithelial neoplasia.

However, most cancers have not been shown to be caused by infectious agents, and to retrieve a safe antigen may not be a simple matter. These tumor-associated antigens may break self-tolerance inducing a dangerous autoimmunity cross-reaction. However, the rapidly-evolving technologies of genetic engineering have progressively improved the synthesis of potential prophylactic vaccines based on non-viral and non-mutated tumor-associated antigens which are able to produce an effective and reasonably safe immune response.

6.1. *HER2 inhibitors*

A Phase II trial testing a single-dose monotherapy with trastuzumab for patients with HER2-positive DCIS did not result in significant, clinically overt, histologic, antiproliferative, or apoptotic changes. However, trastuzumab significantly increased antibody-dependent cell mediated cytotoxicity (ADCC) in 100% of patients; this was demonstrated to be mediated through CD56+ degranulating natural killer cells (Kuerer et al., 2011).

In addition to HER2 inhibitors, HER2 peptide vaccines are being studied as therapeutic agents to induce immune responses in HER2-positive breast cancer. With respect to breast cancer and potential intervention with a cancer vaccine, DCIS offers an ideal setting in which to test cancer vaccination. In a presurgical window-of-opportunity trial, Sharma et al. (Sharma et al., 2012), recently investigated the effects of a vaccine targeting HER-2/neu expression in women with a recent diagnosis of DCIS. At surgery, 5 of 27 (18.5%) vaccinated subjects had no evidence of remaining disease, whereas among 22 subjects with residual DCIS, HER-2/neu expression was down-regulated in 11 subjects (50%). Vaccination was more effective in ER-negative DCIS: no residual disease was found in 40% of ER-negative subjects compared with 5.9% in ER-positive subjects. Results showed that vaccination against HER-2/neu was safe and well-tolerated and induced a decline and/or eradication of HER-2/neu expression.

However, reports regarding HER-2 status as a risk factor in DCIS patients are relatively few in number and controversial (Lazzeroni and Serrano, 2012). Further studies are warranted to test whether these peptides will be particularly useful in high-risk women for the prevention of HER2 positive breast cancer. Nonetheless, breast cancer that does not express ER, PgR, or HER2 will not benefit from these targeted treatments.

Another vaccine with an immunogenic peptide from the HER2/*neu* protein (E75) has been tested in an adjuvant setting. The vaccine is safe and a specific immune response has been observed in all patients enrolled (Peoples et al., 2005). A five-year disease-free survival showed a positive trend for the vaccine group and reached statistical significance for the subjects who completed the vaccine schedules (Mittendorf et al., 2014). HER2/*neu* vaccines may be useful for all the tumors expressing this receptor and are under evaluation for prostate cancer (Hueman et al., 2005).

6.2. *Human mucin 1 (MUC1)*

MUC1, a large glycoprotein, can be abnormally expressed and glycosylated in various malignant and premalignant epithelial lesions. This observation has made MUC1 a candidate target for immunotherapy for over a decade (Beatty and Finn, 2013). A phase I/II study shows that MUC1 vaccine is immunogenic and capable of eliciting long-term memory, in nearly 50% of the subjects. The non-responders had pre-vaccination high circulating suppressor cells. It has been suggested that the vaccination should be made even prior to a premalignant lesion such as adenoma (Kimura et al., 2013). A phase II MUC1 vaccine randomized trial is being set up with the aim of preventing polyp recurrence, and any subsequent new colon cancers.

Immunoprevention strategies, similarly to those regarding many other agents, should be tested in the very early stages of disease, in a "naïve" subject, instead of poly-treated patients with advanced disease. Lately, substantial efforts have been made to apply prevention strategies and vaccines to intraepithelial lesions, such as ductal carcinoma in situ, colonic polyps, and cervical epithelial neoplasms. The premalignant setting is clearly ideal for initial testing of the efficacy and safety of prophylactic vaccines or of any strategies focused on prevention rather than cure.

7. Vitamin D

Low vitamin D levels have also been linked to many chronic diseases including cancer. In a meta-analysis summarizing 5562 deaths out of 62,548 individuals, a significant decrease in mortality risk in the general population has been shown as circulating 25(OH)D increases, with optimal concentrations ~75–87.5 nmol/L. Consistently, a meta-analysis of randomized trials showed a statistically significant reduction of 7% in total mortality in healthy subjects taking vitamin D supplements (Zittermann et al., 2012). Specifically regarding cancer and in particular CRC, a significant risk reduction comparing the highest levels versus the lowest level of 25(OH)D has been observed with a significant dose–response effect (IARC Working Group Reports. Volume 5, 2008) (Gandini et al., 2011).

Genetic variants (polymorphisms) and expression alterations in vitamin D receptor (VDR), CYP27A1, CYP27B1, CYP24A1 and vitamin D-binding protein (GC) may compromise calcitriol levels and signaling activity, thus affecting growth and differentiation of the tumor (Deeb et al., 2007). Several single nucleotide polymorphisms (SNPs) of the VDR gene that may influence cancer risk have been identified. In a meta-analysis, we showed that *FokI* and *BsmI* VDR polymorphisms may affect cancer risk at any site in Caucasians (Raimondi et al., 2009). Furthermore the VDR gene has been shown to be an important regulator of many experimental autoimmune diseases including IBD, Crohn's disease and ulcerative colitis.

A case–control study found that some *GC* polymorphisms are associated with increased CRC risk (Zhou et al., 2012). The epidemiological data clearly show an inverse correlation between vitamin D level and cancer mortality, and for CRC the data hold also for incidence. The trials conducted so far have not sustained these results, but several implications can be considered, the major one being the low dose used, which in one case did not modify the circulating levels of vitamin D. The inclusion of calcium in the supplementation represents another confounding factor in many studies. The ongoing multiple endpoint trial "VITamin D and OmegA-3 TriaL (VITAL)" will provide more definitive data on the role of vitamin D in human cancer and other diseases, but the results will not be available for many years. The supplementation includes vitamin D (2000 IU) or omega-3 fatty acids (1 g of fish oil) or their combination. Lastly, even though there are contradictory results, the role of vitamin D can be personalized, based on the subject genotype. The several genes involved in its pathways, VDR, vitamin D-binding protein and the cytochromes (CYP27A1, CYP27B1, CYP24A1), can have specific polymorphisms that interfere with the physiological functioning of vitamin D (Dong et al., 2009; Gandini et al., 2014a; Hibler et al., 2014).

8. Gut flora-microbiota

The host–environment interaction is considered a key factor in carcinogenesis, and this is even more relevant in colorectal cancer, taking into account the colon acting as a barrier organ and its specific function for nutrient absorption. Within the pathological process, two aspects have shown a very close interdependence: diet and gut flora. They modulate each other with a direct consequence on gut health.

As regards gut flora/microbiota, recent publications have provided mechanistic evidence for the involvement of gut bacteria in the development of CRC (Toprak et al., 2006; Wang et al., 2008; Wu et al., 2009). Altogether, some studies provide the hypothesis for the CRC-associated microbiome and indicate a highly dynamic relationship between intestinal bacteria and developing tumors (Marchesi et al., 2011). A recent study showed for the first time a direct fecal microbiota comparison between 47 CRC patients and 94 control subjects. Overall, the findings were a reduced microbiota diversity in the patients with a selective reduction of the Gram positive (Firmicutes) and an increase of the Gram negative (Ahn et al., 2013). Bacterial diversity is the trademark of a healthy gut. The more diverse the gut bacteria, the less likely can potential pathogens gain the upper hand and lead to infection. This study suggests that reduced gut diversity may also lead to increases in certain bacteria and decreases in others. Firmicutes are involved in the mechanisms to enhance the energy harvest from the diet. Among Firmicutes, the relative depletion was most prominent for the Clostridia class. Gram-positive Clostridia efficiently ferment dietary fiber and other complex carbohydrates to butyrate, a major colonic metabolite that may inhibit colonic inflammation and carcinogenesis.

Many open questions need to be addressed in future studies, including in-depth microbiome analyses of extended groups of tumor samples from different disease stages. Microbiota mediators in the health of the gut may be the short chain fatty acids and the bile acids. Among the short chain fatty acids, butyrate plays a major role in gut mucosa homeostasis with antineoplastic properties. Very importantly, microbiota and its products are elements which are modifiable by diet or supplementation and should be considered in future studies.

In summary, microbiota may promise to become a significant risk biomarker for prevention studies and even a risk modifier itself.

9. Conclusions

Countless agents and nutrients have cancer chemoprevention potential, but before screening any potential candidate, it is mandatory to develop strategies to better identify the proper candidates for chemoprevention and to support and disseminate the very culture of cancer chemoprevention.

The issues that need to be actively studied to reach a transversal consensus to make chemoprevention standard care are few but crucial.

From the clinical point of view: 1) Educational programs: the concept of cancer risk reduction is not sufficiently widespread among physicians (oncologists, surgeons, general practitioners, etc.) who often do not sufficiently select and inform the high-risk subjects who may take advantage of and benefit from a prevention program; 2) Healthy subjects who are adequately and appropriately informed on their risk profile and the risk-benefit balance of an active intervention, can be much more motivated, adhere more favorably and comply to the intervention despite the potential side effects (Waters et al., 2010).

From the biological point of view: 1) Risk factors/categories: risk factors may be the prelude to a specific biology of the potential tumor, and this can determine a better selection of the agent (an example can be provided by SERMs, which are clearly ineffective on ER-negative breast cancer) (Cuzick et al., 2013); 2) Implementation of more studies on intraepithelial neoplasia (IEN) as an intermediate endpoint to be validated as a surrogate endpoint through a prolonged follow up. In order to confirm biomarkers as a surrogate endpoint they should be evaluated within phase III trials in order to obtain a true validation (for instance, the Gail risk model, with all its limitations, was validated through the P1 study) (Costantino et al., 1999).

But an even greater challenge that chemoprevention has to face is the lack of interest shown so far by the pharmaceutical

and biotechnology industries. As a result, the field has experienced limited investments by public institutions and even fewer by private companies. This, despite the fact that cancer prevention has great potential to impact on the social and economic burden of cancer. Key organizations (e.g., the EU) should strongly contribute in the coming years to ameliorate the relationships and collaborations that exist between and among scientists, investors and health providers.

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