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Molecularly targeted agents: Their promise as cancer chemopreventive interventions

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Abstract

Molecular medicine has fully entered in to the oncology arena. The development of targeted therapies is one of the major ongoing efforts in cancer treatment. Targeted therapy refers to treatment strategies directed against molecular targets considered to be involved in neoplastic transformation. Such molecularly targeted agents (MTA) are currently under study in all treatment settings including that of chemoprevention, defined as the use of natural or synthetic agents to interrupt the carcinogenic process, to nip tumours in the bud. This review article aims to provide a general overview of the potential use of some of these MTA in the chemoprevention setting.

Keywords: Chemoprevention; Trials; Targeted agents

1. Introduction

1.1. Chemoprevention

Chemoprevention is defined as the use of natural or synthetic agents to impede, arrest or prevent carcinogenic progression to invasive cancer. The two fundamental concepts underlying chemoprevention are multistep carcinogenesis and field carcinogenesis.

1.1.1. Multistep carcinogenesis

Cancer occurs through a series of steps including the accumulation of molecular changes that allow histological premalignant lesions (*e.g.* metaplasia and dysplasia) to develop into invasive tumours [1]. The earliest genetic modifications (mutations, deletions, amplifications) are not initially translated into cellular or tissue structural changes. Additional events are necessary to induce phenotypical, then physiological modifications in tissue, such as uncontrolled proliferation, invasion, and metastasis. It has been suggested that multiple (10–20 or more) genetic events are necessary for most solid epithelial tumours to arise [2].

1.1.2. Field carcinogenesis

Field carcinogenesis is the extensive multifocal development of premalignant and malignant lesions in the entire carcinogen-exposed area of an epithelial region. This concept explains why diffuse tissue damage resulting from exposure to carcinogens leads to the development of multiple neoplastic lesions in the whole exposed area, either at the same time or at some point in the future. This concept has been clearly established in head and neck, lung and bladder epithelia. It was Slaughter [3]

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who proposed this concept in 1953 to explain the development of second tumours associated with oral cancer. The emergence of any dysplastic lesion in the epithelium of an individual, signals a high risk of developing cancer anywhere in the same epithelium. Treating or controlling precancerous lesions may be a way of avoiding the development of invasive cancers. This hypothesis provides a major rationale for chemoprevention.

1.1.3. Primary, secondary, and tertiary chemoprevention strategies

Chemoprevention approaches target the carcinogenic process at early and potentially reversible stages, focusing on inhibition of one or many elements in the stepwise progression towards cancer. Chemoprevention can be classified into three strategies: (1) primary prevention of cancer in healthy individuals who are at high risk (*e.g.* current or former smokers in case of lung or head and neck cancer); (2) secondary prevention of cancer in individuals with precancerous lesions (*e.g.* intraepithelial neoplasia, leukoplakia, dysplasia; and (3) tertiary prevention aimed at patients who have had a cancer(s) and at preventing the development of second primary tumours or recurrence.

Multiple intervention targets exist that are as diverse as the population implicated in chemoprevention programmes. While MTA are of great promise, effective vaccination programmes are probably one of the chief goals of modern chemoprevention. For instance, we know that persistent infection with oncogenic strains of human papillomavirus (HPV) is a prerequisite for virtually all cases of cervical carcinoma [4,5]. Consequently, pharmaceutical companies such as SmithKline Beecham, and Merck CSL among others and the National Cancer Institute (Bethesda, MD) have developed a number of vaccines, which are either prophylactic, therapeutic, or a combination of both. These drugs are under investigation in phase II trials specifically in developing countries [6–8].

1.2. Molecularly targeted agents

The most rational approach to chemoprevention is to design and test new agents that act on specific molecular and cellular targets. In this respect, the recent development of molecularly targeted agents is a unique opportunity for the chemoprevention field. Recent advances in our understanding of cancer at the molecular level have generated over 500 new agents that are in the clinical development pipeline. These agents can be classified on the basis of the well-established model of hallmarks of cancer popularised by Weinberg and Hanahan (Fig. 1). MTA development should focus on choosing the most appropriate new drug targets and defining the best routes of administration (*e.g.* oral, topical, intradermal, aerosolized or others), as is the case for other chemopreventive agents.

2. Targeted therapies

2.1. Signal transduction inhibitors

2.1.1. HER family

EGFR (HER1) and HER2/neu are members of the HER family of receptors which itself belongs to the

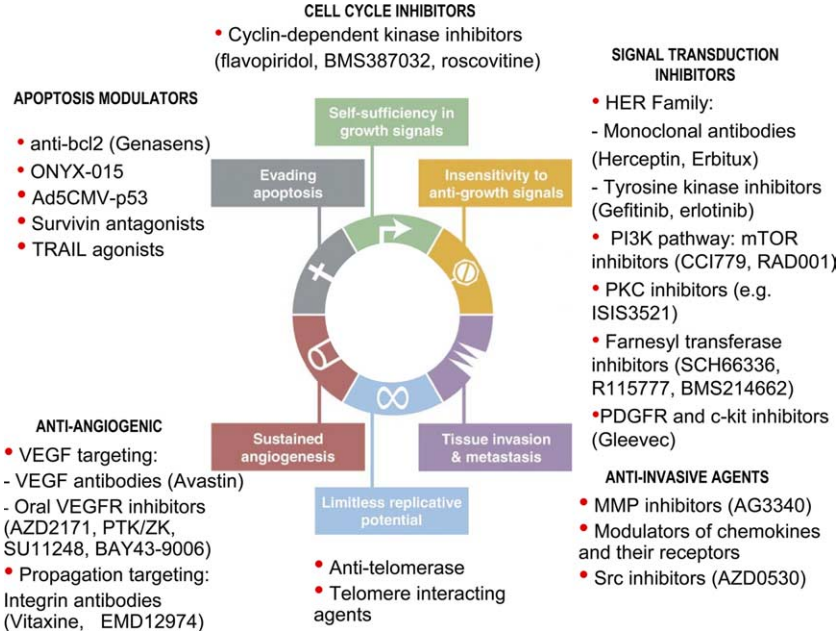

Fig. 1. Classification of molecularly targeted agents according to the hallmarks of cancer cells.

large group of tyrosine kinase receptors. Over the past two decades, comprehensive data strongly supporting a role for the HER family and its ligands in tumour development and growth have been accumulated.

The EGFR signalling pathway regulates cell differentiation, proliferation, migration, angiogenesis, and apoptosis, all of which become deregulated in cancer cells. The importance of the EGFR pathway in tumour biology is suggested by the finding that many human epithelial cancers including head and neck, lung, ovarian, pancreatic, breast cancers and gliomas exhibit EGFR overexpression (and sometimes amplification). Interestingly, overexpression of EGFR has also been established as a major characteristic of numerous precancerous lesions including those arising in the upper and lower airway epithelium. For instance, it has been demonstrated that the level of EGFR expression is 29-fold higher in the normal epithelium of patients with head and neck cancer as compared to the normal epithelium of non-smokers [9]. The level of EGFR expression is also predictive of the evolution of specific precancerous lesions to invasive head and neck tumours [10]. There is an increase in EGFR expression in the bronchial epithelium of smokers from squamous metaplasia to dysplastic lesions to *in situ* carcinoma [11]. EGFR expression is predictive of a poor prognosis and resistance to treatment in many tumour entities and notably head and neck cancers [12–15].

Several drugs targeting EGFR are under development. The chimeric monoclonal blocking antibody C225 (cetuximab, Erbitux®) and two orally available small receptor tyrosine kinase inhibitors (TKI), ZD 1839 (gefitinib, Iressa®) and OSI-774 (erlotinib, Tarceva®) have reached an advanced stage in development and have already shown antitumour activity in a variety of tumour types, alone or in combination with chemotherapy. Other TKI are also being developed as irreversible and/or pan-HER inhibitors (EKB-569, CI-1033, GW57216, PKI-166, BMS599626). Other monoclonal antibodies are also being tested in the clinic (ABX-EGF, York, MDX-447, pertuzumab).

In the setting of chemopreventive trials, The Specialized Programme of Research Excellence (SPORE) Trials of Lung Cancer Prevention (STOP) are 2 parallel studies aimed at investigating the potential effectiveness of gefitinib and tipifarnib (a farnesyl transferase inhibitor) in preventing the emergence and progression of premalignant lesions in former or current smokers with a history of smoking-related cancer. A histologic response, defined as prevention of emergence or progression of premalignant lesions, is the primary endpoint of these trials [16]. They should provide information not only about the potential role of gefitinib and tipifarnib in lung cancer chemoprevention, but also about the molecular changes underlying tumourigenesis which may serve as markers of disease progression. The objectives of the STOP trial are to evaluate the effect of gefitinib and tipifarnib on histologic and biologic parameters in patients with evidence of sputum atypia, to assess various parameters as potential predictors of the effectiveness of these agents, and to evaluate the tolerability of these agents over a 6-month course of treatment. The toxicity profile of HER1 inhibitors make them appealing targets for chemoprevention trials.

Amplification or overexpression of HER2 has been noted in various types of human cancers. In addition to malignant transformation, the activation of HER2 signalling pathways enhances various metastasis-associated properties and may render cancer cells resistant to conventional therapies [17]. HER2 overexpression, which is correlated with particular aggressiveness, is frequent in human epithelial tumours [18]. Stark *et al.* [19] demonstrated that women with benign breast biopsies with both HER2 amplification and a proliferative histopathologic diagnosis may be at a substantially increased risk for subsequent breast cancer. HER2 can be targeted either by monoclonal antibodies (transtuzumab and pertuzumab) or by oral TKI with a pan-HER spectrum (CI-1033, GW57216, PKI-166, BMS599626).

To our knowledge, there are no chemopreventive trials currently ongoing with HER2 targeting agents. However, a secondary chemoprevention trial is planned in patients with oral leukoplakia at the MD Anderson Cancer Centre using either EKB-559, an oral HER1/HER2 inhibitor, or celecoxib or a combination of both.

2.1.2. Ras/Raf/MAPK pathway

The Ras/Raf/MEK pathway is known to control two activities that impact directly on the onset and progression of tumours, namely cell growth and cell survival.

Mutations or alterations in each of the members of this pathway are associated with cancer. Concerning colorectal carcinogenesis, Vogelstein found Ras mutations in 9% of adenomas under 1 cm in size, 58% in adenomas larger than 1 cm, and 47% in carcinomas [20]. Nagasaka have shown in their studies that approximately 40% of colorectal cancers examined harbour genetic alterations in the Ras/Raf/MEK pathway, roughly 10% with B-RAF and 30% with K-RAS mutations [21]. Activating mutations on B-RAF have been found in a high proportion of melanomas, as well as thyroid carcinomas [22]. Ras is known to interact with and activate Raf1, the serine/threonine protein kinase in a GTP-dependent manner. Mutated Raf1, which is constituvely active, is known to possess transforming activities. Raf mutations have been identified in a wide range of human cancers. Independent of its mutational status, Raf is also activated in tumour cells containing the enhanced growth factor signalling pathway, namely those induced by mutant or constitutively-expressed Ras or EGF receptor family members [23].

Ras does not contain a transmembrane domain. Localization is accomplished by the post-translational addition of a lipid (farnesyl) moiety to its carboxy erminal. Many investigations were initiated exploring farnesyltransferase, the enzyme responsible for this modification, as an anticancer drug therapy [23].

Among the many existing farnesyltransferase inhibitors (FTI), R115777 (tipifarnib (Zarnestra), Johnson & Johnson, Beerse, Belgium) is an imidazole-containing heterocyclic compound, which is a potent and selective, orally active, nonpeptidomimetic inhibitor of farnesylproteintransferase. Other FTI include SCH66336 (sarosar) and BMS214662. A phase II trial evaluating tipifarnib for cancer prevention in paediatric patients with neurofibromatosis type 1 and progressive plexiform neurofibromas is ongoing. The STOP trial in lung cancer chemoprevention plans to include an arm with tipafarnib.

BAY43-9006, is bis-aryl urea, which inhibits Raf kinase. This molecule is currently under investigation in phase III trials [24]. It has a double mechanism of action: it inhibits proliferation and acts as an anti-angiogenic compound [25]. There are no ongoing chemopreventive trials with this agent.

2.1.3. PI3-K pathway (mTOR inhibitors)

Many growth factor receptors activate PI3-K, a lipid kinase. Activated PI3-K generates membrane-bound phosphoinositides, which act as second messengers and serve to recruit proteins, such as Akt. After recruitment to the plasma membrane, Akt is activated by phosphorylation [26] and then phosphorylates numerous proteins.

The Akt-activating ability of PI3-K is downregulated by PTEN, a lipid phosphatase [27]. PTEN, located on chromosome 10q23, was first described as a tumour suppressor gene that is commonly deleted in brain, breast, and prostate cancers [28–30].

These three factors Akt, PI3-K and PTEN seem to be required for tumour formation in a wide variety of tissues.

PTEN knockout mice are not viable, whereas PTEN heterozygotes survive and develop endometrial, intestinal, thyroid, adrenal gland, and breast hyperplasia, as well as dysplasias and tumours of the skin, gastrointestinal tract, and prostate [31]. A high frequency of PTEN mutations has been reported in several tumour types such as endometrial carcinoma and brain and breast cancer [32]. The severity of these mutations correlates strongly with the tumour stage and grade. For example, complete loss of PTEN is more frequent in metastatic cancer than in primary tumours [33]. The loss of one copy of PTEN increases the probability of tumour growth, and the level of PTEN expression dramatically affects tumour initiation and progression [34]. PTEN mutations are seen in about 20% of cases of endometrial hyperplasia, a precursor of endometrial carcinoma [35,36]. It was recently shown that PTEN is inactivated by promoter hypermethylation [37].

Numerous studies have demonstrated that the Akt pathway is critical for cell survival *via* phosphorylation of a number of downstream proteins [38]. It plays a very important role in promoting growth and blocking apoptosis in cancer cells such as SCLC [39]. There is increasing evidence that PI3-K/Akt plays an important role in breast cancer tumourigenesis. A recent study found increased Akt2 kinase activity in 40% of breast cancer specimens [40]. Another recent study found that PTEN expression is frequently reduced in advanced breast cancers and reported reduced PTEN protein expression in 38% of invasive cancers and in 11% of *in situ* cancers [41]. Akt activity was found to be constitutive in breast cancer cell lines with either HER2 overexpression or a PTEN mutation [42]. Active Akt can be detected in head and neck squamous cell carcinoma (HNSCC) whose pattern of expression and localization correlate with disease progression [43].

Many transforming events, which do not directly modify PI3-K, Akt, or PTEN genetically, can still cause activation of the PI3-K/Akt/PTEN pathway. Three examples of such transforming events are the BCR/ABL translocation, which is the causative event in chronic myelogenous leukemia, amplification of HER2, and amplification of the EGFR.

Several preclinical studies have indicated that rapamycin or its derivatives specifically inhibit the transforming effect of the PI3-K/Akt pathway. For example, rapamycin inhibits the transforming activity of the oncogenic variants of PI3-K and Akt [44]. There are several mTOR inhibitors under clinical development for cancer therapy. CCI-779 is a rapamycin derivative developed by Wyeth-Ayerst that has completed phase I studies as a single agent administered intravenously or orally. It is currently being investigated in combination studies with other anticancer agents and in a broad spectrum of phase II single agent studies. RAD001 (Novartis) and AP23573 are other mTOR inhibitors undergoing clinical development that are being tested in phase II trials. There is no ongoing chemoprevention trial with these agents.

2.1.4. Other pathways (PDGFR and c-KIT)

c-KIT and *PDGFRA* genes belong to the family of class III receptor protein tyrosine kinases (RTKs), which also includes the colony stimulating factor I receptor, PDGFR, and FMS-related tyrosine kinase 3 [45]. C-KIT and PDGFRA are both located on chromosome 4q12 and have structural similarities with the other PDGFR family members [46–48].

The receptor tyrosine kinase KIT and its ligand stem cell factor (SCF) are essential for germ cell development and spermatogenesis, as well as for the normal development of blood cells (especially erythrocytes), melano-

cytes, mast cells, and the interstitial cells of Cajal (ICC) [49]. Activating KIT mutations are most commonly associated with tumours that arise from cells that are developmentally dependent on an intact SCF/KIT axis, most notably GIST, mastocytosis, seminomas and, much less frequently, acute myelogeous leukemia (AML). There is ample evidence of c-KIT expression in human cancers in the absence of substantial or pathogenetically significant c-KIT activation (*e.g.* melanoma, adenoid cystic carcinoma, small cell lung cancer). In the case of melanoma, loss of c-KIT expression correlates directly with advanced clinical stages [50].

Concerning the involvement of c-KIT in premalignant lesions, we know that 'gain-of-function' mutations of c-KIT occur in up to 90% of GISTs. Affected individuals develop diffuse hyperplasia of ICC and multiple GISTs during adulthood [51–54]. This is unclear for seminomas with c-KIT mutations: such tumours exhibit biochemical evidence of strong c-KIT activation and c-KIT probably plays a role in the initiation or maintenance of seminomas [55].

Inhibition of KIT and PDGFR kinase activity can be achieved with imatinib mesylate (Gleevec, Novartis). Broad-spectrum tyrosine kinase inhibitors (TKI) such as SU11248 (Pfizer) are also known to target PDGFR. No specific chemoprevention strategy has been planned with these agents.

2.2. Cell cycle inhibitors

Molecular mediators of the cell cycle include three families of proteins: (1) cyclins; (2) CDKs which regulate the cell cycle by forming complexes with their cyclin catalytic partners; and (3) endogenous CDK inhibitors which, when activated, inhibit cyclin–CDK complexes. Specific cyclins interact with specific CDKs at different phases of the cell cycle, and the resulting cyclin–CDK complexes control specific cell cycle checkpoints through phosphorylation of specific target substrates. A network of kinases and phosphatases also contribute to the functional regulation of cyclin–CDK complexes. Targeting proteins that control cell cycle checkpoints is an attractive therapeutic strategy in terms of tumour specificity, because aberrant expression of certain cyclins has been causally linked to the development of a variety of human cancers [56].

Cyclin D1 as well as cyclin E are frequently overexpressed in lung cancers and their preneoplastic lesions [57–59]. Immunohistochemical analysis revealed that cyclin deregulation was frequently detected in bronchial preneoplasia [57]. This signifies that altered cyclin expression could play a critical role in the maintenance or progression of a preneoplastic bronchial lesion.

A common downstream target of retinoid and EGFR signalling is cyclin D1 [60]. This highlights the potential clinical value of combining an optimal retinoid with an inhibitor of the EGFR pathway to suppress lung carcinogenesis, but also to the value of identifying chemopreventive mechanisms that are active during clinical trials.

A key regulator of the G1/S phase transition in the cell cycle is the retinoblastoma (pRb) tumour suppressor protein. The retinoblastoma proteins, pRb, p130, and p107 are the three known members of the family designated as "pocket proteins". pRb, a nuclear phosphoprotein is structurally and functionally related to p107 and p130. Members of the retinoblastoma family suppress cell growth, at least in part, by inhibiting E2F-dependent transcription of genes whose products are required for DNA synthesis and/or cell cycle progression.

Loss of pRb has been demonstrated in a variety of cancers, including retinoblastoma, osteosarcoma, small cell lung carcinoma, and bladder cancer [61–63]. Restoring pRb function suppresses the neoplastic properties of pRb-deficient cells [64]. Inactivation of the RB gene is a frequent event in small cell lung cancer [65]. Recent immunohistochemical studies of the expression patterns of Rb family members (pRb/p105, p107, and pRb2/p130) in 235 specimens of lung cancer suggest an independent role for RB2/p130 in the development and/or progression of human lung carcinoma [66,67].

The activity of CDKs can be modulated by targeting these kinases with small molecules that bind to their ATP binding pockets, or by altering the composition of the CDK/CKI complexes through different mechanisms. Based on the functions ascribed to CDKs, inhibition of these kinases may promote different phenotypes including cell cycle arrest, induction of differentiation, apoptosis and inhibition of transcription. The precise phenotype does not only depend on which specific CDK is modulated but also on the growth state of the cell, the presence (or absence) of specific cell cycle components, the tissue type explored and so on [56]. There are currently four direct small molecule CDK modulators undergoing clinical therapeutic trials: flavopiridol, UCN-01, CYC202 and BMS-387032. To our knowledge, no chemoprevention trial has been planned for any of them.

2.3. Apoptosis modulators: p53, bcl-2

The inactivation or mutation of p53, which is associated with decreased apoptosis and carcinogenic progression, is an important event in the growth of most solid tumours. p53 is mutated in more than 50% of all human cancers [68]. p53 mutations have also been found in premalignant lesions in lung tissue and indicate early loss of this tumour suppressor's function in lung carcinogenesis [69–71]. Concerning colorectal cancer carcinogenesis, p53 mutations occur later, signalling the transition from adenoma to dysplasia [72,73]. They are frequent in both head and neck cancers and premalignant lesions [74]. In

addition, specific somatic genome alterations in superficial bladder tumours, such as loss of sequences at 17p accompanied by p53 mutations, may serve as markers of an increased risk of progression [75–77].

Targeting p53 has produced promising results in advanced oral intraepithelial neoplasia (IEN). ONYX-015, a replication-competent adenovirus that selectively replicates in cells with deficient p53 activity and causes lysis thereof, achieved complete histologic resolution of dysplasia in seven (37%) of 19 patients with advanced oral IEN [78]. This activity of ONYX-015 was correlated with a decrease in p53 positivity. p53 replacement therapy with an adenovirus containing wild-type p53 (RPR-INGN-201) yielded promising results in a phase I trial in advanced head and neck cancer [79] and is now being tested clinically in advanced oral IEN.

There is a phase I/II study of oral and intramucosal Ad5CMV-p53 gene in patients with diffuse premalignant carcinoma of the oral cavity or pharynx.

Bcl-2 is an apoptotic inhibitor that may contribute to chemoresistance [80]. Bcl-2 is a member of a large family of related genes that encode both positive and negative apoptotic regulators. Inhibition of apoptosis through overexpression of bcl-2 or related family members has been associated with increased resistance to both chemotherapy and radiation [81,82].

Alterations in bcl-2 have been reported in many neoplasms including cancers of breast [83], lung [84], prostate [85], thyroid [86], gastric [87], bladder [88], ovary [89], cervix [90], melanomas [91], and head and neck tumours [92–96].

The role of bcl-2 in the evolution of head and neck cancer has been the subject of several studies. There are consistent findings suggesting that bcl-2 expression is restricted to stem cells committed to differentiation and morphogenesis in normal epithelial cells [97]. The survival advantage provided by bcl-2 prolongs the life span of these cells allowing proliferation and differentiation to proceed. This has been observed in oral mucosa where bcl-2 positivity was only present in a few basal keratinocytes and dendritic cells [98]. Bcl-2 positive cells have been shown to increase and spread to suprabasal layers in leukoplakia [99] and to increase from mild, moderate, to severe dysplasia [100]. These studies also suggested that bcl-2 expression is less prevalent in carcinomas, and this is also borne out by the reported positivity rate in clinical studies where the range is from 10% to 50% with less than 25% of positive tumours in most studies. In addition, lower positivity in well differentiated compared to poorly differentiated tumours has been evidenced [98,100]. These studies provide proof that bcl-2 plays a significant role in the early stages of oral carcinogenesis.

In addition, Gustavo Bruno Baretto [101] showed that bcl-2 expression is characteristic of the early phase of colorectal carcinogenesis: bcl-2 was expressed in 86% of the adenomas and 67% of the carcinomas analysed in his study.

Oblimersen is an anti-bcl-2 antisense oligonucleotide that downregulates bcl-2. No specific chemoprevention trial has been planned with this agent.

2.4. Anti-angiogenic and vasculo-toxic agents

Angiogenesis is a biological process generated by endothelial cells (EC) that are recruited to form new blood vessels from existing ones. Angiogenesis and the formation of new vasculature are crucial for tumour development and progression. The angiogenic process involves the following sequential steps: (1) activation and formation of the EC angiogenic phenotype; (2) changes in the extracellular matrix and degradation of the basement membrane; (3) proliferation and migration of EC; (4) formation of preliminary tubules; (5) remodelling of newly formed microvessels [102–106].

Imbalanced expression of pro- and anti-angiogenic factors and their receptors on EC may determine generation or regression of new blood vessels. More than 200 proangiogenic factors have been identified. The major factors among them include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), angiopoietin (Ang) and chemokines. These factors initiate angiogenesis by modulating the migration and/or proliferation of EC and the formation of neovasculature. The main targets of angiogenic stimuli are EC from post capillary and small terminal venules [107,108]. The importance of angiogenesis has recently been highlighted by recent publications that showed that early dysplastic lesions are often highly angiogenic. VEGF expression is involved in normal mucosa epithelium and the early stages of premalignant lesions and is down regulated during head and neck tumourigenesis [109]. It can be postulated, first, that VEGF may have a physiological role in upper aerodigestive tract epithelium, and second, that VEGF may play an important role in the early stages of head and neck tumourigenesis. Other genetic factors might play a role in the later stages [110]. A gradual increase in microvessel density was observed during the transition from low dysplasia to high dysplasia and cancer in colorectal carcinogenesis [111]. It may also be an important prognostic factor in resected non-small cell lung cancer [112], and it is present in dysplastic lesions [113].

Several anti-VEGF strategies have been developed, including neutralizing antibodies to VEGF (bevacizumab) or VEGFRs (DC101), soluble VEGFR/VEGFR hybrids (VEGF-TRAP), and tyrosine kinase inhibitors of VEGFRs (BAY43-9006, SU11248, ZD6474, AZD2171, PTK/ZK, and others). Several of these agents are now being investigated in clinical trials.

These attractive drugs, which are being tested in therapeutic trials in advanced solid tumours, need to be

evaluated as chemopreventive agents. However their specific toxicities (hypertension, thrombosis, infarction, microangiopathy, necrosis and bleeding) need to be kept in mind when considering their implementation as chemopreventive strategies.

2.5. Anti-invasive agents

There is strong evidence that matrix metalloproteinases (MMPs), a family of enzymes that degrade the extracellular matrix (ECM), play a significant role in tumour invasion and metastasis. MMPs are present in healthy individuals, and have been shown to be involved in various physiological and pathological processes. The regulation of MMP gene expression is tightly controlled in normal tissues to limit its biological activity. This control is lost in malignancy, and MMPs participate in tumour invasion and spread as a result of their capacity to degrade the ECM and regulate angiogenesis. Two gelatinases, MMP-2 and MMP-9, which degrade basement membrane type IV collagen, appear essential for cellular invasion, and are frequently co-expressed in human cancers [114–116]. In addition, these enzymes have been associated with disease progression in a number of malignancies, including breast, colorectal, lung, prostate, ovarian, pancreatic cancers [117].

There is a general correlation between the stage of tumour progression and the level of MMP expression. For example, in a murine system of chemically-induced squamous cell carcinomas, stromelysin-1 levels are highest in spindle-cell carcinomas that have a high probability of metastasis, whereas stromelysin is found at very low levels in benign papillomas [118]. In melanomas, gelatinase B expression is associated with conversion from the radial growth phase to vertical growth phase and subsequent metastasis [119], whereas gelatinase A expression increases with increasing tumour grade [120]. In addition to higher levels of individual MMPs as the tumour grows, malignant tumours tend to express a wider variety of MMPs than benign tumours. For instance, colon adenocarcinomas express matrilysin, stromelysin-1, stromelysin-3, gelatinase A, and interstitial collagenase, but matrilysin is the only MMP that is found in benign colonic polyps. This suggests that matrilysin may participate in early events in tumour progression and that multiple members of the metalloproteinase family may work in concert to facilitate late-stage tumour invasion and metastasis [121]. Loss of basal membrane type IV collagen, along with elevation of MMP-2 and MMP-9 expression, occurs during colorectal tumourigenesis. This suggests that controlling type IV collagenase activation may be beneficial in preventing human colorectal tumour progression [122]. MMP inhibitors include, among others, AG3340, BMS275291, CGS27023A, marimastat, batimastat, and col-3. Disappointing results in clinical trials in the advanced disease setting have halted the development of most of these compounds. None of them has ever being tested as chemopreventive agents.

2.6. Targeted agents affecting traverse mechanisms

2.6.1. HSP90

The 90-kDa heat shock protein (HSP90) is a ubiquitous, evolutionarily highly conserved, molecular chaperone protein in the eukaryotic cytosol. HSP90, together with a number of other chaperones, promotes the conformational maturation of a wide variety of protein kinases. Inhibition of HSP90 function results in the collapse of the metastable conformation of most of these kinases and leads to their proteolytic elimination by proteasomes [123].

This chaperone is involved in many important aspects of cellular processes and in the folding of signal transduction molecules such as protein kinases (Src, Raf1, CDK4) and steroid receptors [124].

We know that in *Drosophila*, Hsp90 can buffer out the activity of mutant proteins in order to suppress genetic variations and modulate the speed of molecular evolution [125]. These and the fact that increased HSP90 expression is found in different types of human tumours also suggests its possible role in facilitating transformation in different human cancers (breast, prostate, bladder, pancreas, endometrium, brain) [126–133].

Numerous natural and synthetic HSP90 inhibitors have been developed in recent years. They include: 17AAG (geldanamycin), radicicol, novobiocin, and other purine-based inhibitors. Some of these inhibitors also sensitize tumour cells to pro-apoptotic insults [134]. As many growth regulatory signals depend on HSP90 for their functional stability, HSP90 is an appealing molecule for incursions in complex oncogenic pathways. However, HSP90 inhibition affects both normal and tumour cells. Moreover, HSP90 inhibition also induces HSPs including HSP90 itself, by releasing HSF-1 from its HSP90 inhibitory complex. Tumour cells may require a higher level of HSP90, and overcome many of these limitations, but a detailed comparison of the complexity of HSP90 inhibition in normal cells *versus* tumour cells is clearly lacking. Another way to circumvent the general effects of HSP90 inhibitors is to increase their specificity by targeting them to a tumour-specific HSP90 client protein, which is a clear task for future drug development [135]. No specific chemoprevention trial has been planned with these agents.

2.6.2. HDAC inhibition

DNA methylation is a chemical modification, resulting in the addition of a methyl (CH_3) group to the carbon 5 position of the cytosine ring. Most cytosine

methylation occurs in the sequence context 5′CG3′ (also called the CpG dinucleotide) [136].

CpG methylation plays several important roles in tumourigenesis, including the silencing of tumour suppressor genes, loss of imprinting, and failure to express DNA repair enzymes and other enzymes that are important for carcinogen detoxification [137–141]. CpG methylation also silences the expression of differentiation antigens that are critical for immune evasion [142,143]. Finally, epigenetic changes may contribute to resistance to particular therapeutic interventions. For example, silencing of oestrogen receptor expression precludes certain hormonal therapies in breast cancer [142,143]. Thus, pharmacologic reversal of CpG methylation may be therapeutically beneficial insofar as transcription of tumour suppressor genes reactivates sensitization of tumours to immune surveillance or enhances response to other therapeutic interventions. Methylation of numerous tumour suppressor genes has been described in a variety of precancerous states in most solid tumours [breast, prostate, colon, head and neck, lung cancers] [144]. Recent data have demonstrated that aberrant promoter methylation of the p16 tumour suppressor gene, which plays a key role in cell cycle regulation, is an early and frequent event in NSCLC [145]. Methylation of DAPK, the apoptosis-associated gene has recently been described in stage I NSCLC, in which it portends a poor prognosis [146]. Inactivation of the *GSTP1* gene by promoter hypermethylation has been reported in human neoplasia including prostate, breast, renal, and lung tumours [147]. Aberrant promoter methylation of multiple genes was found in bronchial brush samples from former cigarette smokers [148]. Retinoic acid receptor-2 silencing by methylation is an early event in head and neck carcinogenesis [149].

As DNA methylation partially represses gene expression *via* histone deacetylation, HDAC inhibitors have been used to activate expression from methylated genes. Although HDAC inhibitors fail to activate the expression of densely methylated genes when used alone, they can synergize with a demethylating agent to induce the expression of methylated genes. In a phase I trial of depsipeptide (a HDAC inhibitor), three patients with cutaneous T-cell lymphoma achieved a partial response, and one patient with peripheral T-cell lymphoma had a complete response. Clinical trials using a combination of a demethylating agent and HDAC inhibitors in cancer are underway. To our knowledge, none is ongoing in the chemoprevention setting.

2.6.3. Proteasome

Selective elimination of regulatory proteins in the cell is a major mechanism for regulating vital cell processes. Protein degradation is fundamental to cell viability. The primary component of the protein degradation pathway in the cell is the 26S proteasome, which degrades the majority of cellular proteins. The proteasome is a large, multiprotein complex present in both the cytoplasm and the nucleus of all eukaryotic cells. Rapid and irreversible proteasomal protein degradation is central to the activation or repression of many cellular processes, including cell-cycle progression and apoptosis, mainly through nuclear-factor-kappaB (NF-kappaB). This fundamental role singles out the proteasome as a unique target for anticancer therapy and has led to the development of numerous proteasome inhibitors [150]. Several natural and synthetic proteasome inhibitors have been studied. Bortezomib (formerly known as PS-341) was the first proteasome inhibitor to enter clinical trials. It was granted accelerated approval by the FDA for the treatment of advanced myeloma. Due to its broad-spectrum action, bortezomib causes side effects (cumulative neurotoxicity, haematologic toxicity) that are probably not desirable in a chemopreventive agent.

2.6.4. Statins

In lipid disorders, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors or statins are efficient and widely used drugs. There is increasing evidence that they may be useful for the treatment of other diseases such as Alzheimer's disease [151–153], and osteoporosis [154]. The mechanism of action of this class of drugs is still under investigation.

Although inhibition of Ras farnelysation was originally thought to be the mechanism responsible for possible antitumour properties of statins, there is increasing evidence that other mechanisms are involved. *In vivo* and *in vitro* studies have shown different antitumour properties of statins, that can be classified as follows:

1) Growth arrest and apoptosis: cholesterol and dolichol-mediated effects on P21 and P27, CDK inhibitors.
2) Anti-metastatic: reducing matrix metalloproteinase 9 (MMP 9) gene expression results in decreased MMP 9 activity; suppressing the expression of urokinase-type plasminogen activator (u-PA), u-PA receptor, and MMP 9 in monocytes; and reducing the invasiveness of different types of tumours cells (lymphoma cells, melanoma cells, breast cancer cell).
3) Angiogenesis: statins were reported to have antiangiogenic effects as well as proangiogenic effects depending on the dose, the cell type, and the time course [155].

In vitro and *in vivo* studies have suggested a therapeutic effect of statins in cancer, and epidemiological studies (4S, WOSCOPS, AFTEX, LIPID, CARE, ALLHAT-

LLT, PROSPER) have shown a preventive effect of statins against cancer rather than a therapeutic effect [156–159]. No specific chemopreventive trial with such agents is ongoing in the cancer setting. This should be envisioned taking into account the very positive toxicity profile of these agents.

2.7. Selecting targeted agents for chemoprevention trials

Selecting specific targeted agents for chemoprevention trials should be based on a rationale approach taking into consideration a whole set of criterias. Two key issues have to be specially highlighted:

1) The essential principle of chemoprevention is to intervene within the multistep carcinogenic process, thus the target that will be blocked should play an important role in this process.
2) The toxicity profile of a specific drug that may be acceptable for cancer patients will not be the same as the one for patients without cancer. Thus for primary chemopreventive approaches, treating healthy individuals at high risk, the drug to be used should have a very mild or low toxicity profile, proven in very large cohorts (phase III and phase IV data). This is partially true for secondary chemoprevention (patients with pre-cancerous lesions), although a favourable toxicity profile based on phase II trials might be considered enough by some regulatory agencies. Finally in the setting of tertiary chemoprevention, a higher toxicity profile is acceptable, notably when developing such trials under the umbrella of ''adjuvant trials'' [160].

The following represent some of the most relevant criteria to defining an optimal targeted agent for chemoprevention:

- The target should be present and abnormal as compared to normal epithelium.
- The target should influence tumour biology.
- The preclinical data obtained with the drug aiming at the target should be favourable (efficacy, toxicity).
- The key results of the drug in human studies should also be favourable (efficacy, and toxicity from phase I, II and eventually phase III trials).

When using such criteria, the following targeted agents appear as the ones with the best profile:

- NSAID and COX2 inhibitors (treated on a separate manuscript).
- HER signal transduction inhibitors.
- Apoptosis modulators.
- Statins.
- Angiogenesis inhibitors (but with clear toxicity concerns).

3. Conclusions

Chemoprevention should largely benefit from the development and implementation of rationally developed anticancer targeted agents in the clinical setting. Chemoprevention is a clinically proven modality in head and neck, breast and colon carcinogenesis. But there is still a tremendous need to develop even more effective and safer chemopreventive agents. Molecular targeting research has led to a revolution in drug development and is blurring the distinction between malignancy and premalignancy and between cancer therapy and prevention. Now, drugs such as cyclooxygenase 2 inhibitors are leaving the preventive setting and entering the therapeutic arena, and other molecularly targeted agents (*e.g.* epidermal growth factor receptor inhibitors) are also on the verge of doing so.

Notwithstanding its great potential, molecular targeting still has a long way to go in the preventive setting in order to clarify the precise targets and specific side effects of its respective inhibitors.

Finally, developing 'combination chemoprevention' will be essential, just as combination chemotherapy has been so important in the treatment of invasive disease. Surrogate biomarkers for risk and molecular targets for intervention will need to be identified, such that chemoprevention candidates as well as high-risk groups can be thoroughly evaluated for focused clinical studies. This should help avoid conducting costly randomized controlled trials that do not confirm their epidemiologic findings. This point can be illustrated by finding surrounding β-carotene. After strong epidemiologic evidence suggested that β-carotene could reduce lung cancer risk, randomized controlled trials demonstrated that β-carotene actually increased lung cancer risk and mortality in heavy smokers [161,162].

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