

# Cancer prevention by targeting angiogenesis

Adriana Albini, Francesca Tosetti, Vincent W. Li, Douglas M. Noonan and William W. Li

**Abstract** | Healthy individuals can harbour microscopic tumours and dysplastic foci in different organs in an undetectable and asymptomatic state for many years. These lesions do not progress in the absence of angiogenesis or inflammation. Targeting both processes before clinical manifestation can prevent tumour growth and progression. Angioprevention is a chemoprevention approach that interrupts the formation of new blood vessels when tumour cell foci are in an indolent state. Many efficacious chemopreventive drugs function by preventing angiogenesis in the tumour microenvironment. Blocking the vascularization of incipient tumours should maintain a dormancy state such that neoplasia or cancer exist without disease. The current limitations of antiangiogenic cancer therapy may well be related to the use of antiangiogenic agents too late in the disease course. In this Review, we suggest mechanisms and strategies for using antiangiogenesis agents in a safe, preventive clinical angioprevention setting, proposing different levels of clinical angioprevention according to risk, and indicate potential drugs to be employed at these levels. Finally, angioprevention may go well beyond cancer in the prevention of a range of chronic disorders where angiogenesis is crucial, including different forms of inflammatory or autoimmune diseases, ocular disorders, and neurodegeneration.

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## Introduction

Cancer has been identified by the United Nations as a non-communicable disease posing a global health threat with considerable economic consequences.<sup>1,2</sup> The cost of cancer care in the USA alone is projected to rise from US\$125 billion in 2010 to US\$207 billion by 2020.<sup>2</sup> Although great strides have been made in reducing mortality from cardiovascular disease and other non-communicable diseases through preventive efforts, cancer is still usually treated at advanced, often metastatic, disease stages.<sup>3</sup> Screening methods have improved prognosis for some cancers;<sup>4</sup> however, early detection is not yet possible for most malignancies, and the value of screening has been challenged for some tumours.<sup>5</sup> Targeted therapies are emerging as useful therapies, but treating all patients with these costly agents is not economically sustainable.<sup>6,7</sup> As the world population exceeds 7 billion, cancer prevention clearly becomes an urgent goal to pursue.

Many cancers can be prevented by lifestyle changes, such as avoiding tobacco use, excessive UV exposure, infectious agents, poor dietary habits and obesity.<sup>8</sup> Behavioural studies suggest that promotion of healthy dietary habits and exercise is only moderately successful.<sup>9</sup> Thus, cancer prevention remains a difficult task. Preclinical evidence suggests that cancer prevention is feasible, but for the population at large the question is how.

It is estimated that half of men and a third of women will be diagnosed with a cancer in their lifetime.<sup>10</sup> The cancer diagnosis usually comes many years after the transforming events that spawned the cancer. Chemoprevention, the pharmacological prevention of cancer, was proposed over

three decades ago,<sup>11</sup> but little has been invested to advance this life-saving approach, which should be inexpensive, non-toxic, and suitable for chronic administration. In this Review, we suggest a new consideration: limiting chemoprevention to tumour cells may be too specific. To be effective, cancer prevention should ideally impact on the organ microenvironment, boosting physiological defences against tumour development.<sup>12</sup>

## Angiogenesis and angioprevention

Angiogenesis and inflammation are two host-dependent and interdependent hallmarks of cancer<sup>13</sup> that have an early permissive role in tumorigenesis (Figure 1). Angioprevention is the term that we used 10 years ago when we proposed that angiogenesis inhibition was a common target of most cancer chemopreventive drugs.<sup>14</sup> Although epithelial cells that harbour mutations retain distinct, organ-specific phenotypes, endothelial cells are generally untransformed and a common target across many cancers. Increasing evidence supports the angioprevention approach in preclinical models as well as in epidemiological and clinical intervention studies in humans.

Judah Folkman first suggested that tumour dormancy could be maintained by preventing neovascularization of microscopic cancers.<sup>15</sup> However, the clinical validation of antiangiogenic therapy was done in the context of metastatic disease. Although antiangiogenic therapy has improved the standard of care for some cancers, its overall clinical benefit is modest for most patients, mainly observed in the form of prolonged progression-free survival.<sup>16,17</sup> In the setting of heavy disease burden, most patients treated with antiangiogenic agents eventually experience disease progression due to evolution of

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## Competing interests

The authors declare no competing interests.

**Key points**

- Angiogenesis and inflammation are host-dependent hallmarks of cancer that can be targeted using prevention approaches long before tumours initiate and progress
- Several prescription and non-prescription drugs are already available for use in angioprevention, as well as a growing array of nutraceuticals
- We propose four levels of angioprevention: I for the 'healthy' population; II for patients with increased risk of cancer; III for preneoplastic lesions; and IV for prevention of cancer recurrence
- There are several successes in cancer prevention that demonstrate clinical feasibility and levels of intervention, from no to little to intense physician involvement
- To avoid toxicity while maintaining efficacy, angioprevention needs to achieve a degree of angiogenesis control that is not excessively suppressive, such that healthy vascular function is maintained

biological escape mechanisms by tumours.<sup>18</sup> All these data raise the question: 'are angiogenesis inhibitors being used in an optimal scenario?'

Angioprevention applies angiogenesis inhibition to predisposing conditions,<sup>14</sup> such as chronic inflammation, hyperplastic or preneoplastic lesions, and occult tumours. Angiopreventive drugs are chemopreventive compounds that counteract angiogenesis and/or the underlying inflammation. The goal is to influence the tumour microenvironment so that host defence systems are fortified to more durably suppress the development of clinically detectable tumours. Microscopic foci of transformed cells are very common in healthy individuals. Autopsy studies have shown that approximately 40% of women between 40 and 49 years old have occult breast cancers.<sup>19</sup> Similarly, *in situ* prostate cancer has been detected post-mortem in 24% of men aged 60–70 years.<sup>20</sup> Microscopic thyroid cancers are estimated to be present in 98% of individuals by the age of 70.<sup>21,22</sup> Yet, these cancers are diagnosed in only 2%, 8%, and <0.5%, respectively, in these age groups. The body's intrinsic ability to prevent tumour angiogenesis is one mechanism by which cancers are maintained in a prolonged dormant state.<sup>23</sup> Although it is not known which individuals with occult cancers will go on to develop clinical disease, angioprevention has the potential to avert this fate.

In the context of cancer, immune cells act as Janus-like components: on one side they can destroy cancer cells; on the other side they can promote tumour angiogenesis, growth and dissemination.<sup>24–26</sup> The presence of immune cells within tumours has been suggested to contribute to the carcinogenic and metastatic processes,<sup>13,27</sup> and to tumour angiogenesis;<sup>24–26</sup> their polarization seems to determine clinical outcome. A typical example is macrophages: classically-activated M1 macrophages produce antiangiogenic Th1 cytokines (such as interleukin [IL]-12); by contrast, tumour-associated macrophages, M2 macrophages, Tie2-expressing macrophages and myeloid-derived suppressor cells produce Th2 cytokines, allow tissue reconstruction, growth promotion and angiogenesis.<sup>28,29</sup> Similar groupings have been proposed for polymorphonuclear neutrophils (PMNs) as either antitumour 'PMN1' (or N1) or protumour 'PMN2' (or N2) subsets that have a direct role in tumour angiogenesis.<sup>26,30</sup> Protumour and

proangiogenic polarization have been demonstrated for most tumour microenvironment immune cells including dendritic, mast, T and B cells.<sup>25,26,28,29,31</sup> Blocking chronic inflammation can prevent protumour polarization and contribute to angioprevention.

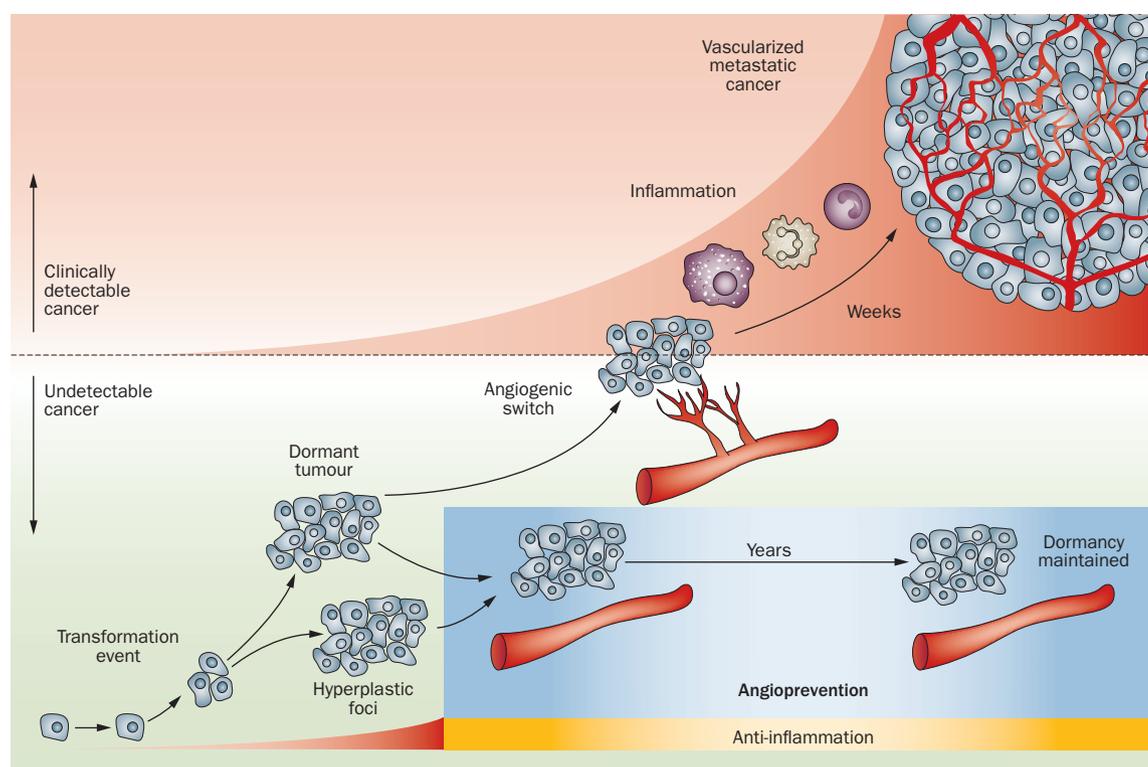
**Mechanisms of angioprevention****Vascular cell migration and invasion**

Inhibition of proteases involved in the degradation of the vascular basement membrane and extracellular matrix suppresses angiogenesis and metastasis.<sup>32</sup> Abnormal matrix remodelling by unchecked protease activity opens the way to sprouting vessels, by breaking the extracellular matrix and favouring detachment, attachment and spatial organization of endothelial cells. Targeting this remodelling is one mechanism of action attributed to the antiangiogenic activity of green tea<sup>33</sup> through inhibition of the matrix metalloproteinases (MMP)-2 and MMP-9. Inhibition of matrix remodelling can occur by direct binding as well as indirect mechanisms including downregulation of proteases and protease stimulators and upregulation of protease inhibitors.<sup>34,35</sup> MMP inhibition has a role in the mechanism of action of numerous angiopreventive agents.<sup>36,37</sup> Flavonoids such as fisetin, apigenin, and luteolin specifically and dose-dependently antagonize MMP-9 gene and protein expression in endothelial cells.<sup>38</sup> Organosulfur compounds such as sulforaphane and phenethyl isothiocyanate are naturally present in dietary cruciferous vegetables, including broccoli and Brussels sprouts. One key target of phenethyl isothiocyanate is protein kinase C, which leads to downstream inhibition of MMP-2 and MMP-9.<sup>39</sup> Eugenol (4-allyl-2-methoxyphenol), a component of basil, also inhibits angiogenesis through modulation of MMP-2 and MMP-9; in addition, eugenol acts through modulation of VEGF, its receptor VEGFR-1, tissue inhibitor of metalloproteinases (CSC-21K, also known as TIMP-2) and RECK.<sup>40</sup> Other angiopreventive compounds, including hyperforin, curcumin, resveratrol, and silibinin, affect metalloproteinases and other matrix proteolytic enzymes, thereby impacting on the migration and invasion of endothelial cells.<sup>35,41,42</sup>

**Endogenous angiogenesis inhibitors and hormones**

Many endogenous angiogenesis inhibitors reside within the host microenvironment, including thrombospondin-1, platelet factor 4, angiostatin and other plasminogen fragments, endostatin, tumstatin, alaphostatin, arresten, canstatin, pigment epithelium-derived factor (PEDF), TIMP-2, ADAM-TS 9 and tetrahydrocortisol as well as some chemokines.<sup>43</sup> Induction of these inhibitors by drugs or diet can 'boost' innate vascular suppression and curb microscopic tumour growth (Figure 2).<sup>43</sup>

Interferons (IFN) have long been known to inhibit angiogenesis.<sup>44</sup> Imiquimod is a topical immunomodulatory drug that is approved by the FDA for the treatment of actinic keratosis to prevent its progression to skin cancer.<sup>45</sup> This agent acts through activation of Toll-like receptor 7 (TLR7) and production of IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , IL-12 and IL-18;<sup>46</sup> repression of MMP activity, and inhibition of tumour angiogenesis.<sup>47</sup>



**Figure 1** | Timing of angioprevention. After a transformation event, most tumours remain clinically indolent for years if not decades. Only after the angiogenic switch, driven by increasing inflammation, and many subsequent cycles of replication will a tumour become clinically detectable. At this point, it will have extensively progressed, is likely to have disseminated tumour cells and may already be metastatic. Angioprevention, which includes anti-inflammatory antiangiogenic mechanisms, needs to be started when a tumour is a dormant or hyperplastic foci or even before this stage, and sustained to maintain the tumour in this state. The observation that angioprevention efficacy increases as a function of time of application with angioprevention drugs, such as aspirin, is not surprising when seen in this context. These concepts must also be kept in mind when developing clinical trials.

As a preventative agent in women who have had oestrogen receptor-positive breast cancer, treatment with tamoxifen reduces the incidence of secondary breast cancer by more than 40%.<sup>48</sup> Tamoxifen has antitumour activity independent of its anti-oestrogen action that is associated with endostatin production.<sup>49</sup> A clinical study of early stage breast cancer showed that tamoxifen (20 mg/day) significantly increased endostatin levels in breast tissue and abdominal subcutaneous fat by 33%, while simultaneously lowering VEGF and angiogenin levels.<sup>50</sup>

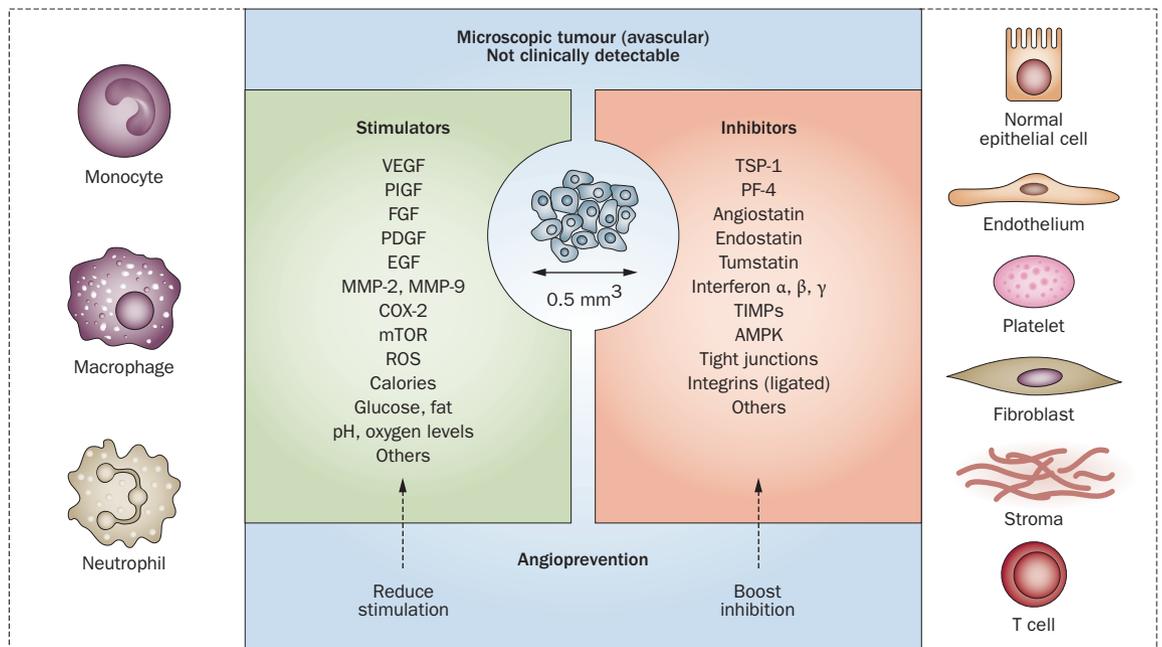
Endothelial cells are sensitive to the hormonal milieu, such as during the menstrual cycle, and hormonal mimetics and antagonists show antiangiogenic activities.<sup>51</sup> A diet high in phytoestrogens, such as genistein and enterolactone, slows breast cancer progression in a fashion similar to tamoxifen,<sup>52</sup> with inhibition of pro-inflammatory cytokines IL-1 $\alpha$  and IL-1 $\beta$  that promote angiogenesis. Indeed, the phytoestrogens enterolactone, quercetin and curcumin alter oestrogen-dependent transcription of genes in breast cancer cells.<sup>53</sup>

#### Inflammation and inflammatory angiogenesis

Chronic inflammation, which promotes angiogenesis and is linked to the development of 30% of all cancers,<sup>25</sup> is a primary target for angioprevention.<sup>24,26</sup> Chronic use of anti-inflammatory agents prevents a wide range of human

tumours.<sup>54–56</sup> The angiopreventive agent resveratrol reduced neutrophil infiltration in mesenteric lymph nodes and lamina propria in a colon cancer model.<sup>57</sup> The signal transducer and activator of transcription (STAT) family member STAT3 is a central signalling hub for immune cells,<sup>58</sup> and is often activated in cancer cells, leading to expression of VEGF, IL-10 and IL-6, which in turn upregulates STAT3 signalling in endothelial and immune cell subsets in the tumour microenvironment.<sup>58</sup> Several angiopreventive phytochemicals—such as apigenin, luteolin, and myricetin—target the STAT3 pathway,<sup>59,60</sup> as well as other key mediators of inflammation, including the NF- $\kappa$ B, PI3K, TNF- $\alpha$ , and HIF-1 $\alpha$  pathways.<sup>41,61</sup>

Cyclooxygenase-2 (COX-2) is another proinflammatory molecule that is overexpressed in many cancers, including breast, colorectal, lung, and prostate and preneoplastic lesions, such as ductal carcinoma *in situ*, cervical intraepithelial neoplasia, prostatic intraepithelial neoplasia, Barrett's oesophagus, and actinic keratosis, but is not highly expressed in healthy tissues.<sup>62</sup> COX-2 promotes carcinogenesis as well as angiogenesis through both prostaglandin-related and non-prostaglandin-related mechanisms.<sup>63</sup> The angiogenic mechanisms include induction of MMP-2 and MMP-9, production of VEGF, bFGF, and PDGF, and enhancement of  $\alpha$ v $\beta$ 3-mediated angiogenesis.<sup>62</sup> COX-2 inhibition interferes with these



**Figure 2** | Angiogenesis is controlled by a balance of endogenous stimulators and inhibitors; in homeostasis, the sum of these effects is null. Stimuli, for example in wound healing, can tip the balance, at first in favour of angiogenesis, where many new vessels are formed, then towards antiangiogenesis, where excess vessels are pruned away. Some angioprevention approaches aim to boost endogenous inhibitor levels and/or lower stimulators. Cell polarization from protumour to antitumour phenotypes, and metabolic repression can also promote a microenvironment resistant to tumour vascularization and tumour expansion. Abbreviations: MMP, matrix metalloproteinase; ROS, reactive oxygen species; TIMP, tissue inhibitor of metalloproteinase.

events,<sup>63</sup> thus aspirin and COX-2 inhibitors are *bona fide* angiopreventive agents. VEGF, MMPs and NF-κB are also targets of the β-adrenergic receptor blocker propranolol that has been shown to be antiangiogenic, and is clinically active in infantile hemangiomas.<sup>64</sup>

**Apoptosis, autophagy, senescence and stem cells**

The induction of autophagy or forcing activated endothelial cells into a quiescent state is also an angiopreventive mechanism.<sup>65</sup> Among chemopreventive compounds, the phytochemicals sulforaphane<sup>66</sup> and resveratrol<sup>67</sup> can activate pathways leading to autophagy. Microarray data suggest that some angioprevention agents, such as N-acetyl-cysteine and 9-cis-retinoic acid, induce a molecular profile in endothelial cells similar to that of *in vitro* senescence.<sup>65,68</sup>

Beyond microscopic tumours, cancer stem cells (also known as cancer initiating cells) reside in, and are dependent on, the tumour microvasculature. Angiogenesis inhibitors have been shown to decrease putative cancer stem cell numbers,<sup>69</sup> suggesting that angiopreventive treatments could not only halt tumour growth but might also restrict cancer stem cell expansion.<sup>69</sup>

**Metabolism and caloric restriction**

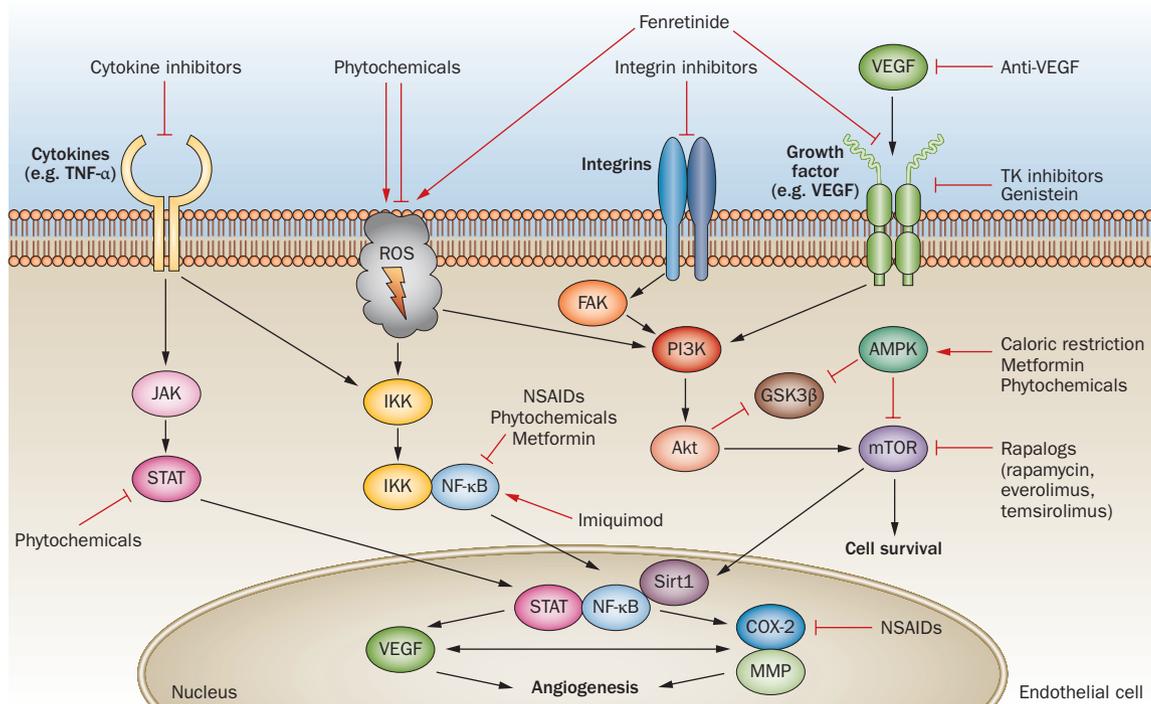
Reduction of caloric intake and energy restriction reduces cancer risk.<sup>70</sup> Pharmacological metabolism regulators are under investigation for cancer prevention. Agents such as 2-deoxyglucose (2-DG), antidiabetic agents targeting glucose metabolism (metformin, rosiglitazone and pioglitazone), and histone deacetylase inhibitors, all exhibit antiangiogenic activity.<sup>71-73</sup>

The mTOR pathway controls protein metabolism in concert with AMPK and Akt; therefore, over-activation of mTOR can induce VEGF-mediated angiogenesis (Figure 3).<sup>74</sup> The mTOR inhibitors, rapamycin, sirolimus, temsirolimus and everolimus (rapalogs) are in clinical use based on dual antiangiogenic and antitumour properties.<sup>75</sup> Rapalogs are undergoing clinical assessment for the secondary chemoprevention of skin cancer and secondary cancers in patients who are immunosuppressed following transplantation.<sup>76,77</sup> Metformin, an AMPK stimulator, inhibits mTOR and demonstrates antiangiogenic activity *in vitro* and *in vivo*.<sup>78-80</sup> Among its unique mechanisms is the upregulation of PEDF, an endogenous angiogenesis inhibitor.<sup>81</sup>

Redox stress is another key component of oncogenic-induced chronic inflammation.<sup>13,25-27</sup> Oxygen radicals can activate endothelial cells and influence cytokine release and vascular permeability.<sup>82</sup> Antioxidants are widely proposed for cancer prevention,<sup>83</sup> and in endothelial cells an antioxidant response may restore redox homeostasis.<sup>84</sup> Many angiopreventive agents exert pleiotropic effects by inhibiting angiogenesis and inflammation mediated by redox-sensitive targets, such as NF-κB, Akt, mTOR (Figure 3).<sup>85,86</sup> Because energy restriction and metabolic regulation have anti-inflammatory and angiosuppressive consequences,<sup>84,87,88</sup> another benefit of angioprevention could be weight control (Box 1).

**Angioprevention in the clinic**

Clinically, angioprevention maintains transformed cells in a dormant state and keeps the microenvironment healthy so that transformation is hampered despite



**Figure 3** | Selected key signalling pathways that are targets for angioprevention. STAT and NF-κB are closely associated with inflammation and angiogenesis. Agents blocking these pathways in endothelial cells could have angiopreventive activity. Angiopreventive compounds induce both free-radical scavenging or transient reactive oxygen species elevation, evoking antioxidant responses. The identification of molecular targets of angioprevention is important for drug design, for identifying predictive biomarkers, and as a rationale for combinatorial angioprevention. Abbreviations: MMP, matrix metalloproteinase; NSAID, non-steroidal anti-inflammatory drug; ROS, reactive oxygen species; TK, tyrosine kinase; STAT; signal transducer and transcription activator.

promoting stimuli. We propose four distinct levels of angioprevention (Figure 4).

**Level I**

This level is aimed at cancer prevention in the general healthy population at ‘lowest’ risk of developing cancer. In these low-risk individuals, intervention must be safe with few, if any, adverse effects. Possible interventions include dietary factors and scientifically supported dietary supplements, caloric restriction, and aspirin, which is already used to prevent heart attack or stroke. Combinations of drugs or foods and nutritional supplements might have synergistic angiopreventive activity.<sup>89</sup> For level I angioprevention, regulatory approval might be unnecessary as long as the interventions are safe and suitable for general application.

**Level II**

Level II angioprevention would be aimed at cancer prevention in individuals at moderate-to-high cancer risk. These include healthy persons with genetic abnormalities that are associated with a higher cancer risk (for example, *BRCA1* and *BRCA2* mutations, Li-Fraumeni syndrome and familial adenomatous polyposis), family history (any heritable cancer), occupational or lifestyle exposure (such as asbestos, toxins, tobacco and heavy alcohol consumption), immunosuppression (for example, transplantation, or HIV) and/or metabolic syndromes

(including diabetes). The higher cancer risk of individuals in this group justifies a higher risk tolerance for the angioprevention agents used than in level I.

Metformin is a well-tolerated drug suitable for level II angioprevention<sup>90</sup> that is widely prescribed for type 2 diabetes and is potentially also usable for level I angioprevention. Difluoromethylornithine (an inhibitor of polyamine synthesis) and sulindac,<sup>54</sup> fenretinide and hormonal mimetics<sup>91</sup> also fall into the level II angioprevention category. Selected phytochemicals (curcumin, hyperforin, xanthohumol, and black raspberry extract) would also be appropriate,<sup>3,92</sup> as well as synthetic triterpenoids, such as bardoxolone, which show potent antiangiogenic and anti-inflammatory activity.<sup>93–95</sup>

**Level III**

Individuals with pre-neoplastic lesions, such as skin actinic keratosis, oral leukoplakia, colon adenomas, cervical dysplasia, prostatic hypertrophy, and Barrett’s oesophagus would be eligible for level III angioprevention strategies. The risk for progression to frank carcinoma warrants a more-aggressive angiopreventive approach, with a greater risk-to-risk-tolerance ratio. A number of prescription drugs with angiogenesis inhibitory properties are available, such as imiquimod (for skin cancer), finasteride (for prostate cancer), and celecoxib (for colon cancer).<sup>96</sup> For the latter, cardiac protective treatment is warranted.<sup>97</sup>

**Box 1** | Angioprevention—not only in cancer

Pathological angiogenesis is a common denominator of many disorders,<sup>96</sup> suggesting that clinical angioprevention may provide benefits in a wide range of conditions beyond cancer. In contrast to cancer, other pathologies often show more dependence on specific angiogenic pathways, further enhancing targeted drug action; the success of angiogenesis inhibition in the treatment of ‘wet’ age-related macular degeneration (AMD) is an example.<sup>145</sup> This can be extended into the angioprevention realm. Epidemiological evidence suggests long-term non-steroidal anti-inflammatory drug (NSAID) use is associated with reduced risk of Alzheimer’s disease, where pathological brain microvessels are characteristic.<sup>146,147</sup> The AREDS (Age-related Eye Disease Study) formula supplementation<sup>148</sup> might implicate angioprevention in the neovascular form of AMD. Pathological angiogenesis driven by hypoxia and inflammation leads to joint destruction in patients with rheumatoid arthritis,<sup>149,150</sup> where angioprevention may avert its progression. Angioprevention could even be used to prevent the growing epidemic of obesity, since controlling angiogenesis regulates adipose mass.<sup>151–153</sup> Genetic polymorphisms of VEGF are associated with childhood obesity,<sup>154</sup> and human studies have correlated BMI with inflammatory biomarkers (CRP) and angiogenesis (VEGF).<sup>143</sup> Green tea intake reduced visceral fat in adults by 6% over 12 weeks in a small, prospective, controlled trial;<sup>155</sup> other compounds such as synthetic triterpenoids are showing promise.<sup>156</sup> Finally, many angiopreventive compounds, including phytochemicals, are showing protective effects on the cardiovascular system, not only by inhibiting atherosclerosis through suppressing plaque neovascularisation, but also by preventing collateral damage during cancer chemotherapy, while often enhancing efficacy.<sup>42</sup> Combinatorial angioprevention regimens are a highly promising but untapped frontier.

**Level IV**

Level IV angioprevention aims to prevent disease recurrence in patients who have achieved cancer remission. An aggressive and sustained angiopreventive approach is necessary, since tumour cell dissemination is likely to exist.<sup>98</sup> Prevention of pathological angiogenesis is of paramount importance for durable cures. Angiopreventive interventions must potently suppress the ‘soil’ (micro-environment) as described by Steven Paget, so that the ‘seed’ (microscopic metastases) will not thrive.<sup>99,100</sup> Level IV therapies include antiangiogenic cancer agents, such as tyrosine kinase inhibitors (axitinib, pazopanib, sorafenib, and sunitinib), mTOR inhibitors (everolimus, and temsirolimus), and certain chemotherapeutic drugs administered at low dose in a ‘metronomic’ schedule.<sup>101</sup> Long-term administration of bevacizumab in the adjuvant setting is currently under clinical investigation.<sup>102,103</sup>

**Regulatory issues, biomarker use**

Regulatory approval of pharmaceuticals for chemoprevention has proved challenging. Both finasteride and dutasteride have been shown in large clinical trials to decrease risk of prostate cancer by 25–35% in treated healthy men, for example, but both were denied FDA approval for cancer prevention, because of uncertainty for applicability in the general population.<sup>104</sup> The discovery of biomarkers for microscopic cancers may pave the way for monitoring the impact of angioprevention.<sup>105</sup> Recent discoveries of proangiogenesis and antiangiogenesis markers in the platelet proteome reflecting dormant cancers are now being translated to clinical studies.<sup>106–108</sup> Several microRNAs are highly expressed during angiogenesis, particularly miR-126 that targets VEGF.<sup>109</sup> Effects of phytochemicals on vascular microRNAs are coming to light,<sup>110</sup> including those with protective effects

on endothelial cells.<sup>111</sup> MicroRNAs might also be a key source of biomarkers useful in angioprevention and cancer prevention in general.<sup>112</sup>

Whether regulatory approval is required for clinical application of angioprevention remains an open question. Dietary and over-the-counter drug angioprevention may not even require physician involvement at level I, while interventions from level II to level IV will need to be prescribed by a clinician.

**Successful angioprevention**

Clinical trials that assess antiangiogenesis and angioprevention as an end point are underway (Table 1), increasing the urgency for identifying biomarkers for angiogenesis.<sup>17</sup> However, a number of chemopreventive angiogenesis inhibitory agents have shown clinical efficacy. Here, we describe selected compounds that demonstrate angiopreventive benefits.

**Fenretinide**

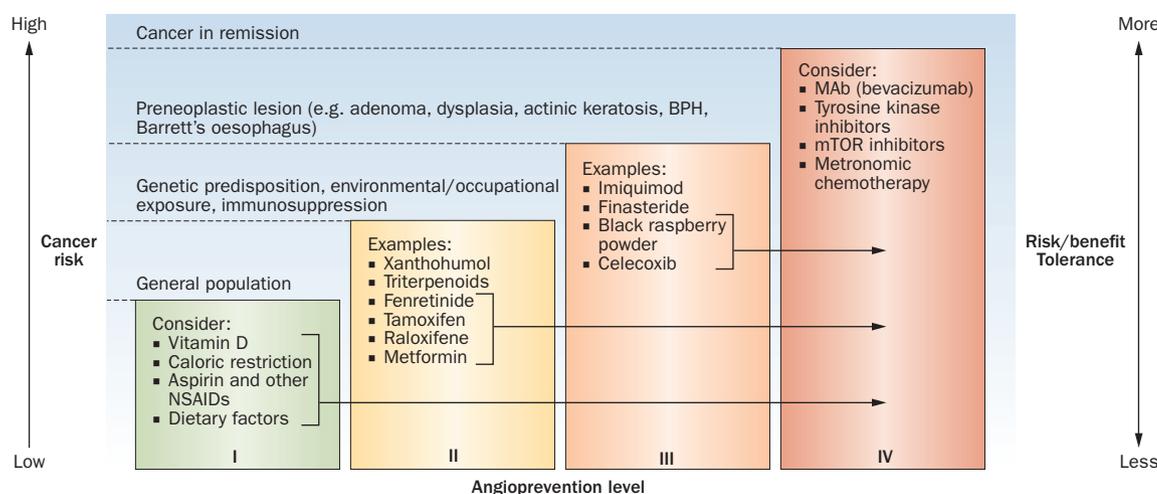
Several retinoids inhibit angiogenesis, including fenretinide.<sup>113</sup> A multicentre, randomized chemoprevention trial evaluated the effect of fenretinide on the incidence of contralateral tumours in women with stage I breast cancer, showing a 35% reduction in second breast cancer incidence in premenopausal women and significantly (95% CI 0.000–0.522;  $P = 0.0327$ ) lower incidence of ovarian cancer in the fenretinide arm.<sup>114</sup> An overall reduction by 17% (hazard ratio [HR] = 0.83; 95% CI 0.67–1.03) in second breast cancer incidence was observed with fenretinide after 14.6 years of follow up, along with a 38% (HR = 0.63; 95% CI 0.46–0.83) reduction in second breast cancer incidence in premenopausal women,<sup>114</sup> reaching 50% ( $P$ -age\*treatment interaction = 0.023) in women aged 40 years or younger. Fenretinide may be active in *BRCA* mutation carriers,<sup>113</sup> and a randomized prevention trial is underway in women who are at high risk for breast and ovarian cancer.<sup>115</sup>

**Metformin**

Metformin, widely used as a treatment for type 2 diabetes,<sup>116</sup> is well tolerated and associated with limited, transient side effects. Retrospective population studies showed a 21% reduced risk and improved survival for all cancers in patients with type 2 diabetes receiving metformin.<sup>117,118</sup> The results were significant even after adjusting for BMI, a cancer risk factor. Conversely, diabetics treated with non-metformin regimens had an increased (1.36–1.42 fold) risk for cancer.<sup>117</sup> Another study demonstrated that patients taking metformin experienced a 62% risk reduction for pancreatic cancer.<sup>119</sup> Metformin upregulates circulating levels of the antiangiogenic extracellular matrix molecule thrombospondin-1 (TSP-1) in insulin-resistant obese women, likely one protective mechanism of action.<sup>87</sup>

**Aspirin and NSAIDs**

Observational studies of long-term use of aspirin or NSAIDs revealed a reduced risk of colon, breast, prostate and lung cancer.<sup>56</sup> Colon cancer and colon polyp



**Figure 4** | Four levels of angioprevention. All humans are at risk for developing cancer. As risk level increases, there is greater tolerance of collateral effects to obtain more benefit. Low toxicity interventions can be continued throughout higher levels. Abbreviations: BPH, benign prostatic hyperplasia; mAb, monoclonal antibody; NSAID, non-steroidal anti-inflammatory drug.

formation have undergone extensive study, because they are frequent lesions, higher risk groups can be identified and treated, and regular colonoscopy can be used to establish an accurate end point for both colon polyps and cancer. Multiple studies have shown that aspirin and NSAIDs, in particular selective COX-2 inhibitors, reduce colonic adenoma formation.<sup>54,120</sup> Three large trials have now shown that regular, long-term aspirin use does reduce cancer incidence. Two of these, studying aspirin for cardioprotection,<sup>55,121</sup> showed that regular aspirin use, independent of dose, decreases incidence and mortality of numerous cancers, with even greater reduction for a subset of cancers, including colorectal, lung, and brain cancer. Long-term usage (>5 years) was needed for these differences to become evident.<sup>55</sup> A large trial in patients with Lynch syndrome at risk for colorectal cancer showed significant differences in colorectal cancer incidence for a subgroup of individuals using aspirin for at least 2 years.<sup>122</sup> Aspirin has antiangiogenic activity that is mediated through both COX-dependent and independent mechanisms.<sup>123–125</sup>

Celecoxib, a selective COX-2 inhibitor and potent anti-inflammatory agent, suppresses angiogenesis and has been clinically validated as a prevention agent for both sporadic colon adenomas and familial adenomatous polyposis.<sup>126,127</sup> To mitigate the cardiovascular toxicities of celecoxib, low circulating levels of C-reactive protein may identify patients for whom the risk–benefit ratio of celecoxib chemoprevention is favourable.<sup>128</sup>

A double-blind, controlled randomized trial of 240 individuals with actinic keratosis showed that treatment with celecoxib for 9 months had a 60% reduction in the mean number of basal-cell and squamous-cell skin cancers.<sup>129</sup> A combination of sulindac and 2-difluoromethylornithine also demonstrated a remarkable reduction in colon adenoma formation.<sup>54</sup>

### Polyphenols

Green tea consumption is associated with a decreased risk of colon, prostate, lung, oesophageal, and other

cancers;<sup>3,41,130</sup> catechins are usually considered to be the active agents. A double-blind, placebo-controlled study in men with high-grade prostatic intraepithelial neoplasia showed that consuming green tea catechins (600 mg/day, equivalent to 4–6 cups of tea/day) over 1 year reduced progression to prostate cancer from 30% in the placebo group to 3% in the treatment arm.<sup>130,131</sup> A Japanese study in patients following removal of colon polyps demonstrated that consumption of polyphenols equivalent to 12 cups of tea/day reduced the risk of adenoma recurrence by 50%.<sup>132</sup> Preventive effects of green tea on precancerous oral and cervical lesions have been documented.<sup>133,134</sup> Extensive literature has associated green tea catechins with the inhibition of several pathways and molecules related to angioprevention. Polyphenon E, a catechin-rich extract from green tea leaves is being evaluated in multiple clinical trials of prostate, bladder, oesophageal, lung, head and neck cancers, as well as leukaemia, and is already commercially available as a topical ointment.<sup>135</sup>

Curcumin targets numerous angiogenesis mediators and inhibitors,<sup>86</sup> with beneficial effects in wound healing, as well as antiangiogenic effects in cancer. Phase I–II clinical trials established that curcumin is well tolerated at doses up to 8 g/day without showing dose-limiting toxicity,<sup>136,137</sup> and pharmacological manipulations are ongoing to increase bioavailability of the natural molecule. Consistent data suggest curcumin is a promising candidate for colorectal cancer prevention. Curcumin in combination with quercetin decreased aberrant crypt foci by 40% in a phase IIa trial,<sup>138</sup> and also decreased the incidence of colon adenomas in patients with familial adenomatous polyposis.<sup>139</sup>

Preclinical evidence of resveratrol activity in colorectal cancer prevention prompted phase I studies to assess the pharmacokinetics, pharmacodynamics and safety of the drug or more bioavailable derivatives in healthy volunteers or cancer patients.<sup>140,141</sup> Remarkably, a randomized double-blind crossover study in healthy obese men showed that resveratrol (150 mg/day for

**Table 1** | Clinical trials of chemoprevention with antiangiogenic principles\*

Compound	Molecular target or mechanism	Condition (ClinicalTrials.gov identifier)	Phase	Study type or design	n
<b>Metabolic regulators</b>					
Metformin	VEGF, mTOR, AMPK, MMP-2, MMP-9 <sup>78-80,87,90,117</sup>	Barretts's metaplasia (NCT01447927)	II	I, R, DB	86
		Colorectal adenoma or high BMI (NCT01312467)	II	I, NR, OL	39
		Prostate cancer (NCT01433913)	II	I, R, DB	50
Pioglitazone	PPAR $\gamma$ agonist, bFGF, VEGF, IL-8, CXCL5, CXCL1 <sup>71</sup>	Lung cancer (NCT00780234)	II	I, R, DB	126
<b>Anti-inflammatory agents</b>					
Aspirin and tea polyphenols	COX inhibitor, VEGF, COX-independent MOA <sup>120-124</sup>	Oesophageal squamous cell carcinoma (NCT01496521)	III	I, R, DB	600
Aspirin and esomeprazole	COX inhibitor, VEGF, COX-independent MOA <sup>120-124</sup>	Barretts's metaplasia (NCT00357682)	III	I, R, OL	2,513
Aspirin and eflornithine	COX inhibitor, VEGF, COX-independent MOA <sup>120-124</sup>	Familial adenomatous polyposis (NCT00983580)	II	I, R, DB	52
Sulindac <sup>†</sup>	COX-2 inhibitor <sup>54,56,60</sup>	Oral premalignant lesions (NCT00299195)	P	I, R, DB	66
Sulindac and eflornithine	COX-2 inhibitor <sup>54,56,60</sup>	Colorectal premalignant or nonmalignant lesions (NCT00118365)	III	I, R, DB	178
Celecoxib <sup>†</sup>	COX-2 inhibitor <sup>54,56,126,127,129</sup>	Cervical intraepithelial neoplasia (NCT00081263)	II	I, R, DB	130
<b>Phytochemicals and derivatives</b>					
Curcumin	NF- $\kappa$ B, VEGF, mTOR, miR-21 <sup>3,53,86,110,138,139</sup>	Familial adenomatous polyposis (NCT00927485)	–	I, R, DB	50
		Colorectal cancer (NCT01333917)	I	I, OL, SG, Ph	40
Polyphenon E	MMPs, STAT3, VEGF <sup>3,34,35,41,130,131</sup>	Prostatic intraepithelial neoplasia (NCT00596011)	II	I, R, DB	272
		Ductal carcinoma <i>in situ</i> (NCT01060345)	P	I, NR, SG, OL	50
		Bronchial dysplasia (NCT00611650)	II	I, R, DB	23
		Cervical intraepithelial neoplasia (NCT00303823)	II	I, R, DB	208
Resveratrol	NF- $\kappa$ B, IGF axis, EGF, VEGF <sup>54,61,140-142</sup>	Healthy adult smokers (NCT01492114)	III	I, R, DB, CS	40
		Impaired glucose tolerance (NCT01375959)	P	I, R, DB, CS	30
Genistein	NF- $\kappa$ B, AP-1, uPA, FAK, VEGF, HIF-1 $\alpha$ , PTEN <sup>42,52,84</sup>	Type II diabetes mellitus (NCT00951912)	–	I, R, DB	180
		High risk for breast cancer (NCT00290758)	IIb	I, R, DB	126
Genistein and vitamin D	NF- $\kappa$ B, AP-1, uPA, FAK, VEGF, HIF-1 $\alpha$ , PTEN <sup>42,52,84</sup>	Early stage prostate cancer (NCT01325311)	II	I, R, DB	50
Black raspberry	COX-2, iNOS <sup>92</sup>	Head and neck cancer (NCT01469429)	I–II	I, R, OL	140
Sulforaphane	HDAC, Nrf2, VEGF, HIF-1 $\alpha$ , MMPs <sup>3,66,95</sup>	Prostate cancer (NCT01265953)	–	I, R, DB	100
		Prostate cancer (NCT00946309)	I–II	I, R, DB	100
<b>Other compounds</b>					
Propranolol	VEGF, MMP-2, MMP-9, NF- $\kappa$ B <sup>64</sup>	Infantile haemangioma (NCT01074437)	II	I, R, DB	60
Imiquimod	MMP-9, interferons <sup>46,47</sup>	Lentigo maligna (NCT01088737)	II–III	I, NR, SG, OL	60
Everolimus	mTOR <sup>75</sup>	Skin cancer (NCT00799188)	III	I, R, OL	175
Fenretinide	IGF-1/IGFBPs <sup>113,114</sup>	High risk for breast cancer (NCT01479192)	III	I, R, DB	764
Tamoxifen	Oestrogen receptor, angiogenin, VEGF, endostatin <sup>49-52</sup>	Breast cancer (NCT01357772)	III	I, R, DB	1,400

\*Selected active clinical trials with known status and prevention as primary purpose are shown. <sup>†</sup>This trial has treatment as primary purpose, but vascular correlates as outcome measurement. Abbreviations: COX, cyclooxygenase; CS, crossover; DB, double-blind; HDAC, histone deacetylase; I, interventional; IL, interleukin; iNOS, inducible nitric oxide synthase; MMP, matrix metalloproteinase; MOA, mechanism of action; NR, non-randomized; OL, open-label; P, pilot; Ph, pharmacodynamics end point; R, randomized; SG, single-group; STAT, signal transducer and activator of transcription; uPA, urokinase-type plasminogen activator.

30 days) exerts caloric-restriction-like effects, improves lipid profiles and decreases inflammatory markers.<sup>142</sup> Considering that metabolic disorders are associated with pathological angiogenesis and chronic inflammation<sup>143</sup> and can be predisposing conditions for some cancers,<sup>70</sup> these results constitute an important premise for further clinical studies.

### Conclusions

A decade after its initial proposal, angioprevention is becoming a clinical reality that further shifts how we approach oncological disease, and uses strategies suitable for healthy individuals as well as those at high risk for cancer. Compared with classic chemoprevention directed at cancer cells, the inhibition of angiogenesis

and inflammation boosts anticancer defence mechanisms and provides protection against a broad spectrum of neoplasms.

We propose four levels of angioprevention: level I for the ‘healthy’ population; level II for those with conditions associated with a raised risk of cancer; level III for treatment of preneoplastic lesions; and level IV for prevention of recurrence of overt cancer. Angioprevention at levels I and II must be fundamentally non-toxic. Angiogenesis is crucial for wound healing and reproductive function, so angioprevention must be titrated to suppress undesirable angiogenesis, but not subvert healthy endothelial maintenance or function. We compare this goal of physiologically compatible angioprevention to the ‘Goldilocks Zone’ concept used by cosmologists seeking planets where life forms may reside.<sup>144</sup> The term originated from the fairy tale about a child seeking porridge that is neither too hot nor too cold, but is ‘just right’. Angioprevention needs to achieve a degree of angiogenesis control that is neither over exuberant nor dangerously suppressed, without interfering with the Goldilocks Zone of healthy vascular function. One point that can be made is that many efficacious angioprevention drugs have multiple targets, yet do not completely inhibit any single pathway. This may lead to lower toxicity when compared with most potent single-target drugs. Several natural backbones of phytochemicals, such as aspirin, have been modified and tested for anticancer or chemopreventive activity. Molecular homologues could be designed from a chemopreventive

backbone to be more antiangiogenic and more suitable as angiopreventives. Synthetic chemistry and molecular tailoring may be used to design angiopreventive diet derivatives or pharmaceuticals.

Clinical trials are now underway to test the efficacy of angiopreventive molecules such as curcumin, artemisinin, resveratrol, genistein, synthetic triterpenoids, and isothiocyanates in neurological, cardiovascular degenerative diseases, and cancer at levels III and IV as defined in this article. Given the global health priorities facing societies today, and with the cancer pandemic in our sights, angioprevention and its clinical development is a concept whose time has arrived.

#### Review criteria

The references selected for this Review were searched in the PubMed database using the terms and Boolean strings: “Angiopreven\*”; “(chemopreven\* OR cancer prevention) AND (angiogen\* AND endothel\*)”; “prevention AND inflammation”; “chemoprevention AND microenvironment”; “antiangiogenic therapy AND clinical trials”; “angiogenesis AND biomarkers”; “antiangiogenesis AND resistance”. We also searched for selected articles mentioning “endothel\* AND “pathways, ROS, cell death, senescence, miRNA, angiogenic gene expression, endogenous inhibitors, metabolism, caloric restriction, redox, antioxidant response”. We apologize to the many colleagues whose work we could not cite owing to space limitations.

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## Author contributions

All authors made a substantial contribution to researching and discussing data for this Review, and to writing the manuscript. All authors reviewed and edited the manuscript prior to submission. D. M. Noonan and W. W. Li contributed equally to this article.