

TNF/VEGF Cross-talk in Chronic Inflammation-related Cancer Initiation and Progression: An Early Target in Anticancer Therapeutic Strategy

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Abstract. *In the last decade a growing body of epidemiological and clinical data has emerged to support the concept that longstanding inflammation potentiates or promotes tumor development, growth and progression. Among pro-inflammatory gene products involved in such interactions are tumor necrosis factor (TNF)- α , interleukin (IL)-6 and vascular endothelial growth factors (VEGFs), whose expression is mainly regulated by the transcription nuclear factor (NF)- κ B. Clinically, several reports have detected abnormally high levels of circulating cytokines in cancer patients, and inflammation is currently being investigated as a target of anticancer therapies. To date three main groups of antiangiogenic drugs approved for clinical use and experimentation can be identified: secreted VEGF inhibitors, tyrosine kinase (TK) inhibitors (mainly VEGFR inhibitors) and drugs that inhibit angiogenesis with a complex mechanism. More recently, TNF- α antagonists have become available. The first clinical data on anti-TNF- α showed that this drug can be used in cancer patients without major side-effects. Further investigations are needed to understand if anti-TNF- α or NF- κ B inhibitors may really represent a novel approach in cancer treatment, probably as adjuvant to other therapies, such as anti-angiogenic or cytotoxic agents.*

The idea that chronic inflammation may play an active role in the process of carcinogenesis dates back to 1863 when

Virchow proposed that cancer originated at sites of chronic inflammation (1). This hypothesis was subsequently abandoned in favor of the concept that inflammation was a consequence of "immune surveillance" against cancer rather than a cause (2). However, in the last decade a growing body of epidemiological and clinical data has emerged to support the concept that longstanding inflammation potentiates or promotes tumor development, growth and progression (3).

To date, more than 15% of malignancies worldwide can be attributed to infections, a global total of 1.2 million cases per year (3, 4), and chronic inflammation is recognized as a risk factor for the development of a wide variety of human cancers. The strongest association of chronic inflammation with malignant diseases is in colon carcinogenesis arising in individuals with inflammatory bowel diseases, *i.e.* chronic ulcerative colitis and Crohn's disease (5). Hepatitis C infection in the liver predisposes to liver carcinoma (6), whereas chronic *Helicobacter pylori* infection is the world's leading cause of stomach cancer (7).

The objectives of this review will be to provide an overview of the current literature on the interplay between inflammation and cancer growth and progression, and to discuss anti-cytokine therapies being pursued in cancer treatment.

The Link between Inflammation and Cancer

While acute inflammation is a part of the defense response, chronic inflammation can lead to a wide variety of diseases, including cancer. Several pro-inflammatory gene products have been identified that mediate a critical role in suppression of apoptosis, proliferation, angiogenesis, invasion and metastasis (8). Among these gene products are the tumor necrosis factor (TNF)- α and members of its

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superfamily, interleukin (IL)-1 β , IL-6, IL-8, chemokines, metalloproteinases (MMPs), vascular endothelial growth factors (VEGFs), cyclooxygenase (COX)-2 and lipooxygenase. The expression of all these genes is mainly regulated by the transcription nuclear factor (NF)- κ B, which is constitutively active in most tumors and is induced by most carcinogens and tumor promoters (8).

Accordingly, a new paradigm is becoming widely accepted, that chronic inflammation, driven in part by chemokines and cytokines at the site of a tumor, may facilitate tumor progression instead of promoting anti-tumor immunity. Tumors and activated stromal cells secrete a wide array of pro-inflammatory molecules that act either directly or indirectly through stimulation of the vascular endothelium to recruit leukocytes to the tumor. After activation, these tumor-associated leukocytes release angiogenic factors, mitogens, proteolytic enzymes and chemotactic factors, recruiting more inflammatory cells and stimulating angiogenesis to sustain tumor growth and facilitate tumor metastasis (9).

Induced by a wide range of pathogenic stimuli, TNF- α plays a crucial role in the initiation and amplification of inflammatory reactions, with actions directed towards both tissue destruction and recovery from damage. Hence, when dysregulated and secreted in the circulation, TNF- α can mediate a wide variety of diseases, including cancer (10). TNF- α is also produced by tumors and can act as an endogenous tumor promoter (11). The role of TNF- α has been linked to all steps involved in cancer initiation and progression, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis and metastasis (Figure 1).

TNF- α and cancer initiation and progression. In addition to acting as a key mediator of inflammation, TNF- α elicits pleiotropic effects in a wide range of cells by binding and activating two cell-surface receptors, designated TNF-receptor 1 (TNF-R1) and TNF-R2. The role of TNF- α in tumor growth and dissemination is complex and apparently paradoxical. As implied in its name, TNF- α was originally identified by its ability to induce the necrosis of transplanted tumors in mice (12, 13), which is an activity that is mostly mediated through increased vascular permeability and subsequent vascular collapse. Subsequent clinical trials have shown that, when administered locally at high concentration, TNF- α exerts powerful anticancer activity by causing selective damage and obliteration of intratumoral blood vessels (14). However, several lines of evidence indicate that TNF- α exerts mitogenic activity (15-18) and induces anchorage-independent growth (19, 20) thus enhancing the capacity of tumor cells to metastasize (15). Moreover, mice deficient in TNF- α are resistant to skin carcinogenesis (21) and antibodies to TNF- α inhibit the development of experimental skin tumors (22). In an

attempt to reconcile the apparently paradoxical activities of TNF- α , it has been proposed that the effects of TNF- α on tumor development are context dependent. Thus, high-dose local delivery of TNF- α can cause tumor regression, yet sustained production of endogenous TNF- α in the tumor microenvironment might actually enhance cancer development and spread (11).

If infection and inflammation enhance tumor development, they must do so through signal-transduction mechanisms that influence factors involved in either malignant conversion or cancer surveillance. The binding of TNF- α to the preassembled TNF-R complexes leads to a change in the orientation of the different TNF-R chains, thus facilitating signal transduction through the rapid recruitment of three types of downstream molecules: TNF-R-associated factors (TRAF), c-inhibitors of apoptosis (c-IAP) and death domain (DD)-containing proteins. Whether a given cell type will undergo apoptosis or induce NF- κ B translocation into the nucleus and induction of gene transcription (Figure 1) (23) will depend on various factors, *i.e.* activation status and differentiation stage.

Of all the different signaling pathways activated by inflammation and infection, NF- κ B might be the most important component of the tumor-promoting machinery. This suggestion is based on the observation, first reported in the context of TNF- α signaling, that NF- κ B is a major activator of anti-apoptotic gene expression (24-28). Despite the presence of death domains (DDs) in the intracellular portion of its major receptor TNF-R1, TNF- α does not trigger apoptosis unless it is combined with inhibitors of RNA or protein synthesis. The requirement for such inhibitors can be alleviated through inactivation of NF- κ B by either deletion of its RelA subunit or expression of the inhibitor of NF- κ B (I κ B)-super repressor (I κ B-SR) (29). Many cytokine-stimulated genes, which include those encoding for cellular adhesion molecules and angiogenic peptides, are regulated at the transcriptional level by transcription factors of the NF- κ B family (30). Normally, NF- κ B members are confined to the cytoplasm through association with inhibitory proteins of the I κ B family (31). TNF- α leads to phosphorylation and degradation of the I κ B proteins, resulting in the release, nuclear translocation and DNA binding of NF- κ B, with subsequent transcriptional activation of responsive genes.

TNF- α -induced angiogenic pathways. Promotion or inhibition of angiogenesis by regulating the levels of cytokine production has been shown *in vitro* and in animal models (32-35). For example, TNF- α stimulated angiogenesis in cultured endothelial cells (EC) and in cornea angiogenesis assays (32, 33), and chronic expression of TNF- α at low levels in a human TNF transgenic mice model induced joint angiogenesis and inflammatory arthritis (34). However, little

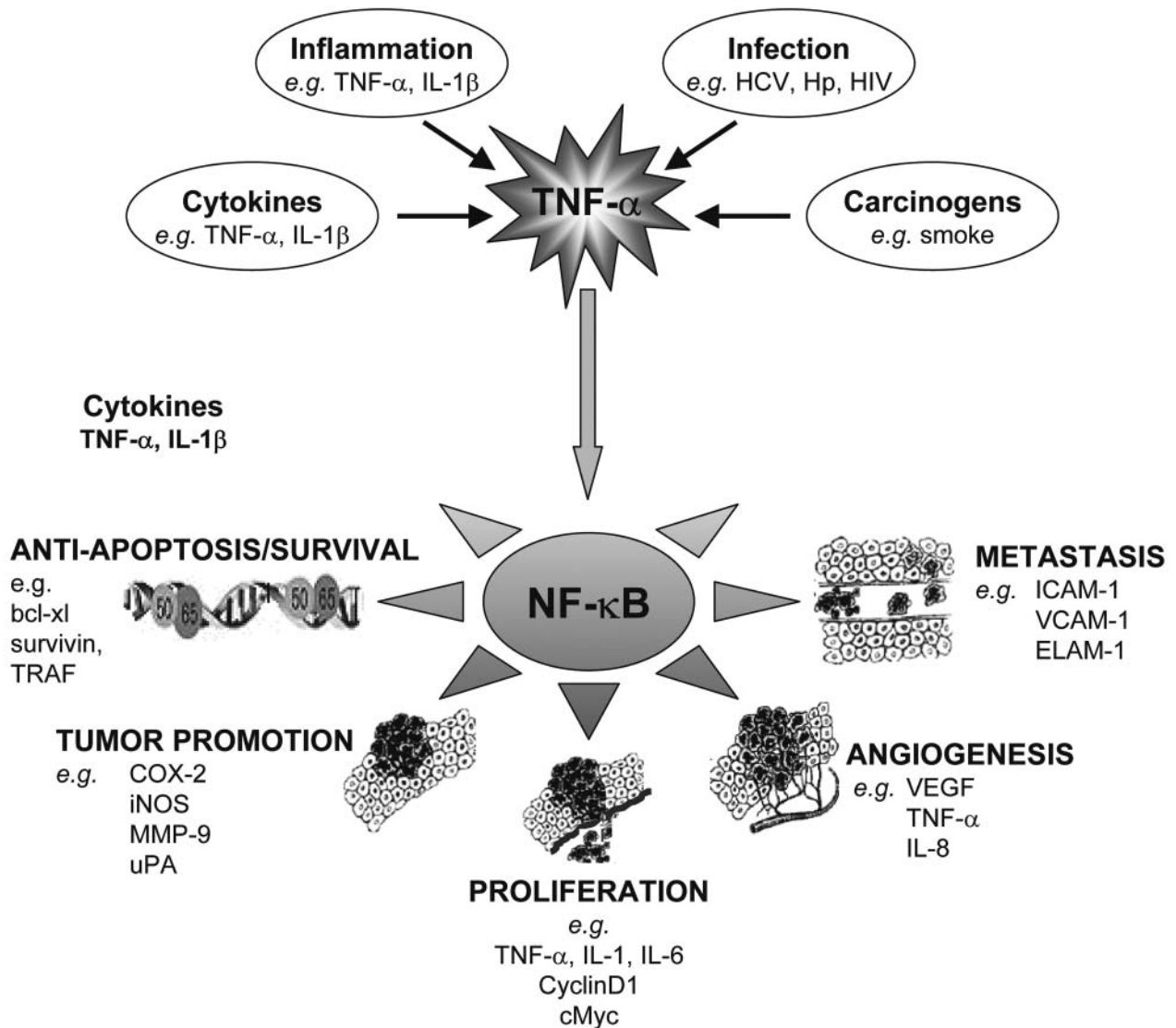


Figure 1. Role of tumor necrosis factor- α (TNF- α) and nuclear factor- κ B (NF- κ B) in cancer initiation and progression. Endogenous TNF- α is produced in response to several stimuli, such as viral infections, carcinogens and sustained inflammation. It is also possible that individuals who naturally produce high levels of TNF- α might show increased susceptibility to, or severity of, certain malignancies. Single nucleotide polymorphisms (SNPs) of cytokine genes are frequently found in regions that regulate transcription or post-transcriptional events and SNPs in the promoter region of TNF- α have been linked to cancer susceptibility and severity (11). TNF- α signaling, in turn, leads to activation of nuclear transcription factors, i.e. NF- κ B, and transcription of many responsive genes, which translate into anti-apoptotic effects, tumor promotion, cell proliferation and angiogenesis. IL: interleukin. HCV: hepatitis C virus. Hp: *Helicobacter pylori*. HIV: human immunodeficiency virus. SNPs: single nucleotide polymorphisms. TRAF: TNF- α receptor-associated factors. COX-2: cyclooxygenase-2. iNOS: inducible nitric oxide synthase. MMP: metalloproteinase. UPA: urokinase-type plasminogen activator. VEGF: vascular endothelial growth factor. ICAM: intercellular adhesion molecule. VCAM: vascular cell adhesion molecule.

is known of the molecular signaling pathways involved in TNF-induced angiogenesis. Among the possible mechanisms, it has been proposed that TNF can directly activate EC migratory pathways (32, 33) through transactivation between the endothelial/epithelial tyrosine kinase Etk and VEGF receptor 2 (VEGFR2) (35). TNF- α has been even reported to mediate macrophage-induced angiogenesis (8). The

angiogenic activity produced by activated murine peritoneal macrophages is completely neutralized by a polyclonal antibody to TNF- α , suggesting that immunological features are common to TNF- α and the protein responsible for macrophage-derived angiogenic activity (8).

In addition, TNF promotes angiogenesis through its ability to synergize VEGF-induced vessel permeability, a

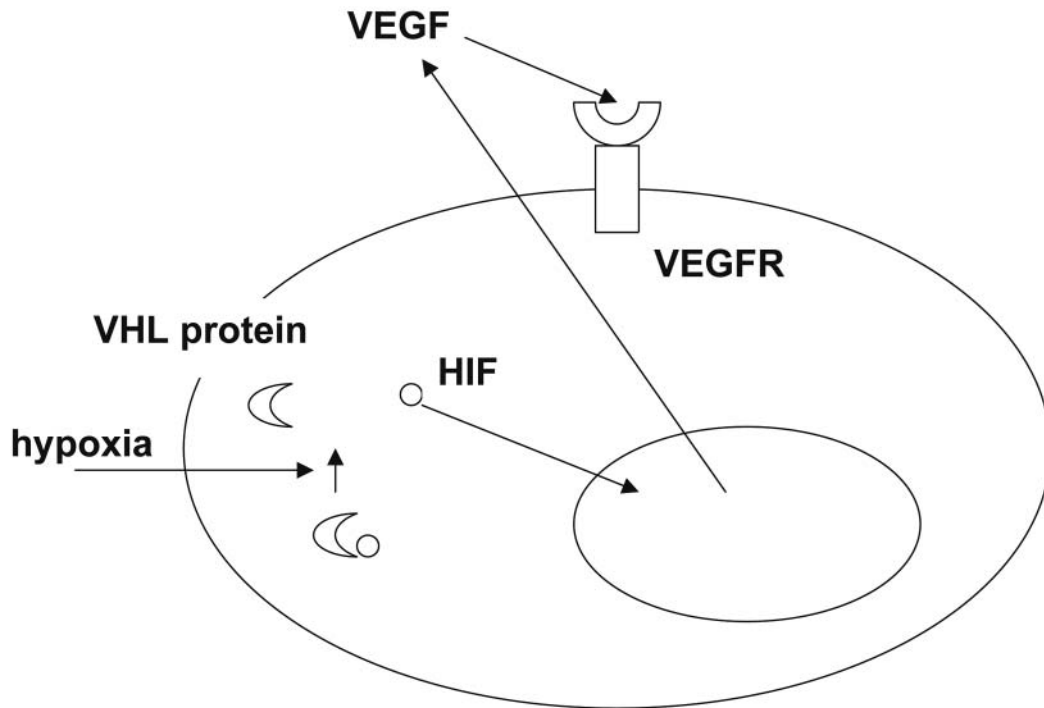


Figure 2. Pathway of vascular endothelial growth factor (VEGF) and its receptors (VEGFR). During hypoxia VHL is unable to bind HIF. Free HIF may translocate to the nucleus and act as a transcription factor for VEGF gene expression. VHL: Von Hippel-Lindau gene; HIF: hypoxia inducible factor.

prerequisite initial event for plasma exudation and fibrin clot formation, a matrix permissive for angiogenesis (36, 37). TNF is also capable of inducing gene expression of proangiogenic molecules, such as VEGF and its receptors (VEGFRs) (38, 39). Indeed, the cellular VEGF mRNA level is potently enhanced in response to TNF- α , probably due to transcriptional activation mediated by the transcription factor SP-1 (40), leading to induction of a paracrine loop for neovascularization under pathological conditions, including cancer.

VEGF in regulation of angiogenesis and lymphangiogenesis. Angiogenesis and lymphangiogenesis are regulated predominantly by several different growth factors and their associated receptor tyrosine kinases. Foremost among these is the VEGF family, which consists of at least five members – VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PlGF). They share a common structure of eight characteristically spaced cysteine residues in a VEGF homology domain. These members have different physical and biological properties and act through specific tyrosine kinase receptors – VEGFR-1, VEGFR-2 and VEGFR-3 (recently reviewed (41)).

The crucial role of new vessel formation in cancer development has been widely demonstrated. Extensive

research by Folkman *et al.* (42-44) has provided evidence that neoplastic tissue needs, as does normal tissue, oxygen and nutrient supply to grow and invade the surrounding structure. The ability of oxygen to diffuse into the extracellular environment only allows it to cover a distance of 150-200 μm from capillaries, hence new vasculature apparatus is required in order for a cancer mass to exceed 200 μm in diameter. Neo-angiogenesis is a complex process, essentially based on pre-existing normal blood vessel remodeling and VEGF-A is in the middle of a balance involving pro-angiogenic stimuli and angiostatic factors (Figure 2) (45).

The role of VEGF-A in tumor angiogenesis, especially in lung, gastrointestinal, ovarian and breast cancer has been investigated and tumor angiogenesis has become a potential target for cancer therapy (46). VEGF-A, also referred to as VPF (vascular permeability factor) has been recognized as the major growth factor that is relatively specific for endothelial cells (47, 48). VEGF-A is a dimeric glycoprotein essential for many angiogenic processes in normal and abnormal states, such as tumor vascularization, mainly by interacting with two tyrosine kinase receptors, VEGFR-1 (also known as Flt-1 (Fms-like tyrosine kinase-1)) and VEGFR-2 (also known as Flk-1 (fetal liver kinase-1) and, in humans, as KDR (kinase insert domain-containing receptor)) (49-51).

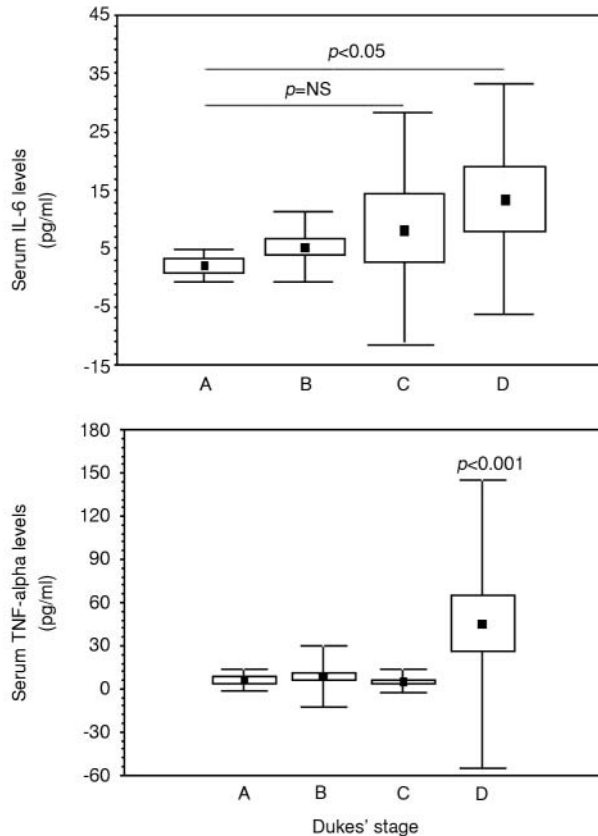


Figure 3. Serum IL-6 and TNF- α levels in colorectal cancer patients stratified on the basis of Dukes' stages. Values are reported as mean (closed squares), standard errors (open columns) and standard deviations (whiskers). (Unpublished personal observations).

The precise role of VEGF-B *in vivo* is not precisely known. A study with mice deficient in VEGF-B reported the development of small hearts and impaired recovery after induced myocardial infarction suggesting that the formation of coronary collaterals might be partly attributed to VEGF-B (41). Moreover, reduced synovial angiogenesis in VEGF-B knockout arthritis models suggest a role of VEGF-B in inflammatory angiogenesis (41). VEGF-B is also suggested to play a role in early tumor development and in oral squamous cell carcinomas, but there is a paucity of conclusive data to indicate a significant role of VEGF-B in tumor progression (52).

VEGF-C is produced as a precursor protein and is proteolytically activated in the extracellular space by proteases to generate a homodimeric protein with high affinity for both VEGFR-2 and VEGFR-3 (41). VEGF-C induces mitogenesis, migration and survival of endothelial cells. Developmental studies, knockout models and gene transfer experiments suggest that VEGF-C is primarily a lymphangiogenic growth factor and its lymphangiogenic

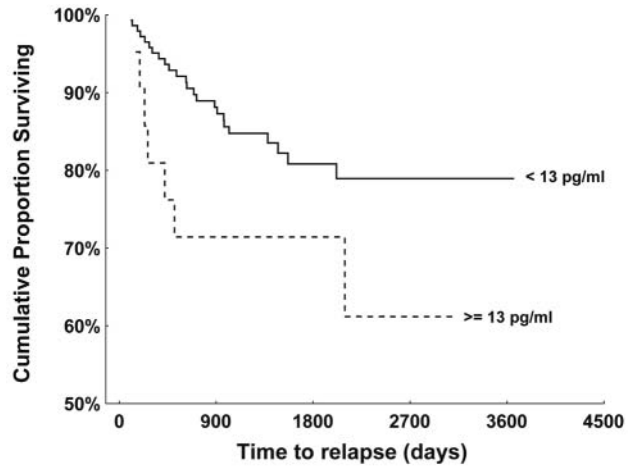


Figure 4. Kaplan-Meier survival analyses of colorectal cancer patients stratified on the basis of IL-6 levels. Serum IL-6 levels were categorized according to a cut-off value calculated on the basis of the mean value + 2SD obtained in patients with benign colorectal diseases. (Unpublished personal observations).

effects are mediated by VEGFR-3 (53-55). VEGF-C is also involved in tumor and inflammation associated lymphangiogenesis (41).

Like VEGF-C, VEGF-D also shows lymphangiogenic potential. The lack of a profound lymphatic vessel defect in VEGF-D deficient mice may reflect a subtle, redundant, or nonexistent role of this growth factor during embryonic development (56). Nonetheless, VEGF-D may induce lymphatic vessel growth in adult life in response to pathological conditions. Lymphatic vasculature provides another route for tumor metastasis. Certain tumors like carcinomas of the breast, lung and gastrointestinal tract have a propensity to metastasize through lymphatic vessels. The production of lymphangiogenic growth factors VEGF-C, VEGF-D and their receptor VEGFR-3 stimulates lymphatic growth in the region of the tumor, enabling cancer cells to gain access to the lymphatic vasculature (57). VEGF-C and VEGF-D have been associated with tumor lymphangiogenesis and metastatic spread of tumor cells and a role of VEGF-A in peritumoral lymphangiogenesis and lymphatic metastasis has also been proposed.

Evidence from Clinical Studies

TNF- α is frequently detected in biopsies from human cancer, produced either by epithelial tumor cells (in ovarian and renal cancer), or stromal cells (in breast cancer) (11) and its production by tumors has been associated with a poor prognosis, loss of hormone therapy responsiveness and cachexia/asthenia (58, 59). Clinically, several reports have associated the detection of abnormally

high levels of circulating TNF in cancer patients with a wide range of tumor types (60), including pancreatic (61), breast (62), lung (63) and prostate cancer (64, 65). Circulating levels of inflammatory cytokines have been associated with the disease status of cancer patients (Figure 3) (63, 66-72) and it has been suggested that IL-6 is an independent negative prognostic marker of survival in certain tumors (Figure 4) (68). In particular, elevated serum IL-6 levels have been found in patients with large tumors (67, 72) and in patients with metastases (68, 70). Moreover, it has been shown that blood concentrations of either IL-6 or TNF- α correlated with the level of serum tumor markers, *i.e.* carcinoembryonic antigen (CEA) or prostate specific antigen (PSA) (70, 72, 73), suggesting that the host inflammatory response to cancer cells and/or their released products could be responsible, through cytokine release, for an up-regulation of adhesion molecules on endothelial cells, which favors attachment and lodging of cancer cells to the site of metastasis.

A recent study examined the associations between baseline levels of C-reactive protein (CRP), IL-6 and TNF- α and the risk of cancer in the Health Aging and Body Composition cohort (74). The results reported suggest that the association between inflammatory markers and cancer may be site specific, being most consistent for lung cancer, with associations noted for all three markers, and colorectal cancer, associated with IL-6 and CRP (74). The most intriguing findings of this analysis were stronger associations with fatal cancers. To explain such associations, the authors offered two possible explanations (nonmutually exclusive). Firstly, this association may be driven by the large contribution of colorectal and lung cancers to this pool, as those cancer types showed associations in site-specific analysis. Secondly, these findings may reflect a role of the inflammatory mediators in the tumor-host interaction related to the development of metastases and of paraneoplastic syndromes, such as cachexia (74).

Inflammation as a Target for Cancer Treatment

Anti-angiogenesis-based cancer therapies. Induction of neo-angiogenesis is a fundamental step in cancer growth and the therapeutic value of inhibiting such a phenomena immediately appeared to be an intriguing area of research. Accordingly, the VEGF network has proven to be an important pathway in the clinical setting, with VEGF-A being the most suitable anti-cancer target (45).

To date three main groups of antiangiogenic drugs approved for clinical use and experimentation can be identified (75): (i) secreted VEGF (sVEGF) inhibitors, (ii) tyrosine kinase (TKs) inhibitors (mainly VEGFR inhibitors) and (iii) drugs that inhibit angiogenesis with a complex mechanism.

Anti-sVEGF drugs. In 1993 Kim *et al.* (76) demonstrated that treatment with a monoclonal antibody specific for VEGF was able to inhibit cancer growth when human rhabdomyosarcoma, glioblastoma multiforme and leiomyosarcoma cell lines were injected into nude mice. Moreover the density of vessels was decreased in the antibody-treated tumors whilst anti-VEGF antibody had no effect on the growth rate of the tumor cells *in vitro*. This evidence prompted the use of the humanized variant of anti-VEGF monoclonal antibody (bevacizumab) in the clinical setting to evaluate its efficacy as an anti-cancer treatment (77).

In a pivotal study, Hurwitz *et al.* (78) randomized 813 patients with previously untreated metastatic colorectal cancer to either irinotecan, bolus fluorouracil, and leucovorin (IFL) plus bevacizumab (5 mg per kilogram of body weight every two weeks) (402 patients), or IFL plus placebo (411 patients). When disease control was obtained, patients were allowed to continue the assigned treatment for the maximum 96-week study period. Treatment was discontinued when disease progression, unacceptable toxicity or patient refusal occurred. The median duration of therapy was 27.6 weeks in the group given IFL plus placebo and 40.4 weeks in the group given IFL plus bevacizumab. The median duration of overall survival (OS), the primary end point, was significantly longer in the group given IFL plus bevacizumab than in the group given IFL plus placebo (20.3 months *vs.* 15.6 months), with a hazard ratio for death of 0.66 ($p < 0.001$) (reduction of 34% in the risk of death in the bevacizumab group). The one-year survival rate was 74.3% in the group given IFL plus bevacizumab and 63.4% in the group given IFL plus placebo ($p < 0.001$). The addition of bevacizumab to IFL was also associated with increases in the median duration of progression-free survival (PFS) (10.6 months *vs.* 6.2 months; hazard ratio for progression 0.54, $p < 0.001$); response rate (44.8% *vs.* 34.8%; $p = 0.004$); and the median duration of response (10.4 months *vs.* 7.1 months; hazard ratio for progression 0.62; $p = 0.001$). Bevacizumab therapeutic improvement was maintained across different patient subgroups according to age, sex, race, performance status, location of the primary tumor, presence or absence of prior adjuvant therapy, serum concentrations of albumin, alkaline phosphatase and lactate dehydrogenase.

The incidence of any grade 3 or 4 adverse events was approximately 10 percentage points higher among patients receiving IFL plus bevacizumab than among patients receiving IFL plus placebo, largely because of an increase in the incidence of grade 3 hypertension (requiring treatment) and small increases in the incidence of grade 4 diarrhea and leukopenia. However, there was no significant difference in the incidence of adverse events leading to hospitalization or to the discontinuation of study treatment (78). Other toxic effects such as hemorrhage, thromboembolism and proteinuria, which were identified as

possible bevacizumab-associated adverse effects in previous phase 1 and 2 trials (79), were not clearly increased in the group given IFL plus bevacizumab (78).

All episodes of hypertension were manageable with standard oral antihypertensive agents (*e.g.*, calcium-channel blockers, angiotensin-converting-enzyme inhibitors and diuretics). There were no discontinuations of bevacizumab therapy, hypertensive crises, or deaths related to hypertension in the bevacizumab group.

The incidence of all venous and arterial thrombotic events was 19.4% in the group given IFL plus bevacizumab and 16.2% in the group given IFL plus placebo ($p=0.26$). Gastrointestinal perforation occurred in six patients (1.5%) receiving IFL plus bevacizumab. Factors other than the study treatment that may have been associated with gastrointestinal perforation were colon surgery within the previous two months in two patients and peptic-ulcer disease in one patient.

Based on these findings the FDA and EMEA have approved the use of bevacizumab in combination with intravenous 5-fluorouracil/folinic acid or intravenous 5-fluorouracil/folinic acid/irinotecan for first-line treatment of patients with metastatic carcinoma of the colon or rectum (www.emea.eu.int, www.fda.gov).

Another study from Yang *et al.* (80) demonstrated the activity of bevacizumab in metastatic renal-cell cancer patients. One hundred and sixteen patients were randomly assigned to low-dose antibody (37 patients, 3 mg per kilogram of body weight, given every two weeks), high-dose antibody (39 patients, 10 mg/kg) or placebo (40 patients) in a double-blind, phase II trial. Crossover from placebo to antibody treatment was allowed. Minimal toxic effects were seen, with hypertension and asymptomatic proteinuria predominating. There was significant prolongation of the time to tumor progression (TTP) in the high-dose-antibody group as compared with the placebo group (hazard ratio 2.55; $p<0.001$). There was a small difference, of borderline significance, between the TTP in the low-dose-antibody group and that in the placebo group (hazard ratio 1.26; $p=0.053$).

The activity of bevacizumab is increasingly being demonstrated in several cancer types and its mechanism of action partly includes the enhancement of the standard chemotherapy effect. In patients with previously untreated advanced pancreatic cancer, biweekly bevacizumab 10 mg/kg associated with a standard dose of weekly gemcitabine 1,000 mg/m² yielded a 21% confirmed partial response with an overall disease control rate of 67%, demonstrating an encouraging synergistic activity that warrants additional studies (81). In advanced hepatocellular carcinoma a phase II study of gemcitabine and oxaliplatin in combination with bevacizumab showed a high PFS rate at 6 months (48%) indicating further investigation with bevacizumab to be worthy in this cancer subtype (82).

In non small-cell lung cancer the combination of bevacizumab with other targeted therapy such as erlotinib or with paclitaxel/carboplatin based regimens, has demonstrated an improved overall response and time to progression with encouraging antitumor activity (83, 84).

Moreover in metastatic breast cancer, the addition of bevacizumab to standard chemotherapy produced a significant increase in tumor response rate, although this did not translate into improved PFS or OS (85).

The property of potentiating the chemotherapy effect seems to be related to "normalization" in tumor vasculature. Antiangiogenic therapy may "normalize" the tumor vasculature for a transient period of time. If used properly, antiangiogenic drugs can paradoxically improve oxygenation and create a more "normalized" environment that may improve drug delivery. Using radiation therapy and a VEGFR-2-specific monoclonal antibody, DC101, individually and in combination to treat mice bearing human glioblastoma xenografts, Jain *et al.* found that giving radiation therapy 4 to 6 days after DC101 treatment delayed tumor doubling time by more than 21 days, which exceeded the expected additive effect (86). Upon further observation, tumor hypoxia decreased 2 days after DC101 treatment, was almost abolished by day 5, and increased again by day 8. This finding suggests that antiangiogenic therapy increases tumor oxygenation, thereby enhancing the tumor's response to radiation.

In addition to reducing hypoxia, DC101 treatment was associated with recruitment of pericytes – cells that help shore up vessel walls – to the tumor blood vessels, which stabilizes the leaky, dilated vasculature, a common characteristic of these vessels. By day 8, pericyte-covered vessels had decreased in number. Vascular normalization thus occurred between days 2 and 5 after blocking VEGF (87-93).

Anti-tyrosin kinase of VEGFR. Sunitinib malate (SUTENT) is an oral multi-tyrosine kinase inhibitor that has shown antiangiogenic and antitumor activities in several *in vitro* and *in vivo* models. Among various TK targets the blockade of all three isoforms of the VEGFRs makes this drug an attractive antiangiogenic agent.

The results of second line treatment with sunitinib in GIST patients who experienced failure with Imatinib mesilate were recently published by Demetri *et al.* (94). Three hundred and twelve patients were randomized in a 2:1 ratio to receive sunitinib (n=207) or placebo (n=105) in a blinded fashion with the possibility, for progressing patients, to cross to the sunitinib arm if placebo had been firstly assigned. The trial was unblinded early when a planned interim analysis showed a significantly longer TTP with sunitinib. Median TTP was 27.3 weeks in patients receiving sunitinib and 6.4 weeks in those on placebo (hazard ratio 0.33; $p<0.0001$). Therapy was reasonably well tolerated; the most common treatment-related adverse

Table I. Improvement of local control rate for in transit melanoma metastases or inoperable soft-tissue sarcomas in patients treated with isolated limb perfusion (ILP) associated with TNF- α , IFN- γ and chemotherapy.

	Numbers of patients	CR (%)	PD (%)	Reference
Melanoma in transit metastasis				
TNF- α +IFN- γ +Melphalan	53	90	10	118
	32	78	22	118
	21	80	10	119
Inoperable soft tissue sarcomas				
TNF- α +Melphalan	10	70	20	120
TNF- α +Melphalan	35	37	54	121
TNF- α +Doxorubicin	16	25	69	122

CR: complete recovery; PD: progressive disease.

events were fatigue, diarrhea, skin discolouration and nausea (94).

In a phase II study with 63 cytokine-refractory metastatic renal cell carcinoma (RCC) patients, 40% of patients treated with sunitinib had a partial response and 27% had stable disease for at least 3 months; the median TTP was nearly 9 months (95). Results from an international randomized phase III trial comparing sunitinib vs. IFN- α in advanced renal cell carcinoma patients will be soon available.

Sorafenib (BAY 43-9006) is another oral kinase inhibitor targeting both tumor cells and the tumor vasculature. It was originally developed as an inhibitor of Raf-1, however it was subsequently found to have activity against B-Raf, VEGFR-2, platelet-derived growth factor receptor, Flt-3, and stem-cell growth factor (c-KIT). In xenograft models (colon, breast, lung) the primary effect of sorafenib is inhibition of tumor growth rather than tumor shrinkage thus hypothesizing a prevalent antiangiogenic action of the drug (96).

In a phase II study, 202 patients with RCC received sorafenib for an initial run-in period, followed by random assignment of responders (*i.e.* patients who maintained stable disease after 12-weeks) to either the study drug or placebo (97). According to the study design, 65 patients with stable disease at 12 weeks were randomly assigned to sorafenib (n=32) or placebo (n=33). At 24 weeks, 50% of the sorafenib-treated patients were progression free versus 18% of the placebo-treated patients ($p=0.007$). Median PFS from randomization was significantly longer with sorafenib (24 weeks) than placebo (6 weeks; $p=0.0087$).

Drugs with a complex mechanism of action. Thalidomide is a drug introduced as a sedative and anti-nausea and vomiting agent in pregnant women more than 50 years ago and subsequently withdrawn from the market because of its teratogenicity. Its potential antiangiogenic effects in cancer

Table II. Clinical applications of TNF- α inhibitors in cancer.

TNF- α inhibitor	Disease	Number of patients	Responses	Reference
Etanercept	Ovarian cancer	16	6 SD	136
	Breast cancer	30	2 SD	130
	Metastatic cancers and fatigue/cachexia	12	reduce fatigue chemotherapy-associated	137
Infliximab	NSCLC		not done	142

SD: stable disease.

treatment have only recently been discovered. Its mechanism of action has not yet been fully defined, but probably includes down-regulation of TNF- α , COX2 inhibition, and modulation of a number of cytokines (IL-6, IL-10, and IL-1 β) (98, 99).

In a pivotal phase II study by Singhal *et al.* (100) involving 84 standard treatment-refractory multiple myeloma patients, thalidomide was associated with a 32% response rate. After 12 months of follow-up, the PFS rate was 22% and the 12-month OS was 58%.

Thalidomide activity was subsequently confirmed in other studies and also in untreated, asymptomatic patients, especially when combined with dexamethasone (101-105), leading many centers to use this combination as first-line therapy.

Other phase II studies have demonstrated a variable activity of thalidomide in solid tumors such as RCC, glioblastoma, prostate cancer, head and neck cancer and melanoma (99, 106-110).

COX-2 inhibitors are also seen as an attractive anti-cancer treatment. In animal models these compounds have shown antiangiogenic properties, beside their antiinflammatory, analgesic and antipyretic activities (111). Phase II-III trials have shown encouraging results when using coxibs in combination with cytotoxic agents (111-113). Coxib development however has been slowed down by the emerging evidence of their cardiovascular and thromboembolic toxicity (114-116).

TNF- α and cancer therapies.

As stated above, several clinical trials have shown that, when administered locally at high concentration, TNF- α exerts powerful anticancer activity by causing selective damage and obliteration of intratumoral blood vessels (14). Recombinant TNF- α , which became available between 1985 and 1988, has been widely used in isolated limb perfusion (ILP) associated with IFN- γ and/or chemotherapy to improve local control rate for both in transit melanoma metastases or soft-tissue sarcomas (Table I) (117-122).

More recently, TNF- α antagonists have become available. Initially it was thought that as TNF- α can act as an endogenous tumor promoter at an early stage of cancer, these agents could be used to prevent tumor development. The increased susceptibility to opportunistic infections, such as *Mycobacterium tuberculosis* in patients taking TNF- α antagonists (59, 123), would preclude wide-scale use of these drugs as preventative agents against sporadic cancer. However, many patients receiving TNF- α antagonist therapy for chronic inflammatory disease are being carefully monitored to see if this therapy has an impact on cancer risk. Early clinical data have shown that TNF- α antagonists determine an increased incidence of lymphoma, when compared with placebo, and an increased risk of non-melanoma skin cancers when associated with steroids and methotrexate (124, 125).

Nonetheless, a number of clinical trials of TNF- α antagonists alone, and in combination with other therapies, are currently ongoing in cancer patients and some preliminary results are available (Table II).

Etanercept has \approx 50-fold greater affinity for TNF- α in a binding inhibition assay and is at least 1,000-fold more efficient than the monomeric-soluble TNF receptor. The half-life of Etanercept is 5-fold that of monomeric-soluble TNF receptor. It is slowly absorbed from a subcutaneous injection site achieving a maximum serum concentration \approx 48 h after a single dose. The elimination half-life is 70 h with a bioavailability of 76%. It is commonly used twice weekly subcutaneous.

In breast cancer, much evidence showed that TNF- α pathways play an important role in tumor pathogenesis, progression and angiogenesis (126-129). A phase II nonrandomized study was performed to evaluate the therapeutic role of Etanercept in 16 patients with metastatic breast cancer (130). Treatment was well tolerated in all patients. There were no treatment related deaths without serious infections in any patient during therapy. The most common side-effects were injection site reactions, fatigue, loss of appetite and headache. Seven patients who completed 12 weeks of treatment were available for disease evaluation. Two patients had radiological stable disease. No substantial improvement in quality of life was seen in patients, as most patients developed symptoms because of progressive disease (130).

Etanercept was also tested in ovarian cancer patients. Convincing data support the link between inflammation and ovarian cancer, and TNF- α is a major inflammation mediator (131, 132). Several preclinical studies confirm the importance of TNF- α in ovarian cancer pathogenesis and progression (133, 134) and positive correlation is seen between tumor grade and the extent of TNF- α expression in ovarian cancer (135). Madhusudan and colleagues evaluated the role of etanercept in patients with recurrent

ovarian cancer. Primary endpoints were the evaluation of toxicity and biological and clinical activity of etanercept in these patient setting. They found that the TNF- α inhibitor was safe and well tolerated in this study. Adverse effects reported were similar to those seen in patients with inflammatory disease without medical intervention, although etanercept was stopped in one patient because of the development of Sjögren's syndrome. Because etanercept is likely to be cytostatic rather than cytotoxic, six patients had disease stabilization as expected. Eighteen patients were evaluated because they completed 12 weeks of therapy. Thirty three percent of patients achieved stable disease. Patients who did not progress also had overall improvements in QOL compared to non-responders, suggesting a clinical benefit with therapy. In both breast and ovarian trials, a significant increase in immunoreactive TNF- α was seen in patients within 24 h of initiation of etanercept therapy. The increase continued at all subsequent time points (days 7, 28, 56 and 84). In addition, in patients with ovarian cancer and stable disease there was a consistent time-dependent decrease in IL-6 and chemokines, suggesting a correlation with response (136).

Etanercept was also employed in an attempt to prevent the development of fatigue and cachexia, which is a syndrome characterized by loss of adipose tissue and skeletal muscle mass. A complex network of proinflammatory mediators triggered by the malignancy or by the chemotherapy itself is thought to be involved in fatigue and cachexia. TNF- α seems to play a central role in the development of this syndrome and NF- κ B is one of the major effectors of this pathway. Because TNF- α antagonism reduces fatigue in rheumatoid arthritis patients, it may also decrease the fatigue caused by chemotherapy, resulting in better tolerability of dose-dense/dose-intense schedules. Monk *et al.* presented their preliminary results of a pilot feasibility study of etanercept and weekly escalating dose of docetaxel in patients with solid malignancies refractory to conventional therapy (137). They showed that a combination of docetaxel and etanercept was feasible and allowed for the maintenance of chemotherapy dose-intensity while profoundly reducing the incidence of toxic fatigue. As a result of grade 4 neutropenia at the second dose level, 43 mg/m² of docetaxel in combination with etanercept 25 mg twice weekly was the maximum tolerated dose. However, in combination with G-CSF, etanercept administration also permitted intensification of weekly docetaxel therapy in most patients.

Several animal models bearing cancer and studies on cancer patients point to TNF- α as a key mediator of cancer-associated weight loss which is, for example, a predictive factor of poor prognosis in patients with non small-cell lung cancer. In order to prevent weight loss in cancer patients, infliximab, an IgG monoclonal antibody that blocks the

binding of TNF- α to its p55 and p75 receptors, was effectively used in association with chemotherapy.

The first clinical data on anti-TNF- α showed that this drug can also be used in cancer patients without major side-effects. Further investigations are needed to understand if anti-TNF- α really represents a novel approach to cancer treatment. It is necessary to test etanercept, infliximab and the other TNF- α blockers, mainly in combination with chemotherapy or other biological drugs such as antiangiogenetic agents, to verify whether they can add clinical benefit in treating cancer patients. Etanercept in combination with low-dose methotrexate chemotherapy has been already evaluated in patients with inflammatory disease and the combination was found to be safe and efficacious (138).

The key role of NF- κ B as a target for cancer prevention and therapy. NF- κ B is increasingly gaining interest as a key regulatory factor in inflammation-driven cancer development. It is a transcriptional complex made up of five proteins with homology domains that work by binding to DNA and activating multiple gene transcription. Constitutive NF- κ B activation leads to a cellular anti-apoptotic status that translates into tumor surveillance escape and treatment resistance in cancer cells, as well as protracted growth factor and cytokine secretion from innate immune response cells (29).

Hyperexpression of NF- κ B has been documented in several human cancers, especially in the inflammation-related ones. Its importance as a potential therapeutic target is therefore under intense investigation.

No effective anti-NF- κ B drug is currently available in the clinical setting, but there is some clinical evidence demonstrating its potential importance in tumor growth and anti-neoplastic treatment efficacy.

In a study from the MD Anderson Cancer Center (139), specimens from 43 patients enrolled onto a clinical trial investigating the role of pre-surgical chemoradiotherapy were studied for the expression of activated NF- κ B protein. The tumor constitutive expression of NF- κ B was detected in only 7% of patients experiencing pathological complete response (pathCR) to the treatment as compared to the 72% of patients achieving less than pathCR ($p \leq 0.001$). Activated NF- κ B was also significantly associated with aggressive pathological features such as perineural, lymphatic, vascular invasion and metastasis development.

NF- κ B also correlated with tumor outcomes. At a median follow-up of 23 months, 48% of NF- κ B-positive patients had died compared to only 5% NF- κ B-negative patients ($p = 0.0013$). In a multivariate risk analysis including stage, pathCR, age, presence of histologically proven regional metastatic lymph nodes and NF- κ B expression as prognostic variables, NF- κ B was the only independent prognostic factor for PFS ($p = 0.010$) and OS ($p = 0.015$).

In another research from Kashani-Sabet *et al.* (140) data from 526 melanoma patients were analyzed in relation to NF- κ B tumor expression, along with parameters of tumor vascularization. Tumor vascularity was a strong predictor of melanoma outcome and a precursor of both tumor vascular invasion and ulceration (two well-known prognostic factors in melanoma patients). A matched-pair tissue array analysis demonstrated significant correlation between the overexpression of NF- κ B and the development of vascular factors. The authors concluded that vascular factors play an important role in the progression of malignant melanoma, ulceration may be a surrogate marker for the interactions between melanoma and the tumor vasculature and NF- κ B seems to play an important role in the development of these factors.

Several I κ B kinases (IKK) and NF- κ B inhibitors are under development, as well as a number of natural products that can inhibit NF- κ B activation when used at high doses but can also affect several other targets (141). Given that NF- κ B in inflammatory cells serves an important immune function and its absence can result in severe immuno-deficiency, prolonged and substantial inhibition of NF- κ B might not be practical in cancer prevention, as already discussed for TNF- α . NF- κ B inhibitors are more likely to be of use in cancer therapy, in which they can be administered intermittently for shorter durations, thereby avoiding immunosuppression associated with long-term inhibition (29). The most likely effect of NF- κ B inhibitors is increased cancer cell apoptosis. Nonetheless, in most cases, this inhibition is insufficient unless combined with apoptosis-inducing drugs or radiation. Thus, NF- κ B inhibitors are most likely to be used as adjuvants along with other cancer therapies (29).

Conclusion

Overall this review provides evidence for a strong link between chronic inflammation and cancer. Thus, inflammatory biomarkers can be used to monitor the progression of the disease and develop new anti-inflammatory drugs to prevent and treat cancer. Numerous anti-inflammatory agents, including those identified from natural sources, have been shown to exhibit chemopreventive activities and thus can be used not only for prevention but also for therapy of cancer. These drugs can also be used as adjuvant to other therapies, such as anti-angiogenic or cytotoxic agents and may provide highly efficacious therapeutic regimens for the treatment of malignancies.

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