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Therapeutic potential of cytokine and chemokine antagonists in cancer therapy

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ABSTRACT

A new paradigm is becoming widely accepted, that chronic inflammation, driven in part by chemokines and cytokines at the site of a tumour, may facilitate tumour progression instead of promoting anti-tumour immunity. Tumours and activated stromal cells secrete pro-inflammatory chemokines and cytokines that act either directly or indirectly through stimulation of the vascular endothelium to recruit leukocytes to the tumour. After activation, these tumour-associated leukocytes release angiogenic factors, mitogens, proteolytic enzymes, and chemotactic factors, recruiting more inflammatory cells and stimulating angiogenesis to sustain tumour growth and facilitate tumour metastasis. Breaking this cycle by inhibiting targets such as cytokines, chemokines and other inflammatory mediators, either alone, or more realistically, in combination with other therapies, such as anti-angiogenic or cytotoxic agents, may provide highly efficacious therapeutic regimens for the treatment of malignancies. This article reviews anti-cytokine and anti-chemokine therapies being pursued in cancer, and discusses in more detail anti-tumour necrosis factor-α (TNF) approaches.

1. Cytokines and chemokines as anti-cancer therapeutic targets

Tumours secrete pro-inflammatory chemokines and cytokines and activate local stromal cell elements to do the same. These chemokines and cytokines act either directly or indirectly through stimulation of the vascular endothelium to recruit leukocytes to the tumour. For some tumour types >70% of the tumour mass consists of infiltrating leukocytes. Upon activation, these tumour-associated leukocytes, especially macrophages, release angiogenic factors, mitogens, proteolytic enzymes and chemotactic factors, recruiting more inflammatory cells and sustaining tumour growth, invasion and angiogenesis. The new vessels that form provide greater access for more inflammatory cells to enter the tumour.

Because of the essential tumour-promoting functions played by the large variety of pro-angiogenic growth factors, cytokines, chemokines and proteinases secreted by tumour-infiltrating leukocytes, it is tempting to explore the utility of drugs targeting cancer inflammation mediators as anti-cancer therapeutics to inhibit tumour angiogenesis and metastasis.

2. Anti-tumour necrosis factor-α (TNF) therapy for cancer

Tumour necrosis factor-α (TNF) is one of the most important mediators of inflammation and has been linked to the stimulation of tumour initiation and progression, in part by inducing the production of angiogenic factors, chemokines, matrix

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metalloproteinases (MMPs) and by stimulating fibroblast growth and function.[1] Therefore, blocking TNF bioactivity or inhibiting TNF production may be an effective anti-cancer therapy.[2] Evidence from animal models and the clinic points increasingly toward the use of anti-TNF therapies to treat cancer or its symptoms.[1] As reviewed by Szlosarek and Balkwill,[3] much evidence from animal and in vitro experimentation supports the mechanistic connections between chronic inflammatory stimulation involving TNF and subsequent initiation and/or eventual promotion of malignancy. Chronic, dysregulated (non-self-limited) inflammation seems to provoke compensatory cellular over-replication, with increased chances for replication errors. Mutagenesis, neoplastic growth, invasion and metastasis involve TNF-mediated production of nitric oxide, induction of angiogenic factors and MMPs, modulation of chemokines and chemokine receptors controlling leukocyte infiltration of tumours, loss of hormonal responsiveness, and acquired resistance to cytotoxic chemotherapy. In his review of cytokines in the pathogenesis and treatment of cancer, Dranoff[4] describes evidence that chemical carcinogens, immunodeficiency, chronic infection and chronic inflammation all have been causally associated with tumourigenesis mediated by overproduction of cytokine growth factors. These imbalances and disease effects arise when cytokine function, which is normally adaptive for response to mild injury induced by carcinogens, pathogens or environmental antigens, is insufficient to limit cellular stress and the resultant cellular damage. The chronic inflammatory stimulation does not lead to repair, but instead leads to persistent and eventually dysregulated cellular function.

3. Clinical and pre-clinical observations and associations implicating TNF in cancer progression

Some of the most detailed pre-clinical work regarding the role of TNF as a tumour promoter was published in series of papers from the laboratory of Fran Balkwill.[5–7] The authors used the induction of skin tumours in mice as a model system to dissect the contribution of TNF and its signalling pathways to skin tumour initiation. In this system a DNA-damaging chemical is used as a topical tumour inducer, followed by the application of a tumour-promoting agent. TNF was first implicated in tumour growth in this model by the observation that TNF knockout mice are highly resistant to the generation of skin tumours by this method. The presence of functional TNF has no effect on DNA mutation rates or tumour initiation, but rather profoundly influences tumour promotion. Anti-tumour effects were observed following pharmacological intervention with a neutralising anti-mouse TNF antibody. The potential role of TNF in the growth of human skin cancers, initiated largely by UV radiation, will be an important area for future investigation.

Recent evidence from another model also strongly supports a role for TNF in tumour growth and promotion. Luo and colleagues[8] showed that TNF was responsible for promoting the growth of lung metastases in a murine model of colon carcinoma. In these experiments tumour cells were seeded in the lung, and systemic treatment with lipopolysaccharide (LPS) resulted in a substantial increase in lung tumour burden. These tumour growth promoting effects were shown to require NF-κB activation in the tumour cells and treatment with a neutralising anti-TNF antibody prevented LPS-mediated NF-κB induction. Mice reconstituted with bone marrow from TNF knockout mice did not experience an LPS-dependent increase in tumour burden, demonstrating that TNF is the critical mediator of tumour growth and progression in this model.

Clinically, several reports have associated detection of abnormally high levels of circulating TNF in cancer patients with a wide range of tumour types,[9] including pancreatic,[10] kidney,[11] breast,[12] lung (asbestosis-induced)[13] and prostate cancers.[14,15] Higher levels of TNF have been correlated with tumour stage, extent of para-neoplastic complications (such as anorexia–cachexia syndrome) and shorter survival time. However, circulating TNF is not always detectable in cancer patients and may vary within individual patients over time and course of disease.[16] Tumour tissue levels may be more relevant than blood levels of TNF and its receptors in explaining pro-tumourigenic associations such as those reported in head/neck squamous cell carcinoma.[17] Significantly elevated pre-operative TNF mRNA transcripts in pancreatic cancer patients were reduced after tumour resection to levels similar to controls.[18]

In a prospective study of 80 patients with prostate cancer (localised or metastatic), but no evidence of active infection or inflammatory disease, serum levels of both IL-6 and TNF correlated directly with the extent of malignant disease. Both cytokines became elevated at the point of prostate-specific antigen (PSA) progression. IL-6 and TNF may prove valuable as prognostic markers for prostate cancer. There was a significantly greater elevation of TNF in patients with metastatic cancer compared with the level in patients with localised disease and in controls. The authors caution that association does not prove causality; studies using agents that target these specific cytokines should determine whether they contribute to malignant progression.[19]

Increasing clinical evidence demonstrating the efficacy of anti-TNF interventions further supports the role of TNF as an important anti-cancer target. Tsimberidou and colleagues reviewed potential clinical indications for agents that block or inactivate TNF, specifically the soluble TNF receptor fusion protein, etanercept, and the anti-TNF monoclonal antibody infliximab, with focus on the functions of TNF in multiple myeloma, myelodysplastic syndrome (MDS), acute myelogenous leukaemia (AML) and myelofibrosis.[20] These authors report evidence of limited therapeutic activity of monotherapy with anti-TNF molecules; with optimisation of dose and schedule, combination with other active biological or cytotoxic agents, and a better understanding of individual cancer patient proteomic patterns of disease and gene polymorphisms (profiling),[21] anti-TNF therapies could provide significant clinical benefit.

In chronic lymphocytic leukaemia (CLL), neoplastic lymphocytes release TNF spontaneously in vitro, and leukaemia lymphocytes are more proliferative and more viable when exposed in vitro to TNF. Is TNF a pro-leukaemic cytokine? The prognostic and clinical significance of TNF was evaluated in 150 consecutive patients with CLL.[22] The mean plasma concentration of TNF (16.4 pg/ml) was significantly higher than

the mean in 20 haematologically normal subjects (8.7 pg/ml; $p < 0.0001$). The results showed correlation of TNF with extent of disease, with serum β_2 microglobulin (β_2-M), and with low haemoglobin and low platelets. There were significantly higher TNF levels in patients with chromosomal abnormalities. TNF levels were predictive of survival in a Cox multivariate analysis, independent of: staging, β_2-macroglobulin, haemoglobin (Hgb), white blood cell (WBC) and platelet count. Inhibition of TNF could be of therapeutic value in CLL, potentially by blocking both a growth signal to the leukaemic clone and a suppressive effect on other haematopoietic lineages.

Similar biology underlies the rationale for the evaluation of anti-TNF agents to inhibit the excessive apoptosis in haematopoietic cells suspected as the cause of cytopaenias in MDS.[23] The pathognomonic, hyperproliferative marrow of MDS, with excessive apoptosis is associated with over-expression of TNF, which when blocked may result in effective disease treatment. Italian investigators described two cases of low/intermediate-risk MDS treated with infliximab. Both patients achieved sustained peripheral erythroid responses, along with marrow response of increased erythroid progenitor cell by BFU-E assay and a clear decrease in CD34+ cells expressing annexin V.[24]

In a separate study, infliximab was given to 37 low risk (international prognostic symptom score (IPSS) <1.0) MDS patients, 17 with normal karyotypes. Therapy was well tolerated and the side-effects included slight myelosuppression, and moderate infusion reactions. Of 28 evaluable patients, 8 (29%) showed a partial response, including 1 patient with a trilineage response, 1 with a bilineage response, 2 with >100% increase in absolute neutrophil count (ANC), 1 with >1 Gm/dl increase in haemoglobin, 1 with decrease in bone marrow blasts from 7% to 1% changing the MDS classification (FAB) from refractory anaemia with excess blasts (RAEB) to refractory anaemia with ringed sideroblasts (RARS). Two patients had minor cytogenetic responses (1 had >50% reduction in trisomy 8 cells and the other in 20q-cells).[25] A multicentre, European Organisation for Research and Treatment of Cancer (EORTC) phase 2 clinical trial of infliximab monotherapy with MDS is ongoing. The pluripotent activity of TNF is implicated again by the variety of modulatory effects achieved with infliximab, and suggests that in addition to the dysplastic haematopoietic cells, the microenvironment of the marrow is also affected. A logical next evaluation could be use of a specific anti-TNF agent to modulate cell–cell signalling and stromal interactions in the marrow, combined with a cytotoxic agent known to be active against the clonal myelodysplastic cells.[26]

Evidence supporting the use of anti-TNF regimens for the treatment of solid tumours is mounting. Immunotherapy with interferon-α and IL-2, standard treatment options for advanced renal cell carcinoma, have low response rates and cause considerable toxicity, related in large part to the high levels of TNF and IL-6 that are induced. Combination therapy with IL-2 and an anti-TNF agent might allow a patient to receive more IL-2 if the TNF-mediated toxicity is abrogated, although one small trial did not report less toxicity of IL2 when rhuTNFR:Fc fusion protein was included in the treatment regimen.[27] Phase II trials in patients with advanced renal cell carcinoma showed moderate efficacy of thalidomide,[28] which has demonstrated apoptotic, immunomodulatory and anti-angiogenic effects. Thalidomide inhibits TNF gene activation by decreasing NF-κB binding.[29] On this basis, a single-arm, phase II study evaluated monotherapy with the anti-TNF antibody infliximab for advanced renal cell carcinoma in 19 patients refractory to prior treatments with IL-2, interferon-α and/or chemotherapy.[30] Three confirmed responses on study were reported, including regression of metastatic hepatic lesions.

Innovative strategies to reduce prolonged systemic cytokine exposure while concentrating cytokine accumulation at the site of the tumour, involve the use of antibody–cytokine fusion proteins (immuno-enhancing cytokines genetically fused to antibodies that target specific antigens over-expressed on tumour cells). Pre-clinical studies that demonstrate tumouricidal activity were reviewed by Dela Cruz.[31]

Etanercept, a TNF receptor fusion protein that effectively neutralises TNF, was tested in a phase II, non-randomised, open-labelled study in 16 patients with progressive metastatic breast cancer refractory to conventional therapy. There were no clinical responses, but biological activity was shown by decreases in serum IL-6 and CCL2, and by a cytokine release assay in peripheral blood cells.[32]

In addition to TNF antagonists, agents that block TNF through inhibiting its production or interfering with its signalling pathway may also have some place in cancer therapy. Thalidomide inhibits the processing of mRNA for cytokines such as TNF and VEGF. Continuous low-dose thalidomide has shown activity in a study of 84 previously treated patients with advanced myeloma.[33] Thalidomide also induced modest anti-tumour responses in malignant glioma.[34]

Further investigation of anti-TNF agents in solid tumours may determine whether a dose–response relationship exists, whether baseline characteristics (i.e., tumour histology, cytokine profiles or gene polymorphisms) can predict response, and whether responses correlate with prolonged survival time. Early results suggest that anti-TNF therapies will find an important place in cancer treatment, both as anti-tumour agents and perhaps much more broadly for supportive care.

4. Potential risks of anti-TNF-α therapy

Because of the adaptive, protective purpose of inflammation, pharmacological inhibition of this pro-inflammatory cytokine can have adverse effects in the host.[35] The risks of opportunistic infections and of reactivation of latent tuberculosis are class effects of anti-TNF agents. Pre-emptive systemic anti-fungal therapy is recommended for patients receiving anti-TNF treatment of graft-versus-host disease (GVHD).[36] Smith and Skelton reported cases of squamous cell carcinoma (SCC) that became evident and grew rapidly during an initial period of etanercept therapy for rheumatoid arthritis (RA).[37] The tumours may have been present but occult and controlled prior to disruption of immunological control. Etanercept could disable innate anti-tumour surveillance by blockade of both lymphotoxin α (TNF-β), and cytotoxic effects of TNF and/or by inhibition of the TH1 cytokine pattern and impairment of cytotoxic T cells. All cases were in chronically ultraviolet (UV)-damaged, actinic skin predisposed to tumourigenesis by long-term, low-level production of TNF. No

new SCCs developed in patients who continued treatment for more than 1 year, suggesting prolonged anti-TNF therapy could be preventive of cutaneous malignancies.

Pharmacovigilance data on etanercept, infliximab and adalimumab (anti-TNF antibody, see Table 1)) were reviewed by the US Food and Drug Administration (FDA) in 2003, with a focus on lymphoproliferative disease in patients treated with these anti-TNF agents, relative to the rate expected in populations with immune-mediated diseases.[38] The potential role of TNF-blocking therapy in the development of malignancies is not known. A prospective study of 18,572 patients with rheumatoid arthritis treated with anti-TNF therapy plus methotrexate reported an increased standard incidence ratio (SIR) compared with patients not receiving methotrexate or biologics, but confidence intervals overlapped for all treatments.[39] Data from a Swedish arthritis registry suggest that TNF blockers may not increase overall tumour risk in patients with rheumatoid arthritis, but may increase the risk of lymphomas.[40] Adams and colleagues reported two fatal cases of aggressive cutaneous and systemic T-cell lymphoma that progressed rapidly in the setting of TNF blockade.[41]

Patients with highly active disease and/or chronic exposure to immunosuppressant therapies may have several-fold higher risk for development of lymphoma, thus caution should be exercised when considering anti-TNF agents in patients with a history of malignancy or who develop malignancy during treatment. The FDA reported on the risks of histoplasmosis,[42] lymphoma,[43] and/or listeriosis.[44] A study of etanercept to treat 174 evaluable patients with Wegener's granulomatosis reported 6 solid cancers in the treated group,

Table 1 – Drugs in clinical development that modulate tumour necrosis factor (TNF) function

Class	Compound (company)	Status/indications
Biological antagonist		
Monoclonal anti-TNF antibody	Infliximab (Centocor, Malvern, PA, USA)	Approved in Europe and United States of America (USA) for rheumatoid arthritis, Crohn's disease, ankylosing spondylitis; under evaluation in cancer patients for supportive care (phase II; cachexia and graft-versus-host disease (GVHD)) and for therapy of multiple myeloma (phase I), renal cell carcinoma (phase II), and myelodysplastic syndrome (MDS) (phase II))
	Adalimumab (Abbott, Chicago, IL, USA)	Approved in Europe and USA for rheumatoid arthritis. Under evaluation for psoriatic arthritis, inflammation, psoriasis, ankylosing spondylitis, heart disease, Crohn's disease
TNF receptor-Ig fusion protein	Etanercept (Amgen Thousand Oaks, CA, USA)	Approved in Europe and USA for rheumatoid arthritis, juvenile arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis; under evaluation in cancer patients for ovarian cancer and refractory multiple myeloma
Pegylated anti-TNF antibody fragment	CDP-870 (UCB Pharma, Brussels, Belgium)	Under evaluation for Crohn's disease (phase III) and rheumatoid arthritis (phase III)
Small molecule antagonists		
Inhibitors of TNF synthesis and signalling	Thalidomide (Celgene Summit, NJ, USA)	Approved in Europe and USA for erythema nodosum leprosum; under evaluation in cancer patients for multiple myeloma (phase III) and renal cell carcinoma (phase II). Under investigation for MDS, basal cell carcinoma, prostate cancer, rheumatoid arthritis, ulcerative colitis, Crohn's disease
	Pentoxifylline (Sanofi-Aventis, Paris, France)	Approved in Europe and USA for peripheral vascular disease; under evaluation in cancer patients for radiation-induced fibrosis (phase II)
p38 MAP kinase inhibitor	SCIO-469 (Johnson & Johnson New Brunswick, NJ, USA)	Under evaluation for rheumatoid arthritis (phase IIa) and multiple myeloma (phase II)
	BIRB796 (Boehringer Ingelheim, Ingelheim, Germany)	Under evaluation for psoriasis (phase IIb/III), rheumatoid arthritis (phase IIa) and Crohn's disease (phase IIa)
	SB-681323 (GlaxoSmithkline, Philadelphia, PA, USA)	Under evaluation for rheumatoid arthritis (phase I), chronic obstructive pulmonary disease (COPD) (phase I) and artherosclerosis (phase I)
	VX-702 (Vertex, Cambridge, MA, USA)	Under evaluation for acute coronary syndrome (phase IIa)
TACE inhibitors	TMI-005 (Wyeth Research, Madison, NJ, USA)	Under evaluation for rheumatoid arthritis (phase II)

Numerous biological and small molecule compounds capable of modulating TNF function are either available commercially, or are under clinical evaluation. Only human TNF is currently approved for a cancer indication, but several of the TNF antagonists are approved for other indications and are under investigation for supportive care or treatment of various malignancies.
TACE, the protease that releases soluble TNF; MAP kinase, mitogen-activated protein kinase.

compared with zero in the placebo control group ($p = 0.01$).[45] Treatment with infliximab has been associated with keratoacanthomas and squamous cell carcinomas.[46] Etanercept has also been associated with squamous cell carcinomas.[47] The strong biological basis of concern warrants heightened vigilance and consideration of the benefit-to-risk ratio in individual patients when prescribing anti-TNF therapies.

5. Anti-TNF as an example of anti-cytokine therapy for supportive care

In addition to approaches aimed at the tumour, anti-TNF therapies are increasingly being tested for supportive care indications. The range of potential indications in this area is extremely broad and diverse due to the pleiotropism of TNF action and includes cancer-associated depression, fatigue, cachexia, treatment of toxicities due to chemotherapy and radiotherapy, treatment of metastatic bone pain, and GVHD.[48,49]

A wealth of evidence implicates TNF as a mediator of cachexia. In fact, prior to its purification and cloning, TNF was termed "cachectin" when it was identified as the soluble factor responsible for severe wasting in rodent models of disease. TNF has also been shown to be important in these processes at the cellular and molecular levels both by increasing destructive proteolysis in mature skeletal muscle and by inhibiting the differentiation of myoblasts necessary for the repair of damaged or stressed muscle tissue.[50] Clinical trials are now testing the ability of anti-TNF agents, such as infliximab, to inhibit wasting in cancer patients.[51–54]

Cancer-related pain remains a significant unmet medical need. TNF appears to be important both for the pain signal itself in some situations and for the metastatic bone erosion that often underlies severe cancer pain. Past research has established that TNF stimulates osteoclastogenesis and osteoclast activation in a variety of immune-mediated diseases and that TNF plays a role in tumour-induced osteolysis concomitant with metastatic invasion of bone.[55,56] This presents an obvious opportunity for clinical application of anti-TNF agents to attenuate tumour-induced destruction of bone and associated neuropathic pain. Tobinick reported two cases of intractable pain from spinal lesions, uncontrolled by narcotics, which were treated with etanercept. One case was of metastatic lung cancer and the other was the result of metastatic mammary ductal carcinoma. Relief of pain was reported to have occurred rapidly, and was sustained for months. Positron emission tomography (PET) scan evidence in the second patient showed diminution of neoplastic activity and restricted invasion.[57] Preliminary evidence such as this warrants the conduct of phase II studies followed by randomised, controlled, and blinded clinical trials to confirm the benefits of anti-TNF therapy for the relief of pain caused by skeletal metastases. Additional pre-clinical studies may be necessary to understand the mechanisms by which TNF promotes neuropathic pain and bone destruction.

Investigative and clinical uses of etanercept or infliximab in GVHD are based on the firmly established role of upregulated TNF as a critical effector cytokine in the multi-organ immunoreactivity to pre-transplant conditioning regimens and/or allogeneic antigens.[58] Infliximab has been reported by transplant clinicians/researchers to be effective in some cases when added to ongoing treatment of refractory acute and chronic GVHD.[59] Added immunosuppression and risk of serious infections in these patients warrant vigilance when using anti-TNF agents in GVHD patients.[36] Notably, no studies have yet elucidated an optimal dose and schedule of the anti-TNF monoclonal antibody and its kinetics in GVHD patients, primarily because of especially high levels of TNF and often severe protein-losing enteropathy.[60–62]

6. Current and future TNF-related therapies

The biological anti-TNF therapies currently approved include etanercept, infliximab and adalimumab (summarised in Table 1). While approvals have been given in chronic inflammatory diseases, such as rheumatoid arthritis, Crohn's disease and psoriasis, these agents are being extensively tested in several areas of cancer therapy and supportive care. Etanercept is a fusion protein consisting of the extracellular domain of TNF-R2 fused to immunoglobulin CH2 and CH3 constant domains, and hence it is capable of neutralising both TNF and lymphotoxin. Both infliximab and adalimumab are monoclonal antibodies that neutralise only TNF and have no effect on lymphotoxin. In addition, several small molecule, oral TNF therapies are under development (reviewed in[63] Table 1) that act to inhibit TNF signalling and synthesis, including inhibitors of TACE (the protease that releases soluble TNF), p38 mitogen-activated protein (MAP) kinase (known to be involved in signalling both TNF synthesis and activity) and NF-κB (a major signalling pathway for TNF activity) and thalidomide (a TNF synthesis inhibitor).

7. Anti-IL-6 therapy for cancer

IL-6 is a secreted, multifunctional acute phase protein that is produced in response to inflammation, stress, injury and infection and is also upregulated in many types of cancer (reviewed by Trikha and colleagues[64]). IL-6 may prove to be an effective target for cancer therapy and several early phase clinical studies with IL-6 antagonists seem to support this hypothesis.[64] IL-6 plays a role in haematopoiesis, and in pathological situations promotes the proliferation and survival of certain haematological malignancies. IL-6 expression is also closely related with some solid tumours and appears to be an autocrine growth factor for solid tumours such as renal cell cancer and prostate tumours. High levels of IL-6 are seen in renal cell carcinoma and the level of its expression correlates directly with tumour burden and survival.[65] IL-6 also has been hypothesised to be a causative factor for cancer-related symptoms including cachexia, flu-like symptoms and fatigue, and anti-IL-6 therapies could potentially alleviate these symptoms to provide quality of life benefits.

Antibodies to IL-6 (BE-8 and CNTO 328) have been safely administered to patients with renal cell carcinoma, B cell lymphoproliferative disease as well as multiple myeloma.[66] CNTO 328 is a mouse-human chimeric anti-IL-6 antibody, in phase II development for the potential treatment of multiple myeloma and renal cell carcinoma. CNTO 328 is currently being studied in a phase I/II study in metastatic renal cell carcinoma patients. The trial has two parts, the first is an

open-label, dose-escalation trial, and the second is a two-arm randomised double-blind, dose-comparison trial. The drug was well-tolerated in both studies. In the first part, patients ($n = 7$) received CNTO-328 (1, 3, 6 or 12 mg/kg) by intravenous infusion on days 1, 29, 43 and 58. All evaluable patients exhibited reduced levels of CRP, 4 showed partial disease response, and 3 showed stable disease. In the second part, CNTO 328 was administered at either 3 mg/kg ($n = 17$) or 6 mg/kg ($n = 20$) doses every 3 weeks for 4 cycles. One patient (3%) achieved a partial response, and 15 patients (40%) showed stable disease[67] (Robert Corringham, Centocor, Malvern, PA, USA).

A murine anti-IL-6 monoclonal antibody, elsilimomab (also known as B-E8) is in phase II for multiple myeloma and has shown preliminary efficacy.[64] Similar therapeutic effects were also observed in the treatment of metastatic renal cell carcinoma. However, there is a limitation for the clinical use of a murine monoclonal antibody as it frequently induces human anti-mouse antibodies (HAMA).

Tocilizumab, a humanised anti-IL-6 receptor antibody (also known as MRA, R-1569, or humanised PM-1), was recently approved in Japan for the treatment of Castleman's disease, a rare lymphoproliferative disease associated with high levels of IL-6 production from enlarged lymph nodes.[68] This therapy is also in clinical development for rheumatoid arthritis, Crohn's disease, myeloproliferative disorder and systemic lupus erythematosus.[69]

Early clinical data shows that IL-6 is a promising cytokine target for oncology. Anti-IL6 therapies may be even more effective when used in patients with less advanced disease and also may be more effective in combination with chemotherapy or possibly with biological agents that inhibit cytokines such as TNF, which appear to have overlapping downstream biological functions in stimulating tumour cell proliferation and causing cancer-related complications such as cachexia and fatigue.

8. Anti-IL8 (CXCL-8) therapy for cancer

The essential role of neutrophils in tumour angiogenesis was demonstrated in neutropenic mice in which IL-8, macrophage inflammatory protein (MIP)-2 (CXCL2) and growth-related oncogene (GRO)α were not able to induce angiogenic reactions.[70] In fact, angiostatin, an anti-angiogenesis factor, may inhibit angiogenesis by blocking chemotaxis of neutrophils to CXCR2 chemokine receptor agonists, including IL-8, MIP-2 and GROα.[70]

IL-8 is an 8-kDa chemokine of the Glu-Leu-Arg (ELR)+CXC family with endothelial cell chemotactic and proliferative activity.[71] Recombinant IL-8 potently stimulated angiogenesis when implanted in the rat cornea and induced proliferation and chemotaxis of human umbilical vein endothelial cells. Angiogenic activity present in the conditioned media of inflamed human rheumatoid synovial tissue macrophages or LPS-stimulated blood monocytes was equally blocked by antibodies to either IL-8 or TNF. An IL-8 antisense oligonucleotide specifically blocked the production of monocyte-induced angiogenic activity. High IL-8 expression levels render tumour cells highly tumourigenic, angiogenic and invasive.[72,73] IL-8 expression in human tumours significantly correlates with angiogenesis, as determined by intra-tumour microvessel density.[74] Finally, expression of IL-8 correlates with angiogenesis in non-small cell lung carcinoma,[75] metastatic growth pattern in melanoma,[76] and with disease staging and prognosis in head and neck squamous cell carcinoma.[77]

Abgenix has developed a fully human anti-IL-8 antibody ABX-IL8 for treating various inflammatory and malignant diseases. This high affinity antibody blocks the binding of IL-8 to IL-8 receptors and inhibits IL-8-dependent neutrophil activation, migration and degranulation.[78] In pre-clinical models, ABX-IL8 inhibits angiogenesis, tumour growth and metastasis of human melanoma,[79] and tumour growth and MMP activity in orthotopic bladder cancer xenografts.[80] Based on preclinical efficacy and drug safety data in phase I study, a phase II study of ABX-IL8 in patients with malignant melanoma was planned in 2002. However, this study was never commenced because ABX-IL8 did not meet its primary endpoint in a separate psoriasis trial. Therefore, the therapeutic value of blocking IL-8 has yet to be assessed in cancer patients.

Given the redundancy of the chemokine network, it might be desirable to target more than one chemokine at a time in order to maximise the effects on disease modulation. Biokine Therapeutics (Rehovot, Israel) has developed a technology platform to identify small molecules that bind to multiple chemokines and neutralise their activities. BKT-RP3 is a small peptide that binds to IL-8 and Mig (CXCL-9) and inhibits angiogenesis, for the potential treatment of melanoma and non-small-cell lung cancer.

9. Other chemokine targets for cancer therapy

CXCR4 is a chemokine receptor that belongs to the seven transmembrane G protein-coupled receptor family, and has SDF-1 (CXCL12) as an endogenous ligand. It has been reported that CXCR4 plays a role in several diseases, such as HIV infection, and various inflammation-related disorders, such as rheumatoid arthritis. This receptor is also involved in cancer progression and especially in metastasis.[81] Furthermore, blockade of the SDF-1/CXCR4 signalling axis by targeting either the ligand or the receptor inhibits cancer metastasis.[82,83] BKT140 and its analogues, peptidic CXCR4 antagonists, inhibit SDF-1-induced migration and invasion of human pancreatic cancer cells, as well as SDF-1-induced chemotaxis. In pre-clinical models, the BKT140 derivative significantly reduced pulmonary metastasis of breast cancer and melanoma cells.[84,85] CTCE-9908 is an analogue of SDF-1 and antagonist of SDF-1 receptors developed by Chemokine Therapeutics (Vancouver, Canada). Pre-clinical studies demonstrated inhibitory activity of murine osteosarcoma lung metastasis. A recently completed phase I study of this compound in healthy adults did not reveal any significant toxicity and warrants further investigation in cancer patient population.[86] The SDF-1 and CXCR4 signalling axis is also being pursed by a number of other companies. These include CXCR4 antagonists AMD3100[87] and AMD070 by AnorMed Inc., (Langley, Canada) which completed phase II studies for stem cell transplantation treatment of multiple myeloma and non-Hodgkin's lymphoma patients,[88] and in phase I for HIV therapy, respectively. These CXCR4 antagonists are now also being investigated for their activity in treating solid

tumours.[89–91] Other CXCR4 antagonists include antibodies to CXCR4, which it is planned will enter phase 1 trial (Northwest Biotherapeutics Inc., Bothell, Washington, USA) and small molecule antagonists being developed by ChemoCentryx (Mountain View, CA, USA).

10. Anti-CCL2 therapy for cancer

In addition to being a key regulatory molecule of monocyte trafficking to sites of inflammation, CC chemokine ligand-2 (CCL2), also known as monocyte chemo-attractant protein 1 (MCP-1) is also a potent pro-angiogenic factor. In a corneal angiogenesis assay, CCL2 elicits angiogenic responses with similar potency to the well-known angiogenic mediator VEGF-A.[92] Angiogenesis induced by CCL2 is associated with prominent recruitment of macrophages, whereas that induced by VEGF is devoid of inflammatory cell recruitment. Tumour expression of CCL2 has been correlated with the degree of tumour-associated macrophage (TAM) infiltration in human gastric carcinomas,[93] oesophageal squamous cell carcinomas,[94] gliomas[95,96] and haemangioma,[97] and breast carcinoma.[92,98,99] Furthermore, high levels of tumour CCL2 were also found to serve as a prognostic biomarker indicating poor prognosis and early relapse.[99,100]

It has been postulated that CCL2 in tumour tissues may stimulate angiogenesis by recruiting tumour infiltrating macrophages and subsequently the production of angiogenic growth factors such as VEGF, TNFα, IL-6 and IL-8.[99,100] In addition, CCL2 may also activate endothelial cells directly via cell surface CCR-2 receptor[101] to regulate MT1-MMP and VEGF activity.[102,103] In JE knockout mice, lack of JE (a mouse orthologue of human CCL2) resulted in reduced tumour macrophage infiltration and suppressed tumour angiogenesis, which in turn led to tumour inhibition.[104] In primary human breast cancer, CCL2 concentration correlated significantly with the levels of VEGF, TP, TNF-α and IL-8, all of which are potent angiogenic factors.[99] However, in the Matrigel angiogenesis assay, tumour angiogenic potential was found to be primarily associated with tumour cell CCL2 expression level.[105] Furthermore, neutralising CCL2 biological activity with function-blocking monoclonal antibodies was able to effectively inhibit in vivo tumour angiogenesis despite the complexity and redundancy of angiogenic growth factors expressed by tumour cells.[105] These same antibodies have also been tested in several xenograft tumour models and tumour inhibition was observed in pancreatic, colon and breast cancer models.[106] In addition to inhibiting tumour angiogenesis, blockade of CCL2 may also modulate anti-cancer immunity by reverting CCL2-dependent T_H-2-polarised immunity to T_H-1 anti-cancer immunity.[107] Given the role of CCL2 in endothelial and fibroblast cell activity,[102,103] it is therefore reasonable to speculate that targeting CCL2/CCR2 axis will also affect tumour vascular and stromal components to provide further benefits.

11. Conclusion

As our understanding and appreciation of the complex role of chemokine and cytokine networks in cancer and inflammation evolve, it is clear that new therapeutic opportunities for treating cancer are emerging. As we consider strategies targeting tumour inflammation mediators for anti-cancer therapies, the potential consequences of inhibiting immune and inflammatory cells, which also participate in anti-tumour immune surveillance, should not be overlooked. With their capacity to mediate cytotoxic responses and phagocytic activity, inflammatory cells undoubtedly are integral components of the first-line defence system of the body's response to fight cancer, and are the basis for anti-cancer immunotherapy. In addition, various proteinases expressed by tumour infiltrating leukocytes are also capable of cleaving endogenous proteins to generate protein fragments with anti-angiogenic properties.[108,109] Therefore, the challenge for clinical oncologists and experimental cancer researchers will be to identify the appropriate molecular targets, cancer types and disease stages for this type of therapy, and to optimise the therapeutic windows and regimens of anti-inflammatory cancer therapies. It is likely that combining these agents with another mode of therapy, such as a cytotoxic, cytostatic, or anti-angiogenic drug, may prove to be most effective. Selecting the appropriate patient population probably will involve the identification of a set of biomarkers to reflect accurately the nature of inflammatory responses in tumour tissues, and to enable selection of patients most likely to benefit from such therapies.

Conflict of interest statement

Authors are employees of Centocor.

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