TIMELINE

Tumour necrosis factor and cancer

Frances Balkwill

Abstract | Tumour necrosis factor (TNF) is a major inflammatory cytokine that was first identified for its ability to induce rapid haemorrhagic necrosis of experimental cancers. When efforts to harness this anti-tumour activity in cancer treatments were underway, a paradoxical tumour-promoting role of TNF became apparent. Now that links between inflammation and cancer are appreciated, is TNF a target or a therapeutic in malignant disease — or both?

In 1975 a paper entitled "An endotoxin-induced serum factor that causes necrosis of tumours" was published in *Proceedings of the National Academy of Sciences of the USA*[1]. In this paper Carswell, Old and colleagues gave an explanation for "one of the best-known enigmas of cancer biology": the haemorrhagic necrosis of tumours. Although the fascinating history of the tumour necrosis factor (TNF) could be traced back more than 80 years[2,3], its isolation in 1975 and subsequent gene cloning in 1984 marked the beginning of an even more surprising story. Over the next 15 years came papers identifying a whole family of related molecules with contradictory roles in cell death, cell survival and organogenesis[4]. The early promise that TNF would be a powerful anticancer cytokine soon faded with the realization that the recombinant cytokine could induce signs and symptoms of endotoxic shock: the therapeutic index was alarmingly small. Moreover, when chronically produced in the tumour microenvironment, TNF was a major mediator of cancer-related inflammation[5–8].

Outside the cancer field, TNF was identified as a master regulator of inflammation and a key player in the cytokine network. This led to the development of antagonists of its action that revolutionized the treatment of rheumatoid arthritis and other inflammatory diseases[9–11]. These TNF antagonists are also in Phase I and II clinical trials in patients with advanced cancer[12–15]. Efforts still persist, however, to refine the undisputed tumour-destructive activities that TNF has under certain circumstances[16].

Whether pro- or anti-tumour, there is no doubt that TNF is important to cancer biology and treatment in the 21st century. However, for this Timeline we need to go back 100 years — or more — to a time when there were no systemic cancer treatments.

A history of tumour necrosis factor

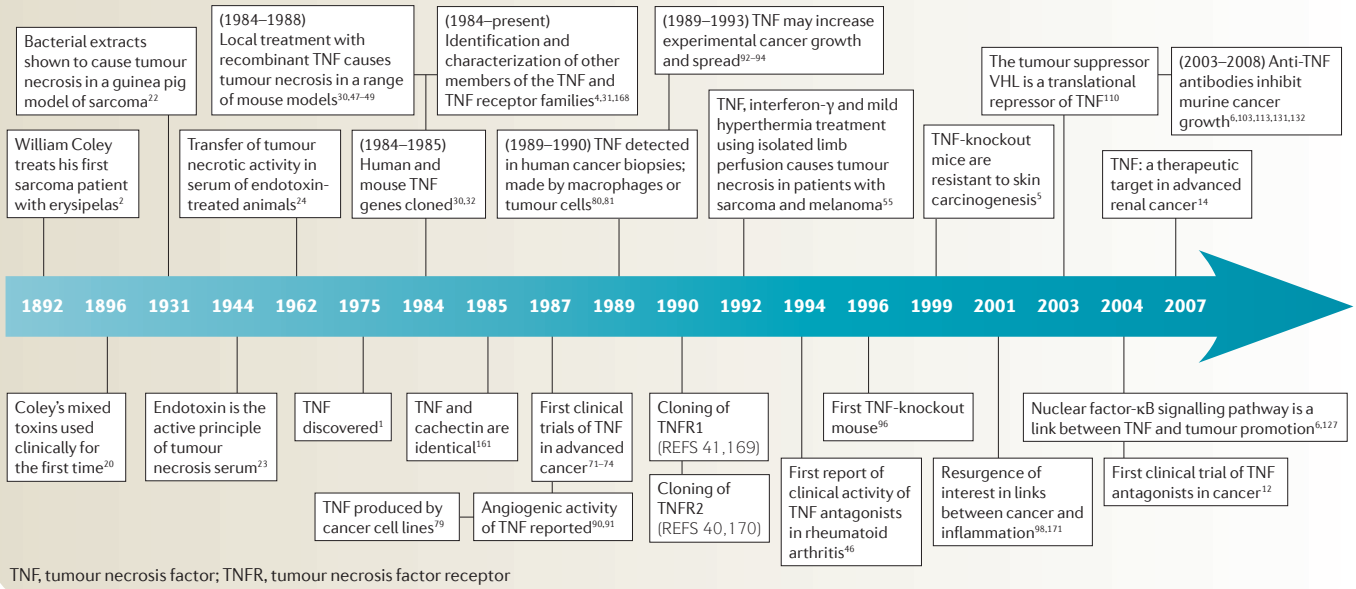
The inspiration for the 1975 Carswell paper was the controversial but fascinating work of New York surgeon William Coley[2,17].

Coley's mixed toxins. In 1890, at the start of his career, Coley was called in to treat a 17 year-old woman with a nagging pain in her right hand. In spite of Coley's undoubted surgical skills, Elizabeth Dashiell died a few months later of an aggressive round cell sarcoma that disseminated at alarming speed throughout her body. (Elizabeth Dashiell was a close friend of John D. Rockefeller Jr. Her death was an inspiration for the philanthropic work of his family, leading to the Rockefeller Institute of Medical Research, now Rockefeller University[18].) Dashiell's death had an equally profound influence on Coley. He immersed himself in hospital records to learn more about these rare but devastating malignancies. Amongst all the sarcoma-induced death and destruction Coley found an intriguing anecdote: the case of a German immigrant who 6 years previously had been dying of a large facial tumour. Fred Stein's fate seemed to be sealed when a post-operative bacterial infection took hold but, as the fever subsided, the sarcoma disappeared. With dogged determination, Coley searched the tenements of the Lower East Side for a man with a scar, and found Stein alive and well 6 years later[18].

This led Coley to a line of clinical research[19] that dominated his entire career. First he infected cancer patients with bacterial isolates[2] (TIMELINE), and then he made "Coley's mixed toxins", slightly less dangerous filtrates from cultures of *Streptoccocus pyogenes* (the bacteria that causes erysipelas) and Gram-negative endotoxin-producing *Serratia marcasens*[20] (FIG. 1). The work was controversial and few were able to reproduce the beneficial effects that Coley obtained but, if the published case histories are to be believed[3,21], Coley was able to obtain rapid and sustained responses in patients who would present a major challenge to medical oncologists in the 21st century.

Endotoxins and TNF. With the advent of radiotherapy and chemotherapy, interest in Coley's mixed toxins waned, but some scientists were still intrigued by his results and attempted to reproduce them in animal models of cancer. For instance, in 1931, Gratia and Linz showed that bacterial extracts caused tumour necrosis in a guinea pig model of sarcoma[22]. In 1944 Shear *et al.* isolated lipopolysaccharide from bacterial extracts and showed this was responsible for tumour regression in a mouse model of cancer[23]. In an attempt to reduce the often lethal effects of endotoxin or other bacterial products in their models, O'Malley *et al.* then took serum from endotoxin-treated animals and gave this to animals with experimental cancers; the serum also caused tumours to necrose, leading to the conclusion it contained a "tumour necrotizing factor"[24]. A major advance came in 1975 when Carswell *et al.* reported that it was a factor made by host cells in response to endotoxin and not bacterial endotoxin itself that destroyed the tumours[1] (TIMELINE). They coined the term "tumour necrosis factor" to describe this activity, reportedly produced by macrophages, which led to necrosis of both mouse and human tumours.

Timeline | **Tumour necrosis factor and cancer**



TNF, tumour necrosis factor; TNFR, tumour necrosis factor receptor

The tumour necrosis factor family

Around the same time, Granger and co-workers described a protein produced by lymphocytes that was toxic to tumour cells[25], but it took another 18 years for two different proteins with related sequences to be isolated from human HL-60 and RPMI-1788 cells. These were named tumour necrosis factor and lymphotoxin respectively[26–28]. The first indication that there might be a family of related cytotoxic proteins came when TNF and lymphotoxin were found to bind to the same cell surface receptor[29]. The availability of the protein sequences soon led to gene cloning of human TNF and lymphotoxin at Genentech in the United States[30,31], and human and mouse TNF in Walter Fier's laboratory in Belgium[32,33] (TIMELINE). In the same year, the first monoclonal antibody to TNF was made by David Wallach's laboratory at the Weizmann Institute in Israel[34].

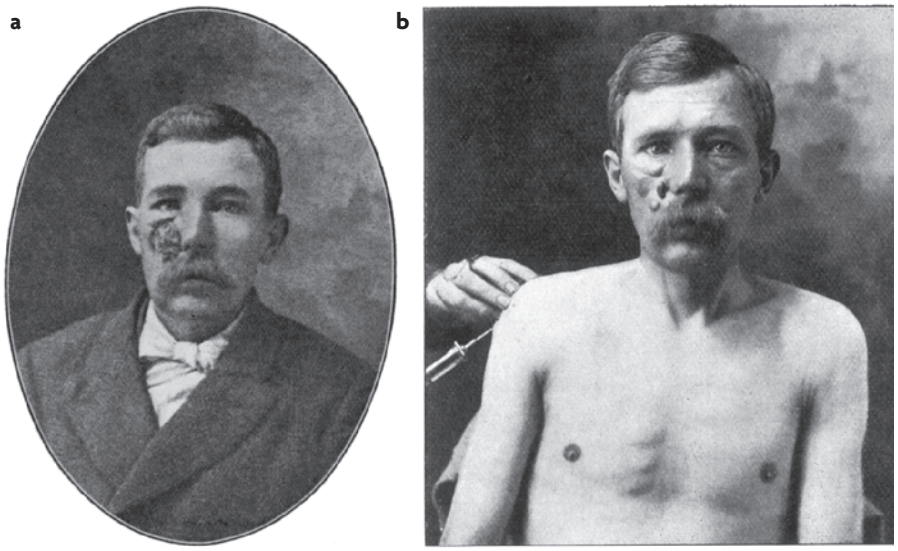


Figure 1 | **Treatment with Coley's toxins.** A patient with round cell sarcoma of the jaw and abdominal metastases seen by Coley in 1899. **a** | Photograph after 63 injections with Coley's toxins; tumour had diminished to about half its original size. **b** | Photograph after further treatment with Coley's toxins. In his 1910 lecture at the Royal Society of Medicine Coley reported that the patient was still alive and well. Images reproduced, with permission, from REF. 17 © (1910) Royal Society of Medicine.

The relationship between TNF and lymphotoxin was the first indication of the existence of a whole superfamily of 19 ligands related to TNF and 29 receptors with a wide range of roles beyond cytotoxicity, being involved in the development and function of the immune system as well as in tissue homeostasis[4,11,35–37] (see Supplementary information S1 (box)). However, within this gene family, TNF (also known as TNFα) was recognized as a uniquely powerful intercellular communicating molecule with crucial and non-redundant roles in innate and adaptive immunity. Lymphotoxin (or TNFβ, as it is now commonly known) has not been studied so extensively in terms of malignant disease and, for reasons of space, will not be considered further in this article.

The next frontier was the identification of cell surface receptors for TNF. In 1985, Aggarwal *et al.* reported that radiolabelled recombinant TNF and lymphotoxin bound to a single class of receptor on carcinoma cells[29]. Proteins that bound TNF were abundant in urine and David Wallach's group correctly surmised that these could be shed surface receptors. Purification was possible because the pharmaceutical company Serono had amassed large quantities of concentrated urine proteins from menopausal women (specifically, from Italian nuns) for their hormone research. Chromatographic purification of a binding protein, now known to be TNFR1 (also known as TNFRSF1A), was achieved in 1989 (REF. 38), and a soluble form of the TNFR2 (also known as TNFRSF1B) was affinity purified

in 1990 (REF. 39). These receptors also have CD numbers now — TNFR1 is CD120a and TNFR2 is CD120b — reflecting the fact that they are both found on haematopoietic cells. TNFR1 has a much wider distribution than TNFR2, being expressed by virtually every cell in the body.

In 1990 the genes for both TNF receptors were cloned: TNFR1 at Hoffmann–La Roche[40] and Genentech[41] and TNFR2 at Immunex[42] and Syntex[43] (TIMELINE). BOXES 1,2 show more detail of TNF receptors and their downstream signalling pathways. The cloning of genes encoding TNF and TNF receptors enabled development of a number of research tools, including gene-deleted mice. Experiments, especially in the early 1990s, revealed that TNF initiates host defence to local injury but that it can also cause acute or chronic tissue damage[44,45]. By the mid 1990s it was becoming clear that neutralizing antibodies and soluble receptor fusion proteins targeting TNF would be successful treatments for a range of human chronic inflammatory diseases[46] (BOX 3; TIMELINE). In parallel with this preclinical and clinical work with TNF antagonists in inflammatory disease, the cytokine itself was under investigation as a cancer therapeutic.

TNF as a cancer treatment

Was the research of the previous 40 years correct? Did recombinant TNF cause tumour necrosis in mouse cancer models and, if so, how did it work?

TNF treatment of experimental rodent cancers. Reassuringly, high doses of human recombinant TNF induced necrosis of both syngeneic and xenografted tumours[30,47–49] (FIG. 2a,b; TIMELINE). For optimal activity, however, TNF had to be injected locally and repeatedly, and there was a risk of regrowth at the periphery of the lesion. An exception was the transplantable murine tumour Meth A sarcoma (which was also used in experiments carried out before recombinant material was available), in which systemic administration of TNF consistently caused haemorrhagic necrosis of vascular subcutaneous, but not avascular intraperitoneal, tumours[30,50,51]. The tumour necrosis caused by TNF was haemorrhagic in nature with major destruction of the vascular bed. Alberto Mantovani's group reported that TNF, and in parallel the cytokine interleukin 1 (IL-1), activated endothelial cells in a gene expression-dependent way, thus changing the perception of the tumour vasculature[52].

Box 1 | **Tumour necrosis factor receptors**

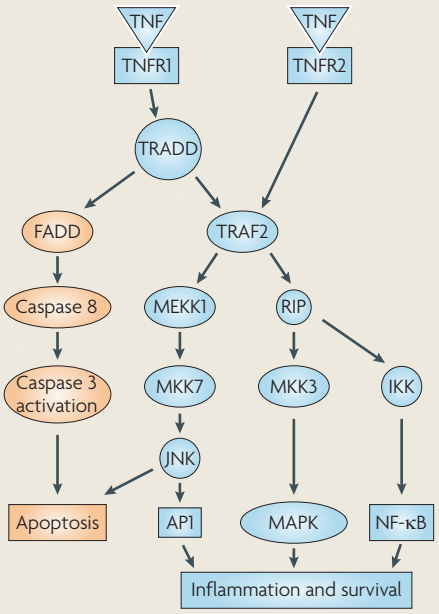
Tumour necrosis factor (TNF; also named TNFα) is a type II transmembrane protein with an intracellular amino terminus. It has signalling potential both as a membrane-integrated protein and as a soluble cytokine released after proteolytic cleavage; its soluble form is a non-covalently bound trimer of 17 kDa components[4,35]. There are two TNF receptors: TNFR1, which is found on most cells in the body, and TNFR2, which is primarily expressed on haematopoietic cells. TNFR1 is activated by soluble ligand, and TNFR2 primarily binds transmembrane TNF. TNF receptors are also shed and act as soluble TNF-binding proteins, inhibiting TNF bioactivity by competing with cell surface receptors for free ligand. In contrast to TNFR1, TNFR2 lacks a death domain. It is often inducible by cytokines such as TNF and interleukin 1 . The biological role of TNFR2 is still not fully understood, although recent evidence suggests that it can modulate the actions of TNFR1 on immune and endothelial cells. Transmembrane TNF can function as both ligand and receptor: soluble TNF receptors can bind to the cytokine on the cell surface and generate reverse signalling.

However, when recombinant mouse TNF was given to mice, it caused similar symptoms to high doses of endotoxin[47,53,54]. This was because of the partial species specificity of human and mouse TNFs. Human TNF binds to murine TNFR1 but not murine TNFR2, whereas murine TNF binds to both murine receptors and this generates a greater *in vivo* response.

To mitigate this toxicity, a local approach to TNF therapy was devised for experimental cancers growing in the extremities: isolated limb perfusion (ILP). As described in the next section, this was actually developed in clinical experiments[55] (TIMELINE), but studies into mechanisms of action and further refinements were carried out in animal models[56]. TNF alone was ineffective in this setting but synergized with melphalan chemotherapy in a rat osteosarcoma ILP model, with mild hyperthermia optimizing the anti-tumour effect[57]. A combination of TNF and doxorubicin had comparable effects in rat sarcoma models[58]. It appears that low doses of TNF increase tumour blood vessel permeability, thus augmenting tissue concentrations of chemotherapy[59] and destroying the tumour vasculature.

Box 2 | **Intracellular tumour necrosis factor signalling**

Tumour necrosis factor (TNF) receptor (TNFR) activation leads to recruitment of intracellular adaptor proteins that activate multiple signal transduction pathways[11,35,153]. TNFR1 activation can have two different end results that are dependent on the cellular context. The default pathway is induction of genes involved in inflammation and cell survival. Ligand binding to TNFR1 induces a range of inflammatory mediators and growth factors through activation of the AP1 transcription factors or IκB kinases (IKKs) that, in turn, activate nuclear factor-κB (NF-κB). NF-κB activation also importantly induces negative regulators of apoptosis such as FLIPL (also known as CFLAR), BCL-2 and superoxide dismutase. If NF-κB activation is inadequate, apoptosis is mediated through caspase 8 and, through accumulation of intracellular reactive oxygen, sustained Jun amino-terminal kinase (JNK) activation and mitochondrial pathways. Apoptosis is a late response to TNF, unlike the rapid apoptosis that is induced by other members of the TNF superfamily such as FAS ligand (FASL) and TRAIL (also known as TNFRSF10C) (see Supplementary information S1 (box)). The signalling pathways downstream of TNFR activation are shown in the figure. FADD, FAS-associated via death domain; MKK, MAPK kinase; RIP, receptor (TNFRSF)-interacting protein; TRADD, TNFR-associated via death domain; TRAF2, TNF receptor-associated factor 2. Figure is modified, with permission, from *Nature Reviews Immunology* REF. 154 © (2003) Macmillan Publishers Ltd. All rights reserved.



Box 3 | **Tumour necrosis factor, cachexia and inflammation**

Bacterial pathogens and many other noxious stimuli induce tumour necrosis factor (TNF) through Toll-like receptors (TLRs) and nuclear factor-κB (NF-κB) signalling[4,11,155]. This TNF is then in the vanguard of a complex biological cascade involving chemokines, cytokines and endothelial adhesions, that recruits and activates neutrophils, macrophages and lymphocytes at sites of damage and infection[4,8]. TNFR1 signalling is essential for defence against infectious agents such as *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Toxoplasma gondii*, *Leishmania* spp., trypanosomes and *Salmonella* spp.[156,157].

In terms of adaptive immunity, TNF and TNF receptor 1 (TNFR1) coordinate the social context of cells, enabling maximal response to pathogens[4,158,159]. TNFR1 is also a co-stimulator of T cell activation and expressed by activated T cells.

It is crucial that TNF is produced in the right place, at the right time and in the appropriate context. Restriction of TNF production to specific cell types may be one of the mechanisms by which its beneficial functions are controlled[160]. Left unregulated, TNF can cause chronic inflammation, generalized wasting and, when high amounts are generated acutely, septic shock. The first indication of this was in 1985 when TNF was found to be identical to cachectin, a circulating factor associated with wasting in parasite-infected animals[161] (TIMELINE). It soon became clear that sustained production of TNF was involved many inflammatory and autoimmune diseases[162,163] and, by the middle of the 1990s, the pioneering work of Marc Feldmann and Ravinder Maini provided clinical proof of this: TNF antagonists were effective treatments for rheumatoid arthritis[9,46]. This was followed by positive results in patients with Crohn's disease[164], psoriasis[165,166], severe chronic asthma[167], psoriatic arthritis, anklosing spondylitis and sarcoidosis (reviewed in REF. 11).

TNF as a cytotoxic protein. At first it was thought that TNF was also directly killing the malignant cells in the animal models of cancer. In tissue culture studies, purified or recombinant TNF was reported to be selectively toxic for malignant cells, as were TNF-containing supernatants from activated macrophages[60]. However, many of these data were generated in the presence of metabolic inhibitors such as actinomycin D, cyclohexamide or mitomycin C[60–62] or in combination with interferon-γ (IFNγ)[63,64]. Alone, TNF could actually induce resistance to these cytotoxic conditions, as first shown by David Wallach[65]. It now seems that, unlike some other members of the TNF family such as TRAIL (also known as TNFSF10; see Supplementary information S1 (box)), TNF is at most weakly cytotoxic or cytostatic to malignant cells. It is only in combination with metabolic inhibitors that its cytotoxic potential is unmasked; the default cell survival and inflammatory pathways downstream of TNF signalling are inactivated by the metabolic inhibitors allowing apoptosis to proceed (BOX 2).

TNF and immune cell killing. The mouse experiments, however, did reveal a role for T cells in the anti-tumour actions of TNF. There was a diminished anti-tumour effect of TNF in T cell-deficient mice[54], and T cell-mediated immunity developed in animals cured of the Meth A sarcoma by TNF[66]. TNF is an important effector molecule in $CD8^+$ T cell and natural killer (NK) cell killing of immunogenic tumour cells[67,68]. NK and IL-2-activated killer cells from *Tnf*$^{-/-}$ mice showed impaired cytotoxic activity[69], and both TNF receptors were recently implicated in tumour surveillance in a genetic model of pancreatic β cell cancer[70].

In conclusion, these preclinical studies showed that anti-tumour effects of TNF were due to destruction of the tumour vasculature with some evidence of a role for TNF in anti-tumour responses[16]. Before these mechanisms were fully appreciated, clinical trials had begun.

Clinical trial of recombinant TNF. The expectation was that recombinant human TNF would be an important new treatment for cancer patients. Unfortunately systemic TNF administration was associated with severe toxicity — induction of a 'cytokine storm' resembling many signs and symptoms of endotoxic shock (not unlike those seen by Coley) — but unlike Coley's toxins there were few tumour responses (for examples see REFS 71–74). For instance, in a review of 219 cancer patients receiving an intravenous infusion of TNF, only two partial responses (greater than 50% tumour shrinkage) were recorded. At lower doses (75–100 mg per m^2 per day) TNF treatment was well tolerated with reversible flu-like symptoms, but at higher doses fever, headache and rigors occurred with hypotension and pulmonary oedema being dose limiting[75]. The side effects seen in the first clinical trials were not surprising, as many of the encouraging preclinical results were obtained using human TNF, which has a lower toxicity in mice. The clinical trials showed that human TNF was as toxic to humans as mouse TNF was to mice.

At this time, the prevailing view was that local administration of TNF would have more chance of success than systemic treatment. In view of this, surgeons Ferdy Lejeune, Alexander Eggermont and their colleagues used ILP to deliver high doses of TNF locoregionally, in combination with IFNγ and melphalan, to patients with cancers of the extremities. This caused specific destruction of tumour vasculature, haemorrhagic necrosis and complete tumour disappearance in patients with advanced soft tissue sarcomas or melanoma[55] (reviewed in REF. 76) (TIMELINE). For instance, in a series of 217 sarcoma cases, the overall response rate was 75% and limb salvage was achieved in 87% of patients[77]. However, this treatment was palliative, preventing the amputation of the affected limb but not affecting distant metastasis. The general understanding of the mechanisms of action in these patients was that TNF increased tumour-selective uptake of the melphalan chemotherapy during the perfusion and that the combination of TNF and IFNγ had a direct and destructive effect on the tumour vasculature. On the strength of these data, in 1999 TNF (tasonermin) was licensed in Europe with a specific indication: "for the treatment of irresectable soft tissue sarcoma of the limbs used in combination melphalan via mild hyperthermic ILP".

The toxicity of systemically administered TNF remained a major impediment to widespread clinical application. The failure of TNF treatment prompted Charlie Starnes to revisit Coley's work in a 1992 *Nature* review[78]. His conclusion, based on re-evaluating the clinical histories of Coley's patients, was that TNF-based therapies should be reserved for patients with soft tissue sarcomas, lymphomas and other tumours of mesodermal origin, but this recommendation was never taken up. Moreover, while the first trials were underway, evidence was accumulating that TNF was not only made by cancer cells in tissue culture but was also present in the tumour microenvironment of many cancers, raising the possibility that it might actually be enhancing cancer growth.

Tumour-promoting factor?

It was at first quite puzzling when, in 1987, Spriggs *et al.*[79] reported that TNF could induce a breast cancer cell line to produce more TNF. This was followed by reports that TNF mRNA and protein could be detected in malignant and stromal cells in human cancer biopsies[80–82] (TIMELINE) and that levels of plasma TNF were increased in some cancer patients, especially those with poor prognosis[83–86] (reviewed in REFS 7,87). To take

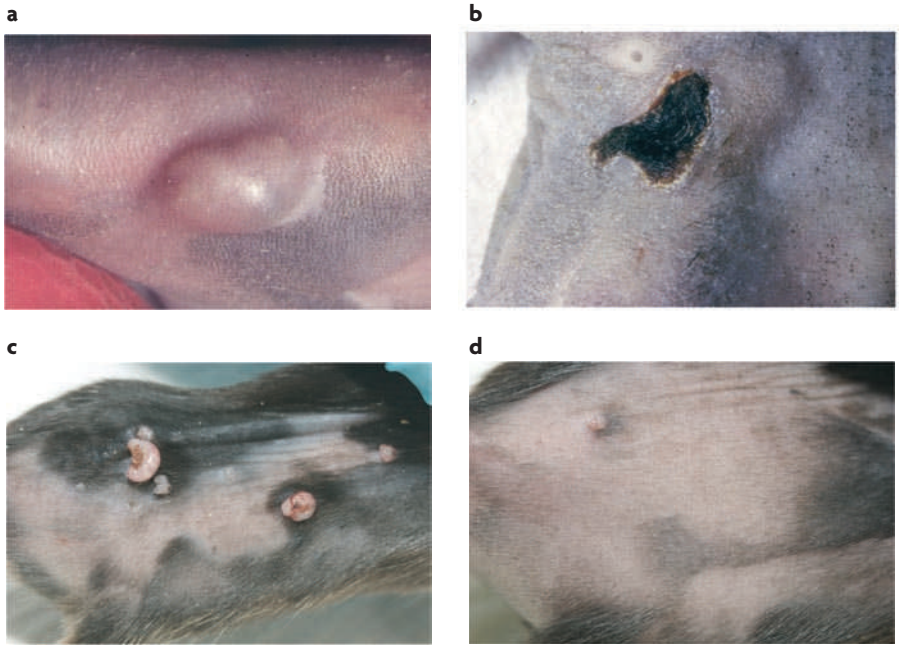


Figure 2 | **The pro- and anti-tumour actions of tumour necrosis factor (TNF) in mouse models of cancer. a** | Mouse bearing subcutaneous human tumour xenograft before treatment. **b** | Haemorrhagic necrosis of tumour after intratumoural injection of TNF. **c** | Wild-type mouse treated with the carcinogen DMBA and the tumour promoter TPA develops skin tumours after 16 weeks. **d** | *Tnf*$^{-/-}$ mouse is highly resistant to 16 weeks of DMBA–TPA treatment. All images are previously unpublished from work of F.B., R. Moore and T. Schioppa to illustrate concepts previously published in REFS 5,48.

the example of prostate cancer, blood TNF concentrations are higher in those patients with advanced, cachectic disease[88], and TNF levels correlate positively with extent of disease[89]. Also, in 1987, when the interest in the tumour-destructive activity of TNF was at its height, came the apparently paradoxical observation that low doses of TNF could have angiogenic activity in both the rabbit cornea and chick chorioallantoic membrane models[90,91]. This led Leibovich *et al.*, in a paper in *Nature*, to suggest that TNF might actually stimulate tumour growth[91]. In 1989, while studying intraperitoneal xenografts of ovarian cancer, we found that TNF treatment could transform ascitic free-floating tumour cells into solid peritoneal deposits with extensive stroma and blood vessels[92]. Moreover we, and others, found that treatment of tumour cells or mice with TNF increased the metastatic activity of transplanted tumour cells[93,94]. Michael Karin's laboratory recently published a molecular explanation for this[95]. They found that Lewis lung carcinoma lines secrete versican, an extracellular matrix proteoglycan, which activates macrophages through Toll-like receptor 2 (TLR2) and TLR6 to produce IL-6 and TNF. Both of these cytokines then act in a paracrine manner to increase lung metastases.

In 1996, the group of George Kollias generated the first TNF-knockout mouse[96] and 2 years later we published a paper that surprised those who were working with TNF as a cancer therapeutic. The paper showed that when *Tnf*$^{-/-}$ mice were treated with a skin carcinogen, they developed fewer, not more, tumours[5] (FIG. 2c,d; TIMELINE).

An explanation for the presence of TNF in the cancer microenvironment came when researchers returned to another historical observation, from Virchow in 1863, that inflammatory cells are found in cancers[97]. We now know that many of the cells and mediators of inflammation are detected in human and experimental cancers and inflammatory conditions increase the risk of cancer (reviewed in REFS 8,98–100). There is strong evidence that this cancer-related inflammation aids the proliferation and survival of malignant cells, stimulates angiogenesis and metastasis, subverts adaptive immunity, and alters response to hormones and chemotherapy. When produced by malignant or host cells in the tumour microenvironment, TNF is a major mediator of cancer-related inflammation[7,8], and research in the past 20 years has begun to reveal some of its mechanisms of action.

Pro-tumour actions of TNF

Unlike their normal counterparts, many malignant cells constitutively produce small amounts of TNF. There is evidence from animal models that this malignant cell-derived TNF enhances the growth and spread of syngeneic, xenogeneic and carcinogen-induced tumours of the skin, ovary, pancreas, pleural cavity and bowel[5,101–105].

Actions of tumour cell-produced TNF. The mechanisms by which tumour cell-produced TNF increases tumour growth are not fully defined. In an ovarian cancer model we found that TNF was an important component of a malignant cell-autonomous network of inflammatory cytokines, including the chemokines stromal cell-derived factor (SDF1, also known as CXCL12) and CCL2 (C-C chemokine ligand 2), the cytokines IL-6 and macrophage inhibitory factor (MIF) as well as vascular endothelial growth factor (VEGF)[102]. This network then acted on the ovarian cancer microenvironment, particularly affecting the leukocyte infiltrate and development of blood vessels in peritoneal tumour deposits. The angiogenic actions of TNF may be due, at least in part, to its ability to cause the differentiation of myeloid progenitor cells into endothelial cells in the tumour microenvironment[106]. TNF produced by malignant cells also caused hyperpermeability of existing blood vessels, stimulating pleural effusion in a lung cancer model[104]. Apart from endothelial cells, other host cells targeted by the paracrine actions of malignant cell-derived TNF are not well characterized. However, in ovarian cancer TNF is important in interactions between tumour cells and macrophages that lead to increased tumour cell invasion and the generation of a tumour-associated macrophage phenotype that has been associated with tumour promotion and poor prognosis[107,108].

Why do malignant cells make TNF? One explanation for constitutive production of TNF by malignant cells is increased *TNF* mRNA stability[109] and this could have a genetic cause. Although evidence that inflammation causes cancer has been accepted for many years, more recently the data show that mediators and signalling pathways of inflammation are downstream of oncogenic mutations; that is, that cancer causes inflammation (reviewed in REF. 100). The first example of this relating to TNF was published in 2003: the tumour suppressor VHL represses translation of TNF[110] (TIMELINE). In renal cancer, cells with

mutated *VHL* produce increased levels of TNF along with other pro-tumour factors such as VEGF. The carcinogenic activity of the bacterium *Helicobacter pylori* is also genetically linked with TNF: members of the Tipα gene family in *H. pylori* are potent TNF inducers and, in combination with activated Ras, can render gastric epithelial cells malignant[111].

Host cell production of TNF in the tumour microenvironment. It is not only malignant cells that can make TNF in the tumour microenvironment. In a genetic model of liver cancer, TNF produced by myeloid cells promoted inflammation-associated tumours[6]; in a model in which chemical damage led to liver cancer, Kupffer cell-derived TNF was one of the mitogens driving the proliferation of hepatocytes in which DNA damage had already been caused by the carcinogenic agent diethylnitrosamine[112]. In both a chemically induced model of colorectal cancer and a genetic model of gastric cancer, macrophage-derived TNF was implicated in inflammation and subsequent tumour development[113,114].

TNF in the tumour microenvironment can cause genetic damage. Whether made by malignant cells or host cells — or both — TNF may directly contribute to oncogene activation and DNA damage. This was first suggested in 1993 when Komori *et al.* reported that long-term TNF treatment of immortalized mouse 3T3 cells rendered them capable of forming tumours in mice[115]. Much later came evidence that TNF stimulated clonal evolution in haematopoietic stem cells with the Fanconi anaemia mutation[116], again increasing the tumorigenicity of these cells. High doses of TNF induced direct DNA damage in *Trp53*$^{-/-}$ malignant cells[117] and even in genetically normal lung epithelial cells[118], suggesting that, when there is chronic inflammation, deregulated and sustained production of TNF could contribute to carcinogenesis and even in some cases be an initiating event.

In terms of the molecular mechanisms of DNA damage, in human cholangiosarcoma cells TNF induced the DNA and RNA editing enzyme, activation-induced cytidine deaminase (AID), that is also increased in human cholangiosarcoma biopsies[119]. The induction of AID by TNF led to mutations of genes such as *TP53* and *MYC*. Through nuclear factor-κB (NF-κB), TNF can also modulate telomerase activity, inducing translocation to the nucleus of the human telomerase catalytic subunit (TERT) bound to p65 (also known as RELA)[120]. Hence TNF may contribute to the immortalization of cells.

Apart from genetic changes, TNF in the tumour microenvironment may also have other direct effects on malignant cells, for instance, inducing the epithelial–mesenchymal transition of malignant cells in an *in vitro* model of colorectal cancer[121]. This may partly explain the ability of TNF to increase the metastatic activity of tumour cells as first reported in the 1990s[93,94,103] and further elucidated by Michael Karin and colleagues in 2009 (REF. 95).

TNF receptor signalling and cancer-related inflammation. Most of these pro-tumour actions of TNF appear to be mediated by TNFR1. This TNF receptor is found on tumour and stromal cells in human cancer biopsies, whereas TNFR2 is generally present on the leukocyte infiltrate, although it is also present on malignant cells in renal cell carcinoma[14]. As might be expected, mice deficient in TNFR1 show attenuated development of primary cancers and metastases. For instance, we found that *Tnfr1*$^{-/-}$ mice are as resistant to DMBA–TPA carcinogenesis as *Tnf*$^{-/-}$ mice[122], and other groups showed that experimental lung and liver metastases were attenuated in *Tnfr1*$^{-/-}$ mice compared with their normal counterparts[123,124]. In wild-type mice whose bone marrow was repopulated with cells from *Tnfr1*$^{-/-}$ mice, the development of colitis and colon cancer was reduced[113], suggesting that TNF in the tumour microenvironment enhanced tumour development through its action on TNFR1-positive myeloid cells. T regulatory (T_{Reg}) cells can suppress specific immune responses against tumours[125], and recently Jo Oppenheim's group reported that TNFR2 is highly expressed on these cells in the tumour microenvironment of murine Lewis lung carcinomas[126]. We previously found some evidence for a role for TNFR2 in tumour development in the skin carcinogenesis model: *Tnfr2*$^{-/-}$ mice were resistant to skin carcinogenesis but the effects were not as strong as in *Tnfr1*$^{-/-}$ mice[122] and we did not study the role of T_{Reg} cells in this model.

Downstream of TNF–TNFR1 and other inflammatory cytokines produced in the tumour microenvironment, NF-κB signalling is a major mediator of the tumour-promoting activity of inflammatory cytokines, as was first demonstrated in seminal papers published in 2004 from Michael Karin's and Yinon Ben-Neriyah's laboratories[6,127] (TIMELINE). NF-κB is a transcription factor the activity of which is triggered by infectious agents and inflammatory cytokines such as TNF through the inhibitor of NF-κB kinase (IKK) complex[128,129] (BOX 2). In resting cells NF-κB dimers are found in the cytoplasm but translocate to the nucleus after activation. NF-κB target genes were already known to be major mediators of inflammation and cell survival but the papers published in 2004 showed that selective inhibition of NF-κB activation in either myeloid cells or epithelial cells attenuated intestinal and liver cancer development[6,127]. These papers firmly established a role for NF-κB in tumour promotion. A more recent paper linked NF-κB, TNF and the tumour suppressor TSC1. IKKβ, a major downstream kinase in the NF-κB signalling pathway, phosphorylates and inhibits the activity of TSC1. This suppression of TSC1 activates the mTOR pathway, enhancing VEGF production and stimulating tumour development[130].

As this Timeline has shown, shortly after TNF was cloned it became clear that this cytokine could enhance many processes of carcinogenesis in ways that were associated with its central role in inflammation. FIGURE 3 summarizes our current knowledge of the tumour-promoting actions of TNF.

If endogenous TNF signalling in the tumour microenvironment is more likely to stimulate than inhibit tumour growth, is TNF a target instead of a treatment?

TNF as a target for cancer treatment

If TNF were involved in growth of experimental tumours, then anti-TNF antibodies or other TNF antagonists would have therapeutic activity in similar mouse models. This is indeed the case, as reported in experiments involving carcinogen-induced, transplantable xenograft and genetic models of common epithelial cancers[6,103,113,131,132]. Anti-TNF antibodies also inhibited experimental metastasis, as was first shown in 1993 (REF. 94).

This raised the possibility that it might be beneficial to neutralize TNF activity in cancer patients. This was tested in Phase I and II clinical cancer trials with TNF antagonists as single agents, with some evidence of clinical activity[12–15] (TIMELINE). For instance, in a Phase I study using the anti-TNF antibody infliximab, stabilization of disease was observed in 7 of 41 patients with previously progressing advanced cancer[15]; in a Phase II study in ovarian cancer, 6 of 30 progressing patients also showed stable disease after treatment with the TNF antagonist etanercept (a soluble TNFR2 fusion protein that binds and neutralizes TNF)[13]; and in renal cell cancer 14 of 39 patients achieved stable disease

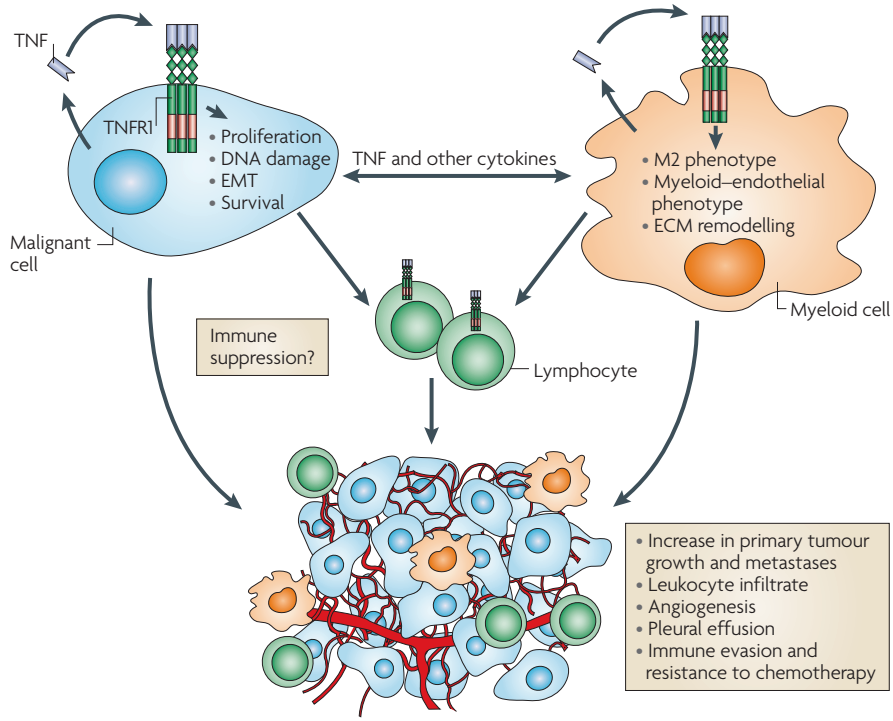


Figure 3 | **Pro-tumour actions of tumour necrosis factor (TNF) in the tumour microenvironment.** TNF, made by malignant cells, myeloid cells and probably other cells in the tumour microenvironment, acts primarily through TNF receptor 1 (TNFR1) in an autocrine and paracrine manner. Documented autocrine actions from the published literature include causing further genetic damage to malignant cells or cells with malignant potential, enhancing malignant cell survival and inducing epithelial–mesenchymal transition (EMT). TNF also induces further TNF expression as well as increasing production of other cytokines, chemokines and C-X-C chemokine receptor 4 by the malignant cells. This combination of cytokines and chemokines also acts on, and is produced by, myeloid cells in the tumour microenvironment and may contribute to maintenance of the phenotype and actions of tumour-associated (M2) macrophages may stimulate remodelling of the extracellular matrix (ECM) and cause differentiation of myeloid–endothelial progenitor cells, contributing to angiogenesis. These actions of TNF, and TNF-related cytokines and chemokines, may also act on lymphocytes contributing to local immunosuppression, although these data are more preliminary. The end result is to enhance primary tumour growth, help facilitate metastatic spread, and to regulate the extent and phenotype of the leukocyte infiltration and angiogenesis. TNF has also been implicated in production of pleural effusion and resistance to chemotherapy. These mechanisms of action have been shown in xenograft, syngeneic, chemically induced and genetic models of a variety of different cancers, and TNF can be detected in human cancer biopsies and in the plasma of patients with some advanced cancers. The pro-tumour actions of TNF may be tumour and tissue specific.

with 3 of 39 obtaining partial responses after infliximab treatment[14] (TIMELINE). Clinical benefit of TNF antagonists has also been seen in the premalignant condition of myelodysplasia[133]. There is as yet no clear idea of the mechanisms of action of anti-TNF in cancer patients, but nearly 20 years of experience in patients with chronic inflammatory disease show that TNF antagonists inhibit cytokine and chemokine production, recruitment of inflammatory cells, angiogenesis and extracellular matrix degradation[11]: all actions that could be useful in a cancer treatment. In addition, binding of TNF antagonists to transmembrane TNF may have direct effects on TNF-producing cells, stimulating a number of cytotoxic pathways. Two specific actions of TNF antagonists on the immune system in patients with inflammatory disease are of particular interest in terms of cancer treatment: modulation of the function of T_{Reg} cells[134] and a reduction in IL-17-producing T helper cell inflammatory responses[135], both of which are implicated in tumour promotion[125,136].

Is there a role for TNF antagonists in cancer prevention? Certainly some of the mouse model experiments described above suggest a role for TNF in the promotion of early cancers (for example REFS 5,6). Both herbal medicines and the polyphenols present in tea inhibit TNF release[137] but, given the role of TNF in regulating innate immunity, increased risk of infection would preclude wider use of current TNF antagonists. However, tens of thousands of people with rheumatoid arthritis or other chronic inflammatory diseases are being monitored for cancer incidence during TNF antagonist treatment. Analyses are complicated by underlying immune system dysfunction in these patients, prior treatment with immunosuppressive and mutagenic drugs, and the small number of malignancies so far recorded. In one meta-analysis of nine double-blinded placebo-controlled trials of anti-TNF antibodies in patients with rheumatoid arthritis, an increased risk of cancer was recorded[138]. However, in a later review the same authors concluded that, with over 50 trials of anti-TNF in inflammatory disease now published, there was no clear evidence for an overall increase in cancer risk. The current view is that caution may be necessary when considering treatment of patients with past or concurrent cancer or premalignant lesions and that there seems to be an increase in rare γδ T cell lymphomas in patients with juvenile Crohn's disease[138,139]. There is no evidence of an increase in overall cancer incidence in patients receiving anti-TNF therapies over a matched cohort of the general public[138,139].

TNF in cancer: target or treatment?

As the TNF timeline moves into the future there are a number of important questions. Can we explain the apparent efficacy of Coley's mixed toxins and the long but anecdotal history of cancer regression associated with acute bacterial infection? Can we harness the tumour-destructive capacity of TNF without promoting cancer or inducing a cytokine storm? Or will TNF antagonists have a more important role in cancer therapy and, if so, at what stage, in which patients and in combination with what other drugs?

Back to Coley: a 21st century perspective. We now realize that Coley's mixed toxins must have been powerful stimulants of TLRs[140], inducing a range of inflammatory mediators, not just TNF. The closest recent approximation to Coley's work is probably the successful local treatment of bladder cancer with bacillus Calmette–Guerin[141]. Current thinking is that both bacillus Calmette–Guerin and Coley's toxins trigger a desirable inflammatory response, through TLRs, that not only stimulates macrophages to kill tumour cells but also promotes the development of sustained and effective adaptive immunity to the tumour[142]. This type of response may also contribute to successful

chemotherapy or radiotherapy, according to recent data from Apetoh *et al.*[143]. They found that dying tumour cells were able to cross-present antigen to dendritic cells in a TLR4-dependent manner, triggering protective immune responses. When tumours were grown in mice with mutant TLR4, the efficacy of chemotherapy and radiotherapy was reduced, and breast cancer patients with a mutation in TLR4 had an increased frequency of metastasis.

Another recent paper on TLR signalling and cancer may also give some clues about desirable immune responses and TNF[144]. The TLR-associated signalling adaptor MYD88 has a crucial role in TLR and inflammatory cytokine signalling. As might be expected, *Myd88*$^{-/-}$ mice were resistant to DMBA–TPA skin carcinogenesis, but they were also resistant to MCA-induced sarcomas — tumours that are very susceptible to immunosurveillance[145]. While searching for downstream effectors of MYD88-induced tumour promotion, Mark Smyth's group found that *Tnf*$^{-/-}$ mice were actually more susceptible to MCA-induced sarcoma. One explanation could be that these sarcomas, like tumour cells that die after chemotherapy, are inherently immunogenic and under such circumstances TNF protects against tumour development[144]. And is it just coincidence that TNF was protective in a sarcoma model, the tumour type that responded most readily to Coley's toxins? It would certainly be interesting to see whether the immune microenvironment of sarcomas is different to that of the more common epithelial cancers.

The exact mechanisms whereby a desirable inflammatory response can be reliably triggered during cancer therapy are not clear, but even before Coley's time there was evidence for cancer regression after some bacterial infections. The priority is to find the best stimuli to change a tumour-promoting microenvironment to a tumour-inhibiting state and to understand the signalling mechanisms involved. And perhaps it is still worth reading Coley's papers and learning from his methods. In 1949, his daughter Helen Coley-Nauts reviewed case histories of 484 patients treated with toxin preparations and recorded that approximately 50% of patients were alive 5 years after treatment began[3]. Importantly, she concluded that the toxins were most effective if the patient was given both local and systemic injections, that it was important that a strong reaction be provoked by each injection, and that treatment should continue for months or even years.

Refining the tumour necrotic activity of TNF. Several new approaches for targeting TNF to cancers are being tested[16], including a radio-inducible TNF-expressing adenoviral vector (TNFerade), which is currently in a Phase III trial for inoperable pancreatic cancer[146]. Animal experiments show that the tumour vasculature is its primary target[147]. Another TNF-based therapeutic is NGF-hTNF, a tumour homing peptide that specifically targets TNF to CD13 (also known as aminopeptidase N) on tumour blood vessels. Responses have been reported in advanced colorectal cancer, liver cancer and mesothelioma, and the agent is currently in Phase II clinical trials (see MolMed website in Further information). There are also attempts to modify TNF to improve safety and efficacy; for instance, a single-chain TNF mutant molecule consisting of three TNF monomers fused by short peptide linkers had reduced systemic toxicity but slightly enhanced anti-tumour activity after intravenous dosing[148].

TNF production by cancer cells: an Achilles heel? Many cancer cells constitutively secrete picogram quantities of TNF and this appears to increase tumour growth. It may, however, be possible to exploit this production of TNF by malignant cells to therapeutic advantage. SMAC (also known as DIABLO) mimetic drugs switch off the survival and inflammatory pathways that are normally induced by autocrine TNF (BOX 2), causing those cancer cell lines that produce TNF to self-destruct[149].

The future of TNF antagonists in cancer treatment. From the clinical experience so far we can conclude that it is safe to give TNF antagonists to cancer patients. In the four Phase I and II cancer trials reported above, TNF antagonist treatment resulted in a period of disease stabilization or better in 20% of patients with advanced cancer[12–15]. To take this forward, we need a greater understanding of the roles of malignant and stromal cell-derived TNF in human cancers and its relative importance in early and late cancers. We also need to identify those patients who are most likely to benefit from TNF antagonist treatment: the anti-TNF antibody trials have suggested that low or absent plasma TNF is a possible biomarker of response[14,15] but the reason for this is not clear. Our unpublished data show that levels of expression of TNF signalling pathway components vary considerably in individual biopsy samples (H. Kulbe and F.B., unpublished data) and this may affect response. The underlying oncogenic mutations of each tumour might determine levels of TNF and response to TNF antagonists, and patients at an earlier stage of disease are more likely to benefit than those with advanced disease.

Information from clinical trials in other diseases will probably help us to understand the mechanisms of TNF action and contribute to patient selection. It is striking that many of the mechanisms by which TNF enhances cancer development — angiogenesis, leukocyte infiltration, and stimulation of other cytokines and chemokines — are inhibited by TNF antagonist treatment in patients with chronic inflammatory diseases[150].

In patients with advanced cancer, TNF antagonists are more likely to be active in combination with other treatments. As TNF induces resistance to BRAF inhibitors[151] and TNF-producing cells have increased resistance to cisplatin chemotherapy[152], TNF antagonists may enhance the action of these approaches. Anti-angiogenic agents such as bevacizumab could also be good candidates to combine with anti-TNF treatments and if, by neutralizing TNF, we can re-educate the host cells in the tumour microenvironment from a pro- to an anti-tumour phenotype[142], then TNF antagonists may contribute to immunotherapy approaches.

Summary

The history of TNF shows us how inflammation can have both positive and negative effects on cancer. Our challenge is to harness the helpful aspects of the inflammatory response in cancer while neutralizing its pro-tumour actions. Is TNF the key to this endeavour?

Frances Balkwill is at the Centre for Cancer and Inflammation, Institute of Cancer, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, UK.
e-mail: Frances.balkwill@cancer.org.uk

doi:10.1038/nrc2628
Published online 3 April 2009

1. Carswell, E. A. *et al.* An endotoxin-induced serum factor that causes necrosis of tumours. *Proc. Natl Acad. Sci. USA* **72**, 3666–3670 (1975).
2. Coley, W. B. The treatment of malignant tumors by repeated inoculations of erysipelas: with a report of ten original cases. *Am. J. Med. Sci.* **105**, 487–511 (1893).
3. Nauts, H. C., Swift, W. E. & Coley, B. L. The treatment of malignant tumors by bacterial toxins as developed by the late William B. Coley, M.D. Reviewed in the light of modern research. *Cancer Res.* **6**, 205–216 (1946).
4. Locksley, R. M., Killeen, N. & Lenardo, M. J. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* **104**, 487–501 (2001).
5. Moore, R. *et al.* Tumour necrosis factor-α deficient mice are resistant to skin carcinogenesis. *Nature Med.* **5**, 828–831 (1999).
6. Pikarsky, E. *et al.* NF-κB functions as a tumour promoter in inflammation-associated cancer. *Nature* **431**, 4461–4466 (2004).

7. Balkwill, F. TNF-α in promotion and progression of cancer. *Cancer Metastasis Rev.* **25**, 409–416 (2006).
8. Sethi, G., Sung, B. & Aggarwal, B. B. TNF: a master switch for inflammation to cancer. *Front. Biosci.* **13**, 5094–5107 (2008).
9. Feldmann, M. Development of anti-TNF therapy for rheumatoid arthritis. *Nature Rev. Immunol.* **2**, 364–371 (2002).
10. Sands, B. E. *et al.* Infliximab maintenance therapy for fistulizing Crohn's disease. *N. Engl. J. Med.* **350**, 876–885 (2004).
11. Tracey, D., Klareskog, L., Sasso, E. H., Salfeld, J. G. & Tak, P. P. Tumor necrosis factor antagonist mechanisms of action: A comprehensive review. *Pharmacol. Therapeut.* **117**, 244–279 (2008).
12. Madhusudan, S. *et al.* A phase II study of Etanercept (Enbrel), a tumour necrosis factor-α inhibitor in patients with metastatic breast cancer. *Clin. Cancer Res.* **10**, 6528–6534 (2004).
13. Madhusudan, S. *et al.* A phase II study of Ethanercept (ENBREL) a tumour necrosis factor- α inhibitor in recurrent ovarian cancer. *J. Clin. Oncol.* **23**, 5950–5959 (2005).
14. Harrison, M. L. *et al.* Tumor necrosis factor α as a new target for renal cell carcinoma: two sequential phase II trials of infliximab at standard and high dose. *J. Clin. Oncol.* **25**, 4542–4549 (2007).
15. Brown, E. R. *et al.* A clinical study assessing the tolerability and biological effects of infliximab, a TNF-α inhibitor, in patients with advanced cancer. *Ann. Oncol.* **19**, 1340–1346 (2008).
16. Daniel, D. & Wilson, N. S. Tumor necrosis factor: renaissance as a cancer therapeutic? *Curr. Cancer Drug Targets.* **8**, 124–131 (2008).
17. Coley, W. B. The treatment of inoperable sarcoma by bacterial toxins (the mixed toxins of the streptococcus erysipelas and the bacillus prodigiosus). *Proc. R. Soc. Med.* **3** (*Surg.* Sect), 1–48 (1909).
18. Hall, S. S. *A Commotion in the Blood* 21–127 (Henry Holt, New York, 1997).
19. Coley, W. B. Contribution to the knowledge of sarcoma. *Ann. Surg.* **14**, 199–220 (1891).
20. Coley, W. B. Late results of the treatment of inoperable sarcoma by the mixed toxins of erysipelas and bacillus prodigiosus. *Am. J. Med. Sci.* **131**, 375–430 (1906).
21. Coley Nauts, H., Fowler, G. A. & Bogatko, F. H. A review of the influence of bacterial infection and of bacterial products (Coley's toxins) on malignant tumors in man. *Acta Med. Scand.*, 29–97 (1953).
22. Gratia, A. & Linz, R. Le phenomenene de Schwartzman dans le sarcome du cobaye. *C R. Soc. Biol.* **108**, 427–428 (1931) (in French).
23. Shear, M. J. & Perrault, A. Chemical treatment of tumors. IX. Reactions of mice with primary subcutaneous tumors to injection of a hemorrhage-producing bacterial polysaccharide. *J. Natl Cancer Inst.* **44**, 461–476 (1944).
24. O'Malley, W. E., Achinstein, B. & Shear, M. J. Action of bacterial polysaccharide on tumours. II. Damage of sarcoma 37 by serum of mice treated with serratia marcescens polysaccharide, and induced tolerance. *J. Natl Cancer Inst.* **29**, 1169–1175 (1962).
25. Granger, G. A. & Kolb, W. P. Lymphocyte *in vitro* cytotoxicity: mechanisms of immune and non-immune small lymphocyte mediated target L cell destruction. *J. Immunol.* **101**, 111–120 (1968).
26. Aggarwal, B. B. *et al.* Human tumor necrosis factor. Production, purification, and characterisation. *J. Biol. Chem.* **260**, 2345–2354 (1985).
27. Aggarwal, B. B., Moffat, B. & Harkins, R. N. Human lymphotoxin. Production by a lymphoblastoid cell line, purification, and initial characterization. *J. Biol. Chem.* **259**, 686–691 (1984).
28. Aggarwal, B. B., Henzel, W. J., Moffat, B., Kohr, W. J. & Harkins, R. N. Primary structure of human lymphotoxin derived from 1788 lymphoblastoid cell line. *J. Biol. Chem.* **260**, 2334–2344 (1985).
29. Aggarwal, B. B., Eessalu, T. E. & Hass, P. E. Characterization of receptors for human tumour necrosis factor and their regulation by γ-interferon. *Nature* **318**, 665–667 (1985).
30. Pennica, D. *et al.* Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature* **312**, 724–729 (1984).
31. Gray, P. W. *et al.* Cloning and expression of cDNA for human lymphotoxin, a lymphokine with tumour necrosis activity. *Nature* **312**, 721–724 (1984).
32. Marmenout, A. *et al.* Molecular cloning and expression of human tumor necrosis factor and comparison with mouse tumor necrosis factor. *Eur. J. Biochem.* **152**, 515–522 (1985).
33. Fransen, L. *et al.* Molecular cloning of mouse tumour necrosis factor cDNA and its eukaryotic expression. *Nucleic Acids Res.* **13**, 4417–4429 (1985).
34. Hahn, T. *et al.* Use of monoclonal antibodies to a human cytotoxin for its isolation and for examining the self induction of resistance to this protein. *Proc. Natl Acad. Sci. USA* **82**, 3814–3818 (1985).
35. Hehlgans, T. & Pfeffer, K. The intriguing biology of the tumor necrosis factor/tumour necrosis factor receptor superfamily: players, rules and the games. *Immunology* **115**, 1–20 (2005).
36. Dillon, S. R., Gross, J. A., Ansell, S. M. & Novak, A. J. An APRIL to remember: novel TNF ligands as therapeutic targets. *Nature Rev. Drug Discov.* **5**, 235–242 (2006).
37. Sabbagh, L., Snell, L. M. & Watts, T. H. TNF family ligands define niches for T cell memory. *Trends Immunol.* **28**, 333–339 (2007).
38. Engelmann, H., Aderka, D., Rubinstein, M., Rotman, D. & Wallach, D. A tumor necrosis factor-binding protein purified to homogeneity from human urine protects cells from tumor necrosis factor toxicity. *J. Biol. Chem.* **264**, 11974–11980 (1989).
39. Engelmann, H., Novick, D. & Wallach, D. Two tumor necrosis factor-binding proteins purified from human urine. Evidence for immunological cross-reactivity with cell surface tumor necrosis factor receptors. *J. Biol. Chem.* **265**, 1531–1536 (1990).
40. Loetscher, H. *et al.* Molecular cloning and expression of the human 55 KD tumor necrosis factor receptor. *Cell* **61**, 351–359 (1990).
41. Schall, T. J. *et al.* Molecular cloning and expression of a receptor for human tumor necrosis factor. *Cell* **61**, 361–370 (1990).
42. Smith, C. A. *et al.* A receptor for tumor necrosis factor defines an unusual family of cellular and viral proteins. *Science* **248**, 1019–1023 (1990).
43. Heller, R. A. *et al.* Complementary DNA cloning of a receptor for tumor necrosis factor and demonstration of a shed form of the receptor. *Proc. Natl Acad. Sci. USA* **87**, 6151–6155 (1990).
44. Beutler, B. A. The role of tumor necrosis factor in health and disease. *J. Rheumatol.* **26**, 16–21 (1999).
45. Feldmann, M. Many cytokines are very useful therapeutic targets in disease. *J. Clin. Invest.* **118**, 3533–3536 (2008).
46. Elliott, M. J. *et al.* Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor a (cA2) versus placebo in rheumatoid arthritis. *Lancet* **344**, 1105–1110 (1994).
47. Brouckaert, P. G. G., Leroux-Roels, G. G., Guisez, Y., Tavernier, J. & Fiers, W. *In vivo* anti-tumour activity of recombinant human and murine TNF, alone and in combination with murine IFN-gamma on a syngeneic murine melanoma. *Int. J. Cancer* **38**, 763–769 (1986).
48. Balkwill, F. R. *et al.* Human tumour xenografts treated with recombinant human tumor necrosis factor alone or in combination with interferons. *Cancer Res.* **46**, 3990–3993 (1986).
49. Talmadge, J. E. *et al.* Immunomodulatory properties of recombinant murine and human tumor necrosis factor. *Cancer Res.* **48**, 544–550 (1988).
50. Watanabe, N. *et al.* Synergistic cytotoxic and antitumour effects of recombinant tumour necrosis factor and hyperthermia. *Cancer Res.* **48**, 650–653 (1988).
51. Nawroth, P. *et al.* Tumor necrosis factor/cachectin-induced intravascular fibrin formation in meth A fibrosarcomas. *J. Exp. Med.* **168**, 637–647 (1988).
52. Mantovani, A. & Dejana, E. Cytokines as communication signals between leukocytes and endothelial cells. *Immunol. Today* **10**, 370–375 (1989).
53. Kettlehut, I. C., Fiers, W. & Goldberg, A. L. The toxic effects of tumor necrosis factor *in vivo* and their prevention by cyclooxygenase inhibitors. *Proc. Natl Acad. Sci. USA* **84**, 4273–4277 (1987).
54. Havell, E. A., Fiers, W. & North, R. J. The antitumor function of tumor necrosis factor (TNF). 1. Therapeutic action of TNF against an established murine sarcoma is indirect, immunologically dependent, and limited by severe toxicity. *J. Exp. Med.* **167**, 1067–1085 (1988).
55. Lienard, D., Ewalenko, P., Delmotte, J.-J., Renard, N. & Lejeune, F. J. High-dose recombinant tumor necrosis factor alpha in combination with interferon gamma and melphalan in isolation perfusion of the limbs for melanoma and sarcoma. *J. Clin. Oncol.* **10**, 52–60 (1992).
56. Nooijen, P. T. *et al.* Synergistic effects of TNF-alpha and melphalan in an isolated limb perfusion model of rat sarcoma: a histopathological, immunohistochemical and electron microscopical study. *Br. J. Cancer* **74**, 1908–1915 (1996).
57. de Wilt, J. H. *et al.* Prerequisites for effective isolated limb perfusion using tumour necrosis factor alpha and melphalan in rats. *Br. J. Cancer* **80**, 161–166 (1999).
58. van der Veen, A. H. *et al.* TNF-α augments intratumoural concentrations of doxorubicin in TNF-α-based isolated limb perfusion in rat sarcoma models and enhances anti-tumour efects. *Br. J. Cancer* **82**, 973–980 (2000).
59. Seynhaeve, A. L. *et al.* Tumor necrosis factor α mediates homogeneous distribution of liposomes in murine melanoma that contributes to a better tumor response. *Cancer Res.* **67**, 9455–9462 (2007).
60. Colotta, F., Peri, G., Villa, A. & Mantovani, A. Rapid killing of actinomycin D-treated tumor cells by human mononuclear cells. 1. Effectors belong to the monocyte-macrophage lineage. *J. Immunol.* **132**, 936–944 (1984).
61. Sugarman, B. J. *et al.* Recombinant human tumor necrosis factor alpha: effects on proliferation of normal and transformed cells *in vitro*. *Science* **230**, 943–945 (1985).
62. Dealtry, G. B., Naylor, M. S., Fiers, W. & Balkwill, F. R. The effect of recombinant human tumour necrosis factor on growth and macromolecular synthesis of human epithelial cells. *Exp. Cell Res.* **170**, 428–438 (1987).
63. Fransen, L., Van der Heyden, J., Ruysschaert, R. & Fiers, W. Recombinant tumor necrosis factor: its effect and its synergism with interferon-gamma on a variety of normal and transformed human cell lines. *Eur. J. Cancer Clin. Oncol.* **22**, 419–426 (1986).
64. Williamson, B. D., Carswell, E. A., Rubin, B. Y. & Prendergast, J. S. Human tumor necrosis factor produced by human B-cell lines: synergistic cytotoxic interaction with human interferons. *Proc. Natl Acad. Sci. USA* **80**, 5397–5401 (1983).
65. Wallach, D. Preparations of lymphotoxin induce resistance to their own cytotoxic effect. *J. Immunol.* **132**, 2464–2469 (1984).
66. Palladino, M. A. Jr *et al.* Characterization of the antitumor activities of human tumor necrosis factor alpha and the comparison with other cytokines: induction of tumor-specific immunity. *J. Immunol.* **138**, 4023–4032 (1987).
67. Kashii, Y., Giorda, R., Herberman, R. B., Whiteside, T. L. & Vujanovic, N. L. Constitutive expression and role of the TNF family ligands in apoptotic killing of tumor cells by human NK cells. *J. Immunol.* **163**, 5358–5366 (1999).
68. Prevost-Blondel, A., Roth, E., Rosenthal, F. M. & Pircher, H. Crucial role of TNF-α in CD8 cell-mediated elimination of 3LL-A9 Lewis lung carcinoma cells *in vivo*. *J. Immunol.* **164**, 3645–3651 (2000).
69. Baxevanis, C. N., Voutsas, I. F., Tsitsilonis, O. E., Tsiatas, D. G. & Papmichail, M. Compromised anti-tumor responses in tumor necrosis factor-α knockout mice. *Eur. J. Immunol.* **30**, 1957–1966 (2000).
70. Calzascia, T. *et al.* TNF-α is critical for antitumor but not antiviral T cell immunity in mice. *J. Clin. Invest.* **117**, 3833–3845 (2007).
71. Selby, P. *et al.* Tumour necrosis factor in man: clinical and biological observations. *Br. J. Cancer* **56**, 803–808 (1987).
72. Creagan, E. T., Kovach, J. S., Moertel, C. G., Frytak, S. & Kvols, L. K. A phase 1 clinical trial of recombinant human tumor necrosis factor. *Cancer* **62**, 2467–2471 (1988).
73. Kimura, K. *et al.* Phase 1 study of recombinant human tumor necrosis factor. *Cancer Chemother. Pharmacol.* **20**, 223–229 (1987).
74. Blick, M., Sherwin, S. A., Rosenblum, M. & Gutterman, J. Phase I study of recombinant tumor necrosis factor in cancer patients. *Cancer Res.* **47**, 2986–2989 (1987).
75. Morice, R. C., Blick, M. B., Ali, M. K. & Gutterman, J. U. Pulmonary toxicity of recombinant tumor necrosis factor (rTNF). *Proc. Am. Soc. Clin. Oncol.* **6**, 29 (1987).
76. Verhoef, C. *et al.* Isolated limb perfusion with melphalan and TNF-α in the treatment of extremity sarcoma. *Curr. Treat. Options Oncol.* **8**, 417–427 (2007).
77. Grunhagen, D. J. *et al.* Outcome and prognostic factor analysis of 217 consecutive isolated limb perfusions with tumor necrosis factor-α and melphalan for limb-threatening soft tissue sarcoma. *Cancer* **106**, 1776–1784 (2006).
78. Starnes, C. O. Coley's toxins in perspective. *Nature* **357**, 11–12 (1992).
79. Spriggs, D., Imamura, K., Rodriguez, C., Horiguchi, J. & Kufe, D. W. Induction of tumor necrosis factor expression and resistance in a human breast tumor cell line. *Proc. Natl Acad. Sci. USA* **84**, 6563–6566 (1987).

80. Beissert, S. *et al.* Regulation of tumor necrosis factor gene expression in colorectal adenocarcinoma: *In vivo* analysis by *in situ* hybridization. *Proc. Natl Acad. Sci. USA* **86**, 5064–5086 (1989).
81. Naylor, M. S., Malik, S. T. A., Stamp, G. W. H., Jobling, T. & Balkwill, F. R. *In situ* detection of tumour necrosis factor in human ovarian cancer specimens. *Eur. J. Cancer* **26**, 1027–1030 (1990).
82. Naylor, M. S., Stamp, G. W. H., Foulkes, W. D., Eccles, D. & Balkwill, F. R. Tumor necrosis factor and its receptors in human ovarian cancer. *J. Clin. Invest.* **91**, 2194–2206 (1993).
83. Karayiannakis, A. J. *et al.* Serum levels of tumor necrosis factor-alpha and nutritional status in pancreatic cancer patients. *Anticancer Res.* **21**, 1355–1358 (2001).
84. Yoshida, N. *et al.* Interleukin-6, tumour necrosis factor α and interleukin-1β in patients with renal cell carcinoma. *Br. J. Cancer* **86**, 1396–1400 (2002).
85. Ferrajoli, A. *et al.* The clinical significance of tumor necrosis factor-a plasma level in patients having chronic lymphocytic leukemia. *Blood* **100**, 1215–1219 (2002).
86. Bozcuk, H. *et al.* Tumour necrosis factor-alpha, interleukin-6, and fasting serum insulin correlate with clinical outcome in metastatic breast cancer patients treated with chemotherapy. *Cytokine* **27**, 58–65 (2004).
87. Anderson, G. M., Nakada, M. T. & DeWitte, M. Tumor necrosis factor-α in the pathogenesis and treatment of cancer. *Curr. Opin. Pharmacol.* **4**, 314–320 (2004).
88. Pfitzenmaier, J. *et al.* Elevation of cytokine levels in cachectic patients with prostate carcinoma. *Cancer* **97**, 1211–1216 (2003).
89. Michalaki, V., Syrigos, K., Charles, P. & Waxman, J. Serum levels of IL-6 and TNF-α correlate with clinicopathological features and patient survival in patients with prostate cancer. *Br. J. Cancer* **91**, 1227 (2004).
90. Frater-Schroder, M., Risau, W., Hallmann, R., Gautschi, P. & Bohlen, P. Tumor necrosis factor type α, a potent inhibitor of endothelial cell growth *in vitro*, is angiogenic *in vivo*. *Proc. Natl Acad. Sci. USA* **84**, 5277–5281 (1987).
91. Leibovich, S. J. *et al.* Macrophage-induced angiogenesis is mediated by tumour necrosis factor alpha. *Nature* **329**, 630–632 (1987).
92. Malik, S. T. A., Griffin, D. B., Fiers, W. & Balkwill, F. R. Paradoxical, effects of tumour necrosis factor in experimental ovarian cancer. *Int. J. Cancer* **44**, 918–925 (1989).
93. Malik, S. T. A., Naylor, S., East, N., Oliff, A. & Balkwill, F. R. Cells secreting tumour necrosis factor show enhanced metastasis in nude mice. *Eur. J. Cancer* **26**, 1031–1034 (1990).
94. Orosz, P. *et al.* Enhancement of experimental metastasis by tumor necrosis factor. *J. Exp. Med.* **177**, 1391–1398 (1993).
95. Kim, S. *et al.* Carcinoma-produced factors activate myeloid cells through TLR2 to stimulate metastasis. *Nature* **457**, 102–106 (2009).
96. Pasparakis, M., Alexopoulou, L., Episkopou, V. & Kollias, G. Immune and inflammatory responses in TNFα-deficient mice: a critical requirement for TNFα in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. *J. Exp. Med.* **184**, 1397–1411 (1996).
97. Virchow, R. *Die krankhaften Geschwulste* (1863).
98. Balkwill, F. & Mantovani, A. Inflammation and cancer: back to Virchow. *Lancet* **357**, 539–545 (2001).
99. Balkwill, F., Charles, K. A. & Mantovani, A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. *Cancer Cell* **7**, 211–217 (2005).
100. Mantovani, A., Allavena, P., Sica, A. & Balkwill, F. Cancer-related inflammation. *Nature* **454**, 436–444 (2008).
101. Suganuma, M. *et al.* Essential role of tumor necrosis factor α (TNF-α) in tumor promotion as revealed by TNF-α-deficient mice. *Cancer Res.* **59**, 4516–4518 (1999).
102. Kulbe, H. *et al.* The inflammatory cytokine TNF-α generates an autocrine tumour-promoting network in epithelial ovarian cancer cells. *Cancer Res.* **67**, 585–592 (2007).
103. Egberts, J.-H. *et al.* Anti-tumor necrosis factor therapy inhibits pancreatic tumor growth and metastasis. *Cancer Res.* **68**, 1443–1450 (2008).
104. Stathopoulos, G. T. *et al.* Tumor necrosis factor-α promotes malignant pleural effusion. *Cancer Res.* **67**, 9825–9834 (2007).
105. Zins, K., Abraham, D., Sioud, M. & Aharinejad, S. Colon cancer cell-derived tumor necrosis factor-α mediates the tumor growth-promoting response in macrophages by up-regulating the colony-stimulating factor-1 pathway. *Cancer Res.* **67**, 1038–1045 (2007).
106. Li, B. *et al.* Low levels of tumor necrosis factor alpha increase tumor growth by inducing an endothelial phenotype of monocytes recruited to the tumor site. *Cancer Res.* **69**, 338–348 (2009).
107. Hagemann, T. *et al.* TNF-α dependent increased c-Jun and NF-κB activity in tumour cell lines upon co-cultivation with macrophages. *J. Immunol.* **175**, 1197–1205 (2005).
108. Hagemann, T. *et al.* Ovarian cancer cells polarize macrophages toward a tumor-associated phenotype. *J. Immunol.* **176**, 5023–5032 (2006).
109. Szlosarek, P. W. *et al.* Expression and regulation of tumor necrosis factor-α in normal and malignant ovarian epithelium. *Mol. Cancer Ther.* **5**, 382–390 (2006).
110. Galban, S. *et al.* von Hippel–Lindau protein-mediated repression of tumor necrosis factor alpha translation revealed through use of cDNA arrays. *Mol. Cell. Biol.* **23**, 2316–2328 (2003).
111. Suganuma, M., Kuzuhara, T., Yamaguchi, K. & Fujiki, H. Carcinogenic role of tumor necrosis factor-α inducing protein of *Helicobacter pylori* in human stomach. *J. Biochem. Mol. Biol.* **39**, 1–8 (2006).
112. Maeda, S., Kamata, H., Luo, J. L., Leffert, H. & Karin, M. IKKβ couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. *Cell* **121**, 977–990 (2005).
113. Popivanova, B. K. *et al.* Blocking TNF-α in mice reduces colorectal carcinogenesis associated with chronic colitis. *J. Clin. Invest.* **118**, 560–570 (2008).
114. Oguma, K. *et al.* Activated macrophages promote Wnt signalling through tumour necrosis factor-α in gastic tumour cells. *EMBO J.* **27**, 1671–1681 (2008).
115. Komori, A. *et al.* Tumor necrosis factor acts as a tumor promoter in BALB/3T3 cell transformation. *Cancer Res.* **53**, 1982–1985 (1993).
116. Li, J. *et al.* TNF-α induces leukemic clonal evolution *ex vivo* in Fanconi anemia group C murine stem cells. *J. Clin. Invest.* **117**, 3283–3295 (2007).
117. Yan, B. *et al.* Tumor necrosis factor-α is a potent endogenous mutagen that promotes cellular transformation. *Cancer Res.* **66**, 11565–11570 (2006).
118. Babbar, N. & Casero, R. A. Jr. Tumor necrosis factor-α increases reactive oxygen species by inducing spermine oxidase in human lung epithelial cells: a potential mechanism for inflammation-induced carcinogenesis. *Cancer Res.* **66**, 11125–11130 (2006).
119. Komori, J. *et al.* Activation-induced cytidine deaminase links bile duct inflammation to human cholangiocarcinoma. *Hepatology* **47**, 888–896 (2008).
120. Akiyama, M. *et al.* Nuclear factor-κB p65 mediates tumor necrosis factor α-induced nuclear translocation of telomerase reverse transcriptase protein. *Cancer Res.* **63**, 18–21 (2003).
121. Bates, R. C. & Mercurio, A. M. Tumor necrosis factor-α stimulates the epithelial-to-mesenchymal transition of human colonic organoids. *Mol. Biol. Cell* **14**, 1790–1800 (2003).
122. Arnott, C. H. *et al.* Expression of both TNF-a receptor subtypes is essential for optimal skin tumour development. *Oncogene* **23**, 1902–1910 (2004).
123. Tomita, Y. *et al*. Spontaneous regression of lung metastasis in the absence of tumour necrosis factor p55. *Int. J. Cancer* **112**, 927–933 (2004).
124. Kitakata, H. *et al.* Essential roles of tumor necrosis factor receptor p55 in liver metastasis of intrasplenic administration of colon 26 cells. *Cancer Res.* **62**, 6682–6687 (2002).
125. Curiel, T. J. Regulatory T cells and treatment of cancer. *Curr. Opin. Immunol.* **20**, 241–246 (2008).
126. Chen, X., Baumel, M., Mannel, D. N., Howard, O. M. Z. & Oppenheim, J. J. Interaction of TNF with TNF receptor type 2 promotes expansion and function of mouse $CD4^+CD25^+$ T regulatory cells. *J. Immunol.* **179**, 154–161 (2007).
127. Greten, F. R. *et al.* IKKβ links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* **118**, 285–296 (2004).
128. Liu, Z.-G., Hsu, H., Goeddel, D. V. & Karin, M. Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-κB activation prevents cell death. *Cell* **87**, 565–576 (1996).
129. Bonizzi, G. & Karin, M. The two NF-κB activation pathways and their role in innate and adaptive immunity. *Trends Immunol.* **25**, 280–288 (2004).
130. Lee, D.-F. *et al.* IKKB suppression of TSC1 links inflammation and tumor angiogenesis via the mTOR pathway. *Cell* **130**, 440–455 (2007).
131. Scott, K. A. *et al.* An anti-TNF-α antibody inhibits the development of experimental skin tumors. *Mol. Cancer Ther.* **2**, 445–451 (2003).
132. Rao, V. P. *et al.* Proinflammatory $CD4^+CD45RB^{hi}$ lymphocytes promote mammary and intestinal carcinogenesis in $Apc^{Min/+}$ mice. *Cancer Res.* **66**, 57–61 (2006).
133. Stasi, R., Amadori, S., Newland, A. C. & Provan, D. Infliximab chimeric antitumor necrosis factor-a monoclonal antibody as potential treatment of myelodysplastic syndromes. *Leuk. Lymphoma* **46**, 509–516 (2005).
134. Nadkarni, S., Mauri, C. & Ehrenstein, M. R. Anti-TNF-α therapy induces a distinct regulatory T cell population in patients with rheumatoid arthritis via TGF-β. *J. Exp. Med.* **204**, 33–39 (2007).
135. Zaba, L. C. *et al.* Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses. *J. Exp. Med.* **204**, 3183–3194 (2007).
136. Langowski, J. L. *et al.* IL-23 promotes tumour incidence and growth. *Nature*, **442**, 461–465 (2006).
137. Fujiki, H. *et al.* New TNF-α releasing inhibitors as cancer preventive agents from traditional herbal medicine and combination cancer prevention study with EGCG and sulindac or tamoxifen. *Mut. Res.* **523**, 119–125 (2003).
138. Bongartz, T. *et al.* Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies. *JAMA* **295**, 2275–2285 (2006).
139. Askling, J. & Bongartz, T. Malignancy and biologic therapy in rheumatoid arthritis. *Curr. Opin. Rheumatol.* **20**, 334–339 (2008).
140. Creagh, E. M. & O'Neill, L. A. J. TLRs, NLRs and RLRs: a trinity of pathogen sensors that co-operate in innate immunity. *Trends Immunol.* **27**, 352–357 (2006).
141. Alexandroff, A. B., Jackson, A. M., O'Donnell, M. A. & James, K. BCG immunotherapy of bladder cancer: 20 years on. *Lancet* **353**, 1689–1694 (1999).
142. Hagemann, T. *et al.* 'Re-educating' tumor-associated macrophages by targeting NF-κB. *J. Exp. Med.* **205**, 1261–1268 (2008).
143. Apetoh, L. *et al.* Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nature Med.* **13**, 1050–1059 (2007).
144. Swann, J. B. *et al.* Demonstration of inflammation-induced cancer and cancer immunoediting during primary tumorigenesis. *Proc. Natl Acad. Sci. USA* **105**, 652–656 (2008).
145. Koebel, M. *et al.* Adaptive immunity maintains occult cancer in an equilibrium state. *Nature* **450**, 903–907 (2007).
146. Senzer, N. *et al.* TNFerade biologic, an adenovector with a radiation-inducible promoter, carrying the human tumor necrosis factor alpha gene: a phase I study in patients with solid tumors. *J. Clin. Oncol.* **22**, 592–601 (2004).
147. Mauceri, H. J. *et al.* Translational strategies exploiting TNF-α that sensitize tumors to radiation therapy. *Cancer Gene Ther.* 31 Oct 2008 (doi:10.1038/cgt.2008.86).
148. Krippner-Heidenreich, A. *et al.* Single chain TNF, a TNF derivative with enhanced stability and antitumoral activity. *J. Immunol.* **180**, 8176–8183 (2008).
149. Petersen, S. L. *et al.* Autocrine TNFα signaling renders human cancer cells susceptible to Smac-mimetic-induced apoptosis. *Cancer Cell* **12**, 445–456 (2007).
150. Charles, P. *et al.* Regulation of cytokines, cytokine inhibitors, and acute-phase proteins following anti-TNF-α therapy in rheumatoid arthritis. *J. Immunol.* **163**, 1521–1528 (1999).
151. Gray-Schopfer, V. C., Karasarides, M., Hayward, R. & Marais, R. Tumor necrosis factor-α blocks apoptosis in melanoma cells when BRAF signaling is inhibited. *Cancer Res.* **67**, 122–129 (2007).
152. Gordon, G. J. *et al.* Inhibitor of apoptosis proteins are regulated by tumour necrosis factor-a in malignant pleural mesothelioma. *J. Pathol.* **211**, 439–446 (2007).
153. Varfolomeev, E. E. & Ashkenazi, A. Tumor necrosis factor: an apoptosis JuNKie? *Cell* **116**, 491–497 (2004).
154. Aggarwal, B. B. Signalling pathways of the TNF superfamily: a double-edged sword. *Nature Rev. Immunol.* **3**, 745–756 (2003).

155. Akira, S. & Takeda, K. Toll-like receptor signalling. *Nature Rev. Immunol.* **4**, 499–511 (2004).
156. Tsenova, L., Bergtold, A., Freedman, V. H., Young, R. A. & Kaplan, G. Tumour necrosis factor α is a determinant of pathogenesis and disease progression in mycobacterial infection in the central nervous system. *Proc. Natl Acad. Sci. USA* **96**, 5657–5662 (1999).
157. Schluter, D. *et al.* Both lymphotoxin-α and TNF are crucial for control of *Toxoplasma gondii* in the central nervous system. *J. Immunol.* **170**, 6172–6182 (2003).
158. Pasparakis, M. *et al.* Peyer's patch organogenesis is intact yet formation of B lymphocyte follicles is defective in peripheral lymphoid organs of mice deficient for tumor necrosis factor and its 55-kDa receptor. *Proc. Natl Acad. Sci. USA* **94**, 6319–6323 (1997).
159. Kuprash, D. V. *et al.* Novel tumor necrosis factor-knockout mice that lack Peyer's patches. *Eur. J. Immunol.* **35**, 1592–1600 (2005).
160. Grivennikov, S. I. *et al.* Distinct and nonredundant *in vivo* functions of TNF produced by T cells and macrophages/neutrophils: protective and deleterious effects. *Immunity* **22**, 93–104 (2005).
161. Beutler, B. *et al.* Identity of tumour necrosis factor and the macrophage-secreted factor cachetin. *Nature* **316**, 552–554 (1985).
162. Brennan, F. M., Jackson, A., Chantry, D., Maini, R. & Feldmann, M. Inhibitory effect of TNF-alpha antibodies on synovial cell interleukin-1 production in rheumatoid arthritis. *Lancet* **2**, 244–247 (1989).
163. Williams, R. O., Feldmann, M. & Maini, R. N. Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis. *Proc. Natl Acad. Sci. USA* **89**, 9784–9788 (1992).
164. van Deventer, S. J. Anti-TNF antibody treatment of Crohn's disease. *Ann. Rheum. Dis.* **58**, 14–20 (1999).
165. Mease, P. J. *et al.* Etanercept in the treatment of psoriatic arthritis and psoriasis: a randomised trial. *Lancet* **356**, 385–390 (2000).
166. Chaudhari, U. *et al.* Efficacy and safety of infliximab monotherapy for plaque-type psoriasis: a randomised trial. *Lancet* **357**, 1842–1847 (2001).
167. Berry, M. A. *et al.* Evidence of a role of tumor necrosis factor α in refractory asthma. *N. Engl. J. Med.* **354**, 697–708 (2006).
168. Ashkenazi, A. Targeting death and decoy receptors of the tumor necrosis factor superfamily. *Nature Rev. Cancer* **2**, 420–430 (2002).
169. Gray, P. W., Barret, K., Chantry, D., Turner, M. & Feldmann, M. Cloning of human tumor necrosis factor (TNF) receptor cDNA and expression of recombinant soluble TNF-binding protein. *Proc. Natl Acad. Sci. USA* **87**, 7380–7384 (1990).
170. Heller, R. A. *et al.* Amplified expression of tumor necrosis factor receptor in cells transfected with Epstein–Barr virus shuttle vector cDNA libraries. *J. Biol. Chem.* **265**, 5708–5717 (1990).
171. Coussens, L. M. & Werb, Z. Inflammation and cancer. *Nature* **420**, 860–867 (2002).

Acknowledgements
The author would like to thank members of the Centre for Cancer and Inflammation at Barts and The London Medical School and also A. Mantovani for useful discussions and criticism.

Competing interests statement
The authors declare competing financial interests: see web version for details.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
MYC | *TP53* | *TSC1* | *VHL*
National Cancer Institute Drug Dictionary: http://www.cancer.gov/drugdictionary/
actinomycin D | bevacizumab | doxorubicin | etanercept | infliximab | melphalan | mitomycin C
UniProtKB: http://www.uniprot.org
AID | BRAF | CCL2 | CXCL12 | CD13 | IKKβ | IL-2 | IL-6 | lymphotoxin | MIF | MYD88 | p65 | SMAC | TERT | TLR2 | TLR4 | TLR6 | TNF | TNFR1 | TNFR2 | TRAIL | TSC1 | versican | VHL

FURTHER INFORMATION
Frances Balkwill's homepage: http://www.cancer.qmul.ac.uk/research/cancer_inflammation/index.html
MolMed: http://www.molmed.com/eng/index.asp

SUPPLEMENTARY INFORMATION
See online article: S1 (box)

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

OPINION

The ups and downs of p53: understanding protein dynamics in single cells

Eric Batchelor, Alexander Loewer and Galit Lahav

Abstract | Cells living in a complex environment must constantly detect, process and appropriately respond to changing signals. Therefore, all cellular information processing is dynamic in nature. As a consequence, understanding the process of signal transduction often requires detailed quantitative analysis of dynamic behaviours. Here, we focus on the oscillatory dynamics of the tumour suppressor protein p53 as a model for studying protein dynamics in single cells to better understand its regulation and function.

How are signals received by a cell translated into decisions such as growth, death and movement? In the past several decades there has been a great deal of success in identifying the proteins and genes that are activated or repressed in response to specific inputs and in assembling them into signal transduction pathways. However, even though we now have maps of many signalling pathways, new questions have arisen owing to the complexity of the pathways they represent. How can we move beyond describing the structure of biological networks to developing a detailed, quantitative understanding of their function and behaviour? One promising approach is to investigate the dynamics of key proteins within the network (FIG. 1). In this context, dynamics is defined as the change of any variable that can be quantitatively measured over time, such as protein concentration, activity, modification state or localization. These data are complementary to the information originally used to describe the network, and have great potential to provide new insight into the relationship between network structure and function. For example, if the activity of a signalling molecule is measured at only a single point in time, the signal could be interpreted as binary: being either on or off. If, however, the signalling activity is quantitatively measured with high temporal resolution over a long period it could show a large number of distinct behaviours. Detailed analysis of dynamic behaviours in diverse systems and under various conditions has the potential to provide new levels of understanding of how cells detect inputs and translate them into outputs.

The analysis of cellular dynamics often requires measurements in single cells, as measurements of averaged dynamics in a population of cells can be misleading. For example, in response to certain doses of antibiotics, some cells live but others die[1]. These different outcomes might reflect differences in the initial state of the cell (such as its cell cycle state, basal level of network components or local environment), which in turn lead to differences in the quantitative behaviour of the information processing network. By visualizing the dynamic behaviour and identifying how it varies among cells (or cell types), we might be able to explain varying behaviours both within cell populations and in different cell types.

Single cell analyses of signalling systems have already revealed important information about the role of dynamics in regulating various cellular responses. For example, in mammalian cells the transcription factor nuclear factor-κB (NF-κB) shows pulses of nuclear localization on stimulation[2,3]. Single-cell analysis of luciferase expression from a synthetic NF-κB-responsive promoter suggested that the pulses are involved in maintaining target gene expression[3,4]. In *Saccharomyces cerevisiae*, the mitogen-activated protein kinase Fus3 shows oscillations in activity in response to mating pheromone[5]. The Fus3 oscillations correlate with oscillations in mating gene expression and the formation of new mating projections, as determined by fluorescence microscopy and flow cytometry using cells expressing fluorescent fusion proteins[5].

In this Perspective, we focus on the p53 network as a model for studying the dynamics of a signal transduction pathway in single