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Clinical targeting of the TNF and TNFR superfamilies

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Abstract

Inhibitors of tumour necrosis factor (TNF) are among the most successful protein-based drugs (biologics) and have proven to be clinically efficacious at reducing inflammation associated with several autoimmune diseases. As a result, attention is focusing on the therapeutic potential of additional members of the TNF superfamily of structurally related cytokines. Many of these TNF-related cytokines or their cognate receptors are now in preclinical or clinical development as possible targets for modulating inflammatory diseases and cancer as well as other indications. This Review focuses on the biologics that are currently in clinical trials for immune-related diseases and other syndromes, discusses the successes and failures to date as well as the expanding therapeutic potential of modulating the activity of this superfamily of molecules.

The tumour necrosis factor (TNF) superfamily (TNFSF) is composed of 19 structurally related proteins (ligands) that bind to one or more molecules from the TNF receptor superfamily (TNFRSF) — a family of 29 structurally similar receptors^{1,2} (TABLE 1). Members of these superfamilies initiate numerous physiological functions¹ and provide key communication systems that regulate the development and homeostasis of the immune system, nervous system, bone as well as ectodermal organs in mammals². The ligands are either membrane-anchored or soluble trimers that cluster their cognate cell surface receptors to initiate signal transduction.

In general terms, TNFSF molecules can promote survival or inflammatory signalling (for example, TNF, lymphotoxin (LT), nerve growth factor (NGF), CD40 ligand (CD40L), OX40 ligand (OX40L; also known as TNFSF4) and B cell activating factor (BAFF)) or they can induce cell death (for example, FAS ligand (FASL) and TNF-related apoptosis-inducing ligand (TRAIL)) (FIG. 1). TNF drives inflammatory activity in immune cells such as T and B lymphocytes as well as in non-immune tissue-resident cells such as fibroblasts and epithelial cells, and NGF controls the growth and maintenance of neurons and the perception of pain. The membrane-expressed form of LT (the LT $\alpha\beta$ complex) is essential for the development and maintenance of lymph node structures, whereas ectodysplasin A (EDA) is

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SUPPLEMENTARY INFORMATION See online article: [S1](#) (table)

required for the normal development of hair, teeth and sweat glands, and receptor activator of NF- κ B ligand (RANKL) has a role in promoting bone metabolism. Other molecules such as BAFF, OX40L, CD40L and LIGHT (also known as TNFSF14) control the responsiveness of many cells within the immune system, including T and B lymphocytes, whereas FASL and TRAIL promote apoptosis in multiple cell types to quell over-exuberant activity.

TNF and the soluble version of LT (LT α), along with their shared receptors TNFR1 and TNFR2, are still the best known and studied TNFSF and TNFRSF members, and many years of research have resulted in considerable success in targeting these molecules in human inflammatory diseases (TABLE 1). Five distinct antibody- or receptor-based drugs directed at blocking TNF, or TNF and LT α , are approved for treating various autoimmune and inflammatory disorders, including rheumatoid arthritis, psoriatic arthritis, juvenile idiopathic arthritis, psoriasis, ankylosing spondylitis, Crohn's disease and ulcerative colitis. Importantly, the success of targeting these molecules in inflammatory disease spurred clinical studies involving many of the other related molecules (TABLE 1). Such studies have led to the successful development of CD30-targeting antibodies that are approved for the treatment of certain cancers, RANKL-targeting antibodies that are approved for patients with osteoporosis, and BAFF-targeting antibodies that are approved for the treatment of systemic lupus erythematosus (SLE).

In this article, we review the current range of biologics targeting TNFSF and TNFRSF molecules that have been or are in clinical trials for autoimmune and inflammatory disease (FIG. 2) and cancer (FIG. 3) as well as several other indications, focusing on the challenges in their development as well as emerging therapeutic targets.

TNFSF functions and roles in disease

The unique structural features of the TNFSF ligands and receptors link these molecules to cell growth, cell survival or cell death, although some molecules can activate both inflammatory and cell death pathways (such as TNF and TNF-related weak inducer of apoptosis (TWEAK; also known as TNFSF12)), with complex regulation depending on target cell types and other extrinsic stimuli (FIG. 1). Ligand–receptor interactions may be mono- and polyvalent. For example, OX40 and its ligand (OX40L) form a monotypic ligand–receptor pair, whereas TNF, LT α , LT β and LIGHT display multivalent interactions that create a complex network of interconnected pathways^{3–7}. The membrane position of TNFSF ligands limits signalling to cells that are in direct contact; however, many of these proteins can also be released in a soluble form to act over a distance. TNFSF members display inducible or constitutive expression patterns, depending on their regulation in a specific cell type. For example, TNF expression is rapidly induced in macrophages following the recognition of pathogens, reflecting its role in early-stage inflammatory responses, whereas LT β is constitutively expressed in B cells, reflecting its role in lymphoid tissue homeostasis. TNFSFRs also show varied expression patterns — from broad cellular distribution to lineage-restricted expression — suggesting that these molecules may have a role in several human diseases.

Many of the molecules are made by — or expressed in — cells of the immune system, which implies that they may be central to autoimmune and inflammatory diseases as well as cancer. However, their function is not restricted to immune cells, as typified by the interactions between NGF and the NGF receptor (NGFR; also known as p75NTR) that regulate pain sensation, and the interactions between RANKL and RANK that control bone metabolism. The genes encoding TNFSF members involved in immune function reside in the major histocompatibility gene complex (chromosome 6) and its paralogous regions².

Furthermore, several TNFRSF molecules involved in immune function are linked on chr12p13 (for example, TNFR1, CD27 and LT β receptor (LT β R)) and its paralogous region in chr1p36 (for example, TNFR2, OX40, glucocorticoid-induced TNFR-related protein (GITR; also known as TNFRSF18), CD30, herpes virus entry mediator (HVEM; also known as TNFRSF14), 4-1BB (also known as TNFRSF9) and death domain receptor 3 (DR3; also known as TNFRSF25)), suggesting similarity in functional activity and/or synergistic regulation. Moreover, many *TNFSF* and *TNFRSF* genes have now been found to display polymorphisms that are linked to human disease (Supplementary information S1 (table)), implying that interventions targeting these molecules may be efficacious in treating several health-related problems.

Therapeutic strategies for targeting TNFSF

Following the first clinical trial of a TNF inhibitor in 1992, several types of biologics targeting TNFSF or TNFRSF molecules have been tested in the clinic. These include mouse, chimeric, humanized and fully human monoclonal antibodies as well as Fc fusion proteins containing the ectodomains of TNFRSF molecules (BOX 1). Several types of agents that bind to TNF, or to TNF and LT α , have now been approved in the United States (TABLE 1). The chimeric antibody infliximab (Remicade; Centocor Ortho Biotech) was the first TNF-directed drug to be approved in August 1998. This was followed by the approval of the TNFR2 Fc fusion protein etanercept (Enbrel; Amgen/Pfizer) in November 1998. The first fully human antibody, adalimumab (Humira; Abbott), was approved in December 2002. Certolizumab pegol (Cimzia; UCB), a pegylated Fab fragment, was approved in April 2008; and another fully human antibody, golimumab (Simponi; Centocor Ortho Biotech), was approved in April 2009 (FIG. 2; TABLE 1). Neutralization of TNF (and LT α in the case of etanercept) was the desired activity, but the molecular structure of the different inhibitors ultimately revealed their mechanism of action.

The intact bivalent antibodies and the receptor–Fc fusion protein all contain an Fc region, which — in the case of the antibodies — has strong cytotoxic activities (antibody-dependent cellular cytotoxicity (ADCC), complement activation and Fc receptor (FcR) binding), and it was widely assumed that the Fc domain had a contributory effector action on the efficacy of the TNF inhibitors. However, etanercept was subsequently found to possess weak cytotoxic activity (its Fc region has reduced FcR binding, ADCC and complement activation); the role of cytotoxicity became less clear when the Fab fragment certolizumab pegol — which is monovalent and lacks the Fc region — showed efficacy in the same clinical indications as the Fc-containing inhibitors. This does not necessarily exclude the contributions of the Fc region to efficacy in some settings, but it does indicate that the primary mechanism of action of the TNF reagents is blockade of ligand binding to the receptors (TNFR1 and TNFR2).

Similar considerations apply to denosumab (Prolia/Xgeva; Amgen), a fully human RANKL-targeting antibody that was approved for the treatment of osteoporosis in June 2010, and belimumab (Benlysta; Human Genome Sciences/GlaxoSmithKline), a fully human BAFF-targeting antibody that was approved in March 2011 for the treatment of lupus (FIG. 2; TABLE 1). The clinical outcome observed with certolizumab pegol has spurred the selection of antibodies that have antagonizing (that is, neutralizing) activity and no Fc effector activity (BOX 1), as well as the development of minimal single-chain variable fragment (scFv) region-only therapeutics (for example, ESBA105 and MEDI-578 against TNF and NGF, respectively) that lack an Fc component.

In addition to direct blockade, biologics are being used to eliminate pathogenic cells expressing TNFRSF members, either as treatments for inflammatory diseases or to directly deplete tumour cells. In this case, antibodies are being selected for natural cytotoxicity or

being engineered in the Fc region for enhanced cytotoxicity from ADCC or complement-dependent cytotoxicity (CDC) activity (BOX 1). Alternatively, they can be conjugated with a cytotoxic drug, as exemplified by brentuximab vedotin (Adcetris; Seattle Genetics), a chimeric CD30-specific antibody that was approved in August 2011 for the treatment of anaplastic large cell lymphoma and Hodgkin's lymphoma (FIG. 3; TABLE 1). Other examples of therapeutic reagents that are being tested in preclinical or clinical studies include agonist antibodies that stimulate TNFRSF signalling, RNA aptamers to TNFRSF molecules, as well as tetrameric or hexameric TNFSF ligand constructs that exert augmented agonist activity, in attempts to enhance antitumour immune responses (FIG. 3; TABLE 1).

TNFSF targets in inflammatory disease

BAFFR, TACI, BCMA–BAFF and APRIL

The discovery that BAFF and its receptors (BAFF receptor (BAFFR), transmembrane activator and CAML interactor (TACI; also known as TNFRSF13B) and B cell maturation antigen (BCMA; also known as TNFRSF17)) control the survival and/or differentiation of both naive and autoreactive B cells^{8,9} led to the emergence of BAFF as a primary target for the treatment of B cell-driven inflammatory diseases. Indeed, transgenic mice overexpressing BAFF have autoimmune symptoms that are reminiscent of SLE, with some characteristics of rheumatoid arthritis and Sjögren's disease. By contrast, mice with lupus-prone backgrounds, BAFF-deficient mouse models of rheumatoid arthritis, and mice treated with TACI–immunoglobulin (Ig) or BAFFR–Ig exhibit reduced signs of disease; TACI–Ig blocks BAFF as well as the related ligand APRIL (a proliferation-inducing ligand; also known as TNFSF13) that also binds to TACI and BCMA, whereas BAFFR–Ig blocks BAFF only. Elevated levels of BAFF have also been observed at inflammatory sites or in serum samples taken from patients with SLE, rheumatoid arthritis, multiple sclerosis and Sjögren's disease^{8,9}. Studies of animals deficient in either BAFFR, TACI or BCMA suggest that BAFFR is the primary receptor controlling BAFF-mediated survival of most peripheral naive B cells, although BAFF or APRIL activity through TACI and BCMA is thought to regulate germinal centre B cell and plasma cell survival^{10,11}.

Based on these data, BAFF antagonists have undergone clinical development and the BAFF-specific antibody belimumab was approved in 2011 in the United States, Europe and Canada for the treatment of SLE⁹. However, only patients categorized as displaying B cell dysfunction in the form of circulating antinuclear antibodies were responsive to the drug, and even within this population 40–60% of individuals did not respond significantly to the drug in Phase III trials^{12,13}. This suggests that selecting patients based on a comprehensive biomarker profile may be essential for therapeutic activity, and that combination therapy with additional reagents targeting other inflammatory molecules may be warranted to increase the efficacy of BAFF antagonists in this disease. Other antibodies have been tested in clinical trials of SLE, including BAFF-targeting antibodies (such as tabalumab; also known as LY2127399), a TACI–Ig dimeric fusion protein that blocks BAFF as well as APRIL (atacept) and a synthetic BAFF-binding polypeptide (blisibimod)^{14,15}.

BAFF antagonists have also been investigated in rheumatoid arthritis. However, because belimumab displayed only mild efficacy in a Phase II trial it was not pursued further⁹. Tabalumab has completed several Phase II trials in rheumatoid arthritis, with patients receiving methotrexate or with non-responders to methotrexate or TNF inhibitors; although no results have been reported, several Phase III trials are currently recruiting participants. Briobacept, another BAFFR–Ig dimeric fusion protein that specifically neutralizes BAFF, was in a Phase I trial in patients with rheumatoid arthritis, but its development was discontinued in 2011 owing to lack of efficacy. Atacept (a TACI–Ig fusion protein) was found to be considerably safe and well tolerated in Phase I trials in patients with rheumatoid

arthritis¹⁶ but primary end points were not met in Phase II trials involving patients who were not responsive to TNF inhibitors or methotrexate, despite evidence of biological activity of the drug^{17,18}.

BAFF has also been targeted for the treatment of multiple sclerosis. Phase II trials of atacicept in relapsing multiple sclerosis were suspended owing to enhanced rather than suppressed inflammatory activity with more lesions and relapses¹⁹. This was surprising, given the positive effects — in patients with multiple sclerosis — of the CD20-specific antibody rituximab (Rituxan; Biogen Idec/Genentech/Roche), which similarly depletes peripheral B cells. These contrasting results of the two agents may be due to the additional effect of atacicept to block APRIL activity, which regulates plasma cell survival. However, BAFFR deficiency in a mouse model of multiple sclerosis correspondingly led to earlier onset and increased severity of the disease²⁰, with the caveat that the pathology in the model used is thought to be B cell-independent — implying an alternative mechanism of action.

BAFF has also been suggested to control a subset of interleukin-10 (IL-10)-producing regulatory B cells that limit inflammation^{21,22}, but it is at present unknown whether inactivation of these cells might account for the deleterious effects observed in multiple sclerosis. Interestingly, BAFF-deficient mice or mice treated with BCMA-Ig displayed less severe experimental autoimmune encephalomyelitis (EAE) symptoms in alternative multiple sclerosis models in which B cells are thought to contribute to disease pathology^{23,24}, suggesting that BAFF blockade alone may still be an option. Tabalumab is currently being tested in an ongoing Phase II trial in relapsing–remitting multiple sclerosis, and the results from this trial may determine any future treatments in this arena. Other indications for BAFF antagonists include chronic immune thrombocytopaenia (ITP), idiopathic membranous glomerulonephropathy, renal transplantation and myasthenia gravis, and Phase II trials with belimumab or blisibimod are planned for all of these indications but have not yet started recruiting participants. BAFFR-targeting antibodies that antagonize BAFF binding and/or deplete BAFFR-expressing cells²⁵ also represent potential therapeutics.

It is not clear whether TACI, APRIL or BCMA are additional direct targets for clinical therapy. Several contrasting and conflicting phenotypes have been described for mice that are deficient in either of these three molecules, depending on the model and disease phenotype assessed^{26–31}. These data suggest that more and varied preclinical studies are required to understand the exact contributions of TACI, APRIL and BCMA to any given immune response before specific reagents for these molecules are pursued.

CD40–CD40L

CD40 is primarily known as a stimulatory receptor that regulates the activity of dendritic cells, macrophages and B cells^{32,33}. CD40 signals can promote survival in these cell types and also induce the production of inflammatory cytokines in macrophages and dendritic cells. CD40 can also participate in upregulating the expression of molecules involved in antigen presentation and T cell stimulation, including ligands in the TNF family such as OX40L and 4-1BB ligand (4-1BBL). Furthermore, CD40 promotes immunoglobulin class switching in B cells and regulates germinal centre reactions. CD40 can thus directly affect disease phenotypes that are dependent on B cell activity and antibody production as well as control diseases that are dependent on the efficient activation of T cell-mediated immunity^{32,33}. CD40 is constitutively expressed on B cells, dendritic cells and macrophages, and thus might not be a useful biomarker. However CD40L expression can be induced on T cells as well as epithelial cells, and levels of soluble CD40L in serum or CD40L expression in inflamed tissue are upregulated in patients with SLE, Sjögren's syndrome, inflammatory bowel disease (IBD), acute coronary disease, rheumatoid arthritis, multiple sclerosis and other indications³³.

Studies of CD40- or CD40L-deficient mice, or treatment with blocking reagents against CD40L, have shown that these molecules have prominent roles in driving several autoimmune and inflammatory phenotypes in models of type 1 diabetes, IBD, psoriasis, multiple sclerosis, rheumatoid arthritis, SLE, transplantation and other diseases^{32,33}. The reduction in inflammation was largely due to the suppression of T and B cell responses, which supported the clinical development of antibodies targeting CD40L. These antibodies primarily worked through neutralization, but depletion of CD40L-expressing cells — particularly T cells — may have enhanced their therapeutic activity. Although humanized or fully human CD40L-targeting antibodies (such as ruplizumab (BG9588), AB1793 and toralizumab (IDEC-131)) demonstrated biological activity in Phase I/II trials of lupus nephritis, immune thrombocytopaenic purpura, pancreatic islet and kidney transplantation, SLE, multiple sclerosis and Crohn's disease, these clinical trials were halted owing to adverse incidents involving thromboembolic events. This detrimental activity is thought to result from the crosslinking of CD40L expressed by platelets, or from the stimulation of FcRs by the antibody bound to platelets, leading to platelet activation and aggregation^{34–39}.

Although the clinical development of CD40–CD40L blockers has since been protracted, alternative feasible approaches for targeting CD40L include non-aggregating CD40L-targeting antibodies lacking Fc regions or mutated antibodies that cannot bind to FcRs on platelets. A more direct approach is to achieve neutralization by targeting CD40, and several chimeric CD40-specific antibodies (such as ch5D12 and chi220 (BMS-224819)) have been shown to be well tolerated and active in prolonging the rejection of renal or islet allografts or xenografts, or in suppressing EAE-type brain lesions, in non-human primates^{40–43}. These antibodies work primarily as antagonists but they may also exhibit some depleting activity. One CD40-specific antibody, ch5D12, was used in an open-label study in patients with Crohn's disease; it was reported to be safe and showed some evidence of clinical activity⁴⁴. A Phase I study of psoriatic arthritis with PG102, a variant of ch5D12, was initiated in 2008 but was terminated owing to poor patient recruitment. ASKP1240 (4D11), a fully human CD40-specific antibody with neutralizing capabilities, also effectively prolonged kidney and liver engraftment in non-human primates^{45,46}. This antibody appeared to be well tolerated in Phase I trials in both healthy individuals and patients who had undergone a kidney transplant, and participants are currently being recruited for a Phase II trial in psoriasis.

HVEM, LT β R–LIGHT and LT α

LIGHT, LT α and LT β are part of a multicomponent network of cytokines that utilize several shared receptors^{6,47}. LIGHT can be formed from several types of activated lymphocytes — either as a membrane-bound or soluble ligand — and binds to the receptors HVEM and LT β R, which are widely expressed on many haematopoietic cells as well as some structural cells. LIGHT (along with TNF ligand-related molecule 1 (TL1A; also known as TNFSF15) and FASL) also binds to soluble decoy receptor 3 (DCR3; also known as TNFRSF6B). Like TNF, soluble LT α binds to TNFR1 and TNFR2, but LT α also exists as a membrane-bound ligand that forms a complex with LT β (LT $\alpha\beta$); LT $\alpha\beta$ binds to LT β R.

LT $\alpha\beta$ on lymphoid tissue inducer cells interacts with LT β R on stromal cells to control lymph node development, and LT $\alpha\beta$ –LT β R crosstalk also organizes the cellular architecture of immune cells in lymphoid and non-lymphoid tissue to allow effective communication among dendritic cells, T cells and B cells in order to initiate and sustain immune responses. B cell-derived LT α and LT β also facilitate germinal centre reactions and antibody production by binding to LT β R on follicular dendritic cells. LT β R signalling can also promote dendritic cell activity by interacting with LT when it is induced on T cells, and LIGHT signalling through HVEM expressed on T cells and non-lymphoid cells can also amplify and sustain T cell responses and other inflammatory activities. In addition, HVEM has emerged as a switch between pro-inflammatory functions and inhibitory signalling, as it

engages the suppressive immunoglobulin superfamily ligands B and T lymphocyte attenuator (BTLA) and CD160.

Blockade of LT α β and LIGHT, or modulation of BTLA-dependent inhibitory pathways, substantially alters the course of inflammatory and autoimmune disease, as shown in several mouse models^{4,6,47,48}. However, the complexity of the LIGHT–LT α β network in activating pro-inflammatory and inhibitory pathways and in controlling lymphoid tissue organization demonstrates the need to design a biologic that is capable of arresting inflammatory signalling while preserving or agonizing the inhibitory pathway and also maintaining protective immunity to pathogens.

A soluble immunoglobulin Fc decoy receptor of LT β R (baminercept; also known as BG9924) entered a Phase II trial in rheumatoid arthritis and was designed to competitively inhibit LIGHT and LT α β from binding to LT β R and HVEM without affecting the inhibitory signalling from HVEM–BTLA (or HVEM–CD160) interactions. Although this manufacturer-sponsored trial was curtailed owing to an inability to achieve an ACR-50 score end point within 3 months, further analysis revealed substantial changes in selected biomarkers and clinical improvement in a subset of patients, and baminercept is currently in a Phase II trial sponsored by the US National Institutes of Health (NIH) for the treatment of Sjögren's syndrome.

Antibodies targeting LIGHT or LT α are also in clinical development for autoimmune diseases. A humanized LT α -targeting antibody (pateclizumab; also known as MLTA3698A), possessing ADCC activity, was used to deplete T cells in a xenogenic model of graft-versus-host disease (GVHD) and resulted in disease amelioration⁴⁹, and in a Phase I study of rheumatoid arthritis it was shown to be safe with mild to moderate adverse events⁵⁰. The development of pateclizumab was triggered based on earlier data in mouse models of delayed-type hypersensitivity, multiple sclerosis and rheumatoid arthritis, which showed that depletion of LT α -expressing T cells ameliorated disease⁵¹. A second Phase I trial is planned to compare pateclizumab with adalimumab, concurrent with leflunomide and methotrexate treatment, in patients with rheumatoid arthritis. A fully human LIGHT-specific antibody (SAR252067) that competitively inhibits the binding of LIGHT to LT β R, HVEM and DCR3 has also been developed⁵². Mouse models and polymorphisms in components of the LIGHT network suggest that inflammatory diseases affecting mucosal tissue may be possible first clinical indications^{47,48,53–55}.

OX40–OX40L

OX40 is a stimulatory receptor that is expressed on activated T cells, natural killer (NK) cells and natural killer T (NKT) cells. A genetic deficiency in *Ox40* or *Ox40l* in mice, or blockade of OX40L, has revealed strong activities of these molecules in driving autoimmunity or inflammation in various different models of asthma, colitis, GVHD, diabetes, multiple sclerosis, rheumatoid arthritis, atherosclerosis and transplantation^{5,7,56}. Preclinical data supported asthma as a primary indication^{57,58}, and oxelumab — a neutralizing fully human OX40L-specific antibody — was tested in a Phase II study of patients with mild allergic asthma. Disappointingly, the primary end point (reduction in forced expiratory volume in 1 second in response to allergen challenge) was not met, which may be due to the short length of the trial and the fact that a non-stratified mild asthmatic patient population was treated. Related to these findings, original data from mouse models showed that OX40L was active in a brief time frame following exposure to a model allergen⁵⁷, and — coinciding with this — recent studies of patients with asthma revealed substantially enhanced levels of soluble OX40L in the serum during an acute asthma attack, with the highest levels seen in those individuals experiencing the most severe reactions^{59,60}. These results suggest that in order to be effective, the neutralization of OX40L might need

to coincide with active asthma exacerbations and that the target population should be patients with moderate to severe asthma rather than mild allergic asthma.

Targeting OX40–OX40L interactions might also be therapeutically relevant in GVHD, transplantation and IBD^{7,56}, but correct timing of treatment and the identification of relevant patients using biomarkers is crucial. Expression of OX40 or OX40L has been reported in tissue or blood samples from patients with inflammatory disease^{7,56}, but a comprehensive study is lacking for any one particular syndrome.

CD30–CD30L

CD30 is another stimulatory receptor that is expressed on activated T cells and other pro-inflammatory immune cells. Knockout or pharmacological neutralization of CD30 or CD30L in mice alleviates diabetes, asthma, colitis and GVHD, and there is strong synergism between CD30 and OX40 in driving immunity^{3,7,61,62}. Levels of soluble CD30 or CD30L are high in serum or tissue samples of patients with atopic dermatitis, systemic sclerosis, IBD, SLE, rheumatoid arthritis, GVHD and other diseases, correlating with disease severity in some instances. It is not clear whether soluble CD30 is representative of the activity of CD30–CD30L interactions, but it is likely that this information will be useful for future directed targeting. As yet, no clinical studies have pursued CD30- or CD30L-specific antagonists, but a Phase I study of a drug-conjugated CD30-specific antibody (brentuximab vedotin (SGN-35)) is currently being planned for GVHD. Brentuximab vedotin can kill CD30-expressing cells and is already approved for the treatment of some cancers (as discussed below), providing a rationale that depleting CD30⁺ effector T cells should inhibit GVHD and possibly result in immunological tolerance to the graft.

FN14–TWEAK

TWEAK and its receptor FGF-inducible 14 (FN14; also known as TWEAKR) are implicated as a key signalling pathway for regulating the complex interactions among immune cells and the epithelium, endothelium and other cell types involved in shaping tissue responses during inflammation^{63–65}. TWEAK is produced by monocytes, dendritic cells and NK cells, among others, primarily as a soluble molecule, whereas its receptor is widely expressed in tissue parenchymal cells. The TWEAK–FN14 pathway is typically dormant but becomes activated in response to injury and disease⁶⁶. Acute activation of FN14 enhances productive tissue repair after insult, whereas excessive or persistent FN14 activation mediates tissue-damaging responses and tissue degeneration⁶⁷. Elevated soluble TWEAK levels have been observed in patients with lupus nephritis, rheumatoid arthritis and IBD, as well as in brain tissue from active multiple sclerosis lesions. Thus, interfering with the TWEAK–FN14 pathway in chronic inflammation may alter tissue damage, and reduced inflammation or pathology has been observed in several mouse models of lupus or kidney disease⁶⁸. In patients with rheumatoid arthritis, a Phase I trial with a TWEAK-specific neutralizing antibody (BIIB023) revealed a trend towards decreased expression of inflammatory biomarkers, and this antibody was well tolerated⁶⁸. As a result, Phase II trials of lupus nephritis involving both glomerular and tubular regions have been initiated and are currently recruiting participants to assess the efficacy of BIIB023.

Emerging novel targets

Substantial preclinical evidence has accumulated to support the potential of several additional TNF family members as therapeutic targets for autoimmune and inflammatory diseases.

For example, GITR-deficient mice or mice treated with GITR ligand (GITRL)-blocking reagents exhibit reduced inflammatory symptoms in models of gut and lung inflammation,

diabetes, pancreatitis, spinal cord injury, allograft rejection and other diseases^{7,69,70}. GITR can be expressed on many lymphoid cells — including T cells, dendritic cells and B cells — and GITRL is inducible on dendritic cells, macrophages and B cells as well as other cells such as endothelial cells. *In vitro* studies show that signalling through GITR or GITRL on macrophages promotes activities associated with atherosclerosis or arthritis, which correlates with the expression of both GITR and GITRL on foam cells and/or macrophages in human atherosclerotic plaques and in synovial tissue sections and synovial fluid from patients with rheumatoid arthritis^{71–73}. Expression of soluble GITRL might represent a useful biomarker signifying the activity of GITR–GITRL interactions. Increased levels of GITRL were found in serum samples taken from patients with rheumatoid arthritis, possibly correlating with mouse models showing that soluble GITRL potentiated arthritis and also correlating with GITR-deficient animals that displayed reduced joint inflammation^{74,75}. Overall, these findings indicate that blocking GITR–GITRL interactions may be applicable for the treatment of inflammatory disease.

4-1BB is also a stimulatory receptor for T cells, particularly CD8⁺ and cytotoxic T lymphocyte (CTL) subsets^{5,7,76}. Mouse studies of rheumatoid arthritis, GVHD, allograft rejection, atherosclerosis and sepsis have implicated 4-1BB–4-1BBL interactions in the development of inflammatory disease^{5,7,76}. Elevated levels of an alternatively spliced form of 4-1BB (produced as a soluble molecule) as well as soluble 4-1BBL have been found in serum samples taken from subsets of patients with rheumatoid arthritis, multiple sclerosis and systemic sclerosis, correlating with active disease in some cases^{77–80}. The functional relevance of these soluble molecules — that is, whether they are pro-inflammatory or anti-inflammatory — is not clear. However, 4-1BB has also been found to be expressed on various pathogenic cells from atopic individuals and from patients with Crohn's disease and atherosclerosis, suggesting that 4-1BB has a pro-inflammatory role^{81,82}. These results suggest that antagonists of 4-1BB or 4-1BBL might have applications in the treatment of inflammatory disease, but as yet these targets have received little clinical attention.

Unexpectedly, targeting 4-1BB with agonist antibodies also strongly suppressed inflammation and autoimmunity in mouse models of rheumatoid arthritis, asthma, GVHD and multiple sclerosis^{5,76,83}. This effect is most likely to be explained by the expansion of a subset of CD8⁺ T cells with a regulatory and suppressive capacity that can block disease driven by CD4⁺ T cells^{84,85}. Potentially in line with these mouse studies, a humanized 4-1BB-specific mouse antibody was found to suppress a T cell-dependent antibody response in baboons⁸⁶. However, the antibody displayed ADCC activity as well as agonistic activity, suggesting that depletion of 4-1BB⁺ cells might have been involved in this therapeutic effect. Although these results are intriguing, there may be strong reservations from the pharmaceutical industry in considering the use of an agonist targeting a T cell-expressed stimulatory receptor in autoimmunity. Indeed, 'cytokine storms' (also known as cytokine release syndrome) were observed in patients treated with a CD28 agonist that binds to a similar stimulatory receptor in an unrelated protein superfamily that is constitutively expressed on T cells⁸⁷. By contrast, 4-1BB is inducible on most T cells, and 4-1BB agonists have already been used to treat patients with cancer (as discussed below) without having any apparent evidence of massive deregulated cytokine production. However, as noted below, some hepatic toxicity was reported in these patients, possibly due to excessive CD8⁺ T cell or NK cell activity, which will probably impede any future development of 4-1BB agonists until further insights are gained.

Additional preclinical studies indicate that TL1A and DR3 may represent promising therapeutic targets. DR3 is another stimulatory receptor for T cells and other lymphoid cells, and TL1A can be expressed by antigen-presenting cells such as dendritic cells^{5,7,88,89}. The interaction of TL1A with DR3 has primarily been linked to forms of IBD, based on strong

genome-wide and selective association studies in humans (Supplementary information S1 (table)), and enhanced expression of these molecules in sera or on T cells or macrophages from intestinal tissues has been observed in patients with ulcerative colitis and Crohn's disease. TL1A also binds to the soluble DCR3 shared with LIGHT, although the significance of this is presently unclear. Studies in TL1A-knockout mice or TL1A-transgenic animals have also shown reduced or spontaneous gut inflammation, respectively^{5,7,88,89}. However, as seen with other TNFSF molecules, increasing evidence suggests that the potential activities of TL1A and DR3 extend to many inflammatory responses. Disease severity is reduced in mouse models of multiple sclerosis, asthma and arthritis when the genes encoding TL1A or DR3 are deleted or when mice are treated with neutralizing agents. Elevated TL1A expression has also been observed in the joint tissue of patients with rheumatoid arthritis, during acute rejection of kidney transplants, on macrophages and/or foam cells in atherosclerotic plaques and in skin biopsy samples taken from patients with psoriasis^{73,90–92}.

Neutralizing the interaction of CD27 with CD70 also has the potential to dampen inflammatory disease activity, either as a stand-alone therapy or in combination with the inhibition of other members of the TNF family. The interaction of CD27 with CD70 can provide proliferative and survival signals to T cells, in the same way as OX40, 4-1BB, DR3 and other molecules, and activate additional lymphoid cells such as B cells. Blockade or knockout of CD27 or CD70 in mouse models have also revealed their key roles in the pathology of multiple sclerosis, rheumatoid arthritis, colitis, GVHD, asthma and allograft rejection^{3,5,7,93}. Soluble CD27 has been found in patients with several diseases but it can be shed from strongly stimulated T cells, suggesting that this may simply be a reflection of immune cell activation. CD70 expression can be induced in dendritic cells, B cells and T cells, and detection of soluble CD70 might arguably be more indicative of CD27–CD70 activity. However, there is a lack of analyses in the literature regarding the role of soluble CD70 in disease.

RANKL and its cell surface receptor RANK may also represent promising therapeutic targets in inflammatory disease. These molecules were originally identified as regulators of bone metabolism and, as indicated previously, RANKL is an approved clinical target for the treatment of osteoporosis. However, RANKL–RANK interactions between T cells and antigen-presenting cells such as dendritic and Langerhans cells also enhance immune cell function, suggesting that this pathway has important roles in regulating aspects of autoimmunity^{94,95}. RANKL expressed on T cells can also stimulate RANK-expressing osteoclasts, leading to bone resorption, which implies that the bone loss seen in patients with T cell-mediated inflammatory diseases such as rheumatoid arthritis may be triggered by these molecules.

Finally, the interactions of FASL with FAS that result in the apoptotic death of FAS-expressing tissue cells might represent another target for the treatment of selected inflammatory diseases. Data from mouse models have suggested that the cytotoxic activity of T cell-expressed FASL contributes to the pathology of GVHD but is not involved in the beneficial graft-versus-leukaemia effect^{96–98}, which indicates that specific blockade of FASL–FAS interactions may offer therapeutic benefits in this setting.

Targeting TNFSF molecules in cancer

BAFFR, TACI, BCMA–BAFF and APRIL

Various studies have supported the notion of blocking APRIL or BAFF as a form of cancer therapy, because these molecules are growth and survival factors for B cells and may directly contribute to the growth of B cell tumours. High levels of BAFF have been found in

patients with B cell malignancies including Hodgkin's lymphoma⁹⁹. APRIL can also be expressed by solid tumours; various tumour cells express the receptors TACI and BCMA, and are thus responsive to growth signals from APRIL or BAFF^{100–102}. Moreover, data from patients with chronic lymphocytic leukaemia (CLL) suggest that increased levels of APRIL correlate with tumour progression. As a result, several trials have already taken place. No safety issues were reported and possible protective effects on tumour progression were observed in Phase I studies of atacept (TACI-Ig) in patients with relapsed or refractory non-Hodgkin's lymphoma, multiple myeloma or Waldenström's macroglobulinaemia (also known as lymphoplasmacytic lymphoma)^{103–105}. A Phase II trial of belimumab for treating Waldenström's macroglobulinaemia is currently recruiting participants, and Phase II/III trials of tabalumab in multiple myeloma are planned but not yet recruiting participants. Specific APRIL antagonists are also being studied to block the survival of B cell lymphoma¹⁰².

CD40–CD40L

Stimulating CD40 to augment the activity of dendritic cells, macrophages, B cells and — indirectly — T cells is an attractive approach for cancer therapy and is highly feasible owing to the constitutive expression of CD40 on such immune cells³³. Several strategies are being, or have been, tested in clinical trials. It is widely acknowledged that stimulating CD40 alone may not be very efficacious, and so combination treatments that include IL-2 or granulocyte-macrophage colony-stimulating factor (GM-CSF) are being tested. These include adoptive immunotherapy with autologous tumour cells or fibroblasts transfected with the gene encoding CD40L, intratumoural injection of adenoviral vectors encoding CD40L or the injection of recombinant CD40L³³. Most of these clinical trials are currently in Phase I development or recruiting participants. A more direct approach is to use CD40-targeting agonistic antibodies. CP-870893, a fully human antibody, induced partial responses in 15–20% of patients with advanced solid tumours, including melanoma and pancreatic adenocarcinoma, in several Phase I trials^{106–108}. It is being tested further in combination with chemotherapy, a neutralizing cytotoxic T lymphocyte antigen 4 (CTLA4)-specific antibody or with the Toll-like receptor 3 (TLR3) ligand poly-ICLC.

An alternative approach is to use CD40-specific reagents to directly kill CD40⁺ tumours, typified by B cell lymphomas. These reagents might be efficacious through ADCC or through direct CD40-mediated signalling that can result in apoptosis in these cancers. Dacetuzumab (SGN-40, S2C6), a humanized depleting and agonistic CD40-specific antibody^{109,110}, has completed Phase I trials in CLL, non-Hodgkin's lymphoma and multiple myeloma; Phase I or II trials alone or with chemotherapy in relapsed large B cell lymphoma; and Phase I trials with a proteasome inhibitor or lenalidomide in multiple myeloma and with rituximab in relapsed CD20⁺ B cell non-Hodgkin's lymphoma. Dacetuzumab was well tolerated but there were some signs of cytokine release syndrome^{111,112}. Lucatumumab (HCD122/CHIR-12.12) is another depleting CD40-specific antibody with ADCC activity¹¹³ that is being tested in a Phase I/II trial of non-Hodgkin's or Hodgkin's lymphoma, and a trial with CD40⁺ relapsed follicular lymphoma is also being planned. However, Phase I trials in advanced CLL and multiple myeloma were terminated, partly owing to minimal biological activity and efficacy¹¹⁴. Finally, a chimeric CD40-specific agonist, Chi Lob 7/4 (REF. 115), which also possesses CDC and ADCC activities, will be tested in a Phase I trial in B cell non-Hodgkin's lymphoma.

OX40–OX40L

As OX40 signals can strongly promote the activity of CD4⁺ and CD8⁺ T cells, as well as NK cells⁵, OX40 is an obvious target for cancer therapy, and numerous tumour studies in mice using agonistic reagents (such as OX40-specific antibodies, OX40L–Fc fusion proteins

and RNA aptamers targeting OX40) or transfection of tumour cells with OX40L have shown feasibility^{5,56}. In models with highly immunogenic tumours, OX40 agonists are highly effective in blocking tumour growth; however, in less immunogenic tumours OX40 reagents need to be combined with other treatments such as GM-CSF, IL-12, chemotherapy or other agonists of T cell-expressed receptors⁵. OX40 is not constitutively expressed, but several reports have found that it is present on tumour-infiltrating T cells in patients with melanoma, breast cancer, colorectal cancer and other cancers^{116–118}. In spite of this, where analysed, only a fraction of patients exhibited OX40⁺ cells associated with the tumour, and a study of sentinel (first tumour-draining) lymph nodes suggested that the number of these cells decreased in more advanced tumours¹¹⁹. Either a method to increase OX40 expression or careful selection of patients for OX40 expression may therefore be required for optimal efficacy.

A mouse OX40-specific agonist exhibited stimulatory activity without any safety issues in non-human primates¹²⁰, leading to a Phase I trial in patients with advanced solid tumours. No toxicity or signs of autoimmunity were reported, and some patients experienced tumour regression (see the [Providence Health & Services](#) website). Currently, a Phase II trial is being planned with the OX40-specific antibody in metastatic melanoma, and a Phase Ib trial is being planned for the treatment of metastatic prostate cancer in combination with cyclophosphamide and radiation therapy. However, as the current reagent is a mouse antibody, second-generation fully human or humanized antibodies need to be developed to allow repeated treatments.

4-1BB–4-1BBL

Agonists of 4-1BB, or forced expression of 4-1BBL on tumour cells or antigen-presenting cells, have shown substantial antitumour effects in several murine models of cancer, enhancing CD4⁺ and CD8⁺ T cell and NK cell activity^{3,5}. A fully human 4-1BB-specific antibody (BMS-663513) showed some bioactivity in an initial Phase I trial of a combined group of patients with melanoma, renal cell carcinoma and ovarian cancer, and the drug had tolerable side effects¹²¹. This led to expanded dose-escalating trials of the agent alone or in combination with chemotherapy or radiation treatment, including a Phase II trial in melanoma.

However, a considerable degree of liver toxicity was observed with higher doses of the antibody, leading to the suspension and termination of several trials. It is not clear why hepatitis was observed. 4-1BB is expressed on NK cells that are commonly present in the liver, and these cells may be activated nonspecifically. High or repeated doses of strong 4-1BB agonists injected into naive or irradiated mice resulted in substantial adverse effects, including altered haematopoiesis, lymphopenia and hepatomegaly, and enhanced the accumulation of CD8 T cells in the liver^{122,123}. Anti-4-1BB injection also augmented CD8 T cell activity in the liver in mouse models of chronic hepatitis¹²⁴, and patients with primary biliary cirrhosis expressed higher levels of soluble 4-1BBL and *4-1BBL* mRNA¹²⁵, perhaps correlating with enhanced 4-1BB–4-1BBL interactions during disease progression.

Currently, a clinical trial is recruiting patients with advanced or metastatic solid tumours to test lower doses of BMS-663513. Another anti-4-1BB agonist (PF-05082566)¹²⁶ is to be tested alone or in combination with rituximab (a CD20-specific antibody) in a planned Phase I trial in patients with CD20⁺ non-Hodgkin's lymphoma. An alternative strategy may be to select less potent agonistic antibodies for clinical use, or to pursue other means of targeting 4-1BB. In mice, a streptavidin conjugate of 4-1BBL, which results in the production of tetramers or oligomers with agonistic activity for 4-1BB, was suggested to have equivalent T cell-stimulatory activity compared to an antibody, but without any apparent toxicity¹²⁷.

Another option could be the use of multivalent RNA aptamers that have also shown activity in mouse tumour models¹²⁸.

A second cancer immunotherapy approach involving 4-1BB has also been tested. This involved the introduction — into T cells — of a chimeric antigen receptor of a CD19-specific antibody scFv region linked to the intracellular signalling domains of CD3 ζ -chain and 4-1BB. This resulted in enhanced T cell stimulation when binding to CD19 and allowed effective and optimal killing of CD19⁺ acute lymphoblastic leukaemia (ALL) cells¹²⁹. The approach was recently translated into the clinic with mixed results. An initial study with a variant vector encoding the HER2 (also known as neu)-specific antibody trastuzumab (Herceptin; Genentech/Roche), the intracellular domain of 4-1BB and the signalling domain of CD28 produced disastrous results; the infusion of a high number of T cells ($\sim 10^{10}$) led to rapid pulmonary infiltrates and death of the patient¹³⁰. It is not clear whether this was due to excessive T cell stimulation as a result of co-expression of the 4-1BB and CD28 signalling domains, whether it was due to co-treatment of the patient with cyclophosphamide (which would have caused lymphodepletion and aided further expansion of the transferred T cells) or simply due to the infusion of too many T cells or as a result of the expression of HER2 in the lung itself. However, encouragingly, a more recent pilot trial using a moderate dose ($\sim 10^7$) of autologous T cells, transfected with a lentivirus encoding the aforementioned CD19–4-1BB construct, showed therapeutic activity in three out of three patients with advanced CLL and resulted in complete remission in two of these patients^{131,132}. Several Phase I trials are now recruiting participants.

GITR–GITRL

GITR agonists also have the potential to augment the antitumour activity of T cells, and their use in clinical cancer immunotherapy is being considered on the basis of strong preclinical data from murine models with anti-GITR treatment or forced expression of GITRL^{69,70,133}. However, signalling through either GITRL on tumour cells or GITR on human NK cells may indirectly or directly impair rather than stimulate the cytotoxic activity of human NK cells⁶⁹, potentially posing an obstacle to future therapy in this area. Results from clinical trials will determine whether any potential negative effects of targeting GITR outweigh the possible positive effects. A Phase I study of a humanized Fc-disabled (aglycosylated), GITR-specific agonist antibody (TRX518)¹³⁴ in unresectable stage III or IV melanoma is currently recruiting participants. Based on preclinical murine studies¹³⁵, a Phase I trial was also recruiting patients to test the activity of autologous dendritic cells transfected with RNA encoding melanoma tumour-associated antigens and GITRL. At the time of publication, this trial was suspended and awaiting funding.

CD27–CD70

As many tumours express CD70, it is a target for depleting antibodies or drug-conjugated antibodies in cancer immunotherapy. Preclinical *in vitro* and *in vivo* experiments have shown the cytotoxic properties of anti-CD70–toxin conjugates against renal cell carcinoma and non-Hodgkin's lymphoma^{136,137}, and several reagents are currently being tested in clinical trials. SGN-75, a humanized CD70-specific antibody (SGN-70)¹³⁸ linked to a toxin of monomethyl auristatin F (MMAF)¹³⁹, is in a Phase I trial in patients with CD70⁺ relapsed–refractory non-Hodgkin's lymphoma and renal cell carcinoma. A Phase I trial with another CD70-specific antibody (MDX-1411) is underway in renal cell carcinoma. However, a trial with a CD70-specific antibody linked to a cytotoxic DNA minor-groove binding agent (MDX-1305) in renal cell carcinoma and relapsed–refractory B cell non-Hodgkin's lymphoma was suspended in 2012. As with other depletion strategies, there is the inherent potential that lymphoid cells expressing CD70 may be inadvertently depleted, putting the patient at risk of developing infection. It was not disclosed whether this was the

reason for suspending the latter study, but as CD70 is generally not constitutively expressed, adverse events might be rare.

Agonists of CD27, by virtue of promoting the activation and expansion of T cells and NK cells, also have potential in the treatment of cancer. As CD27 is constitutively expressed on most T cells, there is no issue with its availability for targeting — unlike some of the inducible TNFRSF molecules such as OX40 or 4-1BB. However, there is a potential for adverse events such as a cytokine storm if overt stimulation occurs. A human CD27-specific antibody (1F5; also known as CDX-1127) displayed a good safety profile in non-human primates. This antibody was reported to have agonistic properties but it suppressed the growth of Raji and Daudi tumours in severe combined immunodeficient (SCID) mice, suggesting that it can also affect deletion through ADCC¹⁴⁰. A Phase I study with CDX-1127 in patients with refractory or relapsed B cell malignancies or solid tumours is currently recruiting participants.

Another approach is to introduce CD70 into dendritic cells to aid antitumour T cell responses by ligating CD27. An ongoing Phase I/II trial in patients with PMEL (also known as GP100)-positive melanoma is testing the safety and efficacy of the adoptive immunotherapy consisting of dendritic cells expressing GP100 and transfected with CD70, CD40L and TLR4 (known as TriMix-DC). Another trial is planned that is combining TriMix-DC with the CTLA4-specific antibody ipilimumab (Yervoy; Bristol-Myers Squibb). Transfection of CD70 alone or in combination with other immunomodulators has been shown to be effective in murine tumour models⁵, and a partial response with no further tumour progression was previously reported in one patient receiving TriMix-DC in a preclinical study¹⁴¹.

CD30–CD30L

Agonistic reagents targeting CD30 may have potential applicability in generalized cancer immunotherapy, as CD30 stimulation should also increase the antitumour activity of T cells. However, CD30 was recognized as a marker for Hodgkin's lymphoma and anaplastic large cell lymphoma (ALCL) many years ago, leading to studies targeting CD30 to directly induce death in these tumour cells. An initial study with a mouse anti-CD30–toxin conjugate showed promising efficacy, but this was short-lived in part owing to the development of human anti-mouse antibodies¹⁴². Various second-generation reagents have been made¹⁴³, including SGN-30 — a chimeric version of a mouse monoclonal antibody (AC10) — which has agonistic activity that results in growth arrest and apoptosis in ALCL and Hodgkin's lymphoma cell lines¹⁴⁴. Several Phase II trials of Hodgkin's lymphoma or ALCL demonstrated a response of up to 70% in patients with ALCL and evidence of disease stabilization over 6–16 months, but only showed modest activity in patients with Hodgkin's lymphoma^{145–147}.

Brentuximab vedotin (SGN-35), the SGN-30 antibody linked to five units of monomethyl auristatin E (MMAE) — a synthetic cytotoxic molecule that blocks division and promotes apoptosis — was then generated to improve toxicity¹⁴⁸; Phase I and II trials with repeated dosing of SGN-35 have shown promising efficacy in patients with Hodgkin's lymphoma and ALCL^{149,150}. Brentuximab vedotin received accelerated approval by the US Food and Drug Administration (FDA) in August 2011 for the treatment of ALCL and Hodgkin's lymphoma, and is the first drug to be approved for Hodgkin's lymphoma in 30 years. Various Phase I and II trials are currently underway or planned to extend the use and maximize the activity of CD30-specific antibodies.

Other antibodies are also in development (for example, XmAb2513 and MDX-1401). As CD30 is largely restricted to activated immune cells, adverse events using toxin-conjugated

antibodies might be limited, as observed in the reported trial data with brentuximab vedotin^{149,150}. However, agonist activity may result in other complications. A Phase II trial of unconjugated SGN-30 with chemotherapy (for example, gemcitabine, vinorelbine and pegylated liposomal doxorubicin) in patients with relapsed Hodgkin's lymphoma was halted owing to pulmonary toxicity in 16% of treated patients¹⁵¹. An alternative approach for targeting CD30 has been considered, which involves introducing a chimeric antigen receptor of a CD30 scFv region and the CD3 ζ -chain into Epstein–Barr virus (EBV)-specific CTLs for adoptive immunotherapy in Hodgkin's lymphoma and non-Hodgkin's lymphoma associated with EBV infection^{152,153}. A Phase I trial is currently recruiting patients to test the efficacy of this therapy.

TRAILR–TRAIL

TRAIL was initially identified as a specific inducer of apoptosis in transformed human cells, and TRAIL-deficient mice showed enhanced tumorigenesis and metastasis, supporting a role for TRAIL in anticancer defence¹⁵⁴. Furthermore, the TRAIL receptors (TRAILRs) are expressed on many tumour cells^{155,156}. TRAIL signalling is complex as TRAIL binds to two death receptors (TRAILR1 (also known as DR4) and TRAILR2 (also known as DR5)) and two 'decoy' receptors that lack death domains (TRAILR3 (also known as DCR1) and TRAILR4 (also known as DCR2)). In addition, TRAIL binds to the soluble ligand osteoprotegerin (OPG), which can inhibit TRAIL-mediated apoptosis¹⁵⁴.

Both recombinant TRAIL and agonistic TRAILR antibodies are in various stages of clinical trials. Dulanermin is one form of TRAIL that is produced as a soluble homotrimer and has displayed no apparent toxicity in animal models or Phase I/II trials^{155–158}. Dulanermin has been tested primarily in combination with other chemotherapeutic drugs and has shown encouraging trends towards antitumour efficacy¹⁵⁸. However, recent Phase II results in combination with chemotherapy showed no improvement in patients with non-small-cell lung cancer with respect to primary end points¹⁵⁹. It remains to be seen whether further stratification of patient populations will reveal a therapeutic benefit of dulanermin. Forms of TRAIL that encode epitope tags or oligomerization domains have also been pursued in preclinical studies. However, their propensity to form higher-order aggregates has hampered their further clinical development owing to their potential for inducing off-target hepatotoxicity¹⁶⁰.

As an alternative, various TRAILR-specific antibodies are also in clinical trials¹⁵⁵. Promising results from Phase I and Phase II trials have been reported with mapatumumab, a TRAILR1-targeting antibody that has been administered as a monotherapy or in combination with chemotherapy to a limited number of patients with non-Hodgkin's lymphoma^{155,161,162}. Phase II trials of mapatumumab in combination with chemotherapy are currently in progress for patients with multiple myeloma, non-small-cell lung cancer and hepatocellular cancer. Several TRAILR2-specific antibodies are also being tested. Conatumumab has completed and is undergoing Phase I/II clinical trials, primarily in combination with chemotherapeutics or other biologics¹⁶³. Phase I trials showed no overt toxicity, with biological markers indicating agonistic activity. However, Phase II results in patients with advanced soft tissue sarcomas showed no additional benefit over doxorubicin¹⁶⁴. Phase II studies of conatumumab in metastatic pancreatic cancer in combination with gemcitabine showed a trend towards increased 6-month survival but no difference from placebo in overall survival or response rate¹⁶⁵. Several additional Phase II trials of conatumumab will reach completion in 2013.

Drozitumab is another fully human TRAILR2 agonist being used as a single agent or in combination therapy that was well tolerated and active in a Phase I trial¹⁶⁶. Drozitumab enhances apoptosis of tumour cells after binding to both inhibitory and activating Fc γ Rs in

mouse tumour xenografts¹⁶⁷, which suggests that it may also exert ADCC activity. Lexatumumab has also completed or is in several Phase I/Ib trials. Similarly to other TRAILR2-targeting agonistic antibodies, lexatumumab was well tolerated but no data have been reported regarding its antitumour activity or patient outcomes. Tigatuzumab is a humanized TRAILR2-specific antibody¹⁶⁸ that was well tolerated as a monotherapy in Phase I trials¹⁶⁹ and is currently in Phase II trials in patients with pancreatic cancer. LBY135 is a chimeric antibody that was also safe as a monotherapy and in combination with capecitabine¹⁷⁰. However, 21% of patients receiving LBY135 did develop human anti-chimeric antibodies.

Last, a Phase I trial with HGSTR2J — another TRAILR2-targeting antibody — was initiated but no results were reported. The development of both LBY135 and HGSTR2J has been discontinued. In general, the current results suggest that TRAILR-targeting antibodies may be useful for cancer therapy but will probably need to be administered as part of a combination therapy to show significant efficacy, and patient stratification will also be required.

FN14–TWEAK

FN14 is expressed on many tumour cells and FN14 signalling can induce apoptosis in some transformed cells, spurring the investigation of this pathway for the killing of solid tumours⁶³. Two humanized agonist antibodies that act as surrogate ligands and directly target cancer cells have been developed to target FN14 (BIIB036 and enavatuzumab (also known as PDL192))^{171–174} and both antibodies effectively inhibit the growth of tumours in multiple xenograft models in mice, including colon, breast and gastric tumours, when given alone or in combination with chemotherapy. Fc-dependent effector function is required for maximal activity, suggesting that agonist signalling and ADCC contribute to *in vivo* tumour-inhibitory activity. A Phase I trial of enavatuzumab in individuals with advanced solid tumours was completed in 2011; however, of concern, some liver and pancreatic enzyme toxicity was observed. In other scenarios, TWEAK can have pro-inflammatory and proangiogenic activity that may favour tumour invasion or migration, leading to the suggestion that blocking its activity could have therapeutic benefits in certain cancers. A Phase I trial of the humanized TWEAK-specific antibody RO5458640 in patients with advanced solid tumours is currently recruiting participants.

Emerging novel targets

The strong activity of LIGHT–HVEM interactions in stimulating immune cells such as T cells and NK cells may also result in the development of future clinical strategies to promote antitumour immunity. LIGHT expressed in cytotoxic T cells, or forced expression of LIGHT in tumour cells, drives a sustained T cell response that has a tumour-eliminating effect in mouse models¹⁷⁵. LIGHT can also activate LT β R on structural cells to induce chemokines such as CC motif chemokine 21 (CCL21) that help to create a microenvironment around the tumour in which antitumour T cells differentiate^{175,176}. Therefore, recombinant LIGHT, forced LIGHT expression or HVEM and LT β R agonists might all represent opportunities for promoting an antitumour immune response during cancer therapy^{177,178}.

Blockade of inhibitory signalling by disrupting BTLA–HVEM interactions is also being considered as a mechanism for enhancing immune responses, akin to CTLA4 blockade (for example, as with ipilimumab, which was recently approved for melanoma therapy). In contrast to therapy that might promote LT β R signalling, several studies in mice have suggested that there may be a link between LT $\alpha\beta$ –LT β R activity that results as a protective mechanism to combat viral infection or injury as well as the development of certain types of cancer, such as liver or prostate cancer^{179,180}. If this applies to the aetiology of some human

tumours, it will indicate that blocking LT α or LT β interactions could be an alternative future therapeutic approach for selected cancers.

The death receptor FAS is similar to the TRAILR and promotes caspase-dependent apoptosis in various cell types, which suggests that it could also be a target for cancer therapy. FAS–FASL interactions are strong regulators of immunity, usually limiting responses^{181,182}. However, several human tumours can express FAS, leading to interest in reagents that agonize the molecule to directly induce death¹⁵⁶. Along with LIGHT and TL1A, FASL also binds to DCR3. DCR3 is upregulated in tumour cells, which suggests that this is a potential means through which tumours might evade death by blocking FASL-induced signals¹⁸³. Agonist antibodies targeting FAS, or FASL expressed through adenoviral vector transfer, have shown antitumour activity in murine models, although this approach has been associated with hepatotoxicity and has therefore generated less interest than TRAILR-targeting antibodies¹⁸⁴. Alternative reagents that might exhibit agonistic activity but low toxicity have been pursued, and a recombinant hexameric form of FASL (APO010) was found to reduce tumour survival *in vitro* and in animal tumour models^{185,186}. A Phase I trial of APO010 was planned in 2007 in patients with solid tumours but the study was not carried out.

Finally, as DR3 is a stimulatory receptor for T cells and NK cells, it may also be amenable for targeting with agonist reagents, or with forced expression of TL1A, to enhance antitumour responses. Little attention has been given to this possibility at present, with only a single murine study investigating this type of activity.

Other indications

Osteoporosis and bone erosion

Early studies of RANK, OPG and RANKL in mice showed that genetic or pharmacological manipulation of this system could modulate various aspects of bone morphogenesis, and these studies laid the groundwork for the current therapeutics targeting these molecules for the treatment of bone disorders such as osteoporosis and bone erosion in patients with rheumatoid arthritis^{94,187}. Osteoclasts promote bone resorption, and RANKL expressed on bone marrow stromal cells stimulates RANK on osteoclast precursors to promote their differentiation. OPG is a soluble decoy receptor that naturally inhibits RANK–RANKL interactions and is thought to preserve bone density by limiting osteoclastogenesis. However, with age its production is not sufficient to prevent osteoporosis. Consequently, blocking RANKL was proposed as a treatment for osteoporosis, with the notion that this would favour the activity of osteoblasts that promote or maintain bone growth and bone density, and thereby restrict osteoporosis. Several very rare human bone genetic disorders have been mapped to mutations in *RANK*^{188–190} and *OPG*¹⁹¹, also supporting clinical development.

Denosumab, a fully human neutralizing RANKL-specific antibody, was approved in 2010 for the treatment of postmenopausal women with osteoporosis, and in 2011 for patients at a high risk of bone fracture after receiving androgen deprivation or adjuvant aromatase therapy for non-metastatic prostate and breast cancer. The results of several Phase I–III trials have been published¹⁸⁷, showing a 20–68% reduction in fracture incidence after therapy, depending on the fracture type^{192,193}. An 8-year follow-up study of patients with osteoporosis enrolled in a Phase II trial also showed encouraging results, with progressive improvements in bone mineral density¹⁹⁴. Denosumab has also been tested in Phase II trials of erosive rheumatoid arthritis, in which it demonstrated substantially lower degrees of bone erosion and an increase in bone density^{195,196}.

An OPG–Fc fusion protein to block RANKL–RANK activity was also assessed in a Phase I study in postmenopausal women¹⁹⁷. Deregulation of the RANK–OPG system is implicated in bone disease in several cancers, including prostate cancer, breast cancer and multiple myeloma¹⁹⁸, and another OPG–Fc fusion protein (AMG-0007) was administered to patients with multiple myeloma or breast cancer in Phase I trials¹⁹⁹. In both cases, the OPG–Fc fusion protein was well tolerated, showing commensurate decreases in markers of bone resorption. However, clinical trials were terminated probably owing to the relatively poor pharmacokinetics that were observed and issues regarding potential immunogenicity. A Phase I trial of recombinant OPG (CEP-37251) was also initiated in healthy postmenopausal women but this study was terminated by the sponsors. As TRAIL also binds to OPG, and OPG can inhibit TRAIL-mediated apoptosis of tumour cells, drugs consisting of OPG may not be as attractive as those directed against RANKL.

Pain

NGF promotes the growth, maintenance and survival of neurons that express its two receptors, NGFR and neurotrophic tyrosine kinase receptor type 1 (NTRK1; also known as TRKA). NGF also increases the pain response to exogenous stimuli in sensory neurons, which led to interest in neutralizing this molecule to reduce chronic pain. The history of clinically targeting NGF has been discussed in the literature^{200,201}. Several NGF-blocking antibodies, including tanezumab (a humanized antibody), have been tested as therapies for relieving pain associated with osteoarthritis and several other chronic conditions. Phase I and II trials of tanezumab demonstrated good efficacy as well as reasonable safety and tolerability profiles, with mild to moderate adverse events^{202–205}. However, several further trials were halted by the FDA in 2010 owing to radio-graphic evidence of bone necrosis in Phase III trials of osteoarthritis, leading to joint replacement of knees, hips or shoulders^{200,201,206}. An advisory panel in 2012 voted for continued pharmaceutical development in this area, based on efficacy in terms of reducing pain and the fact that no obvious biological mechanism has been found that might explain why blocking NGF could lead to greater bone erosion. NGF blockers have the potential to provide pain relief, but more carefully planned and monitored trials will be essential.

Challenges and future directions

In conclusion, the potential for targeting some TNFSF and TNFRSF molecules for disease intervention has already been realized, and there is great promise for other molecules within this family. A primary challenge will be to achieve specificity and efficacy without having any off-target effects. All currently approved TNF inhibitors are effective at treating rheumatoid arthritis and psoriasis or psoriatic arthritis. However, clinical differences between the receptor–Fc inhibitors (such as etanercept) and antibody inhibitors (such as infliximab, adalimumab, and so on) are being recognized²⁰⁷. Etanercept binds to both TNF and LT α , whereas the antibodies only recognize TNF. Here, differences in the clinical efficacy of etanercept versus antibody-based TNF inhibitors may reveal unrecognized aspects of LT α physiology⁴. For example, the antibody-based TNF inhibitors are efficacious in Crohn's disease²⁰⁸, whereas etanercept is ineffective²⁰⁹. LT α binds to both TNFR1 and TNFR2, but — in contrast to TNF — it also binds to HVEM. How this difference in ligand specificity is interpreted in immune function and during treatment is not yet clear²¹⁰.

In addition, within a specific disease (for example, rheumatoid arthritis) about one-third of patients do not respond to any anti-TNF treatment, and for some diseases (for example, multiple sclerosis) TNF inhibitors are contraindicated. The reasons for these differences remain largely unknown, but contributions of the genetic makeup of patients to the underlying mechanisms of pathogenesis are undoubtedly important and the subject of much-needed current research. In this regard, a recent study found that a described risk allele for

multiple sclerosis results in increased production of a soluble form of TNFR1 that can be an endogenous blocker of TNF activity²¹¹. As anti-TNF therapy exacerbated demyelinating disease rather than providing a beneficial effect^{212,213}, this provides a direct demonstration that genetics can inform clinical outcome.

Reagent selection may also be paramount, and for any given molecular target the therapeutic may require optimization as clinical results are obtained. In addition, determining the appropriate stage of disease at which to attempt therapeutic intervention is likely to be crucial to success. Several cell types, particularly within the immune system, can express many of the TNFSF receptors and ligands^{5,7}. An advantage is that a substantial number of receptors and ligands are not constitutively expressed, which probably limits their involvement in normal homeostatic mechanisms. However, this also complicates the timing of treatment, which will obviously need to coincide with the expression and activity of these molecular targets. Expanding the analysis of patient sera to monitor the levels of soluble versions of TNFSF ligands and receptors should aid in determining whether these molecules truly might be useful as bio-markers not only for disease susceptibility, intensity or progression but also for guiding the treatment schedule. Such analyses may lead to the focusing of therapeutic interventions within selected stratified populations and at specific stages of the disease.

Another challenge associated with intervening in inflammatory and autoimmune diseases is achieving the desired modulation of immune cell activity while maintaining a normally protective response. Mouse models and clinical studies have revealed that protective immune function to combat infectious disease is very similar, in terms of molecular control, to the immune function that drives inflammatory and autoimmune diseases^{5,7,214}. This has been particularly illustrated in the treatment of rheumatoid arthritis and Crohn's disease with TNF blockers, where reports of serious bacterial, fungal and viral infections are common^{215,216}. However, antagonists of TNF–TNFR1 and TNF–TNFR2 interactions may be an exception because TNF is a crucial end-stage effector molecule that can participate in the cytotoxic activity that is required for the clearance of pathogens. Therefore, targeting molecules such as OX40L, 4-1BBL, GITRL, CD70 and LIGHT may not dramatically impair resistance to infectious disease, but this could depend on whether the treatment directly coincides with the initial infection event as well as the extent of immunosuppression of T or B cell responses caused by antagonists targeting these molecules.

Second- and third-generation antagonists with a greater neutralizing capacity that results in a shorter course of treatment may in part solve the issue of susceptibility to infection. Similar concerns exist when using agonists to boost immunity in cancer therapy. A major challenge will be to induce the desired antitumour response while maintaining immunological tolerance to self-antigens expressed on normal tissue cells, and to avoid the inadvertent promotion of deregulated inflammation or initiation of an autoimmune response (an immune-related adverse event), as has been seen in patients with cancer receiving other therapies that enhance immune cell responsiveness²¹⁷. The clinical use of agonistic reagents is likely to require optimization in terms of dosing, injection routes and administration schedules. Although co-administration with anti-inflammatory agents might prevent some of the potential side effects associated with augmenting immune function, depending on the nature of the immunosuppressant this may reverse the beneficial effect of the therapeutic intervention.

Combination treatment may also be crucial. Targeting a single TNFSF or TNFRSF molecule or an interaction might potentially be insufficient to dramatically alter the course of disease, regardless of the potential importance of the molecule. The clinical effectiveness of targeting TNF in isolation may again be an exception rather than the rule. Within the immune system,

this could simply reflect the fact that multiple ligand–receptor interactions, both in the TNF family and in other protein families, drive responses — both autoimmune and anti-cancer^{3,5}. Mouse models of disease may be misleading. Dramatic effects on the development of inflammatory or autoimmune disease have been reported with a genetic deficiency in only one protein, following the neutralization of a single interaction, or in relation to tumour therapy following the injection of a single agonist reagent⁵. But these responses may not be typical of therapy in humans.

Some mouse models, particularly those with transplantation of fully MHC-mismatched allografts, have revealed scenarios that are possibly more reflective of human disease. In these cases, neutralization of two or three interactions, such as CD40–CD40L, OX40–OX40L and CD28–CD80 (or CD86) in the immunoglobulin superfamily (which can be targeted by the approved biologics abatacept and belatacept), effectively prevents graft rejection, whereas neutralization of a single interaction has no effect⁵. Similarly, stimulating several receptors such as OX40, 4-1BB, IL-2 receptor and the GM-CSF receptor efficiently suppresses the growth of weakly immunogenic tumours, whereas targeting only one interaction or receptor has little or no effect³. If these are more generalizable results that are applicable to the treatment of human inflammatory disease and cancer, the key will be to determine the appropriate combination of molecules to target for a given disease or stage of disease, and that may be most specific with the fewest off-target effects and adverse events.

Blocking TNF with IL-1 (for example, with pegylated soluble TNFR1 plus anakinra) or TNF with CD80 and/or CD86 (with etanercept plus abatacept) has been attempted in clinical trials of rheumatoid arthritis, but no significant enhancement of clinical activity was reported compared to the individual reagents, and the risk of unwanted side effects and infections was increased²¹⁸ (ClinicalTrials.gov identifier: NCT00537667). Although this is potentially discouraging, targeting the right combinations of interactions, perhaps without modulating TNF activity, may provide greater efficacy in limiting inflammatory disease or promoting antitumour immunity without promoting more adverse events. The development of bispecific biologics, such as tandem scFv–Fc biologics, Fab–scFv biologics, ‘diabodies’ or dual variable domain immunoglobulin G biologics, which target two molecules within a single reagent, will probably be a major advancement in these areas and a focus of future therapies.

Supplementary Material

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Box 1**Types of therapeutic reagents**

Mouse antibodies are highly immunogenic in humans, which reduces their effectiveness and prevents repeated dosing. Chimeric antibodies are denoted by the suffix ‘-ximab’ and consist of the antigen-binding fragment (Fab) regions of a mouse antibody fused with human immunoglobulin constant (Fc) regions (they are 65% human). Humanized antibodies are denoted by the suffix ‘-zumab’. The mouse antibody complementarity-determining regions (the hypervariable regions) are engrafted into a human antibody (which is 95% human), allowing further reduction in immunogenicity. Fully human antibodies are denoted by the suffix ‘-umab’ and are produced either by immunizing transgenic mice that have been engineered to express human immunoglobulin genes or from phage display libraries expressing human antibody fragments. Receptor fusion proteins are another class of biologics, denoted by the suffix ‘-cept’. They are created by recombinant DNA technology. They consist of the ectodomain of a human tumour necrosis factor receptor superfamily (TNFRSF) molecule fused to human immunoglobulin Fc regions, creating a stable, bivalent, high-affinity biologic with specificity for a ligand. These molecules are interchangeably designated with the suffix ‘-Fc’ or ‘-Ig’. Aglycosylation, which prevents some Fc effector functions such as antibody-dependent cellular cytotoxicity (ADCC), is utilized as a modification to limit cell depletion by the antibody and Fc fusion protein reagents. Other modifications include producing Fab fragments of antibodies lacking the Fc region and using strategies such as pegylation to increase their half-life. Additional therapeutic antibodies are selected for natural cytotoxicity (ADCC or complement-dependent cytotoxicity (CDC)) or engineered (Fc-modified) for enhanced ADCC (Fc γ receptor-binding) or CDC (complement-binding) activity by increasing glycosylation or decreasing the fucose content of the Fc region. Conjugation of antibodies with a drug toxin is also being exploited.

Figure 1. Pro-inflammatory and death-inducing activity of TNFSF members

a Tumour necrosis factor superfamily (TNFSF) molecules have canonical TNF homology domains and are active primarily as non-covalently associated homotrimers. TNF receptor superfamily (TNFRSF) molecules contain variable numbers of cysteine-rich domains in their ligand-binding extracellular regions (ectodomains). Soluble or membrane-expressed TNFSF molecules (sTNFSF and mTNFSF, respectively) can promote survival or inflammatory signalling by organizing TNFRSF monomers (for example, TNFR2, CD40, OX40, B cell activating factor receptor (BAFFR) and receptor activator of NF-κB ligand (RANK)) into trimeric or oligomeric configurations, allowing the recruitment of adaptor molecules called TNFR-associated factors (TRAFs) that activate serine/threonine kinase-dependent pathways. **b** | TNFSF molecules can promote apoptotic cell death by organizing TNFRSF monomers containing death domains (for example, TNFR1, FAS and TNF-related apoptosis-inducing ligand receptor (TRAILR)) into trimeric or oligomeric configurations that recruit adaptor molecules containing death effector domains (such as TNFR-associated death domain protein (TRADD) and FAS-associated death domain protein (FADD)), leading to the activation of cysteine/aspartate proteases (caspases). BAX, BCL-2-associated X protein; BID, BH3-interacting domain death agonist; IκBα, inhibitor of κB; IKKα, IκB kinase α; MAPK, mitogen-activated protein kinase; NIK, NF-κB-inducing kinase; p50, NF-κB subunit p50; p100, NF-κB precursor protein p100; RELA, transcription factor p65.

Figure 2. TNFSF targets and therapeutics in autoimmunity and inflammation

This simplified diagram highlights a few of the possible interactions between cells in the immune system (antigen-presenting cells (APCs), B cells and T cells) and tissue cells (such as epithelial cells, endothelial cells, fibroblasts, stromal cells, osteoclasts and neurons). These cells can be controlled by interactions between tumour necrosis factor superfamily (TNFSF) and TNF receptor superfamily (TNFRSF) molecules, which result in stimulatory or pro-inflammatory activity that is characteristic of autoimmune and inflammatory diseases. Several biologics that disrupt TNFSF interactions and that have been or are currently in clinical trials (TABLE 1) are listed. APRIL, a proliferation-inducing ligand; BAFF, B cell activating factor; BAFFR, BAFF receptor; BCMA, B cell maturation antigen; DR3, death domain receptor 3; GITR, glucocorticoid-induced TNFR-related protein; LT α , lymphotoxin- α ; LT β , lymphotoxin- β ; LT β R, LT β receptor; NGF, nerve growth factor; NGFR, NGF receptor; RANK, receptor activator of NF- κ B; RANKL, RANK ligand; TACI, transmembrane activator and CAML interactor; TNF, tumour necrosis factor; TNFR1, TNF receptor 1; TWEAK, TNF-related weak inducer of apoptosis.

Figure 3. TNFRSF targets and therapeutics in cancer

This simplified diagram depicts tumour necrosis factor receptor superfamily (TNFRSF) molecules and ligands that are: being targeted on cells of the immune system (antigen-presenting cells (APCs), T cells and natural killer (NK) cells) to boost immune activity against a tumour cell; or being targeted on tumour cells to either directly promote apoptotic cell death or focus natural or artificial (drug- or toxin-induced) cytotoxic mechanisms towards a tumour cell. Several biologics that have been or are currently in clinical trials (TABLE 1) are listed. APRIL, a proliferation-inducing ligand; BAFF, B cell activating factor; BAFFR, BAFF receptor; FASL, FAS ligand; FN14, FGF-inducible 14; GITR, glucocorticoid-induced TNFR-related protein; TRAIL, TNF-related apoptosis-inducing ligand; TRAILR, TRAIL receptor.

Table 1

Biologics targeting TNF family members in clinical trials

TNF family ligand	TNF family Receptors	Biologic (trade name; developers)	Type of agent	Activity of biologic	Indications	Stage of development
LT α (also known as TNFSF1)	<ul style="list-style-type: none"> • TNFR1 (also known as CD120a and TNFRSF1A) • TNFR2 (also known as CD120b and TNFRSF1B) • HVEM (also known as CD270 and TNFRSF14) 	Etanercept (Enbrel; Amgen/Pfizer)	Human TNFR2–Fc fusion protein	Antagonist	Rheumatoid arthritis, JIA, psoriatic arthritis, psoriasis, ankylosing spondylitis	Approved
		Pateclizumab	Human LT α -targeting antibody	Depleting and antagonist	Rheumatoid arthritis	Phase I
TNF (also known as TNFSF2)	<ul style="list-style-type: none"> • TNFR1 (also known as CD120a and TNFRSF1A) • TNFR2 (also known as CD120b and TNFRSF1B) 	Infliximab (Remicade; Centocor Ortho Biotech)	Chimeric TNF-specific antibody	Antagonist	Rheumatoid arthritis, psoriatic arthritis, psoriasis, ankylosing spondylitis, Crohn's disease, ulcerative colitis	Approved
		Etanercept (Enbrel; Amgen/Pfizer)	Human TNFR2–Fc fusion protein	Antagonist	Rheumatoid arthritis, JIA, psoriatic arthritis, psoriasis, ankylosing spondylitis	Approved
		Adalimumab (Humira; Abbott)	Human TNF-specific antibody	Antagonist	Rheumatoid arthritis, JIA, psoriatic arthritis, psoriasis, ankylosing spondylitis, Crohn's disease	Approved
		Certolizumab pegol (Cimzia; UCB)	Human pegylated Fab TNF-specific antibody	Antagonist	Rheumatoid arthritis, Crohn's disease	Approved
		Golimumab (Simponi; Centocor Ortho Biotech)	Human TNF-specific antibody	Antagonist	Rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis	Approved
		Tasonermin (Beromun; Boehringer Ingelheim)	Recombinant TNF	Agonist	Cancer (sarcoma, solid tumour)	Approved (some cancers); Phase I (other cancers)
		TNFerade	TNF gene therapy	Agonist	Cancer (various)	Phase I/II/III
		TNF Kinoid	Recombinant TNF carrier (self-immunization)	Antagonist	Rheumatoid arthritis, Crohn's disease	Phase II
LT β (also known as TNFSF3)	<ul style="list-style-type: none"> • LTβR (also known as TNFRSF3) 	Baminercept	Human LT β R–Ig fusion protein	Antagonist	Rheumatoid arthritis, Sjögren's syndrome	Phase I/II
		Oxelumab	Human OX40L-specific antibody	Antagonist	Asthma	Phase II
		-	Murine OX40-specific antibody	Agonist	Cancer (solid tumour, prostate)	Phase I
CD27L (also known as CD70 and	<ul style="list-style-type: none"> • CD27 (also known as TNFRSF7) 	SGN-75	Humanized CD70-specific antibody linked to MMAF toxin	Depleting	Cancer (renal, non-Hodgkin's lymphoma)	Phase I

TNF family ligand	TNF family Receptors	Biologic (trade name; developers)	Type of agent	Activity of biologic	Indications	Stage of development
TNFSF7)	• CD40 (also known as TNFRSF5)	MDX-1411	Human CD70-specific antibody	Depleting	Cancer (renal)	Phase I
		MDX-1203	Human CD70-specific antibody linked to DNAMGBA toxin	Depleting	Cancer (renal, non-Hodgkin's lymphoma)	Phase I
		CDX-1127	Human CD27-specific antibody	Agonist (and depleting?)	Multiple cancers	Phase I
		TriMix-DC	CD70-expressing dendritic cells	Agonist	Cancer (melanoma)	Phase I/II
		PG102	Chimeric CD40-specific antibody	Antagonist	Psoriatic arthritis	Phase I (discontinued)
		ASKP1240/4D11	Human CD40-specific antibody	Antagonist	Transplantation, psoriasis	Phase I
		Lucatumumab/HCD122	Human CD40-specific antibody	Depleting	Cancer (Hodgkin's lymphoma, non-Hodgkin's lymphoma, follicular lymphoma)	Phase I/II
		Dacetuzumab/SGN-40	Humanized CD40-specific antibody	Depleting and agonist	Cancer (CLL, non-Hodgkin's lymphoma, multiple myeloma)	Phase I
		Chi Lob 7/4	Chimeric CD40-specific antibody	Agonist and depleting	Cancer (non-Hodgkin's lymphoma)	Phase I
		CP-870893	Human CD40-specific antibody	Agonist	Cancer (melanoma/pancreatic)	Phase I
FASL (also known as CD178 and TNFSF6)	• FAS (also known as CD95 and TNFRSF6) • DCR3 (also known as TNFRSF6B)	-	CD40L gene therapy or trimeric recombinant CD40L	Agonist	Cancer (various)	Phase I
		APO010	Hexameric recombinant FASL	Agonist	Cancer	Phase I (discontinued)
		Brentuximab vedotin (Adcetris; Seattle Genetics)	Chimeric CD30-specific antibody linked to MMAE toxin	Depleting	Cancer (Hodgkin's lymphoma, ALCL)	Approved
		SGN-30	Chimeric CD30-specific antibody	Agonist	Cancer (Hodgkin's lymphoma, ALCL)	Phase II
		XmAb2513	Humanized CD30-specific antibody (Fc-modified)	Agonist and depleting	Cancer (Hodgkin's lymphoma, ALCL)	Phase I
		MDX-1401	Human CD30-specific antibody	Agonist (and depleting?)	Cancer (Hodgkin's lymphoma, ALCL)	Phase I
		BMS-663513	Human 4-1BB-specific antibody	Agonist	Cancer (melanoma, solid tumour)	Phase I/II
		PF-05082566	Human 4-1BB-specific antibody	Agonist	Cancer (non-Hodgkin's lymphoma)	Phase I
		Dulanermin/AMG 951	Recombinant TRAIL	Agonist	Cancer (various)	Phase I/II
		Mapatumumab/HGS-ETR1	Human TRAILR1-specific antibody	Agonist	Cancer (various)	Phase II
CD40L (also known as CD154 and TNFSF5)	• CD30 (also known as TNFRSF8)					
CD30L (also known as CD153 and TNFSF8)	• 4-1BB (also known as CD137 and TNFRSF9)					
4-1BBL (also known as TNFSF9)	• TRAILR1 (also known as DR4, CD26 and TNFRSF10A)					
TRAIL (also known as APO2L, CD253 and TNFSF10)						

TNF family ligand	TNF family Receptors	Biologic (trade name; developers)	Type of agent	Activity of biologic	Indications	Stage of development
	• TRAILR2 (also known as DR5, CD262 and TNFRSF10B)	Conatumumab/ AMG 655	Human TRAILR2-specific antibody	Agonist	Cancer (various)	Phase I/II
	• TRAILR3 (also known as DCR1, CD263 and TNFRSF10C)	Drozitumab/ Apomab/PRO95780	Human TRAILR2-specific antibody	Agonist (and depleting?)	Cancer (various)	Phase I
	• TRAILR4 (also known as DCR2, CD264 and TNFRSF10D)	Lexatumumab/ HGS-ETR2	Human TRAILR2-specific antibody	Agonist	Cancer	Phase I/Ib
	• OPG (also known as TNFRSF11B)	Tigatuzumab/ CS-1008/TRA-8	Humanized TRAILR2-specific antibody	Agonist	Cancer (various)	Phase I
		HGSTR2J/KMTRS	Human TRAILR2-specific antibody	Agonist	Cancer	Phase I (discontinued)
		LBY-135	Chimeric TRAILR2-specific antibody	Agonist	Cancer	Phase I (discontinued)
RANKL (also known as CD254 and TNFSF11)	• RANK (also known as CD265 and TNFRSF11A)	Denosumab (Prolia/ Xgeva; Amgen)	Human RANKL-specific antibody	Antagonist	Osteoporosis	Approved
	• OPG (also known as TNFRSF11B)	-	Human OPG–Fc fusion protein	Antagonist (and depleting?)	Osteoporosis	Phase I (discontinued)
		AMG-0007	Human OPG–Fc fusion protein	Antagonist (and depleting?)	Cancer (bone metastasis)	Phase I (discontinued)
		CEP-37251	Recombinant OPG	Antagonist (and depleting?)	Osteoporosis	Phase I (discontinued)
TWEAK (also known as TNFSF12)	• FN14 (also known as TWEAKR, CD266 and TNFRSF12A)	BIIB023	Humanized TWEAK-specific antibody	Antagonist	Rheumatoid arthritis, lupus nephritis	Phase I/II
		Enavatuzumab/ PDL192	Humanized FN14-specific antibody	Agonist (and depleting?)	Cancer (solid tumour)	Phase I
		RO5458640	Humanized TWEAK-specific antibody	Antagonist	Cancer (solid tumour)	Phase I
APRIL (also known as CD256 and TNFSF13)	• TACI (also known as CD267 and TNFRSF13B)	Atacicept	Human TACI–Ig fusion protein	Antagonist	SLE, rheumatoid arthritis, multiple sclerosis	Phase II/III
	• BCMA (also known as CD269 and TNFRSF17)				Cancer (non-Hodgkin's lymphoma, multiple myeloma, Waldenström's macroglobulinaemia)	Phase I
BAFF (also known as BLYS, CD257 and TNFSF13B)	• BAFFR (also known as BR3, CD268 and TNFRSF13C)	Belimumab (Benlysta; Human Genome Sciences/ GlaxoSmithKline)	Human BAFF-specific antibody	Antagonist	SLE, rheumatoid arthritis	Approved (SLE); Phase II (rheumatoid arthritis)
	• TACI (also known as CD267 and TNFRSF13B)	Tabalumab/ LY2127399	Human BAFF-specific antibody	Antagonist	Cancer (Waldenström's macroglobulinaemia)	Phase II
	• BCMA (also known as CD269 and TNFRSF17)				SLE, rheumatoid arthritis, multiple sclerosis	Phase II/III
		Atacicept	Human TACI–Ig fusion protein	Antagonist	Cancer (multiple myeloma)	Phase II/III
					SLE, rheumatoid arthritis, multiple sclerosis	Phase II/III

TNF family ligand	TNF family Receptors	Biologic (trade name; developers)	Type of agent	Activity of biologic	Indications	Stage of development
					Cancer (non-Hodgkin's lymphoma, multiple myeloma, Waldenström's macroglobulinaemia)	Phase I
		Briobacept	Human BAFFR-Ig fusion protein	Antagonist	Rheumatoid arthritis	Phase I (discontinued)
		Blisibimod/AMG 623	Human BAFF-binding peptibody immunoglobulin	Antagonist	SLE	Phase II
LIGHT (also known as CD258 and TNFSF14)	<ul style="list-style-type: none"> • LTβR (also known as TNFRSF3) • HVEM (also known as CD270 and TNFRSF14) • DCR3 (also known as TNFRSF6B) 	Baminercept/BG9924	Human LTβR-Ig fusion protein	Antagonist	Rheumatoid arthritis, Sjögren's syndrome	Phase I/II
		SAR252067	Human LIGHT-specific antibody	Antagonist (and depleting?)	Inflammatory bowel disease	Phase I
GITRL (also known as TNFSF18)	• GITR (also known as CD357 and TNFRSF18)	TRX518	Humanized GITR-specific antibody (Fc-modified)	Agonist	Cancer (melanoma)	Phase I
NGF (no TNFSF designation)	• NGFR (also known as p75NTR, CD271 and TNFRSF16)	Tanezumab/RN624	Humanized NGF-specific antibody	Antagonist	Osteoarthritis	Phase III
					Lower back pain, bone cancer pain, cystitis	Phase II
		Fulranumab/AMG-403	Human NGF-specific antibody	Antagonist	Osteoarthritis	Phase II
		REGN475/SAR164877	Human NGF-specific antibody	Antagonist	Osteoarthritis, sciatic pain	Phase II
		MEDI-578	Human scFv NGF-specific antibody	Antagonist	Osteoarthritis	Phase I
		ABT-110/PG110	Humanized NGF-specific antibody	Antagonist	Osteoarthritis	Phase I

ALCL, anaplastic large-cell lymphoma; APRIL, a proliferation-inducing ligand; BAFF, B cell activating factor; BAFFR, BAFF receptor; BCMA, B cell maturation antigen; CD27L, CD27 ligand; CD30L, CD30 ligand; DCR3, decoy receptor 3; DNAMGBA, DNA minor groove binding alkylating agent; FASL, FAS ligand; FN14, FGF-inducible 14; GITR, glucocorticoid-induced TNFR-related protein; GITRL GITR ligand; HVEM, herpes virus entry mediator; Ig, immunoglobulin; JIA, juvenile idiopathic arthritis; LTα, lymphotoxin-α; LTβ, lymphotoxin-β; LTβR, LTβ receptor; MMAF, monomethyl auristatin F; NGF, nerve growth factor; NGFR, NGF receptor; OPG, osteoprotegerin; OX40L, OX40 ligand; RANK, receptor activator of NF-κB; RANKL, RANK ligand; scFv, single-chain variable fragment; SLE, systemic lupus erythematosus; TACI, transmembrane activator and CAML interactor; TNF, tumour necrosis factor; TNFR1, TNF receptor 1; TRAIL, TNF-related apoptosis-inducing ligand; TRAILR1, TRAIL receptor 1; TWEAK, TNF-related weak inducer of apoptosis. *No trials have currently been initiated to target the interactions of TNF ligand-related molecule 1 (TL1A; also known as TNFSF15) with death domain receptor 3 (DR3; also known as TNFRSF25) or DCR3 (also known as TNFRSF6B); or of ectodysplasin A1 and ectodysplasin A2 (no TNFSF designation) with ectodysplasin A receptor (no TNFSF designation) or X-linked ectodysplasin A2 receptor (also known as TNFRSF27). For the remaining TNFR superfamily (TNFRSF) members — TROY ('toxicity and JNK inducer'; also known as TNFRSF19), RELT ('receptor expressed in lymphoid tissues'; also known as TNFRSF19L) and DR6 (also known as TNFRSF21) — TNFSF ligands have not yet been discovered, and these members have also not yet been targeted in the clinic.