Advances in targeting cell surface signalling molecules for immune modulation

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Abstract | The past decade has witnessed a surge in the development of immunomodulatory approaches to combat a broad range of human diseases, including cancer, viral infections, autoimmunity and inflammation as well as in the prevention of transplant rejection. Immunomodulatory approaches mostly involve the use of monoclonal antibodies or recombinant fusion proteins that target cell surface signalling molecules on immune cells to drive immune responses towards the desired direction. Advances in our understanding of the human immune system, along with valuable lessons learned from the first generation of therapeutic biologics, are aiding the design of the next generation of immunomodulatory biologics with better therapeutic efficacy, minimized adverse effects and long-lasting clinical benefit. The recent encouraging results from antibodies targeting programmed cell death protein 1 (PD1) and B7 homolog 1 (B7H1; also known as PDL1) for the treatment of various advanced human cancers show that immunomodulatory therapy has come of age.

Immunomodulatory biologics can be utilized to treat immune-related diseases in broad therapeutic areas. The immune response can be dampened in hyperactive immune conditions such as transplant rejection as well as autoimmune or inflammatory diseases, or stimulated to reverse hypoactive immune responses in cancer or chronic bacterial or viral infections. Unlike the traditional and mainstream monoclonal antibody (mAb)- and recombinant fusion protein (RFP)-based therapeutics, which neutralize or deplete targets or target positive cells[1,2], immunomodulatory biologics engage and manipulate cell surface signalling molecules on host immune cells to modulate antigen-specific T cell receptor (TCR) and B cell receptor (BCR) signals to control the direction and magnitude of lymphocyte responses. Such cell surface signalling molecules include those previously known as co-signalling (both co-stimulatory and co-inhibitory) molecules[3] as well as membrane receptors that are involved in adhesion and migration, and can be divided into two major gene families: the immunoglobulin (Ig) superfamily and the tumour necrosis factor (TNF)–TNF receptor (TNFR) superfamily. Among Ig molecules, the B7–CD28 family members have crucial roles in modulating TCR and BCR signals and they influence the outcome of lymphocyte-mediated immune responses[4,5].

Immunomodulatory biologics can generally be classified into two groups based on their mechanisms of action: antagonists (blocking or neutralizing the interaction between receptors and ligands) or agonists (inducing signalling via the receptor by mimicking the ligand). Neutralizing mAbs against disease-facilitating cytokines have become important non-steroidal therapeutic options to treat autoimmune and inflammatory diseases (reviewed in REF. 1).

As a general approach to promote tumour- or pathogen-specific immune responses, one can enhance either lymphocyte priming and maturation in the lymphoid organs or effector functions in the periphery by engaging distinctive co-stimulatory pathways — for example, the CD28, CD137 (also known as 4-1BB or TNFRSF9), CD27 and CD40 pathways — using agonistic reagents (FIG. 1). Alternatively, immune activation can be promoted through the blockade of co-inhibitory pathways by antagonists: for example, the programmed cell death protein 1 (PD1)–B7 homolog 1 (B7H1; also known as PDL1) pathway and the cytotoxic T lymphocyte antigen 4 (CTLA4) pathway (FIG. 1). Conversely, lymphocyte activation can be inhibited to suppress unwanted immunity by either blocking co-stimulatory receptors or triggering a negative regulatory pathway. Immune activation enhanced by co-stimulatory receptors is generally

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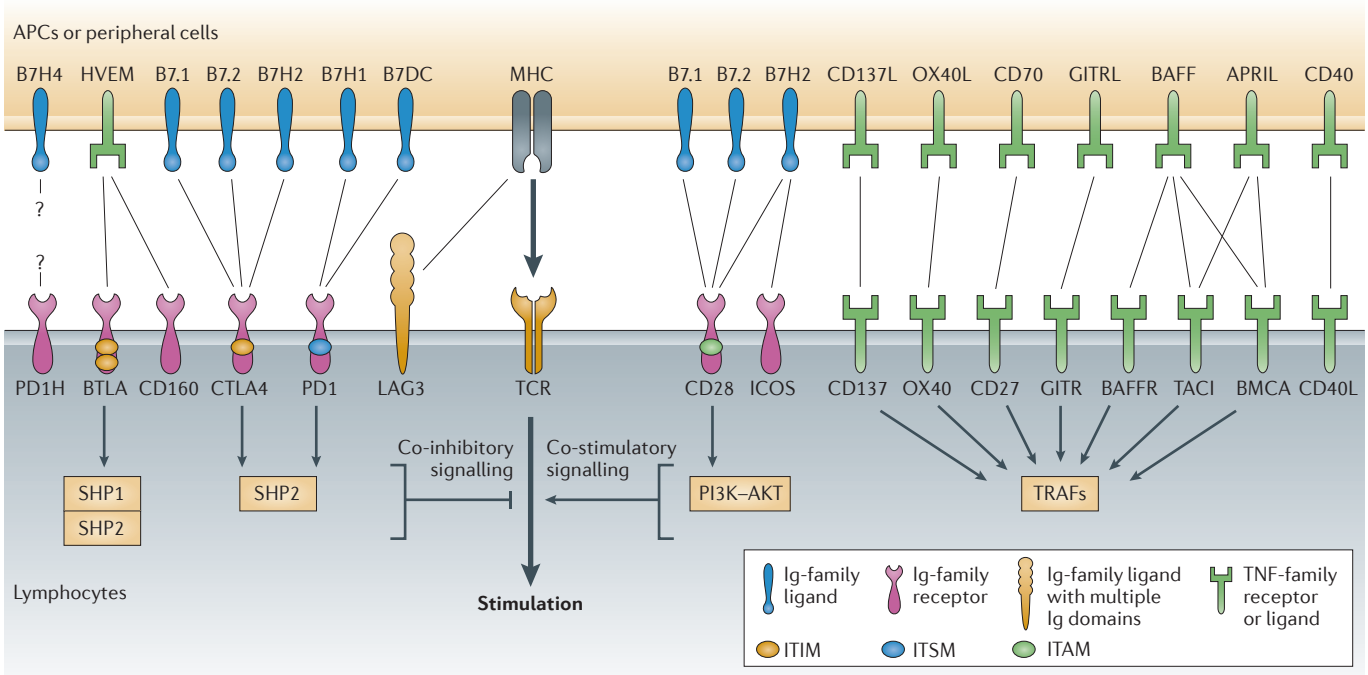

Figure 1 | **Cell surface signalling molecules as important therapeutic targets.** Co-signalling is a complex event that is coordinated through a network of ligand–receptor interactions on the cell surface, with both co-stimulatory and co-inhibitory capacities. The direction and outcome of immune responses are ultimately decided by the interplay of these complicated and often counterbalancing network interactions. Co-signalling pathways are thus important targets for therapeutic intervention. The immunoglobulin (Ig) superfamily and the tumour necrosis factor (TNF)–TNF receptor (TNFR) superfamily are two major gene families of cell surface signalling molecules. Important co-inhibitory receptors that are expressed on lymphocytes include cytotoxic T lymphocyte antigen 4 (CTLA4), programmed cell death protein 1 (PD1), B- and T lymphocyte attenuator (BTLA), lymphocyte activation gene 3 (LAG3), CD160 and the PD1 homolog (PD1H), whereas CD28, inducible co-stimulator (ICOS), CD137 (also known as 4-1BB), CD27, OX40, glucocorticoid-induced TNFR-related protein (GITR), CD40 ligand (CD40L), B cell activation factor receptor (BAFFR), transmembrane activator and CAML interactor (TACI) and B cell maturation antigen (BCMA) are the main co-stimulatory receptors. Co-signalling ligands, including B7 ligand members and TNF ligands, are mainly expressed by antigen-presenting cells (APCs). APRIL, a proliferation-inducing ligand; B7H1, B7 homolog 1; GITRL, GITR ligand; HVEM, herpesvirus entry mediator; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibitory motif; ITSM, immunoreceptor tyrosine-based switch motif; MHC, major histocompatibility complex; OX40L, OX40 ligand; PI3K, phosphoinositide 3-kinase; TCR, T cell receptor; TRAF, TNFR-associated factor.

initiated through membrane proximal kinase activation and followed by phosphorylation cascades, whereas co-inhibitory receptors such as CTLA4, PD1 and B- and T lymphocyte attenuator (BTLA) recruit phosphatases to reverse activation-induced phosphorylation events. However, owing to the temporal and spatial differential expression of co-signalling molecules during immune activation and their differential involvement in cancer or autoimmune diseases, it is crucial to understand the mechanism of individual pathways to design the most efficacious therapeutic biologics with minimized immune-related side effects.

The first immunomodulatory biologic to reach the market was the mAb muromonab (Orthoclone OKT3; Janssen-Cilag), directed against human CD3. Muromonab stimulates an initial T cell expansion followed by T cell depletion resulting from activation-induced cell death; it received approval from the US Food and Drug Administration (FDA) in 1986 for the prevention of transplant rejection. Since then, three additional immunomodulatory biologics have been approved for clinical use in the United States (TABLE 1); all of these target or are derived from the immune checkpoint receptor CTLA4. Furthermore, several immunomodulatory biologics are in different phases of clinical trials for the treatment of cancer as well as autoimmune and inflammatory conditions (TABLE 2).

The FDA approval in 2011 of the CTLA4-specific mAb ipilimumab (Yervoy; Bristol-Myers Squibb) for the treatment of metastatic melanoma represented a major milestone for cancer immunotherapy. More recently, it was shown that an antibody (BMS-936558) targeting the co-inhibitory molecule PD1 induced significant and durable responses in several types of highly refractory tumours[6]. This antibody represents a newer generation of immunomodulatory biologics that stimulate highly effective and long-lasting host tumour immunity with controllable autoimmune toxicities.

Here, we examine the targets and mechanisms by which immunomodulation can be achieved, and discuss the development of immunomodulatory biologics for the treatment of cancer or autoimmune diseases.

Table 1 | **FDA-approved biologics that target immunomodulatory pathways**

Target	Generic name (trade name)	Company	Type of biologic	Pathways	Roles	Indication (for which initially approved)	Year of approval
Monoclonal antibodies							
CD3E	Muromonab-CD3 (Orthoclone OKT3)	Janssen-Cilag	Murine IgG2a	TCR	T cell priming and activation	Kidney transplantation rejection	1986
CTLA4	Ipilimumab (Yervoy)	Bristol-Myers Squibb	Human IgG1	CTLA4	T cell activation	Metastatic melanoma	2011
Recombinant fusion proteins							
CD80 and CD86	Abatacept (Orencia)	Bristol-Myers Squibb	CTLA4-human IgG1	CD28–B7.1, CD28–B7.2, B7H2	T cell activation, T cell tolerance	Rheumatoid arthritis	2005
CD80 and CD86	Belatacept (Nulojix)	Bristol-Myers Squibb	CTLA4-human IgG1	CD28–B7.1, CD28–B7.2, B7H2	T cell activation, T cell tolerance	Kidney transplantation rejection	2011

B7H2, B7 homolog 2; CD3E, T cell surface glycoprotein CD3 ε-chain; CTLA4, cytotoxic T lymphocyte antigen 4; FDA, US Food and Drug Administration; IgG1, immunoglobulin G1; TCR, T cell receptor.

Ig superfamily co-signalling molecules

The first Ig superfamily co-signalling molecules to be identified were the ligand and receptor pair B7–CD28, as reported in 1990 (REFS 7,8), and the family has rapidly expanded ever since. The CD28 receptor family members have a single Ig variable region-like (IgV) motif in their extracellular domains and include CD28, CTLA4, inducible co-stimulator (ICOS), PD1 and BTLA[9]. Their known ligands all belong to the family of B7 ligands, which have a typical structure of two Ig-like extracellular domains, an IgV motif and an Ig constant region-like (IgC) domain. Strictly speaking, these receptors and ligands should be termed 'counter-receptors' because under certain circumstances some of these receptors can also serve as ligands and vice versa.

CD28–CTLA4–ICOS axis. Constitutively expressed on naive T cells, the receptor CD28 provides the primary co-stimulatory signal to promote naive T cell priming following the engagement of B7.1 (also known as CD80) or B7.2 (also known as CD86 and B70) — the CD28 ligands that are mainly expressed on antigen-presenting cells (APCs)[7]. CD28 activates phosphoinositide 3-kinase (PI3K)- and AKT-dependent signalling pathways as well as the mitogen-activated protein kinase (MAPK) cascade. As a result, it induces the expression of high amounts of interleukin-2 (IL-2) and an array of effector cytokines (both pro- and anti-inflammatory), it upregulates the survival factor B cell lymphoma XL (BCL-XL) and downregulates the cell cycle inhibitor cyclin-dependent kinase inhibitor 1B (CDKN1B; also known as $p27^{Kip1}$) to promote T cell activation, differentiation and memory T cell formation[10–12].

Conversely, CTLA4, a CD28 homologue induced on activated T cells, serves as a co-inhibitor to keep T cell responses in check following ligation of B7.1 and/or B7.2 (REFS 13,14). The cytoplasmic domain of CTLA4 contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) that is responsible for the recruitment of SHP family phosphatases, which reverse TCR activation-induced phosphorylation of signalling molecules. The essential negative function of CTLA4 in controlling T cell activation was demonstrated in CTLA4-deficient mice, which had fatal multi-organ destruction caused by uncontrolled lymphocyte proliferation and infiltration[15,16].

ICOS, another homologue of CD28, is induced on activated T cells and constitutively expressed on follicular T helper (T_{FH}) cells. It promotes T cell activation and T cell-dependent B cell responses by interacting with the ligand B7H2 (also known as ICOSL)[17–20]. It was recently found that B7H2 can also bind to CD28 and CTLA4 in humans (but not in mice), indicating a crosstalk and a possible synergistic relationship between CD28 and ICOS co-stimulatory pathways[21] (FIGS 2,3). In humans, the expression of B7.1 and B7.2 is largely restricted to professional APCs, whereas B7H2 is expressed not only on APCs but also induced on parenchymal cells, including endothelial and epithelial cells, under inflammatory conditions[22]. This differential expression pattern indicates that CD28 may regulate effector T cells following B7H2 engagement in the peripheral tissue, in addition to its role during the priming phase in lymphoid organs in response to B7.1 and/or B7.2 binding.

The central role of the CD28–CTLA4–ICOS axis in T cell activation has attracted much effort for therapeutic manipulation. Agonistic biologics that target CD28 or ICOS expand antigen-specific T cells and enhance the antitumour immune response[23]. In an original proof-of-principle experiment carried out two decades ago, ectopic expression of B7.1 in an immunogenic melanoma (K1735) cell line promoted local and systemic antitumour immunity in mice[24]. The B7-mediated antitumour effect has been attributed to the co-stimulation of both direct priming (antigens presented by the tumour)[24] and cross-priming (antigens processed and presented by host APCs)[25] of tumour-specific T cells.

B7.1– and B7.2–Ig RFPs targeting CD28 on naive T cells have also been shown to enhance T cell responses and facilitate tumour regression in several murine tumour models[26–28]. Similarly, a cancer vaccine using B7H2-positive tumour cells or the administration of a B7H2–Ig fusion protein, potentiating positive signals through ICOS and possibly through CD28, induces tumour-specific cytotoxic T lymphocyte (CTL) expansion and leads to tumour regression[23,29,30]. Instead of

Table 2 | **Biologics targeting immunomodulatory pathways in clinical trials**

Name	Companies	Type of biologic	Pathways	Roles	Indications	Trial phase
Immunoglobulin family						
Tremelimumab	MedImmune/ AstraZeneca	CTLA4-specific human IgG2	CTLA4–B7.1, CTLA4–B7.2, B7H2	T cell priming and activation	Solid tumours	II
Galiximab	Cancer and Leukemia Group B (CALGB)/ Biogen Idec	B7.1-specific chimeric IgG1	B7.1	B cell proliferation	Lymphoma	II
BMS-936558	Bristol-Myers Squibb/ Medarex	PD1-specific human IgG4	PD1–B7H1, PD1–B7DC	T cell activation and tolerance	Multiple cancers; HCV	III
CT-011	CureTech	PD1-specific humanized IgG1	PD1–B7H1, PD1–B7DC	T cell activation and tolerance	Advanced solid tumours; HCV	II
MK-3475	Merck/Schering-Plough	PD1-specific IgG4	PD1–B7H1, PD1–B7DC	T cell activation and tolerance	Advanced or metastatic solid tumours	I
AMP224	Amplimmune/ GlaxoSmithKline	B7DC and human IgG1 fusion protein	PD1–B7H1, PD1–B7DC	T cell activation and tolerance	Multiple cancers	I
BMS-936559	Bristol-Myers Squibb	B7H1-specific human IgG4	PD1–B7H1	T cell activation and tolerance	Advanced or recurrent solid tumours	I
MPDL3280A	Genentech/Roche	B7H1-specific engineered human IgG1	PD1–B7H1	T cell activation and tolerance	Solid tumours	I
MEDI4736	MedImmune/ AstraZeneca	B7H1-specific engineered human IgG1	PD1–B7H1	T cell activation and tolerance	Solid tumours	I
MEDI-570	MedImmune/ AstraZeneca	ICOS-specific human IgG	ICOS–B7H2	T cell-dependent B cell response	SLE	I
AMG 557	Amgen	B7H2-specific human IgG	ICOS, CD28, CTLA4	T cell-dependent B cell response	SLE, psoriasis	I
MGA271	Macrogenics	B7H3-specific, ADCC-enhanced humanized IgG1	B7H3	T cell activation and tolerance	Solid tumours	I
IMP321	Immutep	LAG3 and human IgG1 fusion protein	LAG3–MHCII	DC maturation and T cell activation	Multiple cancers	I/II
TNF family						
BMS-663513	Bristol-Myers Squibb	CD137-specific human IgG4	CD137	T cell activation	Solid tumours	I/II
PF-05082566	Pfizer	CD137-specific human IgG	CD137	T cell activation	Lymphoma	I
CDX-1127	Celldex	CD27-specific human IgG1	CD27	T cell activation	Multiple cancers	I
Anti-OX40	Providence Health & Services	OX40-specific mouse IgG	OX40	CD4 T cell activation	Prostate cancer	II
huMAb OX40L	Genentech/Roche	OX40L-specific human IgG1	OX40–OX40L	CD4 T cell activation	Asthma	II
TRX518	GITR Inc.	GITR-specific humanized IgG1	GITR–GITRL	T cell activation	Solid tumours	I
Atacicept	ZymoGenetics/ EMD Serono	TACI and human IgG1 fusion protein	TACI, BCMA and BAFFR	B cell activation and antibody production	SLE, rheumatoid arthritis, multiple sclerosis and optic neuritis	II/III
CP-870,893	Pfizer	CD40-specific human IgG1	CD40	APC activation and B cell maturation	Multiple cancers	I
Lucatumumab	Novartis	CD40-specific human IgG1	CD40	APC activation and B cell maturation	Lymphoma and leukaemia	I/II
Dacetuzumab	Seattle Genetics	CD40-specific humanized IgG1	CD40	APC activation and B cell maturation	Lymphoma and multiple myeloma	II

ADCC, antibody-dependent cell-mediated cytotoxicity; APC, antigen-presenting cell; B7H1, B7 homolog 1; BAFFR, B cell activation factor receptor; BCMA, B cell maturation antigen; CTLA4, cytotoxic T lymphocyte antigen 4; DC, dendritic cell; GITR, glucocorticoid-induced TNFR-related protein; GITRL, GITR ligand; HCV, hepatitis C virus; ICOS, inducible co-stimulator; IgG1, immunoglobulin G1; LAG3, lymphocyte activation gene 3; MHCII, major histocompatibility complex class II; OX40L, OX40 ligand; PD1, programmed cell death protein 1; SLE, systemic lupus erythematosus; TACI, transmembrane activator and CAML interactor; TNF, tumour necrosis factor.

promoting co-stimulatory signals, the blockade of CTLA4 co-inhibitory pathways has also been shown to be effective in treating cancer. Infusion of a CTLA4-blocking mAb results in sustained immune activation and induces potent antitumour responses with limited autoimmunity in mouse models[31–34]. At least two underlying mechanisms have been proposed to explain the antitumour effect: first, the CTLA4-blocking mAb prevents the engagement of B7 ligands to CTLA4 expressed on activated T cells, allowing persistent activation through CD28; second, the CTLA4-blocking mAb binds to and inhibits immunosuppressive $CD4^+CD25^+$ regulatory T (T_{Reg}) cells, which constitutively express a high level of cell surface CTLA4 (REFS 35–37).

Ipilimumab, a human IgG1 CTLA4-specific mAb, was approved by the FDA in 2011 for the treatment of metastatic melanoma. Ipilimumab induced a sustained immune response and objective response rates of 10–15% in patients with advanced melanoma and significantly prolonged the survival of these patients[38]. In Phase III trials with ipilimumab, about 80% of patients had drug-related adverse events and 30% of patients had grade III severe immune-related toxicity with lymphocyte infiltration into multiple organs, causing dermatitis, colitis and hepatitis[39,40]. The adverse events associated with ipilimumab correlate with therapeutic responses, indicating that the drug blocks a central co-inhibitory pathway that affects both antitumour immunity and autoimmunity. Careful evaluation of treatment dosing, frequency and timing is thus critical to minimize immune-related adverse events while preserving antitumour efficacy. Ipilimumab and another CTLA4-specific mAb, tremelimumab, are currently being evaluated for the treatment of several other cancer indications as either a monotherapy or in combination with chemotherapy, an adjuvant, a dendritic cell (DC) vaccine or other biologics.

As an attractive approach to enhance co-stimulation, agonistic mAbs targeting CD28 or ICOS have been shown to strongly promote T cell proliferation and cytokine production in the presence of antigenic signals *in vitro*[17,41]. These mAbs could be especially potent when applied together with an antigen and/or a CTLA4 antagonist. Although co-stimulatory CD28- or ICOS-targeting mAbs have not yet reached clinical development, a 'super-agonistic' CD28-targeting mAb, TGN1412, entered clinical trials based on its ability to stimulate naive human T cells without the need for a TCR signal. TGN1412 induced a severe systemic inflammatory response in healthy volunteers[42], characterized by a massive pro-inflammatory cytokine storm 90 minutes after infusion, which was followed by multi-organ injury and lymphocyte depletion. Fortunately, all volunteers survived after receiving immunosuppressive treatments and cardiopulmonary support in an intensive care unit. The lesson learned from this trial thus cautioned against global non-discriminatory stimulation of naive T cell activation, especially with biologics that have antigen-independent mitogenic activity[42].

Conversely, modulation of the B7–CD28 pathway can also induce strong immunosuppression and immune tolerance. CTLA4–Ig RFP, serving as a decoy receptor that disrupts B7–CD28 interactions, is a potent immunosuppressor. In addition to blocking the interactions between CD28 and B7.1, B7.2 and B7H2, CTLA4–Ig RFP engages B7.1 and B7.2 on DCs and negatively regulates DC function. Crosslinking of B7.1 and B7.2 by CTLA4–Ig on DCs induces the production of indoleamine 2,3-dioxygenase (IDO), an immunosuppressive enzyme involved in tryptophan metabolism, and suppresses T cell activation[43]. Through the inhibition of CD28 pathway activation, together with immune tolerance induction by IDO expression, CTLA4–Ig prolongs allograft survival and alleviates inflammatory conditions in several animal models of autoimmune disease, including diabetes, arthritis, multiple sclerosis, systemic lupus erythematosus (SLE) and colitis[44].

Based on these studies, abatacept (Orencia; Bristol-Myers Squibb), a fusion protein composed of the extracellular domain of CTLA4 and the IgG1 Fc region, was the first biologic targeting the B7–CD28 family to be approved (in 2005, for patients with rheumatoid arthritis who have an inadequate response to TNF neutralization therapy)[45]. It is currently being evaluated in other autoimmune or inflammatory indications in which T cells are hyperactive or T cell activation is undesired, such as psoriasis, ulcerative colitis, type 1 diabetes, lupus nephritis and organ transplantation. Recently, a second-generation CTLA4–Ig product, belatacept (Nulojix; Bristol-Myers Squibb), harbouring two engineered point mutations with improved binding affinity to B7 ligands compared to abatacept, was approved for the prevention of human kidney transplantation rejection. In a Phase III kidney transplantation trial, belatacept had similar graft survival but was associated with better renal function and an improved cardiovascular and metabolic risk profile compared to the standard cyclosporine A treatment[46,47]. Belatacept is also in Phase I/II clinical trials for the treatment of rheumatoid arthritis and type 1 diabetes.

With three therapeutic biologics targeting CTLA4 or based on CTLA4 approved in the clinic, mAbs targeting the B7 ligand — including B7.1-specific and B7H2-specific mAbs — are also being evaluated in clinical trials. In addition to being the co-signalling ligand for CD28 and CTLA4 on professional APCs, B7.1 is expressed on B cells and certain types of lymphomas. Crosslinking of B7.1 by the mAb galiximab (IDEC-114) leads to growth arrest and apoptosis of normal B cells and B cell lymphomas[48]. In a completed Phase I/II trial treating relapsed or refractory follicular lymphoma, galiximab was well tolerated with no dose-limiting toxicities at the dose ranged tested[49]. Galiximab is currently being evaluated in Phase III trials to treat relapsed or refractory Hodgkin's lymphoma alone or in combination with rituximab (Rituxan; Biogen Idec/Genentech/Roche), a CD20-specific mAb.

ICOS was originally thought of as a redundant co-stimulatory receptor to CD28. However, owing to its inducible expression on activated T cells, ICOS has its own unique role in the late phase of T cell activation, memory T cell formation and the T cell-dependent B cell response. Constitutively expressed on T_{FH} cells,

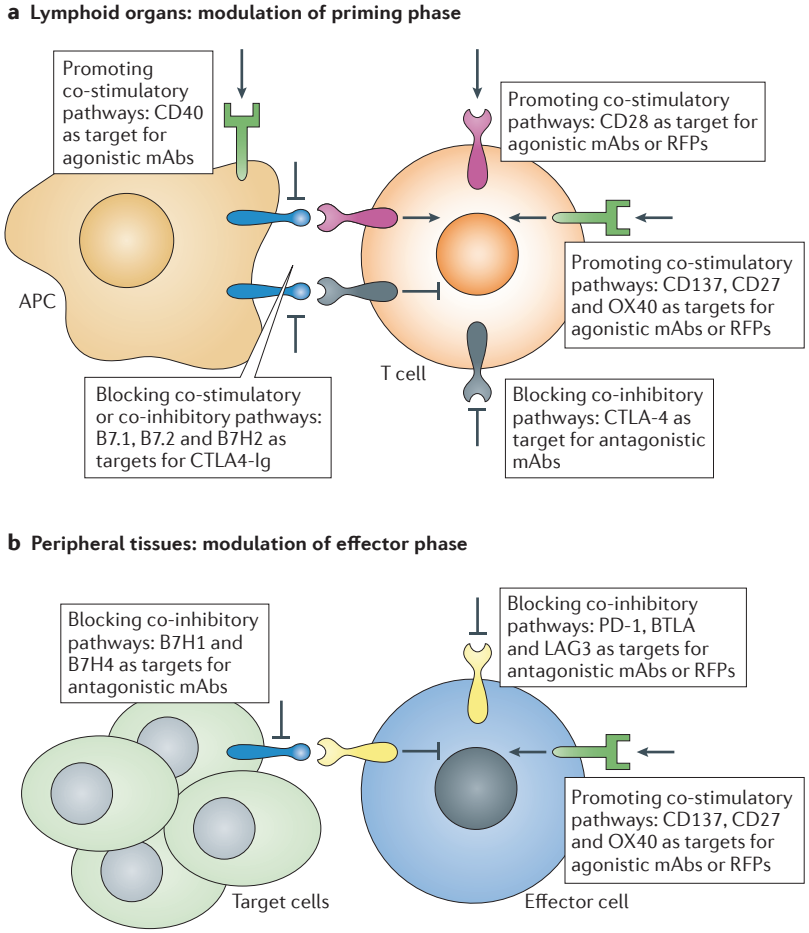

Figure 2 | **Immune modulation of the priming and the effector phase of lymphocyte activation. a** | To promote antigen-specific immune responses during priming in the lymphoid organs, distinctive co-stimulatory pathways can be engaged, such as CD28, CD137 (also known as 4-1BB), CD27, OX40 and CD40, by agonistic reagents or through blockade of the primary early checkpoint receptor cytotoxic T lymphocyte antigen 4 (CTLA4) by an antagonist. Vice versa, the CD28 co-stimulatory pathway can also be blocked by CTLA4–immunoglobulin (Ig) to inhibit T cell activation. **b** | To expand effector T cells or restore exhausted T cells in the peripheral organs, peripheral inhibitory pathways can be blocked, including the pathway mediated by B7 homolog 1 (B7H1) and programmed cell death protein 1 (PD1), B7H4, lymphocyte activation gene 3 (LAG3) as well as B- and T lymphocyte attenuator (BTLA); alternatively, co-stimulatory receptors on effector T cells — such as CD137 and OX40 — can be activated. mAb, monoclonal antibody.

the essential role of ICOS in B cell maturation and the antibody response is demonstrated by ICOS deficiency in both mouse models and humans[20,50,51]. Immunoglobulin class switching and memory B cell formation are severely impaired in ICOS-deficient mice as well as in ICOS-deficient patients, indicating that ICOS pathway blockade suppresses T- and B cell-mediated autoimmune conditions. In the NZB/NZW F_1 mouse model of SLE and in a collagen-induced arthritis (CIA) model, B7H2 antibody treatment indeed led to a decrease in T_{FH} cells as well as germinal centre B cells, and it inhibited disease progression[52]. The ICOS-targeting mAb MEDI-570 and the B7H2-targeting mAb AMG 557 are currently being evaluated in Phase I trials for the treatment of SLE.

NZB/NZW F_1 mouse model
The F_1 generation of the cross between New Zealand black (NZB) mice and New Zealand white (NZW) mice. NZB/NZW F_1 mice have a disease that closely resembles the human disease systemic lupus erythematosus.

B7H1, B7DC and PD1 pathways. B7H1 and its closest homologue B7DC (also known as PDL2) were identified as new B7 family members in 1999 (REF. 53) and 2001 (REF. 54), respectively, both sharing 20–23% protein sequence identity with B7.1 and B7.2 in their extracellular domain[53,54]. The *B7H1* mRNA transcript has broad tissue distribution, including both lymphoid (thymus, bone marrow, spleen and lymph node) and non-lymphoid organs (heart, skeletal muscle, placenta, lung, kidney and liver)[53]. B7H1 is a cell surface protein that is constitutively expressed on APCs (macrophages and DCs). However, it is also inducibly expressed in a large variety of tissues and cell types, including epithelial and endothelial cells, in response to pro-inflammatory cytokines such as interferons (IFNs)[55,56]. By contrast, the expression of B7DC is mainly restricted to DCs and macrophages[54,57].

PD1, a CD28 and CTLA4 homologue that was first identified in a T cell hybridoma undergoing apoptosis, is implicated in promoting programmed cell death[58]. PD1 is inducibly expressed on activated T cells, B cells, macrophages, DCs and monocytes but absent on their naive counterparts[59–61]. Upregulation of PD1 on these cells has been shown to inhibit both adaptive and innate immune responses[59–61]. In 2000, PD1 was found to be a receptor for B7H1 (REF. 62) and in 2001 it was found to be a receptor for B7DC[63]. PD1 contains an ITIM and an immunoreceptor tyrosine-based switch motif (ITSM) in its cytoplasmic region. Experimental data indicate that the ITSM but not the ITIM mediates SHP2 phosphatase recruitment and reverses activation-induced phosphorylation events[58].

Genetic ablation has demonstrated the crucial function of PD1 in controlling lymphocyte activation and in maintaining peripheral tolerance. PD1-deficient mice have various types and degrees of autoimmune conditions, depending on strain backgrounds. PD1-deficient BALB/c mice have dilated cardiomyopathy, congestive heart failure and frequently suffer sudden death[64], which is caused by the generation of high-titre autoantibodies against the heart-specific protein cardiac troponin I[65]. PD1-deficient C57BL/6 mice spontaneously develop arthritis and lupus-like disease at an advanced age (>14 months)[66]. PD1-deficient non-obese diabetic (NOD) mice develop early-onset type 1 diabetes[67]. The suppressive functions of PD1 in the periphery have been shown to be largely mediated through B7H1 rather than B7DC *in vivo*[68,69], as only B7H1 is expressed in peripheral tissue.

The crucial function of the B7H1–PD1 axis in the control of human T cell activation and in maintaining peripheral tolerance appears to be exploited by tumour cells and by viruses during chronic viral infections[70,71]. B7H1 is overexpressed on many freshly isolated human tumours from different tissue origins[71,72]. The expression of B7H1 has been correlated with the progression and poor prognosis of certain types of human malignancies[68,73]. During chronic viral infections, B7H1 is persistently expressed on both lymphoid and peripheral tissues; meanwhile, PD1 is upregulated on virus-specific CTLs. The B7H1–PD1 pathway has been identified to contribute

to T cell exhaustion[74] — a T cell hyporeactive condition that occurs during chronic viral infections. Tumour- or virus-induced B7H1 appears to utilize multiple mechanisms to suppress and facilitate the evasion of host immune surveillance, including the promotion of T cell anergy, exhaustion, unresponsiveness and apoptosis, inducing the expansion of T_{Reg} cells as well as enhancing tumour-intrinsic resistance to killing and apoptosis[70].

In a model of CTL-mediated killing of a human melanoma cell line (624mel), it was shown that B7H1-transfected 624mel cells increased the rate of apoptosis of 624mel-specific CTLs. The inclusion of a B7H1-specific mAb partially suppressed this apoptotic effect[72]. Consistent with this *in vitro* observation, in a mouse P815 tumour model, B7H1 transfectants also induced tumour-specific 2C transgenic T cell apoptosis[72]. Interestingly, when a different tumour-specific TCR transgenic CTL (P1A) was used in the same P815 system, the B7H1-transfected P815 model did not induce P1A apoptosis; rather, it promoted unresponsiveness — a phenomenon also known as 'molecular shielding'. Under this condition, CTLs remain fully functional against B7H1-negative tumours but ignore B7H1-positive tumour cells[75]. Recently, another mechanism of B7H1-mediated immune evasion by tumour cells came to light. Upon PD1 ligation, tumour-associated B7H1 was shown to act as a receptor to deliver an anti-apoptotic signal to tumour cells through its intracellular domain, which renders these cells resistant to CTL lysis and FAS-induced apoptosis[76]. Most importantly, B7H1–PD1 blockade *in vivo* by a B7H1- or PD1-specific mAb promoted CTL expansion[77] and accelerated tumour regression or viral clearance in many murine tumour models or murine models of chronic viral infection[75,78]. Therefore, B7H1 may facilitate multiple mechanisms to evade host antitumour or antiviral immunity *in vivo*.

The recent identification of B7.1 as a second receptor for B7H1 reveals the complexity of B7 family interactions and indicates a potential crosstalk between the CD28–CTLA4 and PD1 pathways[79] (FIG. 3). Induced on activated T cells, B7.1 serves as an inhibitory receptor for B7H1 to suppress T cell responses *in vitro*[79] and contributes to the formation of T cell tolerance *in vivo*[80]. Interestingly, the expression of B7.1 on T_{Reg} cells and its interaction with B7H1 expressed on APCs has a crucial role in T_{Reg} cell expansion during inflammatory responses in a mouse model of graft-versus-host disease (GVHD)[81]. The presence of two ligands (B7H1 and B7DC) for PD1 and two inhibitory receptors (PD1 and B7.1) for B7H1 suggests that biologics that target either PD1 or B7H1 have differential effects. One could speculate that an antagonistic PD1-specific Ab will block the PD1 pathway but leave B7.1–B7H1 interactions untouched, whereas a B7H1-blocking Ab could disrupt both B7H1–PD1 and B7.1–B7H1 pathways without interfering with the B7DC–PD1 interaction. Complete abrogation of B7H1- and PD1-mediated inhibitory pathways thus requires a combination strategy targeting both molecules.

At least seven therapeutic biologics targeting the human B7H1–B7DC–PD1 pathway are currently in clinical trials, and some of these agents have shown promising results. Three PD1-targeting mAbs (BMS-936558/MDX-1106, CT-011 and MK-3475), three B7H1-targeting mAbs (BMS-936559/MDX-1105, MPDL3280A and MEDI4736) and a B7DC–Ig RFP are being evaluated for the treatment of various advanced cancers or hepatitis C virus (HVC) infection. Two published studies of Phase I trials using BMS-936558/MDX-1106 (REF. 82) or CT-011 (REF. 83) as a monotherapy for the treatment of refractory solid tumours or haematological malignancies, respectively, have revealed that both PD1-targeting mAbs were generally well tolerated and had no dose-limiting toxicity up to 10 mg per kg body weight[82,83]. 14% of patients developed grade 3 or grade 4 drug-related adverse events and there were three deaths caused by pulmonary toxicity. Immune-related adverse events included colitis, hypothyroidism and polyarticular arthropathies, but in general these were milder than those observed in the trials with ipilimumab (the CTLA4-specific mAb).

A recent report summarizing the results of 296 patients treated with BMS-936558 showed significant objective responses (partial or complete) in 28% of patients with metastatic melanoma, 27% of patients with renal cell carcinoma and 18% of patients with non-small-cell lung cancer (NSCLC). The result in NSCLC is particularly encouraging, as patients with this type of cancer have responded poorly to immunotherapy in the past[6]. In this unusually large Phase I study, the results of all

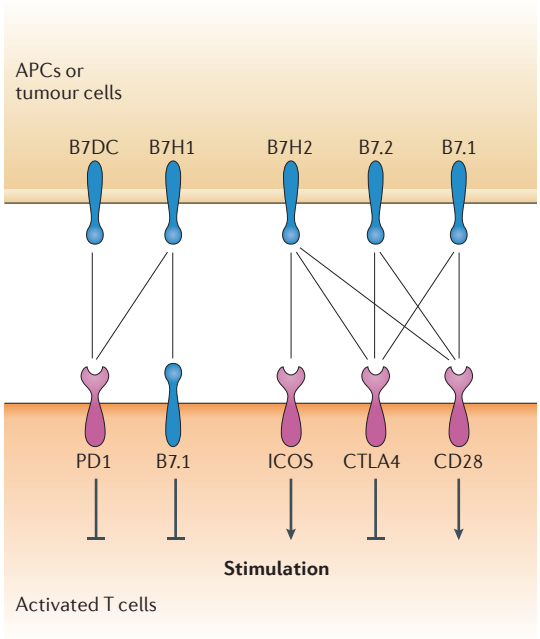

Figure 3 | **B7–CD28 family and newly discovered interactions.** In addition to known interactions (see FIG. 1), B7 homolog 1 (B7H1) was recently found to interact with and deliver a negative signal through B7.1, which is expressed on activated T cells. Human B7H2 — also known as inducible co-stimulator ligand (ICOSL) — was identified to engage both CD28 and cytotoxic T lymphocyte antigen 4 (CTLA4) to modulate T cell activation in addition to ICOS. APC, antigen-presenting cell.

escalating doses were included in the analysis. However, for active doses (determined at 3 mg per kg or 10 mg per kg) the objective response rates were even higher. For example, treatment with 3 mg per kg of anti-PD1 therapy led to objective responses in 41% of patients with melanoma and 32% of patients with NSCLC. The clinical response was shown to be durable with a rare recurrence, indicating the formation of immune memory[172]. B7H1 overexpression in the tumour appeared to correlate with antitumour responses in a small patient cohort[6,82], suggesting the potential use of B7H1 expression as a biomarker or inclusion criteria for this type of treatment. A recent study identified a strong association of melanoma-expressed B7H1 with the presence of tumour-infiltrating lymphocytes[84]. Tumours may thus upregulate B7H1 as an adaptive immune resistance mechanism in response to IFNγ released by tumour-infiltrating lymphocytes to suppress effector T cell function.

The first Phase I clinical trial report of 207 patients treated with a B7H1-targeting mAb (BMS-936559) showed a similar safety profile, with 9% of patients experiencing grade 3 or grade 4 toxicity — slightly lower than the 14% observed in the trials of PD1-targeting mAbs. Importantly, B7H1 blockade also induced durable tumour regression with objective response rates of 6–17% and stable disease rates of 12–41% among patients with cancer[85]. Because B7H1 is shown to be a major inhibitory ligand for PD1 *in vivo*, the lower efficacy of a B7H1-targeting mAb in comparison with a PD1-targeting mAb is somewhat unexpected. However, a direct comparison of the two different mAbs is difficult because the effect of a blocking antibody is also determined by its affinity or avidity, pharmacokinetics, stability, and so on. In addition to mAbs, a B7DC–IgG1 RFP (AMP-224) that targets the PD1 pathway is now in a Phase I trial for the treatment of advanced solid tumours.

The therapeutic principle of B7H1–PD1 blockade appears to be distinct to that of B7.1– or B7.2–CTLA4 blockade. As described above, antibodies targeting PD1 and B7H1 may work mainly by improving effector T cell functions in the tumour microenvironment, where this interaction largely occurs[84]. By contrast, CTLA4-specific mAbs may function by inhibiting naive T cell priming largely in lymphoid organs, where the B7.1– or B7.2–CTLA4 interactions take place. With high rates of durable clinical responses and a reasonable safety profile, B7H1–PD1 blockade could change the paradigm of cancer treatment and may become the building block for future combinations with other therapies. When CTLA4-specific therapy and B7H1–PD1 blockade are carefully combined with direct cancer-killing mechanisms such as traditional chemotherapy or radiation therapy, *de novo* priming and expansion of existing tumour-specific T cells could be greatly enhanced, as shown in animal tumour models[86]. PD1-specific mAbs in combination with tumour antigen-based vaccines, chemotherapy or other biologics (such as ipilimumab or rituximab) targeting multiple pathways are currently in clinical trials for the treatment of late-stage cancers (TABLE 3).

Ig receptors that are emerging as targets

In addition to CTLA4 and PD1, the discovery of several co-inhibitory pathways involving Ig family molecules (FIG. 2) — including BTLA, B7H4 (also known as VCTN1), lymphocyte activation gene 3 (LAG3), T cell immunoglobulin mucin 3 (TIM3) and the PD1 homolog (PD1H) (also known as V-domain Ig suppressor of T cell activation; VISTA) — has opened up new avenues for treating cancer and autoimmunity.

BTLA. BTLA is a CD28 receptor family member that was identified in 2003 (REF. 9). It has a single IgV extracellular domain but limited sequence identity with other CD28 family molecules, such as CD28 (11% sequence identity), CTLA4 (14% sequence identity) and PD1 (13% sequence identity)[9]. BTLA is expressed on T and B lymphocytes as well as subsets of DCs. Herpesvirus entry mediator (HVEM), a TNFR that is widely expressed in the haematopoietic system, was identified as a counter-receptor for BTLA[87]. BTLA contains two ITIMs in its cytoplasmic region, which recruit SHP1 and SHP2 phosphatases upon receptor activation by mAb crosslinking or ligand engagement. BTLA-deficient mice show enhanced T cell activation and exacerbated disease in models of autoimmunity and inflammation, including experimental autoimmune encephalomyelitis (EAE), SLE, airway inflammation in asthma and concanavalin A (ConA)-induced hepatitis[9], indicating a suppressive function of BTLA in controlling T cell activation *in vivo*. Studies of peripheral blood mononuclear cells from patients with melanoma have revealed that BTLA is expressed at high levels on tumour-specific CTLs and inhibits T cell function upon its engagement by tumour-expressed HVEM[88], suggesting that BTLA blockade might potentially improve T cell function and antitumour immunity. By contrast, in murine models of GVHD, BTLA-targeting agonistic mAbs suppress anti-host donor T cell responses[89,90], indicating their potential therapeutic value in transplantation and autoimmune diseases.

B7H4. B7H4 (REFS 91–93) shares 22% and 23% sequence identity with human B7.1 and B7.2, respectively, and was first discovered in 2003. The *B7H4* mRNA has a broad tissue distribution, whereas the B7H4 protein is mainly detectable in peripheral tissues, including the pancreas, ovary and prostate[92]. Although B7H4 is absent from naive haematopoietic cells, high levels of the protein were found in immunosuppressive tumour-associated macrophages and were associated with poor prognosis in patients with ovarian cancer[94,95]. A putative counter-receptor for B7H4 was detected on activated T cells but its identity has yet to be determined[91,92]. The inhibitory function of B7H4 on T cell-mediated immunity was initially reported by three groups[91–93]. An agonistic B7H4–Ig fusion protein inhibits T cell proliferation and cytokine production by inducing cell cycle arrest[92]. B7H4-deficient mice exhibit aggravated disease progression in models of EAE and rheumatoid arthritis[91], further supporting the inhibitory role of B7H4 *in vivo*.

Interestingly, B7H4 was found on a high percentage of freshly isolated melanoma cells as well as ovarian, prostate and lung carcinoma cells[96]. B7H4 expression is associated with tumour progression and poor prognosis in multiple cancer types[97–99], indicating that cancer cells might utilize this inhibitory mechanism to evade the host immune system. Blockade of the B7H4 pathway or tumour targeting using B7H4-specific mAbs is currently being evaluated *in vivo* in cancer models. Conversely, administration of an agonistic B7H4–Ig fusion protein

Table 3 | **Combination therapies involving immunomodulatory biologics in clinical trials**

Biologic	Combination drugs	Targets in combination	Indications	Trial phase
Ipilimumab	Paclitaxel–carboplatin	CTLA4, chemotherapy	Non-small-cell lung cancer	III
Ipilimumab	Melphalan–dactinomycin	CTLA4, chemotherapy	Melanoma	II
Ipilimumab	Leuprolide–goserelin–degarelix	CTLA4, androgen deprivation	Prostate cancer	II
Ipilimumab	Interferon alfa-2b	CTLA4, IFNα	Melanoma	III
Ipilimumab	GM-CSF	CTLA4, GM-CSF	Melanoma	II
Ipilimumab	Bevacizumab	CTLA4, VEGFR	Melanoma	I
Ipilimumab	Dacarbazine	CTLA4, chemotherapy	Melanoma	II
Tremelimumab	CP-870893	CTLA4, CD40	Late-stage melanoma	I
Tremelimumab	DC vaccine	CTLA4, vaccine	Late-stage melanoma	II/III
Tremelimumab	BCG	CTLA4, adjuvant	Bladder cancer	I
Tremelimumab	Bicalutamide	CTLA4, androgen receptor	Prostate cancer	I
Galiximab	Rituximab	B7.1, CD20	Non-Hodgkin's lymphoma	II
BMS-936558	Ipilimumab	PD1, CTLA4	Late-stage melanoma	I
BMS-936558	IPH21	PD1, KIR2DL1, KIR2DL2, KIR2DL3	Advanced solid tumours	I
BMS-936558	Peptide vaccine	PD1, peptide, adjuvant	Late-stage melanoma	I
BMS-936558	Sunitinib and pazopanib	PD1, chemotherapy	Metastatic renal cell carcinoma	I
BMS-936558	Gemcitabine, cisplatin, pemetrexed, paclitaxel, carboplatin, erlotinib and bevacizumab	PD1, chemotherapy, VEGFR	Late-stage non-small-cell lung cancer	I
BMS-936558	Docetaxel	PD1, chemotherapy	Squamous cell non-small-cell lung cancer	III
MK-3475	Carboplatin, paclitaxel, dacarbazine and temozolomide	PD1, chemotherapy	Advanced melanoma	II
CT-011	Rituximab	PD1, CD20	Lymphoma	II
CT-011	FOLFOX	PD1, chemotherapy	Colorectal cancer	II
CT-011	Gemcitabine	PD1, chemotherapy	Pancreatic cancer	II
CT-011	DC vaccine	PD1, vaccine	Multiple myeloma, AML	II
CT-011	Sipuleucel-T and cyclophosphamide	PD1, vaccine, chemotherapy	Prostate cancer	II
CT-011	p53 peptide	PD1, p53 genetic vaccine	Advanced solid tumours	I
IMP321	Peptide vaccine	LAG3, peptide	Melanoma	I/II
IMP321	Gemcitabine	LAG3, chemotherapy	Pancreatic cancer	I
PF-05082566	Rituximab	CD137, CD20	Non-Hodgkin's lymphoma	I
Anti-OX40	Radiation therapy and cyclophosphamide	OX40, chemotherapy, radiation therapy	Prostate cancer	I/II
Atacicept	Adalimumab (Humira)	BAFF–APRIL, TNF	Rheumatoid arthritis	II
CP-870,893	Paclitaxel and carboplatin	CD40, chemotherapy	Advanced solid tumours	I
CP-870,893	Peptide vaccine	CD40, vaccine	Melanoma	I
CP-870,893	Gemcitabine	CD40, chemotherapy	Pancreatic cancer	I
Dacetuzumab	Rituximab, carboplatin and gemcitabine	CD40, CD20, chemotherapy	Lymphoma	II
Dacetuzumab	Lenalidomide and dexamethasone	CD40, chemotherapy	Multiple myeloma	I

AML, acute myeloid leukaemia; APRIL, a proliferation-inducing ligand; BAFF, B cell activation factor; BCG, bacille Calmette–Guérin; CTLA4, cytotoxic T lymphocyte antigen 4; DC, dendritic cell; FOLFOX, folinic acid, 5-fluorouracil, oxaliplatin; GM-CSF, granulocyte–macrophage colony-stimulating factor; IFNα, interferon-α; KIR2DL1, killer cell immunoglobulin-like receptor 2DL1; LAG3, lymphocyte activation gene 3; p53, tumour suppressor p53; PD1, programmed cell death protein 1; TNF, tumour necrosis factor; VEGFR, vascular endothelial growth factor receptor.

alleviates disease phenotypes in the CIA model of rheumatoid arthritis and reduces the incidence of autoimmune diabetes in the NOD mouse model[100,101], indicating its therapeutic potential in autoimmune diseases.

LAG3. First identified in 1990 (REF. 102), LAG3 is an Ig family receptor with a similar domain structure and 20% sequence identity to CD4 (REF. 103). Like CD4, LAG3 interacts with major histocompatibility complex (MHC) class II molecules. LAG3 has a broad expression pattern in the haematopoietic system, including activated T cells and T_{Reg} cells, plasmacytoid DCs, B cells, natural killer (NK) cells, natural killer T (NKT) cells and γδT cells[103]. The inhibitory function of LAG3 on activated T cells has been well documented. Crosslinking LAG3 by mAbs on activated T cells inhibits T cell proliferation and cytokine production induced by CD3 crosslinking *in vitro*[104]. Together with a group of T cell inhibitory receptors, including PD1, CTLA4, CD160 and the NK cell receptor 2B4 (also known as CD244), persistent expression of LAG3 on virus-specific $CD8^+$ T cells has been associated with T cell exhaustion during chronic viral infection[105]. Importantly, a combination of LAG3-blocking mAbs with PD1-specific mAbs reverses the phenotype of exhausted T cells and improves virus clearance more effectively than treatment with a PD1-specific mAb alone in a murine model of chronic lymphocytic choriomeningitis infection[105]. Similarly, co-expression of LAG3 and PD1 on tumour-infiltrating lymphocytes is associated with impaired T cell function in patients with ovarian cancer. Dual blockade of the LAG3 and PD1 pathways improves tumour-specific T cell proliferation and cytokine production *in vitro*[106,107]. Targeting several T cell co-inhibitory pathways that contribute to the suppressive state of tumour-specific T cells might thus yield better clinical outcomes than the blockade of a single pathway.

A combination of a LAG3-specific mAb with a tumour vaccine also increases the number of tumour-infiltrating CTLs and promotes tumour regression in a murine tumour model[108]. A LAG3-targeting mAb thus holds great potential to improve the efficacy of tumour vaccines or PD1-targeting mAbs in patients with cancer. Meanwhile, an RFP of LAG3 and human IgG1 (IMP321) has shown adjuvant properties by activating DCs through MHCII crosslinking[109]. IMP321 enhanced the immunogenicity of influenza and hepatitis B virus (HBV) vaccines in Phase I trials[110,111]. As a monotherapy in a Phase I trial treating patients with advanced renal cell carcinoma, IMP321 showed no dose-limiting toxicity and no clinically significant adverse events in the dose range tested[112]. IMP321 is currently being evaluated in Phase I/II trials for the treatment of advanced melanoma or pancreatic cancer in combination with a peptide vaccine and/or chemotherapy.

PD1H. VISTA[113], also called PD1H[114], has been identified as an Ig family member with immune-inhibitory functions. It has the highest sequence similarity with PD1 and the extracellular domain structure of a single IgV. PD1H has a broad expression pattern in the haematopoietic system, including T cells, NK cells, macrophages, DCs and neutrophils, with the exception of B cells. Overexpression of PD1H on murine tumour cells increased their immunogenicity, and a PD1H-specific mAb exacerbated the development of EAE in mice[113]. Treatment with a single dose of PD1H-specific mAb treatment prevents the induction of GVHD and promotes survival in mouse models[114], indicating the therapeutic value of PD1H-specific mAbs in manipulating immune responses.

The TNF and TNFR superfamilies

Members of the TNF and TNFR family provide divergent signals that are essential for haematopoiesis, the development of secondary lymphoid organs, innate and adaptive immune responses and bone absorption. For T and B lymphocytes, TNF and TNFR family molecules are essential for differentiation, effector functions and the formation of a memory T cell population[115]. Both TNF ligands and receptors have a trimeric structure as their basic units. According to their intracellular domain and cellular functions, TNFRs can be classified into three main groups: death domain-containing TNFRs, decoy TNFRs and TNFR-associated factor (TRAF)-recruiting TNFRs. Death domain-containing TNFR family members include CD95 (also known as FAS), TNFR1, death receptor 3 (DR3), DR4 (also known as TRAILR1), DR5 (also known as TRAILR2) and DR6. Crosslinking of death domain-containing TNFRs by TNF ligands triggers caspase activation and apoptosis, which have been extensively reviewed in the literature[116,117]. Several therapeutic agonistic mAbs targeting tumour-expressed death domain-containing TNFRs induce cancer cell destruction and are currently in clinical trials[118–120]. Decoy TNFRs, including decoy receptor 1 (DCR1; also known as TRAILR3), DCR2 (also known as TRAILR4), DCR3 and osteoprotegerin (OPG), are receptors without intracellular signalling domains. They bind and neutralize TNF ligands and block downstream signalling.

The rest — and the majority — of TNFRs belong to the third family: TRAF-recruiting TNFRs. Upon engagement with TNF ligands, these TNFRs recruit distinctive TRAF family adaptor proteins through intracellular domains to differentially activate MAPK signalling cascades, promote nuclear factor-κB (NF-κB) activation and enhance cellular proliferation and function. In addition to the successful neutralizing biologics against TNF for treating TNF-mediated inflammation, therapeutic biologics targeting other members of the TNF superfamily have been shown to be promising for the treatment of cancer, bacterial and viral infections as well as autoimmune diseases[121].

Death domain-containing TNFR pathways

The engagement of death domain-containing TNFRs by TNF ligands or agonistic mAbs induces apoptosis, representing promising therapeutic strategies if tumour cells or inflammatory effector cells can be specifically targeted. Systematic administration of TNF, CD95 ligand (CD95L; also known as FASL) or agonistic mAbs directed at TNFRs, however, causes severe liver toxicity as a result of massive hepatocyte apoptosis[116]. By contrast, targeting TNF-related apoptosis-inducing ligand (TRAIL; also known as TNFSF10) receptor pathways

has shown encouraging antitumour results in animal models[117]. TRAIL, a soluble TNF ligand, is expressed and secreted by activated lymphocytes and DCs. TRAIL engages two death domain-containing receptors, TRAIL receptor 1 (TRAILR1; also known as DR4) and TRAILR2 (also known as DR5), to induce apoptosis. TRAIL can also be neutralized by binding to three decoy receptors, TRAILR3 (also known as DCR1), TRAILR4 (also known as DCR2) and OPG.

TRAILR1 has a low expression on normal tissues compared with TNFR1 and FAS, and has been shown to negatively regulate the innate immune response. By contrast, TRAILR2 is induced on activated lymphocytes and overexpressed on some tumour cells. In line with the expression pattern of TRAILR1 and TRAILR2, agonistic mAbs directed against TRAILR-positive tumour cells cause limited toxicity to normal tissues. Alternatively, a single-chain variable fragment (scFv) targeting a tumour surface antigen can be fused with CD95L and TRAIL, which can provide tumour-specific targeting and delivers the trimeric CD95L and TRAIL fusion protein locally to the tumour site. Meanwhile, prodrugs consisting of apoptosis-inducing TNF ligands (such as TNF, CD95L and TRAIL) fused with a protease cleavage sequence are being developed. For these drugs, TNF ligands are only activated upon entering the tumour microenvironment, where they are proteolytically released by tumour-associated proteases[117].

Several therapeutic biologics targeting the TRAIL–TRAILR pathway are currently in clinical trials for the treatment of cancer. Two recombinant human TRAIL proteins, dulanermin and AMG 951, in combination with rituximab, bevacizumab (Avastin; Roche/Genentech), cetuximab (Erbitux; Bristol-Myers Squibb/Lilly) or chemotherapy, are being evaluated for the treatment of metastatic colorectal cancers, non-Hodgkin's lymphoma and NSCLC. Mapatumumab (HGS-ETR1), an agonistic human TRAILR1-targeting mAb, is being used to treat solid tumours alone or in combination with chemotherapy. In completed Phase I/II trials treating non-Hodgkin's lymphoma, colorectal cancer and NSCLC, mapatumumab was well tolerated without hepatotoxicity. In a clinical trial of non-Hodgkin's lymphoma, 2 out of 40 patients experienced complete responses and one further patient had a partial response[173]. Similarly, three TRAILR2-targeting mAbs — conatumumab, lexatumumab and CS-1008 — are in clinical trials for multiple cancers, alone or in combination with other mAbs or chemotherapy regimens. Recently, results were reported of Phase I trials using conatumumab and lexatumumab to treat advanced solid tumours[118–120]. Conatumumab and lexatumumab were well tolerated and had no dose-limiting toxicity in the dose range tested. In summary, biologics targeting TRAIL and TRAILRs appear to have promising antitumour efficacy with limited hepatic toxicity.

BAFF–APRIL and BCMA–TACI–BAFFR pathways. B cell activation factor (BAFF; also known as BLYS and TNFSF13B) was first identified as a monocyte-expressed TNF family ligand that promotes B cell activation and was later shown to be one of the most important co-stimulatory ligands for B cell development and function[122]. BAFF and its homologue APRIL (a proliferation-inducing ligand) bind to B cell maturation antigen (BCMA) as well as transmembrane activator and CAML interactor (TACI) to promote B cell development, class switching and survival[123,124]. BAFF, but not APRIL, also binds to BAFF receptor (BAFFR) to promote the development and survival of B2 cells and marginal zone B cells[125].

In 2011, belimumab (Benlysta; Human Genome Sciences/GlaxoSmithKline), a human IgG1 mAb targeting BAFF, was the first drug to be approved by the FDA for the treatment of SLE in 50 years. Belimumab sequesters soluble and membrane-bound BAFF and suppresses B cell proliferation and antibody production, which significantly reduces levels of serum immunoglobulin and autoantibodies in patients with SLE, especially autoantibodies against nuclear and double-stranded DNA. A reduction in various circulating B cell populations was observed in the treatment group, which prompted the evaluation of belimumab in multiple clinical trials for the treatment of other B cell-mediated autoimmune diseases, including rheumatoid arthritis and Sjögren's syndrome, as well as B cell malignancies such as Waldenström's macroglobulinaemia[125]. Moreover, atacicept, a fusion protein of TACI and human Ig with the ability to neutralize BAFF and APRIL and thus block BCMA–TACI–BAFFR activation, is currently in different phases of clinical trials to treat SLE, rheumatoid arthritis, relapsing multiple sclerosis, optic neuritis and B cell malignancies, as a monotherapy or in combination with chemotherapy drugs or other biologics (TNF- and CD20-targeting biologics)[125].

CD40L–CD40 pathway. CD40 is expressed on B cells, DCs and macrophages, and has an essential function in controlling T cell-dependent B cell responses, including B cell proliferation, survival, Ig production, class switching and memory B cell formation[126]. CD40 ligand (CD40L; also known as CD154) is expressed on activated T cells, B cells and other myeloid cells. *CD40L* mutations have been identified in humans and are associated with hyper IgM syndrome, which is characterized by defects in Ig class switching and germinal centre formation. Similarly, mice in which *CD40l* or *CD40* is genetically ablated have profound defects in humoral immune responses[126]. Antagonistic mAbs targeting the CD40L–CD40 pathway have thus been in development for the treatment of autoimmune conditions since 2004.

Conversely, recombinant CD40L protein and CD40-targeting agonistic mAbs have the ability to promote antitumour immunity in animal models and are thus being evaluated in several cancer trials. In clinical trials, CD40L-targeting mAbs have been shown to cause severe thrombosis in patients by unexpectedly inducing massive platelet activation. By contrast, CD40-targeting mAbs have proven to be a safer approach for either blocking the CD40–CD40L pathway (to treat autoimmune conditions) or for inducing CD40 signalling (to promote antitumour immunity).

In completed Phase I trials of two agonistic CD40-targeting mAbs, CP-870,893 and dacetuzumab, treating solid tumours or B cell non-Hodgkin's lymphoma,

respectively, no dose-limiting toxicity and dose-dependent adverse events were observed and the mAbs were associated with objective responses[127,128]. It was originally believed that CD40 crosslinking first activates APCs, which in turn promote the priming and expansion of tumour-specific T cells. However, a new underlying mechanism of agonistic CD40-targeting mAbs in cancer treatment has recently emerged. CP-870,893, in combination with the chemotherapy agent gemcitabine, has shown efficacy against pancreatic ductal adenocarcinoma by directly activating tumour-infiltrating macrophages to destroy the tumour stroma independently of T cell activities[129].

CD137L–CD137 pathway. CD137 was first cloned from a T cell cDNA library in 1989 (REF. 130). Subsequent studies showed that CD137 has a broad inducible expression pattern in both the haematopoietic system (including T and B lymphocytes, NK and NKT cells, monocytes and DCs) and in non-haematopoietic cells (including epithelial, endothelial and smooth muscle cells)[131]. CD137 ligand (CD137L) is mainly expressed on APCs (DCs, B cells and macrophages) and is inducibly expressed on activated T cells and endothelial cells. In line with its expression pattern, CD137 signalling modulates both innate and adaptive immune responses. In the presence of a TCR signal, agonistic CD137-targeting mAbs co-stimulate the proliferation of both $CD4^+$ and $CD8^+$ T cells, induce cytokine production, enhance the cytotoxic activity of CTLs and protect T cells from activation-induced cell death[132]. A recent report shows that a CD137-targeting mAb stimulates memory T cell expansion in the absence of cognate antigens[133] and improves CD8 memory T cell survival. CD137 crosslinking on NK cells stimulates NK cell proliferation and IFNγ production[134], whereas the engagement of CD137 on DCs promotes DC activation by enhancing cytokine production and B7 upregulation[135].

Consistent with their immune-activating properties *in vitro*, agonistic CD137-targeting mAbs have been shown to be potent antitumour reagents that utilize multiple mechanisms in many animal tumour models[136,137]. The antitumour effects of these CD137 agonists have also been validated by CD137L–Fc RFPs[138], CD137 aptamers (agonistic synthetic nucleic acid species)[139] and CD137L-transfected tumour cells[121]. Using targeted depletion, $CD8^+$ T cells have been shown to be essential for the antitumour effects of agonistic CD137-targeting mAbs *in vivo*; $CD4^+$ T cells, NK cells, DCs and non-haematopoietic cells also have roles — depending on the models used[136,137,140]. In addition to the direct effects on immune cells, CD137-targeting mAbs upregulate cell adhesion molecules including intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1) on endothelial cells to recruit and retain activated T cells at the tumour site[141]. Furthermore, in a peptide-induced T cell anergy model, administration of agonistic CD137-targeting mAbs prevented anergy formation and also reversed established anergy[142], suggesting that agonistic CD137-targeting mAbs could potentially disrupt immune tolerance in the tumour microenvironment or during chronic viral infections.

In addition to their profound antitumour effects, agonistic CD137-targeting mAbs surprisingly display anti-inflammatory properties in several autoimmune disease models, including EAE[143], chronic GVHD[144], CIA[145], colitis[146], asthma[147], type 1 diabetes in NOD mice[148] and SLE-like disease in *Fas* (*lpr*)-deficient or NZB/NZW F_1 mice[149,150]. The underlying mechanism of the anti-inflammatory effects of agonistic CD137-targeting mAbs includes apoptosis of autoreactive T cells and B cells as well as expansion of T_{Reg} cells. Conversely, in HBV-transgenic mice, agonistic CD137-targeting mAbs induced liver toxicity mediated by CD8 T cell expansion and the accumulation of CTLs as a result of enhanced resistance to activation-induced cell death[151]. Repetitive injection of CD137-targeting mAbs[152] or systemic transgenic CD137L expression[153] progressively depletes B cells *in vivo*, indicating the potential for inducing B cell toxicity when the CD137 pathway is persistently activated.

In a completed Phase I/II trial treating late-stage cancers including melanoma, renal cell carcinoma and ovarian cancer, BMS-663513 — a fully human agonistic CD137-targeting mAb — caused manageable autoimmune adverse effects when administrated at dose levels ranging from 0.3 mg per kg to 10 mg per kg. Following treatment with BMS-663513, 6% of patients with melanoma had partial responses, and 17% of patients with melanoma and 14% of patients with renal cell carcinoma had no tumour progression for more than 6 months[154]. In a separate Phase I trial treating advanced solid tumours, however, severe liver toxicity (grade 4 hepatitis) was observed with BMS-663513 monotherapy, especially at high doses, leading to the termination of the trial. Detailed biomarker studies from the completed Phase I/II trial indicated that the 0.3 mg per kg dose of BMS-663513 was sufficient to elicit antitumour activity without causing significant liver toxicity, indicating that a low dosing regimen could potentially strike the balance between antitumour efficacy and autoimmunity.

OX40L–OX40 pathway. OX40 shares many functional similarities with the CD137 receptor in the control of T cell activation, expansion, survival and memory T cell formation, but with a preferential effect on $CD4^+$ T cells[115]. This is in line with the OX40 expression pattern, which is restricted to $CD4^+$ cells and $CD4^+FOXP3^+$ (forkhead box P3-positive) T_{Reg} cells. Like biologics targeting CD137, OX40-targeting agonistic mAbs show therapeutic efficacy against tumours in several animal models. Depletion experiments indicate that $CD8^+$ and $CD4^+$ T cells, and sometimes NKT cells, are the cellular components that mediate the antitumour effects of OX40-specific mAbs *in vivo*. With high levels of OX40 expression on T_{Reg} cells, one agonistic OX40-specific mAb has recently been shown to prevent the generation of the inducible T_{Reg} (iT_{Reg}) cell population in the tumour microenvironment and thus improve the antitumour immune response[155]. Furthermore, in a murine B16 tumour model the combination of the chemotherapy drug cyclophosphamide with an OX40-specific agonistic mAb induced profound T_{Reg} cell deletion through activation-induced cell death in the tumour microenvironment and led to the

regression of established tumours[156]. An OX40-specific mAb sponsored by Providence Health & Services is currently in Phase I trials for the treatment of metastatic prostate cancer as a monotherapy or in combination with radiation therapy and cyclophosphamide.

Conversely, owing to its prominent role in controlling $CD4^+$ T cell activation and T cell-dependent B cell responses, blockade of the OX40–OX40L pathway alleviates the severity of inflammatory or autoimmune diseases in several murine models, including EAE, CIA and airway inflammation[115]. RO4989991, a human OX40L-targeting mAb, is being evaluated in Phase II trials for the treatment of asthma.

CD27–CD70 pathway. CD27 (also known as TNFRSF7), another CD137-related TNFR, is mainly expressed on $CD4^+$ T cells, $CD8^+$ T cells, B cells and NK cells, whereas its counter-receptor CD70 is expressed on DCs as well as T and B lymphocytes[115]. In contrast to the absence of CD137 on naive T cells, CD27 is highly expressed on these cells. It is downregulated on activated T cells after repetitive antigenic stimulation, and CD27 downregulation is associated with enhanced effector functions. Crosslinking of CD27 by mAbs or CD70 fusion proteins co-stimulates the proliferation of both $CD4^+$ and $CD8^+$ T cells as well as cytokine production *in vitro* and *in vivo*. It has been shown that CD27 mainly promotes T cell survival rather than stimulating cell cycle progression[157]. In a model of influenza infection, CD27 was crucial for the expansion of virus-specific T cells and the accumulation of CTLs in the lung. Similarly, agonistic CD27-targeting mAbs expanded tumour-specific CTLs and eradicated established tumours in multiple murine tumour models[158].

Engineered human T cells expressing a chimeric antigen receptor (CAR) with an intracellular region consisting of the CD3 ζ-chain signalling domain and a CD27 intracellular co-stimulatory motif show substantially improved expansion, survival and effector functions *in vitro* compared with T cells expressing the CAR–CD3 ζ-chain alone. When infused into tumour-bearing mice in a xenograft model, CAR–CD3–CD27 T cells showed increased survival and enhanced effector functions, leading to tumour regression[159]. Similarly, CAR-expressing T cells with CD3 ζ-chains and a CD137 or CD28 intracellular motif showed increased expansion and eradicated xenograft tumours *in vivo*. Interestingly, among the three constructs, the CD27 and CD137 intracellular motifs conferred better survival *in vivo* than the CD28 construct[159], indicating that targeting multiple co-stimulatory receptors might have added value by enhancing different aspects of T cell activation and effector functions. CDX-1127, a human IgG1 agonistic mAb targeting CD27, is being evaluated in a Phase I trial treating haematological and solid tumours.

GITRL–GITR pathway. Glucocorticoid-induced TNFR-related protein (GITR; also known as AITR and TNFRSF18) has a broad distribution on haematopoietic cells, including natural T_{Reg} cells and activated T, B, NK and myeloid cells[115]. Unlike most other TNF ligands, GITR ligand (GITRL) has limited expression on APCs but it is constitutively expressed on peripheral tissues and presumably engages GITR on tissue-infiltrating immune cells. GITR-targeting agonistic mAbs co-stimulate T cell proliferation and cytokine production *in vitro*, and induce tumour regression *in vivo* through the activation of $CD4^+$ T cells, $CD8^+$ T cells and NK cells in several tumour models[160,161]. With its prominent expression on natural T_{Reg} cells, GITR has been a target for manipulating T_{Reg} function. GITR-targeting agonistic mAbs expand $CD4^+CD25^-$ T cells, abrogate $CD4^+CD25^+$ T_{Reg} cell-mediated suppression and induce autoimmune gastritis and colitis in animal models[162,163]. However, evidence indicates that the expansion of $CD4^+$ effector cells, rather than T_{Reg} inhibition, is the primary mechanism underlying the antitumour effects mediated by GITR-targeting mAbs[164]. TRX518, a humanized GITR-targeting mAb, is currently in Phase I clinical trials treating patients with late-stage melanoma.

Combination therapy

Biologics targeting individual Ig or TNFR family members, as described above, have shown promising therapeutic effects as monotherapies. Meanwhile, several immunomodulatory biologics targeting complementary pathways or combinations of immunomodulatory biologics with traditional chemotherapy, radiation therapy, vaccination or targeted depletion have shown promising synergistic effects in animal models and are being extensively explored in ongoing clinical trials (TABLE 3).

Combinations with direct tumour-killing therapy. Traditional chemotherapy and radiation therapy, together with depleting mAbs or treatment with small-molecule inhibitors, all directly target and kill cancer cells, leading to the destruction of the tumour stroma and the release of tumour antigens. When coupled with these direct killing mechanisms, immunomodulatory biologics promote the priming and expansion of existing tumour-specific T cells and their *de novo* generation, with a potential to form long-lasting and self-sustained antitumour responses. In recent years, small-molecule inhibitors targeting tumours that harbour mutated *BRAF* (vemurafenib (Zelboraf; Plexxikon/Roche)) or translocated *BCR–ABL* (imatinib (Gleevec; Novartis)) have shown high initial response rates in clinical trials[165]. However, the duration of the antitumour response is limited owing to acquired drug resistance. A combination of these fast-acting small-molecule inhibitors with immune co-inhibitory blockade — for example, with CTLA4-specific or PD1-specific mAbs — could promote the priming and expansion of tumour-specific CTLs against multiple tumour antigens and/or epitopes, prevent the generation of escape variants or drug-resistant mutant cancer cells and induce sustained T cell responses.

Combinations with cancer vaccines. As validated in numerous animal models of cancer, tumour vaccine approaches — including whole-tumour lysate, tumour antigen peptide or DC vaccines — greatly enhance the efficacy of immunomodulatory biologics. One main drawback of the vaccine approach is that both

antigen-specific effector T cells and suppressive T_{Reg} cells are expanded following antigenic stimulation. Although antigen-based vaccination was shown to frequently activate tumour-specific CTLs in the bloodstream, with strong antitumour functions *in vitro*, these cells are often inhibited in the tumour microenvironment owing to the presence of inhibitory molecules on tumour cells as well as the presence of suppressive cells in the tumour stroma, such as T_{Reg} cells, tumour-associated macrophages and myeloid suppressor cells. A combination of PD1 blockade with a tumour vaccine plus T_{Reg} cell depletion is being evaluated in several clinical trials (TABLE 3).

Combinations with other immunomodulatory biologics and/or targeting of multiple cellular components. Combining agonistic mAbs targeting co-stimulatory receptors with agents that induce the blockade of co-inhibitory receptors is another highly effective approach for stimulating efficient antitumour immunity and has been validated in various animal tumour models: for example, the combination of CD137-targeting agonistic mAbs with CTLA4 or PD1 blockade[75]. One such combination, a CD40-targeting mAb plus a CTLA4-targeting mAb, is being tested in a Phase I trial for the treatment of late-stage melanoma. However, careful trial design and close clinical monitoring is required to monitor potentially enhanced autoimmunity in such a combination therapy.

In xenograft tumour models, it was recently demonstrated that CD137-targeting agonistic mAbs activate NK cells and synergize with tumour-targeting biologics and/or biologics that induce antibody-dependent cell-mediated cytotoxicity, such as rituximab (a CD20-specific mAb), cetuximab (a mAb targeting epidermal growth factor receptor) and the HER2 (also known as ERBB2)-targeting mAb trastuzumab (Herceptin; Roche/Genentech), thus paving the way for a sequential antibody combination therapy in the clinic[166]. PF-05082566, an agonistic CD137-targeting mAb, is being tested for the treatment of $CD20^+$ non-Hodgkin's lymphoma in combination with rituximab in a Phase I trial.

Combination targeting of several tumour-killing effector cell populations, such as T cells and NK cells, is also being evaluated in clinical trials. IPH21, a blocking antibody against NK inhibitory receptors (killer cell immunoglobulin-like receptor 2DL1 (KIR2DL1), KIR2DL2 and KIR2DL3), induces NK cell activation and was combined with PD1 blockade (using BMS-936558) to treat advanced solid tumours. IPH21–BMS-936558 combination treatment intends to utilize both innate and adaptive tumour-specific killing mechanisms to achieve a better clinical outcome than PD1 blockade alone.

Challenges and perspectives

Over the past decade, cell surface signalling molecules have emerged as crucial targets for the treatment of cancer and immune disorders. Immunomodulatory mAbs or RFPs have had a chequered history as therapeutics but have finally become one of the most promising approaches for the treatment of human diseases. With the promise to induce long-lasting tolerance in autoimmunity or memory responses (immunity) against tumours, immunomodulatory biologics represent a distinctive class of drugs that target and correct aberrant immune responses. They might even hold the key to fully realizing the potential of cancer vaccines, which have resulted in more disappointment than success in recent clinical trials.

mAbs and RFPs are the preferred therapeutics for targeting cell surface signalling molecules because of their specificity, high affinity (in the nanomolar range), stability and the accessibility of their targets. By contrast, small-molecule inhibitors penetrate the cell membrane at ease and thus hold great advantage for targeting intracellular signalling pathways to modulate immune responses; such small-molecule inhibitors include cyclosporin A (targeting calcineurin), sirolimus (targeting mammalian target of rapamycin (mTOR)) and tasocitinib (targeting the Janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathway)[167]. With the aid of structural analysis and high-throughput screening methods, small-molecule inhibitors disrupting immunomodulatory cell surface interactions have also been explored as a means to treat immune disorders[168], including IL-2–IL-2 receptor (IL-2R)[169], B7.1–CD28 (REF. 170) and CD40–CD40L[171] pathway inhibitors, but these have had limited success in moving into the clinic. The common drawbacks for this group of small-molecule inhibitors include lack of specificity, low affinity (in the micromolar range) and the disassociation of biological effect with blocking activity. However, with an expanding repertoire of small molecules and the advancement of crystal structure analyses of surface interactions, the perfect small-molecule inhibitor might be waiting to be identified.

When a given cell surface signalling molecule target is a receptor, it is desirable that both agonistic and antagonistic biologics could be obtained so that all aspects of therapeutic options can be explored. Currently, a major technical hurdle in targeting a receptor is the difficulty of obtaining both agonistic and antagonistic reagents. For example, although a co-stimulatory mAb for CD28 was generated more than 20 years ago, antagonistic CD28-specific antibodies are not yet available for therapeutic manipulation. In theory, such a CD28-specific antagonist will be ideal for the suppression of primary immune responses because this strategy will preserve the interactions of B7.1 and B7.2 with CTLA4 for co-inhibition and would be more efficacious than a CTLA4–Ig fusion protein, which blocks both co-stimulation and co-inhibition. Similarly, although antagonistic PD1-specific mAbs have been generated to enhance immune responses against cancer and viral infections, we have yet to obtain a PD1-specific agonist that can suppress T and B cell immunity to explore its use for dampening inflammation and autoimmunity. Although it is relatively easy to identify and select therapeutic antagonistic mAbs when the interacting receptor and ligand are known, the identification of agonistic mAbs is not as straightforward. This is largely due to the lack of reliable assays in the human system. The recent development of humanized mice or selective human gene knock-in mice may provide possible solutions for overcoming this obstacle.

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