Targeting costimulatory molecules in autoimmune disease

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Abstract

Therapeutic targeting of immune checkpoints has garnered significant attention in the area of cancer immunotherapy, and efforts have focused in particular on the CD28 family members CTLA-4 and PD-1. In autoimmunity, these same pathways can be targeted to opposite effect, to curb the over-exuberant immune response. The CTLA-4 checkpoint serves as an exemplar, whereby CTLA-4 activity is blocked by antibodies in cancer immunotherapy and augmented by the provision of soluble CTLA-4 in autoimmunity. Here we review the targeting of costimulatory molecules in autoimmune disease, focusing in particular on the CD28 family and TNFR family members. We present the state-of-the-art in costimulatory blockade approaches, including rational combinations of immune inhibitory agents, and discuss the future opportunities and challenges in this field.

The risk of autoimmune disease is an inescapable consequence of the manner in which the adaptive immune system operates. To ensure effective immunity against a diverse array of unknown pathogens, antigen recognition systems based on random gene rearrangement and mutagenesis have evolved to anticipate the antigenic universe. These include B cell receptors, which can see native antigen, and T cell receptors (TCRs) that can see antigen presented by major histocompatibility complex (MHC). An inevitable consequence of this approach is that cells capable of recognising self-proteins can arise, potentially triggering autoimmunity. Furthermore, since T cells recognise antigen in the context of self-MHC molecules, they are by definition self-reactive.

Although both T cells and B cells are endowed with diverse recognition potential, the responsibility for maintaining tolerance, and thereby avoiding autoimmunity, lies mainly with T cells. Indeed, most B cell responses require "help" from the T cell compartment, generating a permissions hierarchy in which the T cell is the final arbiter of the response.

T cell tolerance is maintained by a number of mechanisms, broadly divided into deleting certain clones during development (central tolerance) and curbing the activity of mature clones that have the potential to cause harm (peripheral tolerance). Central tolerance governs entry to the mature T cell compartment, but is imperfect since healthy individuals harbour self-reactive T cells[1]. The continuous operation of peripheral tolerance mechanisms is therefore required to protect us from autoimmunity.

Arguably, the central tenet of peripheral tolerance is that the context of T cell antigen encounter dictates the immunological outcome. The maintenance of peripheral tolerance thus relies on T cell encounter with self-antigen occurring in a non-immunogenic context. On a practical level, a major contributor to "context" is the engagement of costimulatory receptors alongside TCR-mediated antigen detection. In the broadest sense, discrimination between a tolerogenic and immunogenic encounter with antigen rests on the differential upregulation of costimulatory ligands and concomitant provision of costimulatory signals.

In the light of this, a major therapeutic goal in autoimmune disease is to alter the context of T cell stimulation by the targeted manipulation of costimulatory pathways. Building on the overwhelming success of checkpoint blockade in cancer, immunotherapy is gaining traction in autoimmune settings[2], providing an exciting landscape for the development of novel costimulation blockade approaches.

In this Review, we first outline the biology of co-stimulation, focusing on the CD28 and tumour necrosis factor receptor (TNFR) superfamilies, and then discuss the therapeutic targeting of these molecules in autoimmunity, drawing examples from the transplantation literature where appropriate.

Costimulatory molecules

Costimulation helps the immune system to identify which antigenic stimuli are worthy of a response[3]. CD28 was one of the first costimulatory molecules to be identified and is a T cell surface protein, which must be engaged by its ligands, CD80 (B7.1) and CD86 (B7.2), to induce T cell activation and differentiation. Expression of CD80 and CD86 is upregulated on antigen-presenting cells (APCs) following ligation of APC-expressed CD40 with T cell-expressed CD40 ligand (CD40L). Since CD28 ligation upregulates CD40L, this generates a feedforward dialogue (**Figure 1a**) that co-ordinately drives T cell activation and dendritic cell (DC) maturation. Thus, the CD28 and CD40 pathways can be considered primary costimulatory interactions. Indeed, naïve CD4 T cells already express some CD40L[4], emphasizing the early role played by CD40 engagement.

Following initial activation, additional costimulatory molecules, many of which are structurally related to either CD28 or TNFR, equip T cells with other ways to sense their environment. These include inducible T-cell costimulator (ICOS), OX40 (also known as TNFRSF4) and 4-1BB (also known as TNFRSF9), as well as receptors that negatively regulate T cell activation, such as cytotoxic T-lymphocyte protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) (**Figure 1b**, **Supplementary Figure 1**). Engagement of these receptors by their respective ligands regulates survival, differentiation, migration and function in cell-type and tissue-specific contexts.

The CD28 superfamily (**Table 1**) comprises the receptors CD28, CTLA-4, ICOS, PD-1, B- and T-lymphocyte attenuator (BTLA), which will be discussed in turn, and CD28H (also known as TMIGD2), which is not covered here because comparatively little is known about its function. For the TNFR superfamily (**Table 2**), membership is more extensive so we have taken a selective approach, focusing on the CD40 and OX40 pathways and referring only briefly to other family members.

The CD28 pathway

Expression, signalling and structure

The CD28 pathway comprises CD28 and CTLA-4 and their shared ligands, CD80 and CD86. CD28 is a 44 kDa transmembrane glycoprotein with a single extracellular IgV-like domain, and is expressed on the cell surface as a homodimer. Most CD4 T cells and about half of the CD8 T cells constitutively express CD28 (**Figure 2**) and it can also be found on activated natural killer (NK) cells[5,6] and innate lymphoid cells[7]. CD28 has been reported on plasma cells, neutrophils and eosinophils in certain settings, however its functional significance on these cell types is still being investigated[8-10].

CD28 lacks intrinsic catalytic activity, however its short cytoplasmic tail contains one YMNM and two PxxP (PYAP and PRRP) motifs, as well as two additional tyrosine residues. The tyrosine residues, particularly the tyrosines within the YMNM and PYAP motifs, are phosphorylated upon ligand binding. Adaptor molecules containing SH2 domains, including the p85 subunit of phosphatidylinositol 3-OH kinase (PI(3)K), Grb2 and Gads, which bind the phosphorylated YMNM, as well as SH3 domain-containing molecules, which bind the PYAP motif, are recruited to phosphorylated CD28. These and other molecules activate signalling pathways that include mitogen-activated protein kinase (MAPK), AKT, nuclear factor of activated T-cells (NFAT) and nuclear factor κB (NF-κB)[11].

CTLA-4 is the inhibitory counterpart to CD28, binding to their shared ligands with the same conserved MYPPPY motif[12]. The cytoplasmic domain of CTLA-4 is almost 100% conserved in mammals, but lacks an immunoreceptor tyrosine-based inhibition motif (ITIM) or immunoreceptor tyrosine-based switch motif (ITSM), unlike other inhibitory members of the CD28 family such as PD-1 and BTLA. Like CD28, CTLA-4 is a transmembrane glycoprotein expressed as a dimer, however CTLA-4 expression is limited to activated T cells and regulatory T (Treg) cells. CTLA-4 is highly endocytic — only 10% of CTLA-4 molecules are found at the cell surface[13], the remainder are in recycling endosomes or lysosomes[14,15].

The ligands, CD80 and CD86, are predominantly expressed on APCs, such as B cells, DCs, macrophages and monocytes, and are upregulated in response to inflammatory stimuli and engagement of pattern recognition receptors. Both ligands are transmembrane glycoproteins with two extracellular domains, an IgV-like and an IgC-like domain. CD86 is expressed as a monomer, and CD80 is a dimer with stronger binding affinities to both CD28 and CTLA-4 than those of

CD86[16]. Although both ligands can mediate CD28-dependent and CTLA-4-dependent functions, mice deficient for CD80 are largely normal, whereas CD86-deficient mice show defects in antibody isotype switching and germinal centre formation[17]. Furthermore, the timing and pattern of expression of the two ligands are distinct; CD86 is expressed at moderate levels at steady state and upregulated rapidly in response to inflammatory stimuli, whereas CD80 is found at low levels in the absence of inflammation and is upregulated more slowly after activation[18,19]. There are also numerous cell type-specific differences in the regulation of CD80 and CD86 expression.

Pathway functions and link to disease

Engagement of CD28 lowers the threshold for TCR mediated T cell activation, which results in enhanced proliferation, cytokine production and cell survival[20]. Conversely, a lack of CD28 costimulation at the time of TCR engagement can lead to unresponsiveness, anergy and cell death through apoptosis. Mice deficient in CD28 show low basal immunoglobulin levels[21] and impaired germinal centre formation,[22] consistent with a requirement for CD28 costimulation in follicular helper T cell (T_{FH}) formation[23,24]. CD28 dose-dependently induces ICOS and the microRNA miR-17~92, either of which could underlie its capacity to promote T_{FH} differentiation[24,25].

Numerous T cell-mediated disease models show amelioration in the CD28 deficient setting. CD28 knockout mice show minimal signs of experimental autoimmune encephalomyelitis (EAE) after immunisation with myelin oligodendrocyte glycoprotein (MOG) 35-55 peptide, with reduced inflammatory infiltrates in the brain and spinal cord[26]. CD28 costimulation is also required for collagen induced arthritis[27] and T cells that lack CD28 fail to induce mucosal inflammation and colitis after adoptive transfer into recipients with severe combined immunodeficiency (SCID)[28]. In the MRL/lpr model of lupus, findings were mixed, with increased splenomegaly but decreased IgG autoantibodies in the absence of CD28[29]. Although vasculitis and arthritis were abolished, levels of IgM autoantibodies were unchanged and mild glomerular lesions were still apparent, especially in older animals[29].

Further insight into the complexity associated with CD28 deficiency came from studies of CD28-deficient non-obese diabetic (NOD) mice, in which, surprisingly, diabetes was exacerbated rather than reduced[30]. This observation led to the discovery that, aside from its role in T cell activation, CD28 is also important for Treg homeostasis, and CD28 deficiency or signalling blockade results in fewer peripheral Tregs[30,31]. This is in part due to a defect in thymic selection; however, inducible and Treg-specific CD28 knockout mice revealed a role for CD28 signalling in mature Treg homeostasis[32,33].

CD28 costimulation is tightly regulated by CTLA-4, which is constitutively expressed by Tregs. Mice lacking CTLA-4 in Tregs exhibit lethal immune activation and systemic immune dysregulation[34], suggesting a critical role for CTLA-4 in normal Treg function. CTLA-4 acts as a competitive inhibitor of CD28 by virtue of its higher affinity for their shared ligands[16], and through its capacity to physically remove these ligands from the surface of APCs[35,36]. By controlling T cell CD28 engagement, CTLA-4 sets immune activation thresholds that are thought to prevent the aberrant priming of self-reactive T cells. The ability of the CTLA-4 pathway to control the behaviour of autoreactive T cells has been well documented in mice, with a rampant and fatal multi-organ tissue inflammation seen in CTLA-4-deficient animals[37,38]. This is also the case in humans, as individuals with heterozygous deficiency in CTLA-4 frequently develop an immune dysregulation syndrome with multiple autoimmune features[39,40]. Furthermore, it is increasingly evident that autoimmune responses can be unleashed following the therapeutic blockade of CTLA-4 in cancer patients.

The CD28 pathway has been extensively targeted in an attempt to downregulate the immune response in autoimmune and transplantation settings. Until recently, dogma maintained that

memory T cells were independent of CD28 suggesting that costimulation blockade would be ineffective in autoimmune settings where responses are chronic. However, this view has been called into question, and it is now clear that CD28 also contributes to memory T cell responses [41]. This may explain why therapies that interrupt CD28 signalling can still be effective when administered during an ongoing pathogenic immune response[42].

Therapeutic targeting with soluble CTLA-4

CTLA-4 is the natural regulator of the CD28 pathway: fusion proteins including the extracellular domain of CTLA4 and a modified Fc portion of human immunoglobulin (CTLA4–Ig) have therefore been developed to mimic this immune regulatory activity. Abatacept is the first generation soluble CTLA-4-Ig fusion protein[43] and attracted early attention in a phase I trial in psoriasis vulgaris, in which nearly half of the patients achieved a 50% or greater sustained improvement in clinical disease[44]. Subsequent studies in rheumatoid arthritis led to FDA approval in 2005 for use in this indication, where it continues to be an effective therapeutic[45]. A recent Phase III open label study of subcutaneously administered abatacept in juvenile idiopathic arthritis showed robust efficacy[46], with the majority of children experiencing a marked improvement in disease score. By treatment month 4, inactive disease status was achieved in 30% of 6-17 year olds and 50% of 2-5 year olds [46].

Promising results from abatacept have led to the development of next generation variants with improved characteristics. Belatacept (LEA29Y) differs from abatacept by two amino acids in the ligand binding region, and binds approximately 4-fold more avidly to CD86 and approximately 2-fold more avidly to CD80[47]. In non-human primates, belatacept was more effective at suppressing humoral immunity after sheep red blood cell immunization and was better able to prevent renal allograft rejection than abatacept[47]. Belatacept received FDA approval in 2011 for renal transplantation, and its use may avoid the nephrotoxicity associated with immunosuppression via calcineurin inhibitors such as cyclosporine. It is associated with significantly higher rates of patient and graft survival at seven years than cyclosporine, despite a higher risk of early acute rejection[48]. Further studies are required to clarify how it compares with the more recent tacrolimus-based immunosuppressive protocols.

Another CTLA-4-Ig variant is XPro9523[49], in which 4 amino acid substitutions improve binding to CD86 by 23-fold and to CD80 by 5.9-fold, and an additional 2 amino acid changes enhance binding to the neonatal Fc receptor (FcRn) with the aim of increasing the *in vivo* half-life of the molecule. Indeed, in non-human primates, at high doses XPro9523 had a longer serum half-life than belatacept, however this effect was lost at low doses[49]. XPro9523 was more effective at suppressing humoral immunity after keyhole limpet hemocyanin (KLH) immunization than both abatacept and belatacept. The most recently developed CTLA-4-Ig variant, MEDI5256, is the first attempt to preferentially increase binding to CD80 over CD86. As a result, MEDI5256 exhibits a 128-fold increase in monovalent binding of CD80 compared with abatacept and a 5-fold improved monovalent affinity for CD86[50]. In KLH immunized non-human primates, a 10-fold lower dose of MEDI5265 was shown to be equipotent to abatacept, and MEDI5256 had a significantly longer plasma half-life due to Fc region modifications. It will be of interest to assess how altering the bias of CTLA-4-Ig binding, towards either CD86 or CD80, influences its immunosuppressive properties in different disease settings.

The mechanism of action of CTLA-4-Ig molecules centres on their binding to the costimulatory ligands CD80 and CD86 and thus inhibiting CD28 costimulation in T cells. This process also inhibits DC activation[51] by interrupting the early feedback between naïve T cells and APCs (see **Figure 1a**). Early suggestions that CTLA-4-Ig would induce anergy and clonal tolerance have not been supported with data and it is now clear that CD28 blockade is not necessarily sufficient for these outcomes; for example IL-2 signalling can inhibit anergy in the absence of CD28

costimulation[52]. It was also proposed that CTLA-4-Ig signals through CD80/86 on APCs to upregulate indoleamine 2,3-dioxygenase (IDO) activity leading to local tryptophan depletion[53] however this remains controversial[54,55,56].

With an armament of soluble CTLA-4 molecules at hand, a key challenge is now to understand the variability in response profiles and predict patients who are likely to respond best. In rheumatoid arthritis, the most robust marker of response to abatacept is the presence of anti-citrullinated protein antibody (ACPA)[57,58], while in psoriatic arthritis, baseline markers of poor prognosis appear to correlate with abatacept responsiveness[59]. In type 1 diabetes, emerging data suggest that follicular helper T cell markers on circulating T cells may predict the clinical response to abatacept[60]. The ability to select patient groups who are likely to respond to CTLA-4-Ig molecules would help inform treatment decisions as well as aid in the design of new clinical trials.

Therapeutic targeting with CD28 antibodies

Agonistic anti-CD28 antibodies. Therapeutic targeting of CD28 has a chequered history, with the ill-fated trial of superagonistic anti-CD28 antibody (TGN1412) making headlines in 2006. The premise for this trial was that continuous TCR-derived signals, which could be complemented by CD28, are important for Treg proliferation [61], and that boosting this suppressive population could be useful in autoimmune disease settings. Since CD28 has traditionally been recognized for its role in conventional T cell responses, it follows that preferential expansion of Tregs would likely require careful dosing. The disastrous consequence of a bolus injection of TGN1412 in healthy volunteers has been well documented[62] and the reasons that rodent, primate and human experiments all failed to predict the cytokine release syndrome that occurred have been extensively investigated. Briefly, each system had an unfortunate confounder: laboratory housing of rodents may have made them "too clean" [63], the macaques used for preclinical testing lack CD28 on effector memory T cells (unlike, humans, baboons and rodents)[64,65], and human cells taken from the blood responded differently to human cells packed closely together in lymphoid tissues *in vivo*, such that *in vitro* testing failed to recapitulate *in vivo* behaviour.

This antibody has since returned to the fore under a new name, TAB08, and doses that preferentially activate Treg cells, rather than conventional T cells, have been identified *in vitro*[66]. On the basis of this, a second healthy volunteer study was initiated involving a careful dose titration, starting with 1000-fold less than in the original trial and this time applied by slow infusion. No cytokine storm was observed and the individuals receiving the two highest doses of antibody exhibited a transient increase in serum IL-10 levels, consistent with Treg activation[66]. The results of a subsequent Phase Ib trial in 18 patients with rheumatoid arthritis are not yet published but reportedly show an acceptable level of adverse events and a clinical response in most patients[67]. Thus, with dosing regimens that preferentially target Tregs, it may be possible to treat autoimmune diseases by engaging the CD28 receptor with a superagonist antibody.

Antagonistic anti-CD28 antibodies. Conceptually, antagonizing CD28 is easier to rationalize, since it is an intuitive extension of the use of CTLA-4-Ig fusion proteins to block CD28 ligands. Antagonistic CD28 antibodies inhibit skin graft rejection in mice[68] and prevent rejection of transplanted kidneys in non-human primates in combination with tacrolimus, mycophenolate mofetil (MMF) or rapamycin[69]. Extensive preclinical studies[70] paved the way for a first-in-human study of a pegylated antigen-binding fragment (Fab) antibody (FR104) that targets an epitope on CD28 that overlaps with the MYPPPY ligand-binding motif[71]. This antibody was well tolerated in healthy volunteers and inhibited the formation of antibodies in response to KLH challenge[71]. Further studies with FR104 in non-human-primate graft-versus-host disease (GVHD) models demonstrated improved GVHD-free survival in a FR104 plus sirolimus cohort compared to a CTLA-4-Ig plus sirolimus cohort, however, this did not translate into improved overall survival due to infection-related complications[72]. The tested regimen could potentially be of considerable clinical

value, provided risks are effectively mitigated, particularly for transplant recipients at high risk of GVHD[72].

It has been suggested that an antagonistic CD28 antibody, which blocks CD28 costimulation but leaves CTLA-4 mediated responses intact, may be superior to CTLA-4-Ig molecules [68,73]. Indeed, it was postulated that preserved CTLA-4 signalling after treatment with antagonistic CD28 antibodies upregulated the inhibitory SLAM family member 2B4 (CD244), which then played a critical role in the observed immunoregulation[68] ; however, 2B4 upregulation was not observed in a subsequent study in non-human primates[72]. It has also been suggested that an antagonistic CD28 antibody may promote Treg homeostasis[74], unlike CTLA-4-Ig, which impairs Treg homeostasis in both mice[30,31] and humans[75,76]. However, treatment with FR104 impaired Treg homeostasis, consistent with a positive role for CD28 signalling in Treg biology[72].

Conceptually, if CTLA-4 works by competing with CD28 for their shared ligands, interactions between CTLA-4 and its ligands should have little biological effect when CD28 is fully blocked. The superiority of the antagonistic CD28 antibody in side-by-side comparisons with CTLA-4-Ig molecules[68,77,78] is therefore more likely to reflect a higher binding affinity, particularly in light of the relatively weak interaction between CTLA-4-Ig and CD86. Thus, antagonistic anti-CD28 antibodies are a promising immunomodulatory approach, and their biological mechanism of action likely resembles CTLA-4-Ig-based inhibition of the CD28 pathway.

Overall, therapeutic targeting of the CD28 pathway has entered a new era, with a host of novel modalities in the pipeline. It is worth reflecting on the unusual prospect of having both antagonistic and agonistic anti-CD28 antibodies in the clinic for autoimmune indications, the latter targeted selectively at the Treg population with a requirement for careful dosing considerations.

The ICOS pathway

Expression, signalling and structure

The inducible costimulator (ICOS) protein plays an important and nonredundant role in the regulation of adaptive immune responses. Unlike CD28, which is constitutively expressed on T cells, ICOS is rapidly upregulated upon activation of $CD4^+$ effector T cells [79,80]. Although increased levels correlate with the differentiation of T helper 1 (Th1) and Th17 cells[81,82], ICOS expression is highest on T_{FH} cells within the germinal centre (GC), where ICOS regulates humoral responses[79,83].

ICOS signalling on T cells is mediated through interaction with a cell surface ligand, ICOSL (also called B7 related protein 1 or B7RP-1). The ligand is constitutively expressed on myeloid and plasmacytoid DCs, macrophages and B cells, as well as on non-hematopoietic tissues such as vascular and alveolar epithelial cells[80,84]. Although ICOSL expression on B cells is increased by CD40 engagement and B cell-activating factor (BAFF)[85], the strength of BCR signalling also modulates ICOSL expression and ultimately controls the development of T_{FH} cells [86]. ICOSL is also detected at low levels on monocytes, where it can be induced by IFN-γ[87].

Phosphatidylinositol 3-kinase (PI3K) is recruited in response to ICOS activation[88,89]. Although PI3K is also activated by CD28, ICOS ligation recruits the p50α regulatory subunit of PI3K that results in stronger downstream signalling, as measured by levels of the lipid messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3) and subsequent AKT phopshorylation[88]. One downstream target of AKT is the transcription factor forkhead box protein O1 (FOXO1), which fine tunes multiple biological functions that are crucial for differentiation of T cells, including T_{FH}[90,91].

ICOS-mediated PI3K-AKT signalling events promote early T cell activation, T_{FH} differentiation and migration to the follicle[92], and also the physiological maintenance of germinal centres. Within the follicles, engagement of ICOS stabilizes the T_{FH} cell phenotype through the zing-finger transcription factor Krüppel-like factor 2 (KLF2) and the intracellular protein osteopontin (OPN)[93,94].

ICOS signalling via PI3K also profoundly reprogrammes T cell metabolism through activation of mammalian target of rapamycin (mTOR). mTOR, a conserved serine/threonine kinase, mediates AKT phosphorylation that drives the differentiation and function of T_{FH} cells, so this protein is central to immunological and metabolic signalling events emanated by ICOS.

Pathway functions and link to disease

The relationship between increased numbers of ICOS-bearing T_{FH} cells and clinical disease status has been substantiated in numerous autoimmune disorders including systemic lupus erythematosus (SLE)[95], Sjögren's syndrome[96], rheumatoid arthritis[97] and type 1 diabetes[98]. Increased ICOS expression has also been detected on CD4 and CD8 T cells in patients with SLE[99], and ICOS-positive T_{FH}-like cells contribute to the pathophysiology of fibrosis in systemic sclerosis[100]. Alterations in the post-transcriptional regulation of ICOS by RNA-binding proteins, including roquin and endoribonuclease ZC3H12A (also known as Regnase), have been linked to uncontrolled T_{FH} cell proliferation and autoimmunity in mice[101].

ICOS ligation induces the expression of numerous cytokines, including IL-21, which is critically required for the formation and the function of T_{FH} cells[89]. Engagement of ICOS exacerbates the elevated secretion of IL-21 by T_{FH}, permitting the formation of abnormal humoral responses that contribute to the development of autoimmunity. The correlation between T_{FH} cell numbers and IL-21 levels has been preclinically documented and clinically validated in numerous autoimmune conditions[98,102,103]. Conversely, deficiency in ICOS is associated with a severe reduction in circulating memory T helper cells, which results in a progressive loss of B cell repertoire and an increased rate of opportunistic infections[104,105].

Therapeutic targeting of ICOS–ICOSL

Several preclinical animal models that recapitulate autoimmune conditions as well as GVHD have demonstrated the therapeutic benefit of abrogating the ICOS–ICOSL pathway, confirming the importance of this pathway in T cell activation[106-115].

An ICOSL-blocking mAb[116] was developed and tested in two preclinical autoimmune models. Blockade of ICOSL reduced the progression of lupus nephritis in NZB/NZW (F1) and dampened neo-antigen humoral responses in the collagen induced arthritis model[117].

This preclinical work led to the development of AMG 557 (also known as prezalumab), a human IgG2 monoclonal antibody that binds with high affinity to ICOSL and prevents its interaction with ICOS. The safety, tolerability, pharmacokinetics and pharmacodynamics of AMG 557 were evaluated in individuals with mild and stable SLE in two phase 1 clinical trials (NCT02391259 and NCT00774943)[118]. In both studies, AMG 557 was well tolerated and exhibited an acceptable safety profile. Patients treated with AMG 557 in the multiple-ascending dose study had significantly lower IgG titers against KLH administered by intradermal immunization, whereas the IgM anti-KLH titer remained unchanged. The disease score (BILAG, SELENA-SLEDAI), and levels of anti-nuclear antibodies (ANAs) or complement were not altered in SLE patients who received AMG 557. A randomized, phase 1b double-blind study to evaluate the safety, tolerability, pharmacokinetics, pharmacodynamics and clinical effects of AMG 557 in 20 SLE patients with active lupus arthritis was also recently completed (NCT01683695)[119]. Given the disease complexity and high heterogeneity that characterizes patients with SLE, the clinical response was focused on the lupus

arthritis response index (LARI), which is primarily composed of an improvement of the joint count, discontinuation or tapering of immunosuppressants (methotrexate, azathioprine and etanercept) or prednisone, and on the SLEDAI and BILAG index scores. Although the number of LARI responders was relatively small (3/10 and 1/10 in the AMG 557 and placebo cohorts, respectively), more patients in the AMG 557-treated group showed improvement in SLEDAI score (7/10 patients) than in the placebo group (2/10 patients). A Phase 2a randomized, double-blind placebo-controlled study to evaluate the clinical and biological efficacy of AMG 557 in individuals with primary Sjögren's Syndrome was recently completed (NCT02334306). Notably, the disclosed Phase 2a results have indicated that despite a statistical reduction of rheumatoid factor level over placebo, no improvement of clinical or other biomarker measures of disease activity were met by AMG 557.

A recombinant murine mAb engineered with antibody dependent cellular cytotoxicity (ADCC) function has also been investigated to ascertain the role of autoreactive ICOS-positive T cells. This pharmacologic approach relies on the restricted expression of ICOS on antigen-experienced helper memory T cells and on T_{FH} cells. In studies conducted in the lupus-prone B6.Sle1 or the sclerodermatous GVHD murine model, treatment with the ADCC-enhanced ICOS mAb inhibited autoimmunity or suppressed skin fibrosis, respectively[100,120].

Similar to the above antibody, MEDI-570 is an afucosylated human IgG1κ monoclonal antibody, directed against the ligand binding domain of human ICOS. MEDI-570 displays relatively high binding to ICOS (pM level), which abrogates ICOS-mediated T-cell proliferation and cytokine production[121]. The lack of fucose in the carbohydrate moiety of the constant region fragment (Fc) enhances binding to the low affinity immunoglobulin gamma Fc region receptor III-A (FcγRIIIA, also known as CD16), which is expressed on NK cells and macrophages resulting in significantly increased ADCC activity of MEDI-570 against T cells expressing ICOS[122]. A phase 1 study to evaluate the safety and tolerability of MEDI-570 was initiated in SLE (NCT01127321). A total of 17 patients were dosed with MEDI-570, but enrolment into higher doses was stopped even though dose-limiting toxicities were not identified. Although the clinical pharmacological activity of MEDI-570 has not been disclosed, the rationale behind the selective elimination of ICOS-positive T cells still holds promise for the treatment of autoreactive T cell-dependent autoimmune disorders and for chronic GVHD.

One unresolved question is whether the mechanism of anti-ICOS therapy primarily relies on targeting $ICOS^{high}$ T_{FH} or whether inhibiting ICOS+ memory T cells is also key. Preclinical and clinical studies have demonstrated that pharmacological abrogation of ICOS signalling on T_{FH} cells profoundly impacts both short- and long-term humoral responses. Other animal studies have also suggested that ICOS regulates the ontogeny and maintenance of a subset CD4+ memory T cells and their antigen responses can in some cases be impaired by anti-ICOS therapies[123]. Additional studies will be required to decipher the relative contribution of T_{FH} and memory T cells as targets for ICOS-directed therapies.

Other CD28 superfamily members

PD-1

Blockade of the PD-1 pathway, by targeting either PD-1 or PD-L1, can successfully augment the immune response in cancer settings. Emerging preclinical studies now support the opposing strategy in autoimmunity: agonising PD-1 to elicit immune suppression. The cytoplasmic domain of PD-1 contains both an ITIM and an ITSM, and recruitment of the tyrosine phosphatase SHP-2 to the latter is believed to inhibit effector molecules such as Zap70 in T cells and Syk in B cells. PD-1 is particularly effective at inhibiting CD28 signalling, as PD-1-recruited SHP-2 dephosphorylates CD28 more efficiently than it dephosphorylates other TCR signalling components such as Zap70, CD3-zeta or LAT[124]. Together with the observation that anti-PD-L1 mediated rescue of T cell

exhaustion depends on CD28[125], this raises the surprising possibility that both major checkpoints targeted in cancer immunotherapy converge on the CD28 pathway, as CTLA-4 restricts CD28 engagement and PD-1 inhibits CD28 signalling[126]. It should be noted, however, that the ability of PD-1 to inhibit targets other than CD28[127], and the differing expression patterns of PD-1 and CTLA-4, mean that, in practise, their biological functions are distinct[126]. Accordingly, agonising the PD-1 receptor is not functionally equivalent to inhibiting CD28 costimulation.

Efforts to agonise PD-1 to suppress immune responses in preclinical settings include the use of a PD-L1-Fc fusion protein which decreased disease severity in the mouse collagen induced arthritis model[128,129]. In addition, recombinant PD-L1-Fc fusion proteins and adenovirus expressing PD-L1-Fc both significantly decreased intestinal inflammation and clinical symptoms in two mouse colitis models, in which colitis is induced by either dextran sodium sulfate or T cell transfer[130].

Agonists against PD-1 are at an early stage of clinical development and include the antibody CC-90006, which is currently in Phase I trials in psoriasis (NCT03337022). In addition, Immunocore are developing Immune Mobilising Monoclonal TCRs Against Autoimmunity (IMMTAAI) molecules comprising a TCR coupled to an agonistic anti-PD-1 effector arm (Dr Peter Weber, personal communication). The rationale for this approach is that by binding to tissue-specific peptide-MHC targets, these bi-specific constructs will elicit localised immune suppression.

The natural role of PD-1 in dampening potentially harmful immune responses is exemplified by the immune-mediated adverse events seen in recipients of checkpoint inhibitors targeting this pathway[131]. The above approaches will test whether deliberate engagement of the PD-1 pathway can reverse pathological responses and restore immune regulation in autoimmune settings.

BTLA

BTLA is an inhibitory member of the CD28 family that is upregulated on T cells during activation and is also expressed on B cells, NK cells and APCs[132]. The cytoplasmic domain of BTLA contains an ITIM, an ITSM and a growth factor receptor-bound protein 2 (Grb2) binding motif and most, although not all, data suggest that BTLA negatively regulates immune responses[133]. BTLA-deficient mice show increased susceptibility to autoimmune diseases[134].

Agonistic anti-BTLA antibodies decrease contact hypersensitivity in mice[135] and inhibit GVHD in an allogeneic hematopoietic stem cell transplantation model[136]. Fusion proteins comprising the BTLA ligand HVEM and an Fc domain can inhibit anti-CD3-induced T cell proliferation in vitro when crosslinked[137]. The concept of agonistic antibodies to BTLA for autoimmune conditions such as SLE has been patented[138], so further development of these agents as therapeutics is likely.

The CD40 pathway

Expression, signalling and structure

CD40 (TNFRSF5) [139] is a member of the TNFR superfamily (**Table 2**) which are predominantly Type I transmembrane proteins and share a conserved structure with one or more cysteine-rich domains in the N-terminal region[150]. It was originally identified and functionally characterized on B-lymphocytes[140], and it is also expressed by a wide spectrum of cell types including macrophages[141], DCs[142], platelets[143], activated epithelial cells and vascular endothelium[144,145]. In addition to being expressed on the plasma membrane of these cell types, soluble CD40 can be found in sera of normal individuals, and may function as a negative regulator of CD40 signalling[146].

CD40 pathway signalling is triggered following binding of CD40 ligand (CD40L or CD154). CD40L was initially described as an early activation marker of CD4+ T-lymphocytes, but is also

expressed by other cell types, including activated platelets, macrophages, mast cells, and vascular endothelial cells[147-149]. Like CD40, CD40L exists in a biologically active soluble form (sCD40L), generated by proteolytic cleavage.

CD40L adopts a trimeric structure, and crystallographic studies indicate that initiation of CD40 signalling requires ligand-dependent dimerization or trimerization of CD40 by CD40L[150]. Structurally, CD40 consists of an extracellular domain comprising four cysteine-rich domains[151,152], a single transmembrane domain, and an intracellular domain. CD40L binds a well-defined epitope in the extracellular portion of CD40 to trigger pathway activation. Although the cytoplasmic tail of CD40 lacks intrinsic kinase activity, the engagement of CD40 by CD40L results in the recruitment of TNFR-associated factors (TRAFs),[153,154] adapter proteins that then recruit downstream signalling molecules such as NIK (also known as MAP3K14), inhibitor of NF-κB kinase (IKK) and TPL2 (also known as MAP3K8). These trigger the downstream activation of multiple kinase cascades involving JNK, ERK and p38, some of which culminate in the activation of transcription factors (such as NF-κB and AP1), which induce the expression of genes involved in cell survival, activation and differentiation[153,155].

Pathway functions and link to disease
The cellular diversity of CD40 expression and potential for triggering multiple signalling pathways contribute to a broad array of immunological functions induced downstream of this receptor. For example, CD40 signalling is required for the generation of T cell-dependent antibody responses, germinal centre formation and memory B cell differentiation[156,157]. CD40 signalling in macrophages and DCs induces cytokine secretion and expression of surface activation molecules, including CD69, CD80 and CD86, that are involved in the regulation of CD4+ T cell help and CD8+ T-cell cross-priming and activation[158]. CD40 pathway activation in epithelial or endothelial cells induces cytokine and chemokine secretion that, in the context of tissue inflammation, could trigger additional leukocytic infiltration. The potential for co-expression of CD40 and CD154 on the same cells suggests the possibility of autocrine signalling, however data on this are limited and the *in vivo* relevance unclear.

Further insights into the role of CD40–CD40L interactions come from the X-linked immunodeficiency syndrome, hyper-IgM (HIGM) syndrome. Patients with loss of function mutations in CD40 or CD40L lack germinal centres as well as a memory B cell repertoire, the consequences of which are a severe impairment of T cell-dependent antibody responses and an increased susceptibility to certain opportunistic infections[159,160]. B cells from patients with HIGM are unable to undergo Ig class switching or affinity maturation, thus patients present with little to no circulating IgG, IgA or IgE antibodies. These immunological defects are phenocopied in CD40 and CD40L-deficient mice[157,161].

The above data suggested that inhibition of CD40–CD40L interactions would inhibit both B cell and APC activation and subsequent T cell priming, representing a potentially viable approach for treating disorders for which pathology is dependent on APC-driven T cell activation. This notion is supported by pharmacological inhibition of CD40–CD40L interactions in preclinical models of autoimmune disease and solid organ transplantation, in which pathway blockade reduces autoimmune disease pathology and prolongs allograft survival[162-166]. In contrast, pathway activation by stimulating CD40 pathway signalling could be a promising approach for cancer immunotherapy, stimulating various immune effector functions thought to be important for immune-mediated anti-tumour responses[167].

Therapeutic targeting of CD40-CD40L
Initial approaches to block CD40–CD40L interactions focused on anti-CD40L monoclonal antibodies including IDEC131 and BG9588[162,168], in part because targeting CD40L should

specifically target activated T cells. Despite initial data suggesting potential for clinical efficacy[162], thromboembolic events were reported, consistent with expression of CD40L on platelets[148], and clinical development was halted. It emerged that CD40L can interact with αIIbβ3 integrin to promote platelet aggregation and thrombus stability independently of CD40[169]. Additionally, thromboembolic liabilities of anti-CD40L mAbs are reported to be dependent on FcγR cross-linking, therefore current biologics targeting CD40L utilize Fc-silenced antibodies and F(ab')2 fragments. There is some evidence, however, that Fc-silencing of anti-CD40L antibodies can alter their *in vivo* efficacy[170].

A number of modified anti-CD40L approaches are currently being evaluated in the clinic (**Table 3**). For example, dapirolizumab pegol is a pegylated anti-CD40L Fab' fragment initially reported to be effective in individuals with moderately to severely active SLE[171]; this activity was not confirmed in a subsequent Phase 2 study (NCT02804763). More recently, a study with the CD40L-specific fusion protein VIB4920 reported reductions in rheumatoid factor and some outcomes of disease activity in patients with rheumatoid arthritis[172].

Another approach for targeting CD40–CD40L has focused on monoclonal antibodies targeting CD40, supported by preclinical studies in autoimmune disease and transplantation[163,173]. There are now several anti-CD40 antibodies in clinical development (**Table 3**), and some have been modified by Fc mutations to be Fc-silent, presumably to avoid depletion of CD40 expressing non-target cells. In contrast to biologics targeting CD40L, Fc silencing of anti-CD40 antibodies does not appear to affect their inhibitory potential[173]. Furthermore, there are no reports of thromboembolic events associated with these anti-CD40 antibodies in preclinical or clinical studies[163,173,174].

While still early in clinical development, results with some anti-CD40 mAbs have been encouraging. For example, iscalimab (CFZ533) is a pathway blocking, non-depleting anti-CD40 antibody[175] that has recently been shown to improve clinical outcomes in patients suffering primary Sjogren's syndrome[176], and prolong kidney allograft survival, function and morphology as a calcineurin-inhibitor (CNI) treatment[177]. In contrast, the frequency of acute rejections was increased in patients dosed with Bleselumab, another anti-CD40 antibody, as a part of a CNI-free treatment regimen[178]. The underlying basis for these differences in efficacy remains to be fully understood, and will be critical in order to understand how to optimally use these biologics in the clinic.

One important consideration for clinical use of antibodies targeting CD40 (and CD40L) is dosing, particularly in the context of autoimmunity and transplantation. This is because the overall expression of CD40 can be elevated in patients with disease[179], which would affect target-mediated clearance of the antibody and highlights the importance of understanding the pharmacokinetic and pharmacodynamic relationships in disease-relevant conditions (a notion that will likely apply to therapeutics targeting other TNFR family members). Furthermore, recent data indicates that the pharmacokinetic and pharmacodynamic relationships of anti-CD40 mAbs in the peripheral blood may not reflect the concentrations of anti-CD40 antibodies required for pathway blockade in affected tissue[175], the likely site of action of these biologics in vivo.

The OX40 pathway

Expression, signalling and structure

OX40 (CD134) is a type 1 transmembrane protein initially identified on activated CD4+ T cells[180]. It has since been found on Tregs and memory (but not naive) T cells[181], and at lower levels on other cell types including activated CD8+ T cells, NKT cells, NK cells, and neutrophils[182]. With the exception of constitutive expression on murine Treg cells, OX40 is upregulated following cellular activation. Signalling downstream of the TCR can induce OX40 expression, and levels of OX40 are

further augmented by co-stimulation via CD28 and IL-2[23,183]. Consistent with its restricted expression on activated T cells, OX40 is often confined to sites of inflammation in human disease and has been reported to demarcate autoreactive cells in individuals with type 1 diabetes[184].

Like OX40, OX40L (CD252) expression is also induced following cellular activation[185] and can be upregulated following stimulation via CD40–CD40L interactions[186,187], as well as by cytokines including IFN-γ, thymic stromal lymphopoietin (TSLP) and IL18 [188,189]. Although OX40L was initially thought to be restricted to professional APCs such as B cells, macrophages and DCs[187,190], OX40L has also been found on mast cells, bronchial smooth muscle cells, Langerhans cells, vascular endothelial cells as well as activated CD4+ and CD8+ T cells[182].

The structure of the human OX40–OX40L complex revealed that three copies of OX40 bind to the trimeric ligand via monomer–monomer receptor–ligand interfaces[191]. Mutagenesis studies indicate that the OX40–OX40L interface is not contiguous, but is distributed over the surface of OX40L in at least two general areas. Furthermore, residues in three OX40 complementarity determining regions contribute to binding affinity that, at ~150 nM, is somewhat lower than described for other multi-domain TNFR–ligand interactions. The structure of the OX40–OX40L interaction complex could have implications for blocking or activating anti-OX40 or anti-OX40L mAbs. For blocking mAbs, failure to occupy the extensive receptor–ligand binding site could result in residual signalling, particularly in the context of membrane-bound receptor–ligand interactions. To date there are no published structures of anti-OX40 or anti-OX40L mAbs (or fragments thereof) bound to their respective antigens, but in vitro data suggest that complete blockade of OX40–OX40L interactions and downstream signalling is possible with such antibodies.

A variety of signalling pathways have been reported to be induced downstream of OX40 binding, including PI3K–PKB, MAPK, NF-kB and NFAT[182]. One of the challenges inherent to studying signalling downstream of OX40 is that both the ligand and the receptor are inducibly expressed, making it difficult to distinguish between signalling pathways induced by the stimulators (e.g. IFN-γ) or by ligation of OX40. Nevertheless, the cytoplasmic tail of OX40 has been demonstrated to recruit TRAF2, TRAF3 and TRAF5, which directly interact with components of the canonical and non-canonical NF-kB signalling pathways[192]. Genes induced downstream of OX40 (which often augment signalling from other costimuli such as TCR ligation) encode proteins that promote cell survival, cell cycle progression and cytokine production.

Pathway functions and link to disease

OX40–OX40L interactions regulate immune cell functions that have been implicated in the pathogenesis of autoimmune diseases[182,193]. Major effects of OX40 ligation on CD4+ T cells include enhanced proliferation and T helper subset differentiation, increased cell survival and augmented cytokine production. Transgenic overexpression of OX40L on DCs or OX40 on T cells results in increased effector T cell numbers, and mice deficient in either receptor or ligand display reduced CD4+ T cell expansion[194-196]. OX40 signals are also required for the generation, maintenance, and optimal reactivation of memory CD4+ T cells[195]. OX40–OX40L similarly regulate the activation and survival of CD8+ T cells, although OX40-deficient animals can mount primary and memory cytotoxic T cell responses[194]. Interestingly, deficiency in either receptor or ligand has minimal effect on the humoral immune response[194], so combination approaches may be needed in indications where both T and B cells should be targeted.

The outcome of OX40–OX40L interactions on a given cell type is influenced by activation of other pathways (e.g. TCR signals, cytokines). For example, TSLP induces OX40L on DCs that subsequently promote IL4-driven Th2 differentiation[188,197], potentially linking this pathway to pathology in asthma, and atopic dermatitis (AD). Additionally, the proliferation and survival of Th1 and Th17 cells has been observed following OX40 stimulation in vitro. These data suggest that

targeting OX40–OX40L interactions could be most effective in combination with therapies specific for other immune pathways linked to pathology in specific diseases (e.g. anti-IL4Rα antibodies in AD).

OX40 signals can also influence the generation, function and survival of Treg cells, notably inducible Tregs[182,198]. OX40 pathway activation can stimulate Treg expansion in vitro, although it has also been reported to block their suppressive function[199]. Conversely, OX40 deficient humans and mice have reduced numbers of Tregs. Similar to T effector cells, the cytokine environment plays an important role in shaping the outcome of OX40–OX40L interactions on Treg proliferation, survival and function.

Several recent reviews have highlighted the role of OX40–OX40L interactions in autoimmune and inflammatory diseases[193,200]. Expression of receptor and ligand have been reported on leukocytes in synovial fluid from patients with rheumatoid arthritis, glomeruli from patients with lupus nephritis, lesions from patients with atopic dermatitis or psoriasis, and bronchial submucosa in patients with mild asthma. Furthermore, in preclinical studies, OX40–OX40L blocking antibodies reduced pathology in models of colitis, rheumatoid arthritis, uveitis and type 1 diabetes[193]. Collectively, these results suggest that OX40–OX40L signals could be linked to pathology in numerous diseases.

Therapeutic targeting of OX40–OX40L
Similar to the CTLA-4 system, the OX40 pathway is targeted to opposite effect in cancer versus autoimmunity. Accordingly, OX40 stimulation using agonistic antibodies has been reported to boost T cell anti-tumour immune responses in preclinical models[201,202], although clinical trial results have been mixed, suggesting further optimisation is required[203]. In the autoimmune setting, the goal is to antagonise the OX40 pathway and thereby suppress the immune response.

There are several therapeutics targeting OX40 or OX40L in development (**Table 4**), however clinical efficacy data on the effects of these drugs is currently limited. A randomized, placebo controlled clinical study with oxelumab, an anti-OX40L antibody, did not provide benefit in patients with mild asthma despite partial and transient reductions in total IgE and sputum eosinophils[204]. A randomized, controlled Phase 2 study of an anti-OX40 antibody, GBR 830, reported significant clinical improvement in patients with moderate to severe atopic dermatitis, together with decreases from baseline in OX40 expressing T-cell and OX40L expressing DCs in lesional skin[205].

Like many other TNFR family proteins, OX40 and its ligand exhibit cellular, tissue-type and temporal variation in expression. Expression of these proteins is largely restricted to activated cells in an inflammatory environment (including autoantigen-specific lymphocytes), so targeting OX40–OX40L interactions may inhibit pathology in autoimmune and inflammatory diseases with organ-specific manifestations. Although tissue-specific expression has supported the rationale for development of several therapeutics targeting OX40–OX40L, evidence of expression may simply reflect the presence of activated cells rather than pathway activation or contribution to pathology. Thus, a combination of expression of receptor and ligand on pathologically relevant cell types in affected tissue, coupled with some evidence of pathway-specific activation, is important to inform indication selection for OX40–OX40L targeted therapeutics.

This inducible expression enables tissue-specific targeting, but it also presents challenges for drug development. For example, pharmacokinetic and pharmacodynamic information from a first-in-human study with healthy volunteers may not be predictive of the doses required for optimal pathway blockade and activation in patients, nor provide conclusions about potential on-target safety or tolerability. Early clinical studies could therefore be more informative if done in patient populations, a strategy that has been adopted by several companies[204,206]. A second advantage of

inducible expression could be reduced target-mediated clearance of anti-OX40 or anti-OX40L antibodies from receptor-mediated endocytosis, potentially translating into prolonged exposure and thus less frequent dosing. However, this presents another challenge: defining the tissue phamacodynamics. The recent clinical trial failure of oxelumab in asthma could be explained in part by inadequate dosing for optimal pathway blockade in tissue[204]. Finally, given the inducible nature of expression, the timing of clinical intervention could be crucial.

Another consideration for development of OX40–OX40L pathway modulators is the multiple effects of OX40 ligation on both Tregs and effector T cells. Although the in vivo relevance of some of these data is not clear, it is possible that such immunological dualism might compromise the efficacy of both blocking and agonistic approaches. Blocking OX40–OX40L interactions (in the absence of cell depletion) might be expected to decrease effector T cells and increase Treg cell function, and agonistic therapeutics the opposite. Data from a recent clinical trial with oxelumab in subjects with mild asthma indicated that there were no reductions in T cells specific for sensitizing allergen or recall antigens or in the number of circulating DCs[204]. Interestingly OX40L has also been reported to deplete OX40L expressing cells such as DCs[207], so the absence of a substantial pharmacodynamic effect could be related to a number of factors including under-dosing, limited translatability or monitoring at an inappropriate time point. Despite no changes in DC or T cell numbers a decrease in eosinophils was observed, but it remains unclear if this was a direct or indirect consequence of anti-OX40L treatment. Another OX40L antibody (KY1005) in development for atopic dermatitis was engineered to be non-depleting, prudent given the fact that this receptor is inducibly expressed on a variety of cells[208]. It may also make sense to engineer anti-OX40 antibodies to be non-depleting, to avoid depletion of memory CD4+ T cells, which would potentially compromise recall responses.

Consistent with the absence of severe side effects in in vivo preclinical studies, current clinical data indicates that targeting OX40–OX40L interactions is probably safe and well tolerated. However, few patients have been dosed, and as mentioned above, it is unclear whether full pathway blockade has been achieved, nor whether effects of pathway blockade on both pro- and anti-inflammatory immune cell types might compromise efficacy. Additional long-term data in the context of appropriate posology is required to make definitive conclusions on the safety profile of therapies targeting OX40–OX40L interactions.

Other TNFR family members

In addition to CD40 and OX40, numerous other TNF / TNF receptor family members have been investigated as potential therapeutic targets. As well as the receptors and ligands discussed below, therapies are in development for two other pathways. The anti-LIGHT (TNFSF14) antibody AEVI-002 (SAR252067, MDGN-002) is being investigated for Crohn's disease in a phase I clinical trial (NCT03169894); a LTβR-Ig fusion protein, baminercept (BG9924), that targets LIGHT and LTαβ failed to show efficacy in clinical trials in rheumatoid arthritis (NCT00458861) or primary Sjögren's syndrome[209]. CD30 is being targeted in systemic sclerosis (NCT03222492, NCT03198689) using brentuximab vedotin, a chimeric anti-CD30 antibody conjugated to the cytotoxic drug MMAE, which is approved for the treatment of Hodgkin lymphoma and anaplastic large cell lymphoma[210].

TNF

Although TNF was first identified as a factor capable of inducing necrosis of tumour cells in vitro and in vivo[211,212], hopes to use TNF for cancer therapy were dashed by the systemic toxicity observed in clinical trials[213,214]. Targeting the TNF pathway has, however, revolutionised the treatment of rheumatoid arthritis and, to date, five biologicals that inhibit binding of TNF to its

receptors TNFR1 and TNFR2 have received FDA and EMA approval: infliximab, a chimeric murine/human IgG1 monoclonal anti-TNF antibody[215]; etanercept, a recombinant fusion protein of TNFR2 linked to the Fc region of human IgG1[216]; adalimumab, a recombinant human IgG1 monoclonal anti-TNF antibody[217]; golimumab, a human IgG1 monoclonal anti-TNF antibody[218] and certolizumab pegol, a pegylated recombinant humanized Fab′ fragment of an anti-TNF antibody with improved plasma half-life[219]. The use of TNF inhibitors has also been approved for other autoimmune and inflammatory diseases including Crohn's disease, ulcerative colitis, psoriasis, psoriatic arthritis, juvenile idiopathic arthritis, ankylosing spondylitis, axial spondyloarthritis, hidradenitis suppurativa and uveitis. No improvement was observed in clinical trials of infliximab in multiple sclerosis (MS)[220], however administration of a TNFR2 agonist recently showed promising results in the EAE model[221], possibly reflecting the role of TNFR2 engagement in expanding Treg[222,223]. In 2018, another 151 TNF inhibitors were in the clinical pipeline, promising further developments in the field[224].

BAFF

BAFF (*TNFSF13B*)[225,226] binds to three receptors: BAFFR (*TNFRSDF13C*), TACI (*TNFRSF13B*) and BCMA (*TNFRSF17*)[227,228]. Additionally, there is evidence that BAFF interacts with the Nogo-66 receptor (also known as reticulon-4 receptor), which is expressed in the central nervous system[229]. BAFF is initially expressed as a membrane-bound protein but is rapidly cleaved from the cell membrane into a soluble form[230]. It is expressed by monocytes, macrophages, DCs, T cells, bone marrow stromal cells and astrocytes and its expression is upregulated in response to pro-inflammatory stimuli[225,231-234].

The three receptors show distinct expression patterns and have discrete functions in B cell homeostasis, survival and activation. BAFFR is expressed on most mature B cell subsets and its interaction with BAFF is important in B cell homeostasis and the development of mature B cells. Mice deficient in BAFFR or BAFF show a significant decrease in peripheral B cell numbers and their mature B cells do not fully develop[235,236]. Similarly, B cells from humans carrying a homozygous deletion in the gene that encodes BAFFR have arrested B cell development[237]. In contrast, TACI and BCMA are only expressed on plasma cells, where they support the generation and homeostasis of this cell subset[238,239]. TACI can also be found on switched memory B cells and marginal zone B cells and supports immunoglobulin class switching, but also functions as a decoy receptor[240,241]. In addition to its importance in B cell biology, BAFF also has a role in T cell costimulation[242].

There is a wealth of evidence connecting BAFF and its receptors to autoimmune pathology. Overexpression of BAFF in mice leads to development of SLE-like autoimmune symptoms[227,243,244], and elevated levels of BAFF, which correlate with disease activity, can be found in the serum of patients with SLE[245,246] or Wegeners granulomatosis[247], in the synovial fluid or serum of patients with rheumatoid arthritis[245] and in CNS lesions in MS[233].

As a consequence, several therapeutics targeting BAFF and its receptors have been developed. The most successful to date is belimumab, a recombinant anti-BAFF humanized monoclonal IgG1λ antibody that was, in 2011, the first biological treatment for SLE to be approved by the FDA[248-250]. Belimumab is generally efficacious, although not all SLE patients benefit from treatment. Promising results have also been observed for belimumab in phase II clinical trials in primary Sjögren's syndrome[251], rheumatoid arthritis[252], diffuse cutaneous systemic sclerosis[253], primary membranous nephropathy[254] and trials are also being conducted in myositis and lupus nephritis (NCT02347891, NCT01639339). Endpoints for clinical trials of belimumab treatment in myasthenia gravis and vasculitis were not met[255,256]. Belimumab is frequently described to only bind the soluble form of BAFF, although there is evidence from in vitro assays that belimumab also has some reactivity with membrane-bound BAFF[257,258].

Following the success of belimumab, two additional therapeutics were engineered to bind both the soluble and membrane-bound forms of BAFF: tabalumab[259], a fully human monoclonal IgG4 antibody, and blisibimod (AMG 623/A-623)[260], a peptibody consisting of a BAFF binding domain fused to the Fc region of human antibody. Blisibimod showed promising results in a phase II trial for IgA nephropathy and received orphan drug designation for treatment of this disease (NCT02062684, https://www.globenewswire.com/news-release/2017/08/09/1082711/0/en/Anthera-Announces-FDA-Orphan-Drug-Designation-for-Blisibimod-for-the-Treatment-of-IgA-Nephropathy.html). Both drugs were, however, unable to reach primary endpoints in clinical trials in SLE and rheumatoid arthritis[261,262]. Development of tabalumab has since been discontinued, although post-hoc analysis of one of the trials indicates positive responses in Japanese SLE patients[263].

Ianalumab (VAY736) is a human IgG1κ monoclonal antibody that binds to BAFFR. It showed promising results in a phase II trial in primary Sjögren's syndrome[264] and its efficacy is currently being investigated in a larger phase II trial (NCT02962895). It is also being trialled for the treatment for rheumatoid arthritis, MS, SLE, pemphigus vulgaris, autoimmune hepatitis and idiopathic pulmonary fibrosis (NCT03574545, NCT02038049, NCT03656562, NCT03656562, NCT01930175, NCT03217422, NCT03287414).

APRIL
APRIL (*TNFSF13*), a close homologue of BAFF, also interacts with TACI and BCMA but not BAFFR[230]. Atacicept is a recombinant fusion molecule of the extracellular region of TACI and the human IgG1 Fc domain[265] that binds to both BAFF and APRIL. Clinical trials in SLE[266], MS[267] and optic neuritis[268] were terminated early due to adverse events, and trials in rheumatoid arthritis did not meet their primary endpoints[269]. A more recent phase II trial in patients with active autoantibody-positive SLE did, however, show a reduction in disease activity and severe flares, particularly in predefined subpopulations with high disease activity[270].

RANK
RANKL (*TNFSF11*) is produced by activated T cells, osteoblasts and stromal cells and binds to RANK, or to a decoy receptor osteoprotegerin (OPG). The ratio of RANKL/OPG has been shown to be altered in the cerebrospinal fluid of MS patients at clinical onset and mRNA levels of RANK, RANKL and OPG are elevated in PBMC from individuals with relapsing-remitting MS[271]. Given the role of RANKL in bone erosion, attempts have been made to target this pathway in rheumatoid arthritis. The anti-RANKL antibody denosumab was shown to have a positive effect on the progression of joint destruction in rheumatoid arthritis patients treated with conventional synthetic disease-modifying antirheumatic drugs[272].

Combination therapy

Considerations for combination therapy
Regulation of the immune system is complex and multi-facetted. Pathways important to immune defence can frequently be triggered in multiple ways and reinforced by feedback loops. Such complexity and redundancy means that blockade of a single target may be insufficient to achieve the desired immunomodulatory effect. Combination therapy is increasingly being implemented in both immune stimulatory and immune suppressive settings, as exemplified by the use of an anti-CTLA-4 mAb alongside an anti-PD-1 mAb in cancer immunotherapy. It is possible to achieve combination therapy either *via* the independent delivery of two reagents, or through the use of bispecific antibodies that permit dual pathway targeting (**Text Box 1**).

Text Box 1: Bispecific antibodies

Bispecific antibodies have been engineered to express antibody fragments that recognize two (and in some cases more) antigens. Some examples include AMG 570, a bispecific antibody–peptide conjugate that targets ICOSL and BAFF and is in clinical development for the treatment of rheumatoid arthritis and SLE, and ABT-122, a bispecific antibody that targets both TNF and IL-17A, development of which has been discontinued. Bispecific molecules come with particular considerations. For example, the stoichiometry of antigen-targeting arms in a bispecific antibody is fixed, and may not reflect (or optimally target) the concentrations of the targets in vivo. Additionally, it is important to consider whether membrane and/or soluble proteins are being targeted. If two membrane proteins are targeted, it is possible that the bispecific antibody may enable cell–cell interactions, or facilitate receptor cross-linking. Finally, there are reports of increased immunogenicity of bispecifics relative to monoclonal antibodies.

Safety considerations are of particular importance when contemplating combination therapy in the autoimmune setting, since the presenting condition is generally not life threatening. Of note, the safety profile associated with combined treatments is not necessarily predictable from the safety profiles of the individual agents. A central concern is that the simultaneous blockade of multiple immune pathways may increase the risk of infection. Indeed, combined targeting of TNFα and IL-1 with etanercept and anakinra, in rheumatoid arthritis patients who were refractory to methotrexate, showed no benefit over etanercept alone but was associated with a higher incidence of serious infections [273]. In another smaller study, however, the combination of etanercept and anakinra resulted in a safety profile that was not markedly different from either drug alone[274]. Limited information on the safety profile of abatacept and anakinra in combination also appears favourable: in a retrospective review of 4 children with refractory systemic juvenile idiopathic arthritis treated concurrently with these agents, outcomes appeared positive with evidence of disease control and no significant infections or adverse events[275].

Targeting of TNF and IL-17A with a bispecific antibody (ABT-122) in rheumatoid arthritis patients responding poorly to methotrexate showed a safety profile comparable to that of targeting TNF alone (adulimumab)[276]. No serious infections were observed, although efficacy was not meaningfully different from that seen in the adulimumab comparator arm. A similar study in Psoriatic Arthritis patients also found that the safety profiles and efficacy of ABT-122 resembled those of adulimumab[277].

Collectively these studies suggest that although safety remains a major consideration for combination therapy, this barrier may not be insurmountable. However, if efficacy is driven mainly by blockade of one of the two pathways, dual targeting may provide no benefit over monotherapy.

A key challenge is the rational selection of complementary interventions. Using interventional clinical trials to identify pathways that may still be active in partial or non-responders may enable data-driven identification of potential combinations. Understanding the relative impact of costimulatory pathways on different cell types, and at different sites, will also help guide rational intervention strategies. Selecting agents that target different cell subsets maybe an effective approach; for example, in cancer immunotherapy, the CTLA-4 Ab predominantly targets CD4 T cells and the PD-1 Ab targets CD8 cells, which may be one factor underlying the success of this combination therapy.

Knowledge of the functional hierarchies that exist between different costimulatory receptors may also help inform combination strategies. For example, one strategy would be to shut down the early bidirectional interactions between T cells and APCs (**Figure 1a**) by simultaneously inhibiting the primary costimulatory interactions between CD28 and CD40 and their ligands. To tackle ongoing

immune responses, targeting a primary costimulator alongside an inducible receptor or inducible cytokine that acts at a later point in the immune response might be a better approach. Combining T cell inhibition with cytokine targeting may be particularly relevant when considering the continuum that exists between autoimmunity and autoinflammation. While there are conditions that sit at each end of the spectrum – the NLRP3-associated cryopyrinopathies representing autoinflammation, and IPEX caused by Foxp3 mutations being autoimmune – it is clear that many diseases have elements of both autoimmunity and autoinflammation[278,279]. Since the autoimmune component reflects the actions of the adaptive immune system, while autoinflammation stems from innate immune activity, dual targeting of adaptive and innate elements may be an effective strategy.

A further strategy for successful combination therapy in autoimmune settings might be to partner a drug that targets the immune system with tailored regenerative treatments that counteract local damage at the target organ. In type 1 diabetes this could involve combining an immunosuppressive therapy, such as costimulation blockade, with a drug that promotes beta cell regeneration and function. In rheumatoid arthritis, targeting of T cells or inflammatory cytokines could be combined with administration of therapies that counter pathological bone loss.

Below, and in **Table 5**, we provide selected illustrations of how combination therapies targeting costimulatory pathways are being developed.

CTLA-4-Ig and CD40-CD40L blockade
As highlighted in **Figure 1a**, the initial interaction between T cells and APCs is driven by the CD28 and CD40 pathways. It therefore follows that interventions targeting these two pathways could extinguish immune responses before secondary costimulatory interactions come into play (**Figure 3**). Consistent with this, the combination of CTLA-4-Ig and an anti-CD40L Ab (MR1) prolonged survival of both skin and cardiac allografts in mice[280]. Similarly, the combination of CTLA-4-Ig and another anti-CD40L Ab (5C8) was 100-fold more efficient than either drug alone in a primate renal allograft model [281], although this combination was associated with primary CMV infection in seronegative recipients of CMV+ transplants[282]. The thromboembolic events associated with the anti-CD40L Ab[283] called an untimely halt to exploration of this reagent combination. With the development of new drugs targeting the CD40–CD40L pathway that lack thromboembolic liabilities, this combination is once again under investigation. Accordingly, the combination of CTLA-4-Ig and an anti-CD40 Ab (that is not associated with thrombosis) promotes allogeneic bone marrow chimerism and skin graft survival in mouse models[284]. Subsequent testing in non-human primates revealed the same strategy could support long-term islet allograft survival in conjunction with basiliximab and sirolimus administration[285]. We expect to see this combination taken forward as new reagents targeting the CD40–CD40L pathway, as well as new CD28-directed molecules, progress though development.

Cellular depletion and costimulation blockade
Controlling memory responses is challenging, so debulking autoreactive lymphocytes *via* cellular depletion strategies prior to costimulation blockade is an attractive proposition. The overarching concept here is to remove activated lymphocytes and allow lymphocyte reconstitution to occur in a costimulation-poor environment. Moreover, depletion strategies can remove CD28-null T cells[286] that are refractory to agents targeting this pathway but could contribute to pathogenesis. Lymphopenia following depletion of mature T cells and B cells with agents such as the anti-CD52 antibody, alemtuzumab, can be a predisposing factor for autoimmunity, due to homeostatic proliferation that leads to a restricted T cell repertoire[287]. Of note, CD28[288] and its ligands[289] contribute to lymphopenia-induced T cell proliferation, suggesting that agents targeting the CD28 pathway (e.g. abatacept) would be a rational choice for use in combination with lymphocyte depletion approaches (**Figure 4**). Lymphocyte deletion can also be achieved with rituximab, which targets CD20+ B cells, however rituximab unexpectedly also reduces T cell numbers, likely by an

indirect mechanism[290]. The combination of rituximab and abatacept has been tested in 3 patients, two with refractory juvenile-onset SLE and one with Copa syndrome (presenting with polyarthritis, nephrothapy and interstitial lung disease). In all 3 patients, use of abatacept within 1 month of the last round of rituximab treatment resulted in a decrease in autoantibody levels and complete remission for over 2 years[291]. Use of rituximab and abatacept in combination is planned in the setting of type 1 diabetes (NCT03929601).

Future outlook

Despite therapeutic targeting of the TNF pathway in rheumatic disease for nearly 20 years, more widespread targeting of other members of the TNFR and CD28 superfamilies in autoimmunity has taken longer to emerge. There is now growing excitement at the prospect of translating our understanding of costimulatory pathways into new therapeutics for autoimmunity, mirroring the success of immunotherapy for the treatment of cancer. By integrating information from multiple genome wide association studies with epigenetic data, it has become clear that susceptibility to nearly all autoimmune diseases maps to promoters that are active in CD4 T cells[292]. Together with the fact that single nucleotide polymorphisms associated with autoimmunity encompass many costimulatory molecules (such as CD28, CTLA-4, ICOS, CD40 and OX40L), control of CD4 T cell activation is clearly at the heart of genetic susceptibility to autoimmune disease. Costimulatory pathways are therefore a natural target for immunotherapy in autoimmunity, and increasingly refined genetic analysis may fine-tune therapeutic targeting in a disease-specific manner.

The targeting of costimulatory molecules complements a host of other approaches in development for the treatment of autoimmune diseases. These include expanding Treg populations using modified IL-2 molecules or adoptive cell therapy, blocking specific cytokines implicated in immune pathology and tolerance-inducing protocols such as peptide immunotherapy.

All of these approaches will benefit from improved mechanistic understanding of immune cell interactions and function. Furthermore, the revolution in single cell analysis has provided a platform for unprecedented molecular understanding of patient-specific immune pathways and their activity over time, which presents opportunities for data-informed tailored interventions. Technological advances, such as bispecific antibody engineering, will bolster our capacity to deliver such interventions. Finally, an increasing emphasis on characterisation of the pre-symptomatic phases of autoimmunity will allow therapies that meet the required safety threshold to be deployed earlier, potentially enhancing the prospect of success.

Tables

Table 1 | Overview of the CD28 receptor and the B7 ligand families

Gene	Protein name and aliases	Ligands	Main functions
CD28 receptor family			
CD28	CD28, Tp44	CD80, CD86, ICOSL	Promotes T cell activation, proliferation and survival
ICOS	ICOS, CD278, AILIM, CVID1	ICOSL	Promotes T_{FH} cell differentiation and maintenance Promotes anabolic metabolism and lipogenesis
CTLA4	CTLA-4, CD152, CD, GSE, GRD4, ALPS5, IDDM12, CELIAC3	CD80, CD86	Negatively regulates immune responses Regulates CD28 signalling by removing ligands (CD80 and CD86) from APCs via transendocytosis
PDCD1	PD-1, CD279, SLEB2, hSLE1	PD-L1, PD-L2	Negatively regulates of immune responses Suppresses CD28 and, to lesser extent, TCR signalling Prevents follicular recruitment of T cells
BTLA	BTLA, CD272	HVEM	Negative regulator of immune responses, although costimulatory functions have also been reported
TMIGD2	CD28H, IGPR-1	B7-H7	Both T cell costimulatory and coinhibitory functions have been reported Only expressed in higher primates
B7 ligand family			
CD80	CD80, B7-1, BB1, LAB7, CD28LG1	CD28, CTLA-4, PD-L1	Ligand for CD28, CTLA-4 and PD-L1 Cis interaction with PD-L1 blocks PD-1/PD-L1 binding (in mice)
CD86	CD86, B7-2, B70, LAB72, CD28LG2	CD28, CTLA-4	Ligand for CD28 and CTLA-4 Dominant ligand for T_{FH} cell differentiation
PDCD1LG2	PD-L2, B7-DC, CD273,	PD-1, RGMb	Ligand for PD-1 and RGMb
CD274	PD-L1, B7-H1, PDCD1LG1	PD-1, CD80	Ligand for PD-1 and CD80
ICOSLG	ICOSL, B7-H2, GL50, B7RP1, CD275, LICOS, B7RP-1	ICOS, CD28	Ligand for ICOS
CD276	B7-H3, B7RP-2	Unidentified	Reports of both costimulatory (T cells) and coinhibitory (T cells and NK cells) functions Role in osteoclast differentiation and bone mineralization
VTCN1	B7-H4, B7x, B7S1, B7h.5	Unidentified	T cell coinhibitory
VSIR	VISTA, B7-H5, GI24, SISP1, PD-1H, DD1α	VSIG-3	T cell coinhibitory
NCR3LG1	B7-H6, NR3L1	NKp30	Involved in NK cell activation but also suggested to have inhibitory function
HHLA2	B7-H7, B7-H5, B7y	CD28H	Ligand for CD28H Only expressed in higher primates

APC, antigen-presenting cell; BTLA, B and T lymphocyte attenuator; CTLA4, cytotoxic T lymphocyte antigen 4; ICOS, inducible T cell co-stimulator; ICOSL, ICOS ligand; TCR, T cell receptor; T_{FH} cell, follicular helper T cell; VISTA, V-domain immunoglobulin suppressor of T cell activation.

Table 2 | Overview of selected members of the TNF superfamily

Gene	Protein name and aliases	Interacting proteins	Functions
TNF receptor family			
TNFRSF1A	TNFR1, TNFR, FPF, p55, p60, TBP1, TNFAR, CD120a, TNFR55, TNFR60, TNF-R-I, p55-R	TNF (soluble and membrane bound), LTα	Pro-inflammatory Pro-apoptotic Immune-regulatory when shed
TNFRSF1B	TNFR2, p75, TBPII, TNFBR, CD120b, TNFR1B, TNFR80, TNF-R75, p75TNFR, TNF-R-II	TNF (membrane bound), LTα	Anti-inflammatory Immune regulatory
LTBR	TNFRSF3, TNFR3, TNFCR, D12S370, TNFR-RP, TNFR2-RP, LT-BETA-R, TNF-R-III	LTαβ, LIGHT	Essential for peripheral lymphoid organ development
TNFRSF4	OX40, CD134, ACT35, IMD16, TXGP1L	OX40L	T cell costimulatory Enhances CD4+ T cell proliferation, survival, cytokine production and Th cell differentiation Required for generation, maintenance and optimal reactivation of memory CD4+ T cells Important for Treg cell generation and survival, though it may block Treg cell suppressive functions
CD40	TNFRSF5, Bp50, CDW40, p50	CD40L	Important for T cell-dependent antibody responses, germinal centre formation and memory B cell differentiation Induces cytokine secretion and expression of activation molecules like CD69, CD80 and CD86 in macrophages and dendritic cells
CD27	TNFRSF7, S152, LPFS2, T14, Tp55	CD70, Siva	Enhances cell proliferation and survival Involved in CD8+ memory formation Enhances Th1 and plasma cell differentiation Promotes thymic Treg development Inhibitory effect on Th17 cells reported
TNFRSF8	CD30, Ki-1, D1S166E	CD153	Costimulatory Involved in memory T cell expansion
TNFRSF9	4-1BB, CD137, ILA, CDw137	4-1BBL	Costimulatory for CD8+ T cells and (to lesser extent) for CD4+ T cells May promote B cell expansion and survival May enhance survival of dendritic cells Induces Treg cell expansion, though it may reduce Treg cell suppressive function
TNFRSF11A	RANK, CD265, TRANCER, OPTB7, FEO, OFE, ODFR, OSTS, PDB2, LOH18CR1	RANKL	T cell–dendritic cell interactions Osteoclast and lymph node development
TNFRSF13B	TACI, CD267, TNFRSF14B, CVID, RYZN, CVID2, IGAD2	APRIL, BAFF	Immunoglobulin class-switching Maintenance/generation of plasma cells Immune-regulatory when shed
TNFRSF13C	BAFFR, CD268, CVID4, BROMIX, prolixin	BAFF	B cell homeostasis and development, Immunoglobulin class-switching T cell costimulation
TNFRSF14	HVEM, ATAR, TR2, CD270, HVEA, LIGHTR	LIGHT, LT-α3, BTLA, CD160, SALM5	Crosslinking induces costimulatory signals
TNFRSF17	BCMA, CD269, TNFRSF13A, BCM	APRIL, BAFF	Maintenance of long-lived plasma cells
TNFRSF18	GITR, AITR, CD357	GITRL	T cell costimulatory
TNFRSF21	DR6, CD358, BM-018	Syndecan-1	Coinhibitory Suggested to negatively regulate NF-KB

			and NFAT activation
TNF ligand family			
TNF	TNF, TNFA, TNFSF2, DIF, TNLG1F	TNFR1, TNFR2	Ligand for TNFR1 and TNFR2
TNFSF4	OX40L, CD252, Gp34, CD134L, TNLG2b, TXGP1	OX40	Ligand for OX40 Reverse signalling has been reported
CD40LG	CD40L, CD154, HIGM1, IGM, IMD3, T-BAM, TRAP, gp39, TNFSF5	CD40	Ligand for CD40
CD70	CD70, CD27L, LPFS3, TNLG8A, TNFSF7	CD27	Ligand for CD27 Reverse signalling has been reported
TNFSF8	CD153, CD30L, TNLG3A	CD30	Ligand for CD30
TNFSF9	4-1BBL, CD137L, TNLG5A	4-1BB	Ligand for 4-1BB Reverse signalling has been reported
TNFSF11	RANKL, CD254, TRANCE, OPTB2, ODF, OPGL, TNLG6B, hRANKL2	RANK, OPG	Ligand for RANK and OPG Reverse signalling has been reported
TNFSF13	APRIL, CD256, TALL2, ZTNF2, TNLG7B, TRDL-1	TACI, BCMA	Ligand for TACI and BCMA Reverse signalling has been reported
TNFSF13B	BAFF, CD257, BLYS, TALL1, THANK, ZTNF4, TNLG7A, TNFSF20, DTL	BAFFR, TACI, BCMA, Nogo-66	Ligand for BAFFR, TACI, BCMA and Nogo-66 Reverse signalling has been reported
TNFSF14	LIGHT, CD258, HVEML, LTg	HVEM, LTBR, DCR3	Ligand for HVEM and LTBR T cell costimulatory when ligated
TNFSF18	GITRL, TL6, AITRL, TNLG2A	GITR	Ligand for GITR Reverse signalling has been reported

APRIL, a proliferation-inducing ligand; BAFF, B cell-activating factor; BTLA, B and T lymphocyte attenuator; CD40L, CD40 ligand; DCR3, decoy receptor 3; GITR, glucocorticoid-induced TNFR-related protein; HVEM, herpesvirus entry mediator; LTα, lymphotoxin-α; LTβR, lymphotoxin-β receptor; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB; OPG, osteoprotegerin; OX40L, OX40 ligand; RANK, receptor activator of NF-κB; RANKL, RANK ligand; TNF, tumour necrosis factor; TNFR, TNF receptor; Treg cell, regulatory T cell.

Table 3 | Therapeutics targeting CD40-CD40L interactions

Therapeutic (company)	Drug characteristics	Reported mechanism of action	Clinical status
Anti-CD40 mAbs			
ABBV-323 (AbbVie)	Unknown	Blocking antibody	Ongoing Phase1 in ulcerative colitis (est. completion 2020) (NCT03695158)
ASKP-1240/bleselumab (Astellas/Kyowa)	Stabilized IgG4	Blocking, minimal agonistic activity, non-depleting (humans)	Negative results in Phase 2 in kidney allograft transplantation (NCT01780844) Ongoing Phase2 study in focal segmental glomerulonephritis (est. completion 2019) (NCT02921789)
BI-655064 (Boehringer Ingelheim)	IgG1, LALA	Blocking, minimal agonistic activity, non-depleting	Ongoing Phase 2 in lupus nephritis (est. completion 2020) (NCT03385564) Negative results in Phase 2 in rheumatoid arthritis (NCT01751776) Phase 1 terminated in idiopathic thrombocytopenic purpura (NCT02009761)
FFP-104/PG-102 (Fast Forward Pharma)	Humanized wild-type IgG4	Blocking, minimal agonistic activity, non-depleting	Phase 2 completed in Crohn's disease (no outcome reported) (NCT02465944) Phase 1/2 completed in primary biliary cirrhosis (NCT02193360)
BMS-986090 (Bristol Myers Squibb)	Diabody with modified IgG4	Not reported; likely blocking	Phase 1 healthy volunteer study terminated (programme discontinued) (NCT02079480)
Iscalimab (CFZ533)	N297A IgG1, fully human	Blocking, minimal agonistic activity, non-depleting	Positive Phase 2 study in kidney allograft transplantation, dose range finding study ongoing (NCT03663335) Positive Phase 2 study in primary Sjögren's Syndrome (NCT02291029) Ongoing Phase 2 studies in lupus nephritis (NCT03610516) and hidradenitis suppurativa (NCT03827798 Completed Phase 2 studies in Grave's disease (NCT02713256) and myasthenia gravis (NCT02565576)
Anti-CD40L mAbs			
Dapirolizumab pegol (UCB / Biogen)	Humanized pegylated anti-CD40L Fab′	Blocking, non-depleting	Discontinued after negative Phase 2 study in SLE (NCT02804763)
BMS-986004/letolizumab (Bristol Myers Squibb)	Anti-CD40L domain antibody-modified IgG1 Fc fusion	Blocking, non-depleting	Open label Phase 2 completed in immune thrombocytic purpura (NCT02273960) Ongoing Phase 2 study in GvHD (est. completion 2024)

	protein		(NCT03605927)
MEDI4920/VIB4920 (AstraZeneca/Viela Bio)	Anti-CD40L Tn3 fusion protein	Blocking, non-depleting	Completed Phase 1 in healthy volunteers (NCT02151110) and rheumatoid arthritis (NCT02780388) Ongoing Phase 2 study in kidney allograft transplantation (NCT04046549)
AT-1501 (Anelixis)	Humanized anti-CD40L IgG1 kappa	Blocking, non-depleting	Ongoing Phase 2a study in amyotrophic lateral sclerosis (NCT04322149)

CD40L, CD40 ligand; GVHD, graft-versus-host disease; LALA, Leu234Ala and Leu235Ala; mAb, monoclonal antibody; SLE, systemic lupus erythematosus.

Table 4 | Therapeutic targeting of OX40-OX40L interactions

Therapeutic (company)	Drug characteristics	Reported mechanism of action	Clinical status [a]
Anti-OX40 mAbs			
KHK4083 (Kyowa Kirin)	Human, non-fucosylated IgG1	Prevents OX40–OX40L interactions	Completed Phase 2 in moderate ulcerative colitis (NCT02647866) Ongoing Phase 2 in atopic dermatitis (NCT03703102)
ISB830/GBR830 (Ichnos/Glenmark)	Humanized IgG1	Prevents OX40–OX40L interactions	Ongoing Phase 2b in atopic dermatitis (NCT03568162)
Anti-OX40L mAbs			
Oxelumab/LC.001 (Genentech/Roche)	Human modified IgG1	Prevents OX40–OX40L interactions; has lytic activity	Completed Phase 2 in asthma (completed, NCT00983658) Discontinued due to lack of efficacy
KY1005 (Kymab)	Human IgG4	Prevents OX40–OX40L interactions with OX40; non-lytic	Ongoing Phase 2 in atopic dermatitis (NCT03754309)

mAb, monoclonal antibody; OX40L, OX40 ligand.

Table 5 | Overview of selected combination therapies targeting costimulatory pathways

Combination therapy	Disease or preclinical model	Results
Anti-TNF antibody (adalimumab or etanercept) plus methotrexate	Rheumatoid arthritis	Adalimumab: more patients had an ACR50 response with combination therapy (59%) than with either therapy alone (37%, 43%); combination associated with less radiographic progression and higher remission rates[293]
		Etanercept: more patients had an ACR50 response with combination therapy (71%) than with either alone (54%, 42%); disability significantly improved and joint damage reduced by combination therapy versus monotherapy[294]
CTLA-4-Ig fusion protein (abatacept) plus methotrexate	Rheumatoid arthritis	Improved clinical response with combination therapy (ACR20 in 75.4% and 27.7% of patients receiving the combination or placebo plus methotrexate respectively at week 16)[295]
CTLA-4-Ig fusion protein (abatacept) plus non-methotrexate DMARDs	Rheumatoid arthritis	Clinical benefit of abatacept appears similar whether combined with methotrexate or other DMARDs; ACR20 responses were comparable between patients receiving Abatacept plus methotrexate (58.8%) or other DMARDS (55.8%)[296]
CTLA-4-Ig fusion protein (belatacept) plus rapamycin	Renal transplantation (following induction therapy with ATG or alemtuzumab)	Combination therapy associated with low acute rejection rates, good graft function & acceptable side effect profile[286,297]
CTLA-4-Ig fusion protein (abatacept) plus anti-CD20 antibody (rituximab)	Juvenile-onset SLE	Clinical remission in 2 patients[291]
	Copa syndrome	Clinical remission in one patient[291]
Antagonistic anti-CD28 antibody plus rapamycin	Mouse model of type 1 diabetes (NOD)	Combination delayed the development of diabetes[298]
PD-1 agonist plus CD40L blocking antibody	Mouse model of islet transplantation	The combination (but not either reagent alone) induced long term survival of islet allografts[299]
ICOSL–BAFF bispecific mAb–peptide conjugate (AMG 570)	Rheumatoid arthritis (NCT03156023)	Phase I ongoing[300,301]

	SLE (NCT04058028)	Phase II ongoing
BAFF–IL-17A bispecific mAb (tibulizumab/LY3090106)	Sjögren's syndrome (NCT02614716)	Antagonised both BAFF and IL-17A in cell-based models
		Triggered dose-dependent decrease in B cells in cynomologus monkeys (consistent with BAFF neutralization)[302]; Phase I ongoing
TNFα–IL-17A bispecific mAb (JNJ-61178104)	ND	Well tolerated in first-in-human trials
		Half-life (4.3-9.7 days) was shorter than for parent antibodies[303]
TNFα–IL23 bispecific mAb (from Boehringer Ingelheim)	ND	Preclinical
TNFα–IL23 bispecific mAb (CMX-02)	ND	Preclinical
Anti-BAFF antibody (belimumab) plus anti-CD20 antibody (rituximab)[a]	SLE (NCT03312907)	Plans for Phase III trial published[304]
	Sjögren's syndrome (NCT02631538)	Phase II ongoing
	Lupus nephritis (NCT02260934)	Phase II completed
		Interim results suggest belimumab delays B cell reconstitution after rituximab treatment compared to rituximab only, however no improvement in clinical outcome was observed (see Related Links)
	SLE (ISRCTN47873003)	Phase II ongoing
NGF–TNF bispecific mAb (MEDI7352)	Osteoarthritis (NCT02508155)	Dose-escalation study ongoing
	Painful diabetic neuropathy (NCT03755934)	Phase II ongoing

ACR20/50, American College of Rheumatology criteria, 20%/50% improvement; ATG, antithymocyte globulin; BAFF, B cell-activating factor; CD40L, CD40 ligand; CTLA4, cytotoxic T lymphocyte antigen 4; DMARD, disease-modifying anti-rheumatic drug; ICOSL, inducible T cell co-stimulator ligand; mAb, monoclonal antibody; ND, not determined; NGF, nerve growth factor; NOD, non-obese diabetic; SLE, systemic lupus erythematosus; TNF, tumour necrosis factor. [a]The rationale for this combination is that rituximab causes elevations in BAFF; SLE patients have higher BAFF levels and neutralizing BAFF mobilizes B cells from tissues — rituximab efficiently depletes circulating B cells but is less effective on B cells in tissues.

Figure Legends

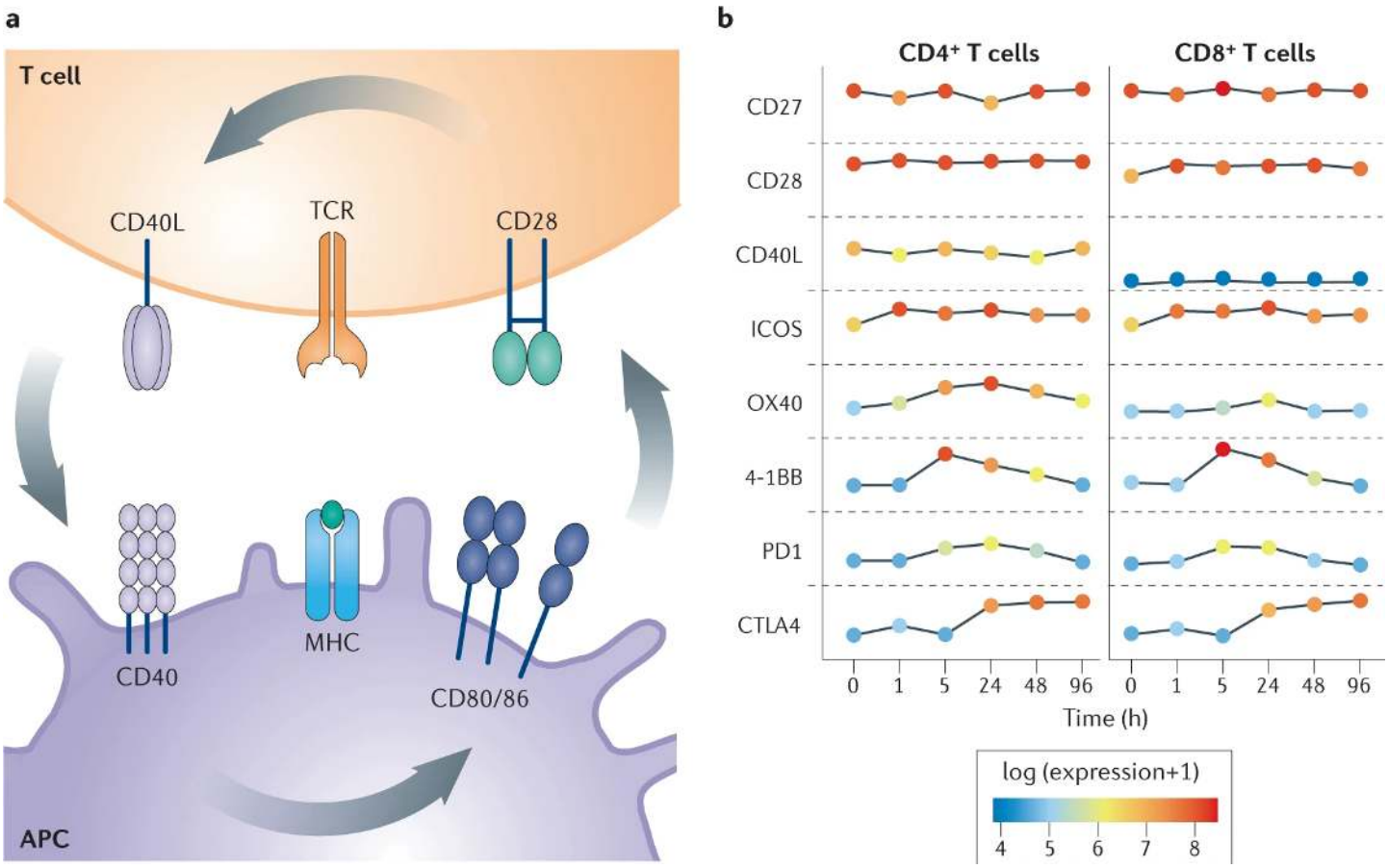
Figure 1 Hierarchy of costimulatory interactions (a) Schematic representation of feedforward loop in interactions between T cells and antigen-presenting cells (APCs). CD80 and CD86 are upregulated on APCs in response to CD40 signalling. In turn, the binding of CD80/86 to CD28 on T cells promotes upregulation of CD40L. (b) Expression of costimulatory molecules changes upon T cell activation. Shown are gene expression levels of a selection of CD28 or TNF family members in murine CD4+ or CD8+ T cells at the indicated time points after *in vitro* activation with anti-CD3/anti-CD28 beads. CD27 and CD28 are expressed constitutively on both CD4+ and CD8+ T cells. CD40L expression is typically restricted to CD4+ T cells. Expression of ICOS, OX40, 4-1BB, PD1 and CTLA4 is induced upon T cell activation. Normalized microarray data in series matrix files was obtained from the ImmGen project (GSE37448[305]) and mean of replicates (log(Expression)+1)), is shown. MHC, major histocompatibility complex; TCR, T cell receptor.

Figure 2 Therapeutic targeting of costimulatory molecules expressed on T cells and APCs. Shown are selected members of the CD28 and TNFR family and their ligands on T cells and APCs. CD80 has been shown to bind PD-L1 in cis (not shown). ICOSL has also been reported to bind CD28 (not shown). Also depicted are select therapeutics discussed in this review along with their mechanism of action. TAB08 acts as a superagonist. VIB4920 is a fusion of two Tn3 molecules (human tenascin C) and human serum albumin, with each Tn3 domain engineered to bind specifically to CD40L thereby disrupting interactions with CD40. ADCC, antibody-dependent cellular cytotoxicity; CTLA4, cytotoxic T lymphocyte antigen 4; MHC, major histocompatibility complex; OX40L, OX40 ligand; TCR, T cell receptor.

Figure 3 Combining CTLA-4-Ig and CD40-CD40L blockade for the treatment of autoimmunity. With no intervention, the feedforward loop of CD28 and CD40 signalling drives T cell activation and dendritic cell maturation (top) as shown in Fig. 1. Blockade of CD28, through cytotoxic T lymphocyte antigen 4 (CTLA4)–Ig, and of CD40, through anti-CD40 or anti-CD40L antibodies, can prevent T cell priming and interrupt T cell-driven activation of antigen-presenting cells, such as dendritic cells, expunging immune responses at an early stage (bottom). CD40L, CD40 ligand; MHC, major histocompatibility complex; TCR, T cell receptor.

Figure 4 Combination therapy in autoimmunity using Alemtuzumab and Abatacept Top: Cellular depletion using Alemtuzumab removes CD52 expressing T cells, some of which are autoreactive. The resulting lymphopenia can be a predisposing factor for autoimmunity due to CD28-driven homeostatic proliferation of a pool of T cells with a restricted TCR repertoire. Middle: Abatacept binds to the ligands of CD28, CD80 and CD86, thereby making them unavailable for support of CD28-dependent T cell activation and proliferation. However, CD28-null T cells are refractory to this effect and T cell subsets less reliant on CD28, including memory cells, will be less affected. Bottom: By debulking primed autoreactive T cells, including CD28-null and memory T cells, with Alemtuzumab and allowing immune reconstitution under the cover of Abatacept, CD28-dependent homeostatic proliferation is blunted thereby decreasing the risk of autoimmunity. APC, antigen-presenting cell.

Figure 1


a
T cell
CD40L
TCR
CD28
CD40
MHC
CD80/86
APC
b
CD4+ T cells
CD8+ T cells
CD27
CD28
CD40L
ICOS
OX40
4-1BB
PD1
CTLA4
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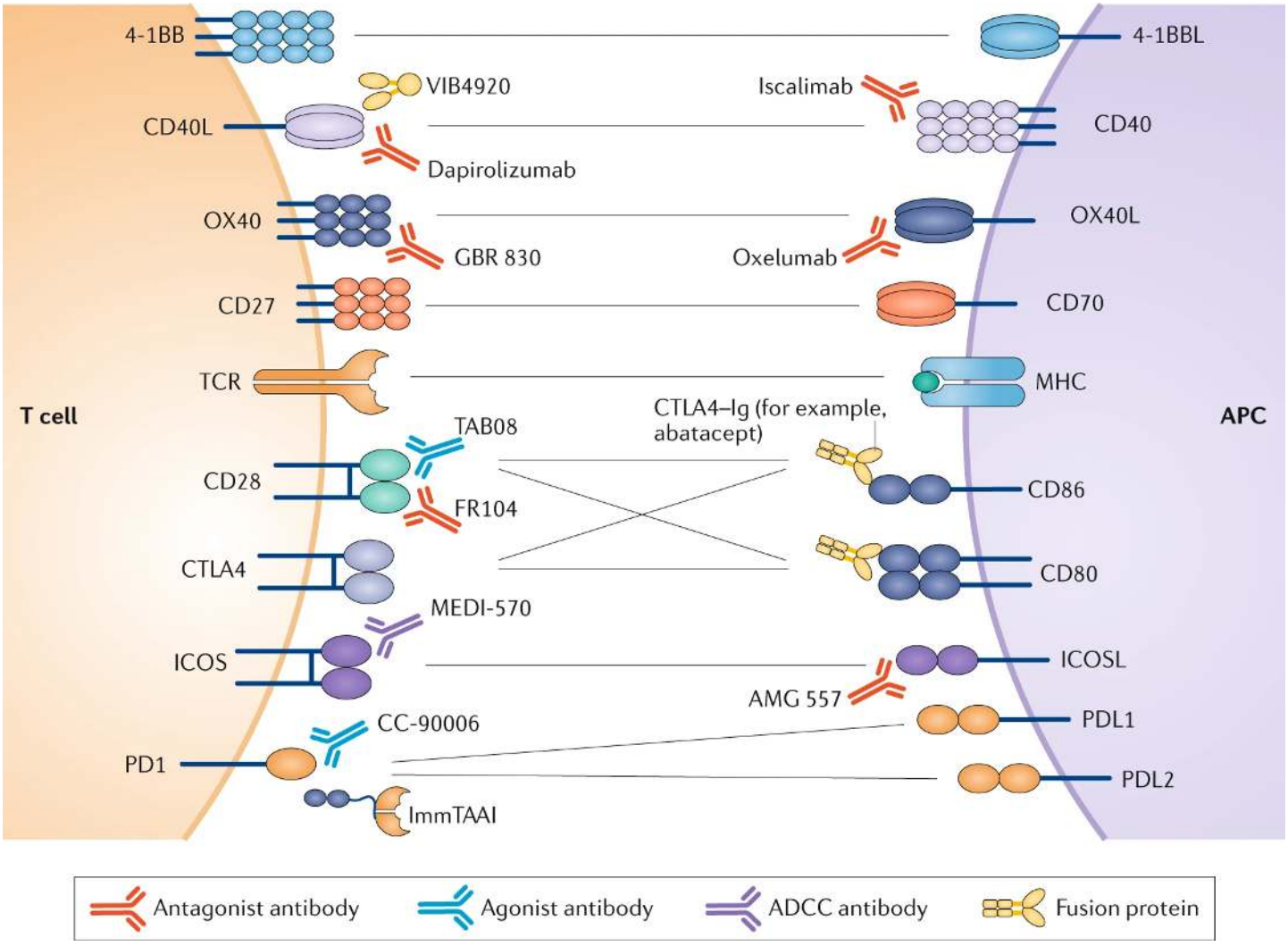
Time (h)
log (expression+1)
4 5 6 7 8

Figure 2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4-1BBL
VIB4920
Iscalimab
CD40L
CD40
Dapirolizumab
OX40
OX40L
GBR 830
Oxelumab
CD27
CD70
TCR
MHC
T cell
APC
CTLA4–Ig (for example, abatacept)
TAB08
CD28
CD86
FR104
CTLA4
CD80
MEDI-570
ICOS
ICOSL
AMG 557
CC-90006
PDL1
PD1
PDL2
ImmTAAI
Antagonist antibody
Agonist antibody
ADCC antibody
Fusion protein

Figure 3

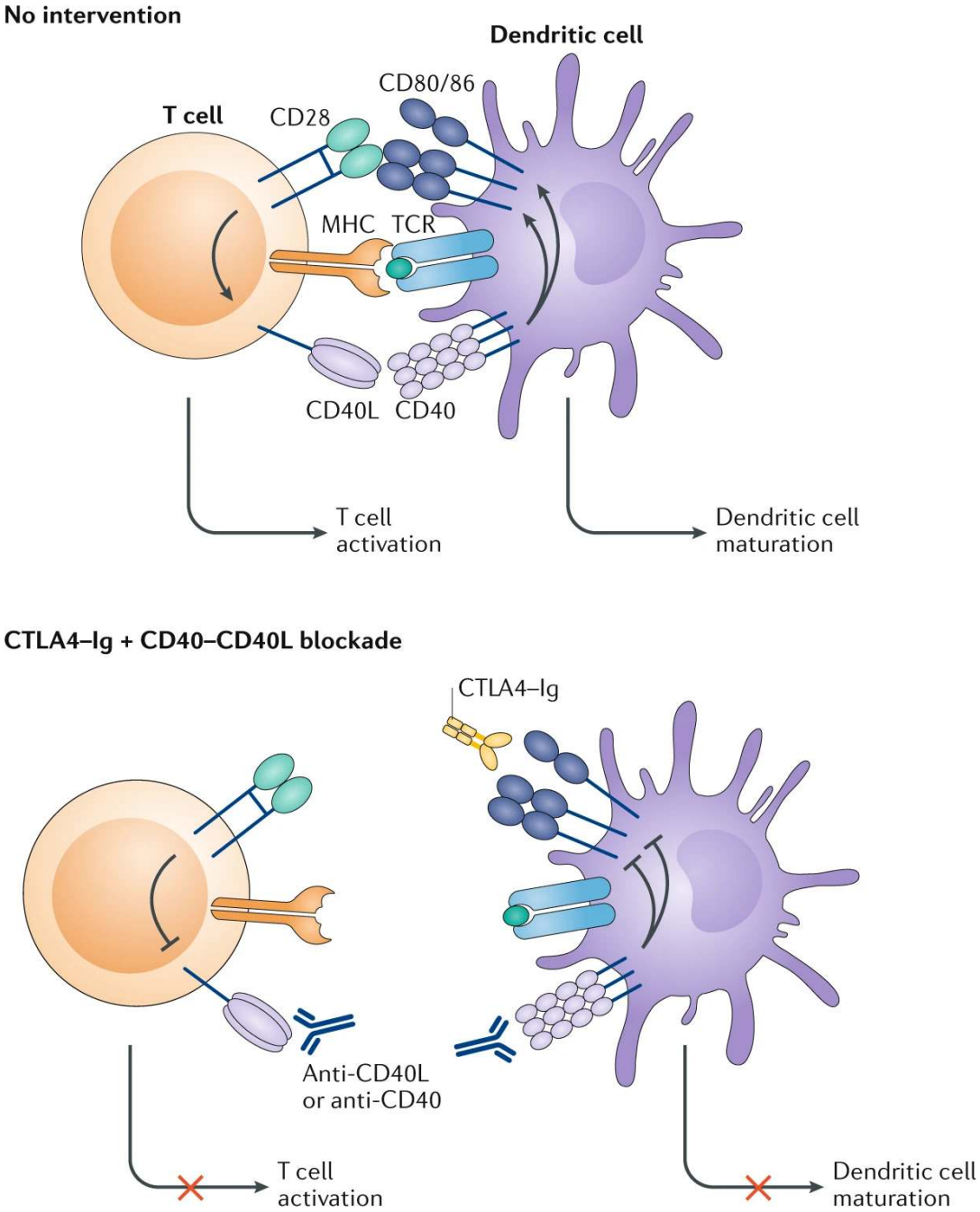
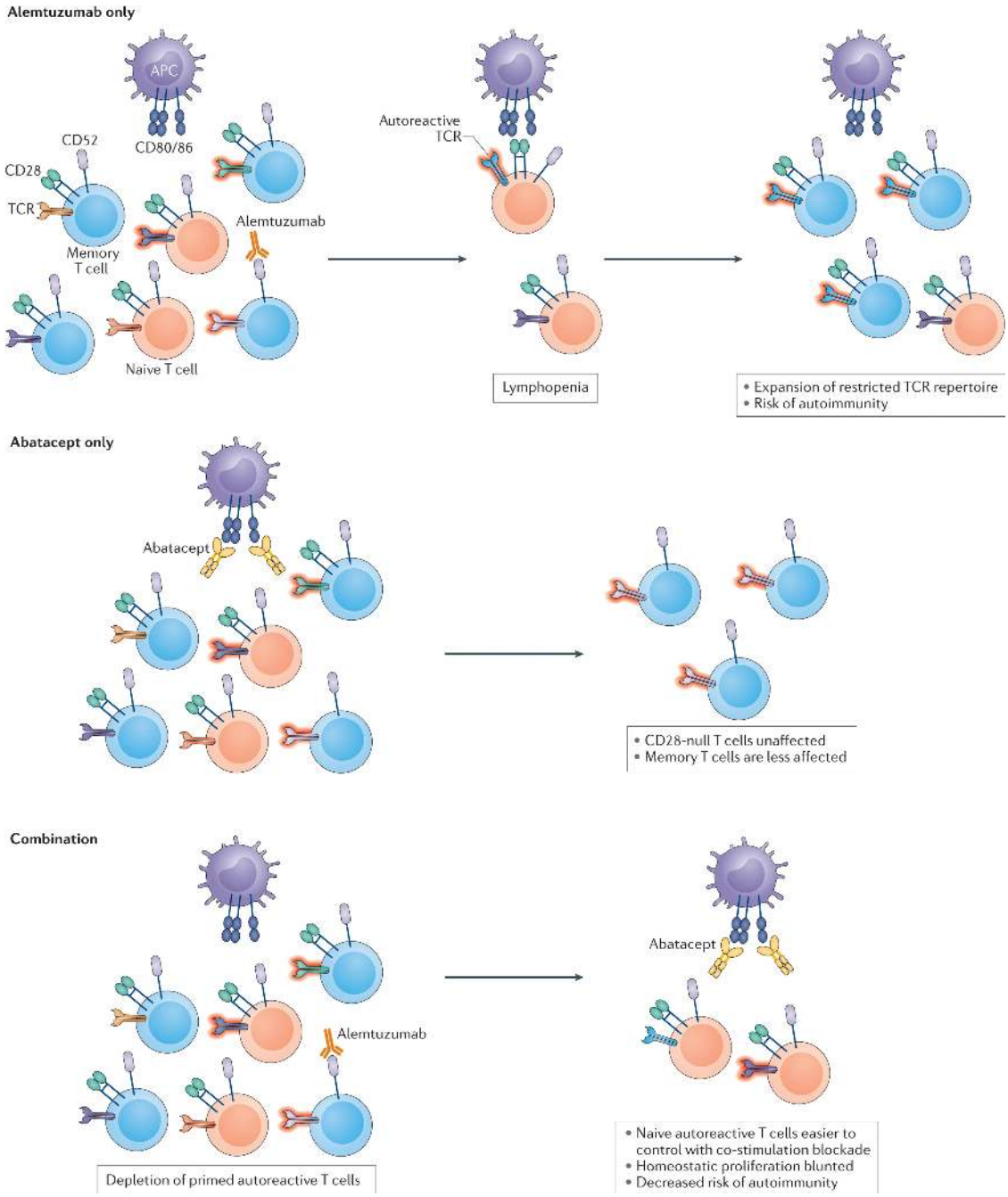

No intervention
Dendritic cell
CD80/86
T cell
CD28
MHC
TCR
CD40L
CD40
T cell activation
Dendritic cell maturation
CTLA4–Ig + CD40–CD40L blockade
CTLA4–Ig
Anti-CD40L or anti-CD40
T cell activation
Dendritic cell maturation

Figure 4


Alemtuzumab only
APC
CD52
CD80/86
CD28
TCR
Memory T cell
Alemtuzumab
Naive T cell
Autoreactive TCR
Lymphopenia
• Expansion of restricted TCR repertoire
• Risk of autoimmunity
Abatacept only
Abatacept
• CD28-null T cells unaffected
• Memory T cells are less affected
Combination
Alemtuzumab
Depletion of primed autoreactive T cells
Abatacept
• Naive autoreactive T cells easier to control with co-stimulation blockade
• Homeostatic proliferation blunted
• Decreased risk of autoimmunity

Related links

Phase IIa results for AMG 557: https://acrabstracts.org/abstract/a-phase-2a-study-of-medi5872-amg557-a-fully-human-anti-icos-ligand-monoclonal-antibody-in-patients-with-primary-sjogrens-syndrome/

Phase II interim results for belimumab plus rituximab in lupus nephritis: https://acrabstracts.org/abstract/phase-2-trial-of-induction-therapy-with-anti-cd20-rituximab-followed-by-maintenance-therapy-with-anti-baff-belimumab-in-patients-with-active-lupus-nephritis/

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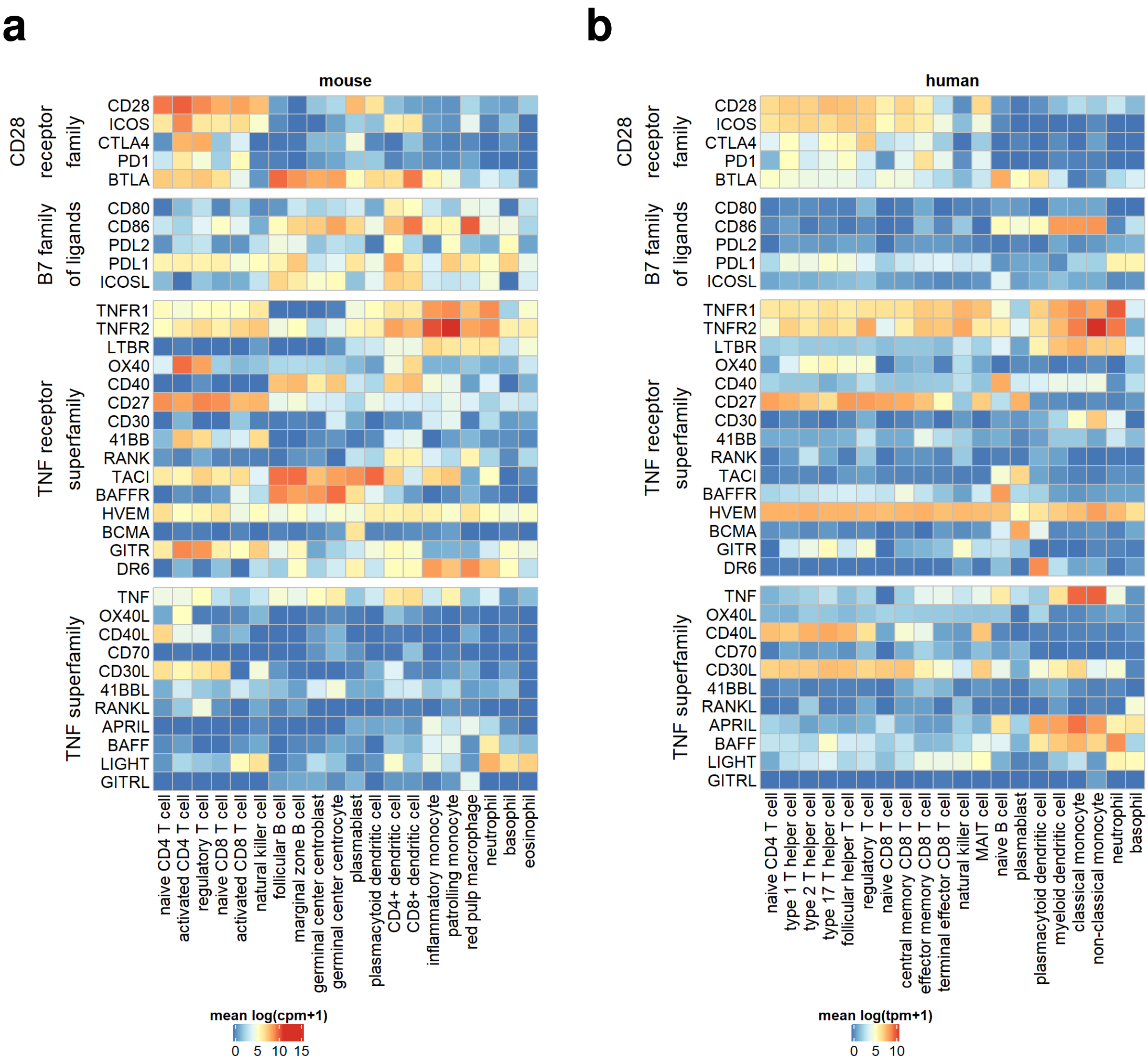
Supplementary Information



Supplementary Figure | Expression patterns of selected CD28 and TNF family members in mouse (a) and human (b) immune cell subsets. Data was taken from publicly available datasets (GSE109125 and GSE107011[1,2]) and mean of replicates, where available, are shown.

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