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Rationale for CD40 pathway blockade in autoimmune rheumatic disorders

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Abstract

CD40 and CD40L (CD154) belong to the tumour necrosis factor receptor superfamily and are expressed by immune and non-immune cells. CD40L plays a central role in co-stimulation and regulation of the immune response via activation of CD40-expressing cells. Imbalance of the CD40/CD40L costimulatory pathway is reported in many autoimmune diseases including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and Sjögren's syndrome (SS) thus supporting its role in the breach of immune tolerance typical of these diseases. Targeting CD40/CD40L signalling may represent a novel therapeutic option for several autoimmune disorders.

Key points:

1. CD40/CD40L signalling pathway regulates immune and non-immune cell responses
2. CD40/CD40L signalling is altered in autoimmune diseases such as SLE, RA, and SS
3. Early clinical trials programmes targeting CD40L were halted due to thrombotic adverse events
4. New therapeutic approaches targeting CD40 or using modified molecules against CD40L are currently being tested
5. Targeting CD40/CD40L signalling is a promising novel therapeutic strategy for reducing inflammation in autoimmune rheumatic disorders

1. Introduction

CD40L/CD40 interactions exert profound effects on immune and non-immune cells (1-3).

In a pathogenic setting, the deregulation of CD40 signalling has been observed in multiple autoimmune diseases (1-3).

Conversely, therapeutic up-regulation of the CD40 pathway in cancer may have potent anti-tumor effects. Thus the CD40 pathway has long been an attractive therapeutic target for treating autoimmune diseases; however, early clinical trials of monoclonal antibodies blocking CD40 ligand (CD40L) were halted due to platelet-related thromboembolic complications (4).

CD40 is a transmembrane type I glycoprotein which belongs to the tumour necrosis factor (TNF) gene superfamily and behaves as a co-stimulatory molecule. It is constitutively expressed by B cells and antigen presenting cells including monocytes, neutrophils and dendritic cells, and may be expressed on other cell types such as epithelial cells, endothelial cells, smooth muscle cells, fibroblasts, and platelets (2). Its ligand, CD40L (CD154), is a type II transmembrane protein which exists in soluble (sCD40L) or membrane-bound form. CD40L is mainly expressed on activated T cells, B cells, natural killer, platelets, endothelial, epithelial, and smooth muscle cells (1, 2). Soluble CD40L is mainly produced by platelets (5) and activated T cells (6) and is functional, being capable of enhancing platelet activation and B cell proliferation (7). The wide expression of this co-stimulatory machinery indicates the pivotal roles they play in different cellular immune processes.

In this review, we will first outline the role of CD40–CD40L in the biology of immune cells. We will then review published data on the role of the CD40–CD40L signalling pathway in autoimmune rheumatic diseases and, finally, novel approaches to targeting the pathway for clinical efficacy.

2. Co-stimulation

The requirement for B and T cell co-stimulation during the initiation of adaptive immune responses acts as a checkpoint that is involved in maintaining immune tolerance. A failure to regulate these

signals can underlie the development of autoimmunity which makes them an attractive therapeutic target. Similarly, in cancer therapy, the inhibition of regulatory pathways such as CTLA-4 and PD-1 has been utilised successfully with the aim of propagating anti-tumor immunity.

The requirement for CD28 is of fundamental importance to the initiation of adaptive immune responses in that naïve T cells are dependent upon CD28-signalling for activation and proliferation. The CD28 pathway has been effectively targeted in the treatment of rheumatoid arthritis (RA) with abatacept [Orencia; CTLA-4Ig; (8)]. Although patient responses to therapy can be variable, a subpopulation of patients appear to respond particularly well to treatment. Nevertheless, a key problem associated with CD28-blockade is the inability to effectively time the treatment. Specifically, patients are unlikely to present in the clinic until many years after tolerance mechanisms were initially bypassed and autoimmune responses are initiated. At this stage, activated/memory T cells become less dependent upon CD28-costimulation and are located at sites of inflammation where features of the local microenvironment diminish the efficacy of therapy. Indeed, attention has turned towards treating patients at earlier stages of the disease (9), even so far as to treat individuals who are at risk of RA development prior to the onset of active synovitis (10).

Although, CD28 can continue to play a role in an ongoing immune response, the upregulation of other costimulatory pathways implies some redundancy. These alternate co-stimulatory pathways include other members of the CD28 family and wider Immunoglobulin superfamily and TNF-receptor superfamily members which cooperate with/take over from CD28 in driving the maintenance, differentiation and effector function of activated T cell populations (11).

This hierarchical arrangement in the relative contributions of various costimulatory and inhibitory pathways brings additional targets for biological therapies in autoimmunity with the capacity to modulate features of lymphocyte effector function which are the basis of immune mediated pathologies (**Figure 1**). For example, (ICOS (Inducible T cell co-stimulator) expression is driven by CD28 signaling in activated T cells (12). This mediates signalling that is important to the germinal centre reaction via the maintenance of T follicular helper cells (Tfh) (13, 14) which places the ICOS-

ICOS-L pathway at the center of B/T Cell crosstalk and therefore an appealing target in B cell mediated immune pathologies. Indeed, ICOS-L has been targeted via a fully human monoclonal antibody (AMG-557; prezalumab) although a phase 2a clinical trial to evaluate the safety and efficacy of AMG 557 in subjects with primary Sjögren's syndrome showed no efficacy. It is possible that this is associated with redundancy created by the presence of additional costimulatory pathways.

The CD40-CD40L pathway fulfils multiple roles within this co-stimulation hierarchy. By driving Antigen Presenting Cell (APC) activation, including the expression of the CD28 ligands CD80 and CD86 (15, 16), CD40 signaling could be seen to sit above CD28 in the hierarchy by licensing effective CD28 co-stimulation. However, the CD40-CD40-L also forms an integral part of T cell effector function through a key role that is played in B/T cell crosstalk. This multifaceted role that is played in the generation of an adaptive immune responses has generated significant interest in the pathway as a target for therapy in autoimmune/inflammatory diseases.

3. CD40L/CD40 signalling in immune cells

The engagement of CD40 by CD40L promotes an intracellular signalling cascade, including the recruitment of proteins called TNFR-associated factors (TRAFs) and the activation of the nuclear factor κB (NFκB)-signaling pathway which culminates in the activation of transcription factors and cytokine production (17).

CD40 co-stimulation, in concert with cytokines or other stimuli, promotes B cell activation, proliferation, differentiation and survival via the upregulation of co-stimulatory molecules CD80 and CD86 which interact with the B cell receptor (BCR) on human peripheral and tissue resident naïve and memory B cells (**Figure 2**). CD40 signals also support the differentiation of B cells into antibody-secreting plasma cells and, in combination with additional signals, drive switching to various antibody isotypes (18-21). CD40 signals have been implicated in the development of germinal centres (**Figure 2**, GCs) (22). Indeed, lymph nodes from patients with X-linked hyper IgM syndrome

(HIGM), arising due to genetic defects in CD40L, have normal primary follicles but GCs are largely absent (23). Interestingly patients with HIGM, despite a high incidence of infections due to impairment of the immune system, are prone to autoimmune manifestations, especially hematologic abnormalities, arthritis, and inflammatory bowel disease. The mechanisms by which HIGM is associated with autoimmunity are not completely elucidated. A defective development of regulatory T cells as well as an impaired peripheral B-cell tolerance checkpoint may be important (24). Conversely, the CD40 pathway contributes to the 'licencing' of dendritic cells though the upregulation of other co-stimulation molecules and cytokine production, The presentation of peptide by an 'unlicensed' APC to a cognate T cell receptor leads to T cell non-responsiveness or deletion, helping to maintain peripheral tolerance (25).

CD40-CD40L interactions have been shown to influence the behaviour of other immune cells including T cells (26), natural killer cells (27), dendritic cells (28) and macrophages (29) favouring their maturation, survival and effector functions (**Figure 2**). For instance, recent evidence has shown that macrophages and neutrophils from CD40L-deficient patients show a decreased oxidative burst and microbicide activity (30, 31).

CD4 help for CD8 T cell responses also involves CD40–CD40L interactions, since the 'licensing' of dendritic cells by CD40L-expressing CD4 T cells is required to drive CD8 responses (**Figure 2**). Additionally, stimulations by an agonist anti-CD40 Ab have been proven to be sufficient to induce efficient CD8 responses in the absence of CD4 T cell help *in vitro* and *in vivo* (32, 33).

4. CD40/CD40L signalling in autoimmunity

4.1 Systemic Lupus Erythematosus (SLE)

SLE is a systemic autoimmune disease characterized by autoantibodies to nuclear antigens and immune complex deposits in small blood vessels, affecting the skin, joints, lungs, heart, brain, and kidney (34). Several studies have shown a dysregulation of CD40/CD40L signalling in SLE.

CD40L is up-regulated on several immune cells isolated from SLE patients with active disease (35-38). B cells expressing CD40L from SLE patients spontaneously produce antibodies *in vitro* (35) and transgenic mice overexpressing CD40L develop a lupus-like disease with age, suggesting a key role of CD40L in promoting autoimmunity (39). Treatment with rituximab, an anti-CD20 mAb which depletes B cells, decreases the frequency of B cells expressing CD40 and T cells expressing CD40L, suggesting that some of the benefit of this drug in treating lupus patients may be secondary to decreased activation of the CD40 signalling pathway (40, 41). SLE patients also have elevated levels of sCD40L in serum which correlates with disease activity (42).

Altered CD40 signals have been associated with kidney involvement as confirmed by CD40 upregulation in the kidney of SLE patients which in turn leads to the production of the pro-fibrotic cytokine TGF-β, which may contribute to kidney disease (43).

SLE patients display increased apoptosis of $CD34^+$ hematopoietic progenitor cells, contributing to the cytopenias often seen in lupus, and which may, in part, be driven by activation of the CD40 pathway via the upregulation of the death receptor Fas (44).

A role for the CD40 pathway in pathogenesis has been further validated in spontaneous mouse models of SLE. Treatment with anti-CD40L antibody prior to onset of symptoms reduces proteinuria, prolongs survival, ameliorates kidney disease and decreases anti-dsDNA Ab titres (45-47). Treatment commenced after the onset of moderate to severe proteinuria also ameliorates kidney disease and immune complex deposition as well as prolongs survival. Interestingly, greater benefits have been observed when combining CD40 pathway blockade with CTLA4-Ig especially on survival, anti-dsDNA Ab production, and kidney disease (47).

Multiple genome-wide linkage analyses have identified regions in humans and in mice which are associated with SLE (48). The CD40 gene lies on human chromosome 20q11.2-13.1, a region with

linkage to SLE incidence (48, 49). A missense SNP, rs11086998 G, which results in an amino acid substitution within the cytoplasmic tail of CD40 and three residues upstream of the TRAF6 binding site (CD40-P227A), has been associated with risk of SLE (50). Similarly the rs481085 SNP major allele G is increased in SLE compared to controls, while the minor allele T is associated with reduced CD40 expression and is under-represented in patients with SLE (51).

4.2 Rheumatoid arthritis (RA)

RA is a chronic inflammatory disease of synovial joints. It is characterized by accumulation of adaptive and innate immune cells within the synovium, autoantibody production [anti-citrullinated protein antibodies (ACPA)] and proliferation of resident stromal cells leading to degradation of the underlying cartilage and bone (52). GWAS studies have shown an association between the CD40 locus in both juvenile and adult arthritis (53-57). Indeed, RA patients homozygous for the risk allele rs4810485 have a third more CD40 expression on $CD19^+$ B cells in peripheral blood than those homozygous for the non-risk allele (56). Studies have also identified an association between polymorphisms in the CD40 locus and increased rate of joint destruction in patients with ACPA-positive RA (58) and response to TNF inhibitor treatments (59).

From the immunological point of view, CD40 is functionally expressed on several stromal and synovial immune cell populations including fibroblasts, B cells, T cells and monocytes (60-62).Once activated, CD40 signalling stimulates immune cells to proliferate, to upregulate adhesion molecules and to secrete pro-inflammatory cytokines and chemokines (60, 63). In addition, CD40 signalling in fibroblast-like synovial cells induces the expression of RANKL, involved in the osteoclast-mediated bone resorption (64) suggesting a possible role of CD40 signalling in the onset of bone erosions. CD40 expression on immune cells is upregulated by pro-inflammatory cytokines, including IFN-γ and TNF-α (60-62).

Therapeutically, treatment with anti-CD40 blocking antibody reduced TNF-α production from RA synovial fibroblasts *in vitro*, whether in the presence or absence of other immune cells, (62).

Regarding CD40L, RA synovial and peripheral T cells and B cells express CD40L at higher levels than peripheral cells isolated from healthy controls (62) and treatment with an anti-CD40L mAb prevented or ameliorated arthritis in pre-clinical models (65). However, the drug did not reverse the disease when arthritis was already established (65) indicating a more pivotal role for blocking this pathway in the earliest phase of the disease.

By contrast, a transcriptomic study of human RA synovium taken at different stages of disease found an increased expression of CD40L related gene signature in both pre-RA (arthralgia and undifferentiated arthritis) and RA samples (early RA, and established RA) suggesting that the pathway is active in both early and established human disease (61).

CD40L expression levels were found to be positively correlated with disease activity index (DAS28), and C-reactive protein (CRP) and treatment with TNF-α inhibitors decreased the expression of CD40L, ameliorated disease activity, as well as reduced ACPA antibody production by RA peripheral blood mononuclear cells (PBMCs) (66).

Although there is some conflicting data (67) the effect of activating the CD40 pathway with agonist antibodies in animal models resulted in earlier onset and more severe disease (68), supporting the hypothesis of an adjuvant property of CD40 on the initiating pathogenic mechanisms.

Altogether, these data suggest that blocking CD40/CD40L may be crucial at the initial stages of arthritis but with more variable and unpredictable efficacy in later stages.

4.3 Sjögren's syndrome (SS)

SS is a systemic autoimmune disease with a female-to-male predominance of 9:1 that primarily involves the salivary and lacrimal glands with dry eyes and mouth as common symptoms. It can also involve other major organs, and is associated with an increased risk of developing B cell lymphomas

(69). Around 25% of SS patients develop GCs within salivary gland (SG) lymphocytic foci and which are associated with more severe disease (70). The very definition of SS as a disease characterised by ectopic lymphocytic structures associated with B cell hyperactivity, together with organisation of the lymphocytic structures and germinal centre formation, makes the CD40 pathway an attractive target for this population. Indeed, the presence of both CD40 and CD40L has been confirmed within SS lymphocytic foci (42).

Constitutive expression of CD40, CD40L, and other co-stimulatory markers (i.e. CD80 and CD86) has also been described on SG ductal epithelial and endothelial cells in SS thus suggesting the potential to activate effector immune cells (71-73). The expression of CD40 on SG is regulated by pro-inflammatory stimuli such as interferon-gamma (IFN-γ) and IL-1β. In turn CD40 enhances the surface expression of the adhesion molecule intercellular adhesion molecule-1 (ICAM-1)/CD54 on SG epithelial cells (73) promoting leukocyte recruitment and the establishment of inflammation. Evidence suggests a possible role of CD40 signalling pathway in promoting SS epithelial cell apoptosis but this mechanism needs to be further elucidated (74). Increased levels of sCD40L have also been reported in SS (42).

Therapeutic treatment with systemic anti-CD40L antibody reduced sialadenitis, inhibited ectopic lymphoid structure formation and autoantibody production, as well as decreased the frequency of SG antibody-secreting cells in mouse models (75)

4.4 Systemic sclerosis (SSc)

Systemic sclerosis is an immune-mediated rheumatic disease that is characterised by fibrosis of the skin and internal organs as well as vasculopathy and systemic complications such as scleroderma renal crisis, pulmonary arterial hypertension, digital ulceration, and gastro-oesophageal reflux. Treatments for specific complications have emerged and a growing evidence base supports the use of immune suppression for the treatment of skin and lung fibrosis (76). Increased expression of

CD40L has been reported on activated $CD4^+$ T lymphocytes in systemic sclerosis and blocking anti-CD40 antibody reduced the expression of the co-stimulatory molecule, CD80, in SSc activated monocytes (77).

Soluble CD40L concentration is higher in systemic sclerosis than in controls and correlates with clinical and laboratory features (78, 79).

The blockade of CD40/CD40L interactions by anti-CD40L monoclonal antibody significantly reduced cutaneous fibrosis, anti-topoisomerase I autoantibody and normalised B lymphocyte activation, as evidenced by reduced levels of immunoglobulin, in a mouse model (TSK/+ mice) (79).

CD40 mRNA was found to be constitutively expressed in both SSc and normal human fibroblasts but CD40 protein expression was higher on SSc fibroblasts. In addition, ligation of CD40 by recombinant human CD40L resulted in increased production of IL-6, IL-8, and monocyte chemoattractant protein-1 in SSc but not normal fibroblasts in a dose-dependent manner. The co-stimulatory molecule CD80, was also induced on SSc fibroblasts by CD40 ligation (80). Although CD40/CD40L may to be implicated in the pathogenesis of SSs, there have been no clinical trials to date.

5. Clinical trials

Given the key role that the CD40 pathway may have in the pathogenesis of autoimmune disease, it has long been considered an attractive therapeutic target. Several clinical trials targeting the CD40/CD40L signalling pathway have been completed or are ongoing. Early trials using anti-CD40L mAb were discontinued due to thrombotic side effects. New molecules targeting CD40 or novel engineering approaches to CD40L have been identified to minimize the collateral effects (**Table 1**).

5.1 Targeting CD40/CD40L in SLE

Ruplizumab (Hu5c8, BG9588), a CD40L-specific humanized IgG1 mAb, was one of the first molecules generated to target the CD40 pathway. Treatment with ruplizumab resulted in a significant decrease in anti-dsDNA antibody level and haematuria as well as a significant increase in complement C3 concentration in an open label Phase 2 study in patients with proliferative lupus nephritis (81). $CD38^{high}$ B cells and IgM and IgG anti-dsDNA secreting plasma cells were decreased (82). In addition, the reduction of anti-dsDNA antibodies was also associated with improvement in the SLE disease activity index (SLEDAI) (82).

However, despite this promising evidence of clinical effect, further development of hu5c8 was discontinued because of treatment-emergent cardiovascular thrombotic events (TEs) (83). Numerous TEs including pulmonary vascular thrombi and vasculopathy were subsequently found after administration of hu5c8 in Rhesus monkeys (4, 83).

Toralizumab (IDEC-131), a humanized anti-CD40L mAb, was evaluated in a number of early clinical trials, including Phase 1 and Phase 2 studies in SLE (84, 85). Even at higher doses of 0.05-15.0 mg/kg toralizumab was demonstrated to be safe and well tolerated with no adverse event in both studies (85). Unfortunately, the efficacy of IDEC-131 compared to placebo was not demonstrated in SLE patients with mild to moderate disease activity (85). Similar to ruplizumab, further development of this agent was stopped due to increased thrombosis in other trials (86).

The TEs of ruplizumab and toralizumab appear to require a functional Fc portion (87, 88). Activated platelets express CD40L and *in vitro* analyses have shown that immune complexes consisting of sCD40L and an anti-CD40L monoclonal antibody can trigger platelet aggregation (87, 88). Inhibition of platelet Fc receptors can block this antibody mediated platelet aggregation.

One approach to reducing the TE risk is to remove the Fc portion, and an anti-CD40L Fab' antibody fragment conjugated to polyethylene glycol (PEG), ***dapirolizumab pegol (CDP7657)*****,** was subsequently developed and showed no evidence of pro-thrombotic effects in pre-clinical studies (4). Dapirolizumab was found to inhibit humoral immune responses in monkeys without thromboembolic complications (4). In a Phase I study, dapirolizumab pegol showed no serious treatment-emergent

adverse events. Exploratory analyses of patients with high disease activity at baseline indicated the potential for clinical improvement and reduction in anti-dsDNA antibodies, with down-modulation of peripheral blood plasma cell and B cell genes, particularly those related to immunoglobulins. (89). A Phase 2 dose-ranging study (NCT02804763) in adults with moderately-to-severely active SLE failed to achieve the primary end point of establishing a dose response at week 24 (P = 0.06), although numerical improvements in clinical endpoints were observed with dapirolizumab pegol in comparison to placebo, as were improvements in complement levels and reductions in anti-dsDNA antibodies. Four TEs were observed but three of these were in the placebo-treated arm (90).

5.2 Targeting CD40/CD40L in RA

VIB4920 (MEDI4920) is a novel CD40L binding protein comprised of two Tn3 proteins derived from fibronectin type III domain, fused to human serum albumin. VIB4920 targets CD40L but does not possess an Fc domain and therefore is unlikely to induce thrombotic effects. A phase 1, randomized, blinded, placebo-controlled, single-ascending dose study confirmed safety and tolerability of MEDI4920 in healthy adults (91). In a Phase 1b, randomized, double-blind, placebo-controlled, multiple-ascending dose study, VIB4920 significantly reduced RA disease activity, as measured by DAS28-CRP, at day 85 as well as additional clinical parameters including tender/swollen joint counts, CRP, and patient and physician global assessments (3, 86). Improvements in clinical activity in this trial were accompanied by reductions in rheumatoid factor of approximately 50% at the higher doses and in other circulating biomarkers associated with pathways that drive RA disease activity (86). Lower doses of VIB4920 were associated with a high prevalence of anti-drug antibodies. Although these were not detected at the highest dose, consistent with effective suppression of the CD40 pathway, the implications of this finding for future clinical utility remain to be determined (86). A Phase 2 study to evaluate the efficacy, safety, and pharmacokinetics is currently ongoing (NCT04163991).

An alternative approach to reducing TE risk is to utilise blocking antibodies to the more widely expressed CD40. ***BI-655064*** is a humanized antagonistic IgG1 mAb that binds CD40 and is modified to reduce Fc mediated effector functions including cytotoxicity and platelet activation (92). BI-655064 demonstrated safety and was well tolerated in a cohort of healthy volunteers (92). In a double-blind, randomized Phase 2a trial (NCT01751776), patients with RA received either weekly BI-655064 (120 mg) or placebo as add-on therapy to methotrexate (93). The primary endpoint of an ACR20 response at week 12 was seen in 68.2% in the active arm (n=44) compared to 45.5% in placebo (p=0.06). Interestingly, a significant difference in efficacy between active drug and placebo was seen in those patients with a disease duration less than 2.5 years (p=0.009). Treatment with BI-655064 also reduced the frequency of activated B cells, specifically class switched ($CD19^+IgD^-CD27^+CD95^+$), pre-switched ($CD19^+IgD^+CD27^+CD95^+$) and double-negative ($CD19^+IgD^-CD27^-CD95^+$) cells. Reductions in IgG and IgA rheumatoid factor, total IgG and IgM, IL6, metalloproteinase (MMP)-3 and RANKL levels were also observed. Amongst T allele carriers of the CD40 SNP rs4810485, ACR50 responses were greater in the active treatment arm compared with placebo (54.5% vs 16.7%; p=0.04) (93). Pharmacokinetic variability was observed with steady state only being reached towards the end of the 12 week intervention period, raising the possibility of inadequate dosing. A Phase 2 trial to evaluate the long term efficacy and safety of different doses of BI 655064 versus placebo as add-on therapy to Standard of Care (SOC) during maintenance therapy for lupus nephritis is currently ongoing (NCT03385564).

5.3 Targeting CD40/CD40L in SS

Iscalimab (CFZ533), is a human non-agonistic anti-CD40 monoclonal IgG1 blocking antibody, containing a modified Fc domain that renders it unable to mediate Fcγ-dependent effector functions and is therefore non-depleting (94). Iscalimab reduces humoral responses as well as GC formation in monkeys following kidney transplantation (95). In a Phase 1/2 study in de novo renal transplant,

iscalimab in combination with mycophenolate mofetil (MMF) and corticosteroids (CS) demonstrated comparable efficacy to tacrolimus, MMF and CS in terms of graft rejection, graft loss or death. However, compared to tacrolimus, iscalimab demonstrated improved renal function with fewer serious adverse events and infectious complications (96) as well as normal histology in the allografts (97)

Iscalimab also demonstrated efficacy in a Phase 2a randomized controlled trial (NCT02291029) in patients with primary SS (98). Forty-four SS patients were enrolled: 8 patients received 3 mg/kg s.c. iscalimab and 4 placebo in cohort 1 and 21 received 10 mg/kg i.v. iscalimab and 11 placebo in cohort 2. Iscalimab was safe and well tolerated. In cohort 1, high target-mediated drug disposition (TMDD) was observed with correspondingly low plasma PK values and no evidence of a treatment difference between iscalimab and placebo on EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) scores [baseline-adjusted reduction from placebo at week 12 was only 0·41 points (95% CI -2·89 to 3·70)]. However in cohort 2, there was a statistically significant and clinically meaningful improvement in clinical disease activity as measured by improvement in ESSDAI in patients receiving iscalimabwith a baseline-adjusted mean reduction in ESSDAI at week 12 of 5·21 points (95% CI 0·96 to 9·46; one-sided p=0·009). The high-dose iscalimab arm showed statistically significant improvements in physician's global assessment compared with placebo, and other measures such as the EULAR syndrome patient reported index (ESSPRI), multidimensional fatigue inventory (MFI), and patient's global assessment showed trends to improvement Data suggest that the chemokine CXCL13 may be a biomarker of germinal centre formation and, in keeping with the histological definition of SS as a focal lymphocytic sialadenitis, we have previously found that serum levels of CXCL13 correlate with the extent of histological SG inflammation (99). It is therefore of interest that iscalimab 10 mg/kg was associated with a marked reduction in CXCL13 levels. The TMDD observed with subcutaneous dosing, which may also be relevant to other anti-CD40 mAbs, can be overcome with either i.v. or s.c loading with corresponding evidence of clinical efficacy and reduction in symptoms and CXCL13, albeit from an open-label cohort (100).

Any beneficial effect of anti-CD40–CD40L blockade on lymphoma risk in SS remains to be seen.

6. Conclusions

Progressive disability, systemic complications and early death are still a reality leading to socioeconomic costs and unmet needs for several rheumatic autoimmune disorders. Indeed, some patients still fail to respond to current conventional and biologic disease modifying therapies.

The CD40-CD40L axis modulates many immune responses, and alterations in this signalling pathway have been reported in several autoimmune rheumatic disorders.

Early attempts to modulate this pathway through CD40L blockade were terminated due to thromboembolic events. Recent developments have focussed on targeting CD40 or on modifying anti-CD40L molecules to prevent Fc receptor binding. Several molecules blocking CD40 or CD40L have been tested in humans, with encouraging data emerging and new clinical trials ongoing in multiple autoimmune diseases. More data is required to be certain that newer approaches to CD40L blockade do not increase risk for thromboembolic events. Aside from this safety consideration, in making the choice between targeting CD40 or CD40L, one key distinction is the more widespread nature of CD40 expression and susceptibility to TMDD, necessitating higher dosing, with implications for cost, and more extensive receptor occupancy.

The use of monoclonal antibodies in autoimmune disease is often effective through tuning down excessive target signalling, rather than by completely eliminating it. However, given the importance and multi-faceted roles of CD40-CD40L interactions, it is plausible that persistent pathway blockade may be associated with infectious adverse events. Effective CD40 pathway blockade would also be anticipated to impair vaccination responses. Given the potential roles of the CD40 pathway in facilitating immune responses to cancer, it is unknown if persistent pathway blockade might increase cancer risk. Although the safety data from recent trials has been encouraging, ongoing study is required as programmes progress.

It is still is not clear at which stage of disease this therapy will be most efficacious. Many pre-clinical studies reviewed here suggest greater efficacy in the very early stages of autoimmunity. Indeed, short-term deep pathway blockade in this setting might hypothetically allow efficacy with a lower overall risk infection compared with long-term therapy. However, the results of iscalimab in established SS are encouraging, and may reflect disruption of pathogenically important B-T cell interactions within glandular lymphocytic foci/ectopic germinal centres. Results require confirmation in larger studies. It remains to be determined if stratification by pathotype in RA or by CD40 genotype may identify patients more likely to benefit. Given the important role of CD40/CD40L at multiple levels within the immune system, further studies should also determine the mechanisms of action most relevant to human autoimmune disease modification with this promising therapeutic approach.

Contributors

All authors contributed to the conception and content of the Review. All authors critically revised and edited the first draft and approved the final version.

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Conflict of Interest

BF is paid instructor/consultant for: Novartis, Roche, BMS and Servier.

Search strategy and selection criteria

References for this Review were identified through searches of PubMed and ClinicalTrials.gov with the search terms “CD40 and CD40L in SLE, RA, SS, SSc, psoriasis” from 1991 until 2019. We

largely selected publications from the past 5 years, but did not exclude commonly referenced and highly regarded older publications. We also searched the reference lists of articles identified by this search strategy and selected those we judged relevant.

Figure 1 – Co-stimulatory pathways relevant to current drug development in rheumatic autoimmune disorders

Only those immune co-stimulation targets referred to in the review are illustrated. Antigen-specific interaction between TCR and MHC expressed on APC provides the first signal for adequate T cell activation. Different stimulatory (green) or inhibitory (red) co-stimulatory signals may influence T cell-dependent immune responses. Dysregulation of these pathways is reported in several autoimmune diseases and novel pharmacological agents (e.g. Iscalimab, AMG 557) targeting molecules involved in the co-stimulatory machinery are being developed.

Abbreviations: APC, antigen presenting cell; CTLA4, cytotoxic T-lymphocyte-associated protein 4; ICOS, inducible co-stimulator; MHC, major histocompatibility complex; PD-1, programmed cell death-1; TCR, T cell receptor

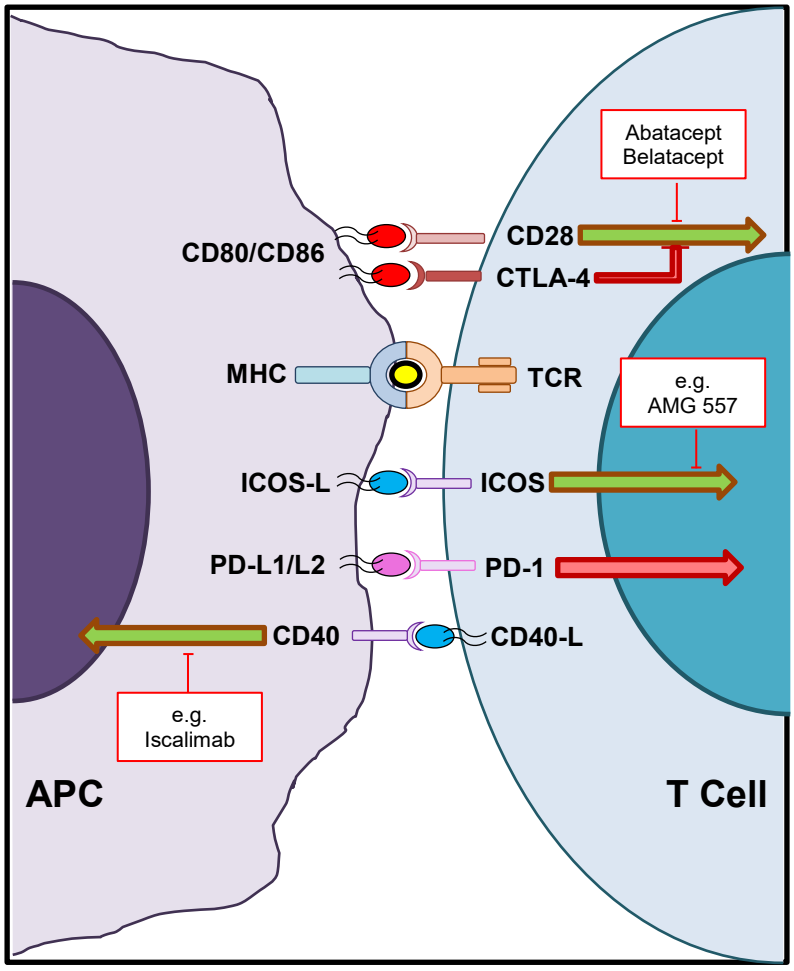
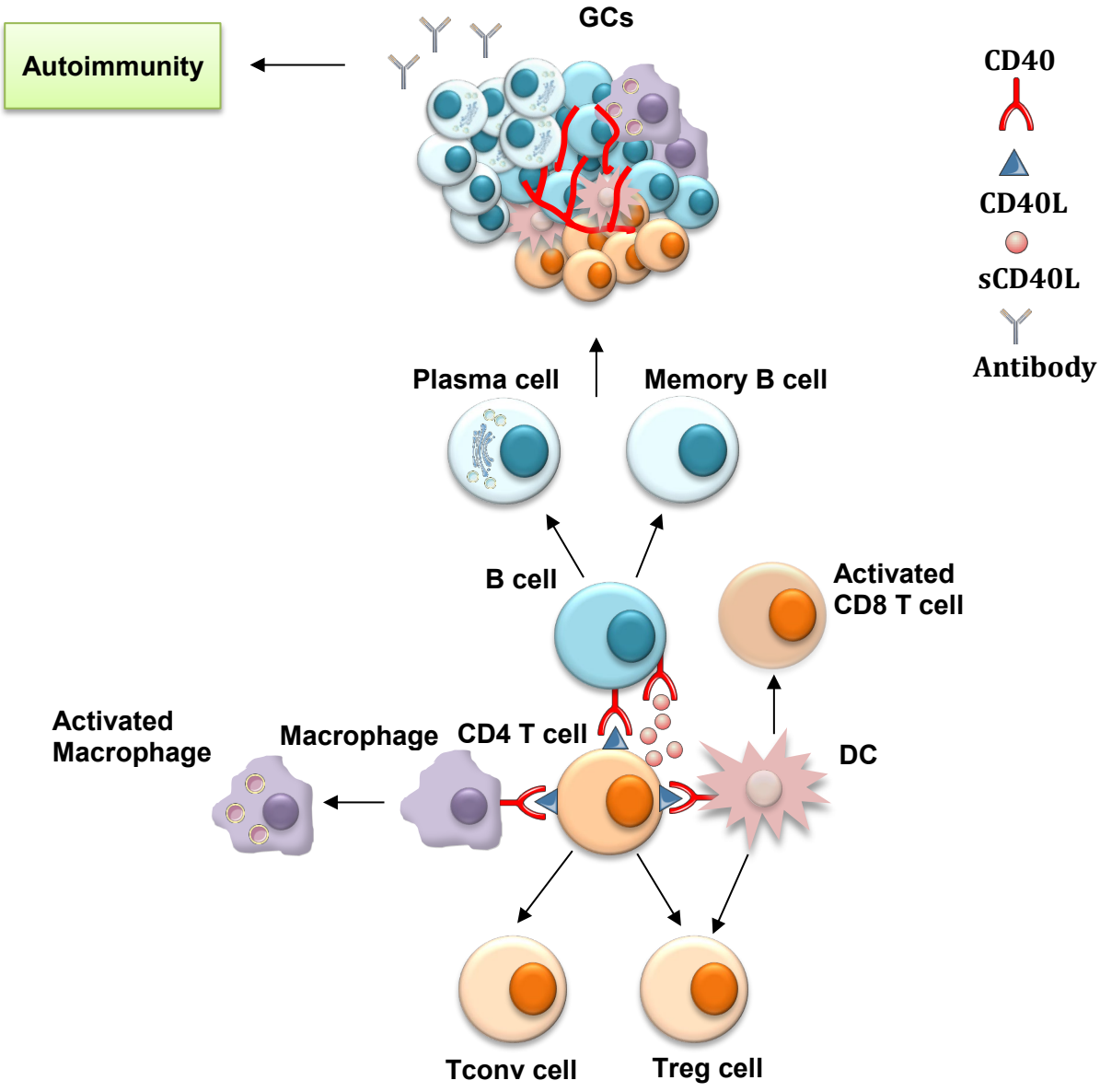


Figure 2 - CD40/CD40L in autoimmunity

CD40L expressed on T cells interacts with CD40 on APCs and regulates different effector functions. CD40–CD40L interaction regulates T-cell co-stimulation and differentiation of conventional and regulatory T cells as well as contributes to the activation of macrophages, dendritic cells (DCs) and B cells. On B cells, the interaction between CD40 and CD40L induces their differentiation to memory B cells and antibody-producing plasma cells. All these events may contribute to the formation of germinal centres (GCs) and in the development of autoimmunity.



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1 Table 1 CD40/CD40L targeting molecules

Target	Commercial name	Molecule	Diseases	Clinical trial stage	Outcome in rheumatic disorders	References
CD40L	Ruplizumab (BG9588, hu5c8)	Humanized IgG1 mAb	SLE, transplantation	Stopped after phase 2 (thromboembolism)	Reduced disease activity (SLE)	(83)
	Toralizumab	Humanized IgG1 mAb	SLE, AT, CD, MS	Stopped after phase 2 (thromboembolism)	No superiority compared to placebo (SLE)	(84, 85, 87)
	Dapirolizumab	Fab' fragment	SLE	Phase 2b completed (NCT02804763)	Clinical response rate higher in dapirolizumab group vs placebo (P=0.06)	(90)
	Letolizumab (BMS-986004)	Human IgG1 fusion protein	Transplantation, AT	Phase 1 and 2 completed (AT, NCT02273960) Phase 1 and 2 ongoing (GVHD, NCT02273960)		
	VIB4920 (MEDI4920)	Tn3 fusion protein	RA, SS, transplantation	Phase 1b completed (RA, NCT02780388) Phase 2 ongoing (RA, NCT04163991) Phase 2 ongoing (SS, NCT04129164) Phase 2 ongoing (transplantation, NCT04046549, NCT04174677)	Clinical and laboratory response (NCT02780388)	(86)
CD40	Iscalimab (CFZ533)	Fc-modified human IgG1 mAb	SS, RA, SLE, MG, transplantation	Phase 1 completed (RA) (NCT02089087) Phase 2a completed (SS) (NCT02291029) Phase 2 completed (GD) (NCT02713256) Phase 2 completed (MG) (NCT02565576)	Safe and tolerated (NCT02089087) Safe and tolerated. Clinical and laboratory response (NCT02291029)	(98)

				Phase 2b ongoing (SS) (NCT03905525) Phase 2 ongoing (SLE, LN) (NCT03656562, NCT03610516) Phase 2 ongoing (LT, KT) (NCT03781414, NCT03663335) Phase 2 ongoing (T1DM) NCT04129528		
	Bleselumab	Human IgG4 mAb	Psoriasis, transplantation, FSGS	Phase 1 completed (KT) (NCT01279538) Phase 2a completed (psoriasis) Phase 2 ongoing (KT and FSGS) (NCT02921789)		
	BI-655064	Humanized IgG1 mAb	RA, AT, SLE	Phase 2 completed (RA, AT) (NCT01751776, NCT02009761) Phase 2 ongoing (SLE) (NCT03385564)	Safe and tolerated. Clinical and laboratory response (NCT01751776)	(92)
	Ch5D12	Human IgG4 mAb	CD	Phase 1 and 2 completed		
	FFP104	Human IgG4 mAb	CD, PBC	Phase 1 and 2 ongoing (PBC) (NCT02193360) Phase 2 ongoing (CD) (NCT02465944)		

Abbreviations: AT, autoimmune thrombocytopenia; CD, Crohn's disease; DM, Type 1 Diabetes; FSGS, focal segmental glomerulosclerosis; GD, Graves' disease; KT, kidney transplant; LN, lupus nephritis; LT, liver transplant; MG, myasthenia gravis; MS, multiple sclerosis; PBC, primary biliary cirrhosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SS, Sjögren's syndrome