Molecular basis and therapeutic implications of CD40/CD40L immune checkpoint

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Abstract

The CD40 receptor and its ligand CD40L is one of the most critical molecular pairs of the stimulatory immune checkpoints. Both CD40 and CD40 L have a membrane form and a soluble form generated by proteolytic cleavage or alternative splicing. CD40 and CD40L are widely expressed in various types of cells, among which B cells and myeloid cells constitutively express high levels of CD40, and T cells and platelets express high levels of CD40L upon activation. CD40L self-assembles into functional trimers which induces CD40 trimerization and downstream signaling. The canonical CD40/CD40L signaling is mediated by recruitment of TRAFs and NF-κB activation, which is supplemented by signal pathways such as PI3K/AKT, MAPKs and JAK3/STATs. CD40/CD40L immune checkpoint leads to activation of both innate and adaptive immune cells via two-way signaling. CD40/CD40L interaction also participates in regulating thrombosis, tissue inflammation, hematopoiesis and tumor cell fate. Because of its essential role in immune activation, CD40/CD40L interaction has been regarded as an attractive immunotherapy target. In recent years, significant advance has been made in CD40/CD40L-targeted therapy. Various types of agents, including agonistic/antagonistic monoclonal antibodies, cellular vaccines, adenoviral vectors and protein antagonist, have been developed and evaluated in early-stage clinical trials for treating malignancies, autoimmune diseases and allograft rejection. In general, these agents have demonstrated favorable safety and some of them show promising clinical efficacy. The

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mechanisms of benefits include immune cell activation and tumor cell lysis/apoptosis in malignancies, or immune cell inactivation in autoimmune diseases and allograft rejection. This review provides a comprehensive overview of the structure, processing, cellular expression pattern, signaling and effector function of CD40/CD40L checkpoint molecules. In addition, we summarize the progress, targeted diseases and outcomes of current ongoing and completed clinical trials of CD40/CD40L-targeted therapy.

Keywords

CD40/CD40L; immune checkpoint; molecular basis; effector function; therapeutic implications

Introduction

Since the development of expression cloning in the late 1980s, a number of stimulatory and inhibitory immunoreceptors with their natural ligands, have been identified as gatekeepers of immune responses known as "immune checkpoints". Stimulatory immune checkpoints serve to boost the magnitude of cell activation initiated by T cell receptor (TCR)/ B cell receptor (BCR) engagements, which are necessary in generating effective immune responses against infections and tumors. On the contrary, inhibitory immune checkpoints negatively regulate immune cell activation, which limit excessive immune-mediated tissue damage and play a major role in immune tolerance.[1–2] In the past decades, immune checkpoint-based therapy has emerged as a promising immunotherapeutic in the clinic. Blockade of stimulatory immune checkpoints or activation of inhibitory immune checkpoints has been used to ameliorate immune responses in autoimmune and inflammatory diseases and to prevent rejection after allogeneic transplantation. In contrast, blockade of inhibitory immune checkpoints or activation of stimulatory immune checkpoints has been used to boost immune responses against tumors. Recently, the most successful immune checkpoint-based therapy is targeting on inhibitory immune checkpoint programmed cell death protein 1(PD1)/programmed cell death ligand 1 (PDL1). Anti-PD1/PDL1 therapy has been approved to treat a wide variety of malignancies, including melanoma, lung cancer, liver cancer, gastric cancer, kidney cancer, bladder cancer, head and neck tumor, Hodgkin's lymphoma, etc.[3] However, the response rate of anti-PD1/PDL1 therapy alone or in combination, is only ranging from 10% to 50% in most malignancies. Thus, other immune checkpoints are under active investigation for their therapeutic potential.

CD40 and CD40 ligand (CD40L) have been well-documented as stimulatory immune checkpoint and play a broad role in various immunological processes contributing to both humoral and cell-mediated immune responses. CD40 belongs to the tumor necrosis factor receptor superfamily (TNFRSF). CD40L is a member of TNF superfamily (TNFSF). They are under intense investigation as an attractive target for immunotherapy, with several drugs enhancing or blocking their activities in clinical trials. In this review, we focus on describing the molecular basis and therapeutic implications of CD40/CD40L checkpoint molecules. We summarize the understanding regarding to structure, interaction, cell-type expression profile and signaling of CD40/CD40L checkpoint molecules. CD40/CD40L interaction in various

cells and consequential effector functions are categorized. We also discuss the current development of therapeutic strategies targeting CD40/CD40L interaction.

CD40/CD40L checkpoint molecular structure

We summarize CD40/CD40L checkpoint molecular structure, processing and interaction in Figure 1. CD40, also known as Bp50 and TNFRSF5, is a type I membrane glycoprotein with a 62 amino acid (aa) cytoplasmic tail, a 22 aa transmembrane domain and a 173 aa extracellular domain in human. It was first discovered in 1987 as a 50-kDa protein expressed on B cells which regulates B cell proliferation.[4] CD40 locus is on human chromosome 20 and on mouse chromosome 2. Similar to other members of TNFRSF, the extracellular domain of CD40 is characterized by the presence of 4 cysteine-rich domains (CRDs) engaged in oligomerization and ligand binding.[5] There are 2 forms of CD40: membrane CD40 (mCD40) and soluble CD40 (sCD40). A fraction of mCD40 and sCD40 self-assemble into noncovalent homodimers through CRD1 in the extracellular domain.[6] sCD40 can be generated by alternative splicing in the cytoplasm [7] or from a proteolytic process in which mCD40 is cleaved via tumor necrosis factor-α-converting enzyme on the cell surface after its ligation with CD40L.[8] sCD40 acts as a natural antagonist of CD40 by shedding CD40/CD40L interaction.[9] In addition to its natural ligand CD40L, microbial proteins heat shock protein 70 and borrelial OspA have been reported to bind to CD40 as well.[10–11]

CD40L, also known as gp39, CD154 and TNFSF5, is a type II membrane glycoprotein with a 22 aa cytoplasmic tail a 24 aa transmembrane domain and a 215 aa extracellular domain in human. It was firstly recognized in 1992 on murine activated T helper (Th) cell clone for its capacity to bind to CD40.[12] CD40L locus is on both human and mouse chromosome X. Similar to other members of the TNFSF, CD40L contains a conserved TNF-homology domain in extracellular region that enables trimerization and receptor binding.[13] CD40L assembles as noncovalent homotrimers or heterotrimers consisting of full-length and truncated forms of CD40L. Upon cell activation, CD40L translocates to the cell surface known as membrane CD40L (mCD40L).[14] A soluble form of CD40L (sCD40L) co-exists with mCD40L. Several mechanisms have been shown to contribute to the generation of sCD40L. sCD40L can be generated by enzymatic cleavage of mCD40L on the cell surface via a disintegrin and metalloprotease (ADMA) 10 and ADAM17[15] or via matrix metalloproteinase-2 (MMP-2).[16] sCD40L can also result from enzymatic cleavage of full-length CD40L intracellularly.[17] sCD40L is trimeric and biologically active.[18] In addition to its classical receptor CD40, CD40L also binds to αIIbβ3, α5β1, αMβ2 and αvβ3 integrals.[19–20]

CD40/CD40L interaction has been analyzed through molecular modeling, mutagenesis and X-ray crystallography. The binding of trimeric mCD40L or sCD40L to mCD40 on the cell surface triggers the trimerization of the receptor which is necessary for downstream signaling.[21] The ligand binding sites are located in the inter-subunit grooves of CD40L trimer and associate with CDR1, CDR2 and CDR3 regions of CD40.[22] The interface of CD40/CD40L interaction shows charge complementarity, with the CD40L chain being positively charged and the CD40 chain being negatively charged. Several charged residues in the interface of CD40L chain (Y82, D84, N86, K143, R203, R207, Y146, R203 and Q220)

and CD40 chain (K143, Y145, D84, E114, E117, E74 and E117) have been reported to contribute to the stability of the complex.[23–25]

CD40/CD40L checkpoint expression profiles and regulation

To date, most of the CD40/CD40L studies have been focused on a specific cell type without addressing their systemic expression profiles. By virtue of bioinformatic analysis and database mining, we profiled the cell-type expression of CD40L and CD40 genes in various types of primary human cells. In a normalized transcriptional dataset gathering 714 RNA-seq studies,[26] we selected data from human primary cells without risk factor challenges and found that mRNA levels of CD40L are relatively higher in T cells and platelets, whereas that of CD40 are relatively higher in B cells and myeloid cell lineages (Figure 2A). Analysis of a single cell RNA-seq dataset of human lung[27] also demonstrated high levels of CD40 in myeloid cells and B cells, and CD40L in T cells (Figure 2B). Therefore, CD40/CD40L checkpoint molecules may be especially important in mediating T cell, B cell, myeloid, and platelet interactions. The current understanding for biological function of the CD40/CD40L interaction emphasizes its crucial roles in T cell-dependent B cell differentiation and activation which are indispensable for humoral response, and in mediating a bi-directional dialogue between T cells and monocytes (MCs)/macrophage (MΦ) which provides an amplification loop in cellular immunity, as well as in T cell-mediated platelet activation.

mCD40 is constitutively expressed on B cells, and appears to be reduced on end-stage plasma cells.[28] In MCs, MΦ and dendritic cells (DCs), the expression of mCD40 increase with cell development and maturation.[29–31] The expression of mCD40 has found induced by "danger" signals including pathogen-associated molecular patterns (PAMP), such as lipopolysaccharide.[31] and damage-associated molecular patterns (DAMP), such as high mobility group box protein 1.[32] We and others reported that metabolite-associated danger signal (MADS) homocysteine,[33–34] induces the expression of mCD40 on MCs. Inflammatory cytokines such as granulocyte/macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3) and interferon-γ (IFN-γ)[35] can increase mCD40 expression.

CD40L is highly expressed in activated CD4$^+$ T cells. Resting CD4$^+$ T cells have little or no detectable mCD40L. Activation-induced mCD40L expression has been reported in Th1, Th2, Th17 and follicular helper T (Tfh) cells, as well as regulatory T cells, albeit at much lower levels compared to Th cells.[36–37] The rapid and transit expression of mCD40L on CD4$^+$ T cells is tightly regulated by 4 mechanisms. First, preformed CD40L in secretory lysosomes translocates to the cell surface in effector and memory CD4$^+$ T cells within a few minutes upon activation.[38] Second, CD40L mRNA expression is regulated at both the transcriptional and posttranscriptional levels.[39] Third, mCD40L is proteolytically cleaved into sCD40L after cell activation. Lastly, CD40 ligation induces internalization of mCD40L followed by lysosomal degradation.[40] Release of sCD40L and induction of mCD40L occur in concert but were found to differ in kinetics following cell activation.[41] Various stimuli can promote CD40L expression in CD4$^+$ T cells, including signals through TCR,[42] co-stimulatory molecules[43] and inflammatory cytokines such as IL-12.[44]

Platelets are another important source of CD40L. Resting platelets have no detectable mCD40L. After stimulation with a wide range of platelet activators, such as thrombin, collagen or adenosine di-phosphate (ADP) plus adrenaline, mCD40L is rapidly and transiently expressed due to the translocation of CD40L from intraplatelet stores.[45] Meanwhile, sCD40L is released by proteolytical cleavage after platelet activation, resulting in the down-regulation of mCD40L.[46] Furthermore, mCD40L expression is inducible on other immune cells, such as CD8+ T cells, mast cells, basophils, natural killer (NK) cells, eosinophils and innate lymphoid cells (ILCs), as well as conditional innate immune cells such as endothelial cells (ECs) and vascular smooth muscle cells (VSMCs).[47–52]

CD40/CD40L intracellular signaling

CD40 signaling is primarily mediated through different members of adapter protein TNF receptor associated factor (TRAF) on a cell type and stimuli dependent manner (Figure 3 and Table 1). TRAF family proteins have 7 recognized members which include TRAF1-7. All TRAF family members, with the exception of TRAF7, have a TRAF domain located at the C terminus, contributing to the formation of TRAF homotrimers or heterooligomers and the interaction with cytoplasmic domain of CD40 and downstream effectors. TRAF1, TRAF2, TRAF3, TRAF5 and TRAF6 have been reported to bind to CD40. Trimeric TRAFs are recruited to CD40 trimer.[53] Trimeric TRAF2/3 bind strongly, whereas TRAF1/5/6 bind weakly to CD40.[24,54] TRAF2/3 have been reported to translocate to lipid rafts by CD40 ligation, which is essential for downstream signaling.[55] There are two nonoverlapping TRAF binding sites in CD40 cytoplasmic domain.[56–57] One is located at the membrane distal region and interacts with TRAF 1/2/3/5. The other is located at the membrane proximal region and binds to TRAF6. Both binding sites have been reported to regulate signaling activity in concert or independently. Germinal center (GC) formation in response to immunization is arrested only in mutagenesis of both the two binding sites.[58] Disrupting CD40-TRAF6 but not CD40-TRAF2/3/5 interaction attenuates atherosclerosis and promotes plaque fibrosis in apolipoprotein E (ApoE)$^{-/-}$ mice,[59] and inhibiting CD40-TRAF6 signaling in MΦ reduces atherosclerosis.[60]

Depending on cell types, CD40-TRAF complexes regulate a wide range of signal pathways. The most known signal pathway initiated by CD40-TRAF complexes is the nuclear factor kappa-B (NF-κB) pathway. The canonical NF-κB pathway involves activation of trimeric inhibitor of NF-κB kinase (IKK) complex (IKKα/β/γ) by TGFβ-activated kinase 1 (TAK1) and subsequent IKK complex-mediated NF-κB inhibitor (IκB) α degradation, resulting in rapid and transient translocation of NF-κB hetero- or homo-dimers to the nucleus. The non-canonical NF-κB pathway has been elucidated by NF-κB inducing kinase (NIK)-mediated activation of IKKα and subsequent proteolysis of p100 into p52, which associates with RelB and then translocates into the nucleus.[61] In 293T cells, TRAF2/5 activates both pathways after recruitment to CD40, whereas TRAF6 only activates the canonical NF-κB signaling and TRAF3 acts as a negative regulator of the noncanonical NF-κB pathway.[62] In mouse mature B cells, TRAF2 mediates activation of the canonical NF-κB pathway but serves as an inhibitor of the noncanonical pathway.[63] In airway epithelial cells, TRAF3-mediated enhancement of the canonical NF-κB signaling is essential for CD40-induced RANTES (regulated on activation, normal T cell expressed and secreted) expression.[64]

Other well-documented effector events downstream of CD40-TRAF engagement include the three mitogen-activated protein kinase (MAPK) pathways (extracellular-signal-regulated kinase (ERK) pathway, JUN N-terminal kinase (JNK) pathway and p38 pathway), the Src tyrosine kinase (represented by Lyn) pathway and the phosphoinositide 3-kinase (PI3K)/ protein kinase B (PKB, also known as AKT) pathway. In mouse B cells, MAPK kinase kinase 1 is recruited to CD40-TRAF2 complex, followed by JNK and p38 activation as well as activator protein 1 (AP1) translocation, ultimately resulting in cell proliferation and antibody production.[65] In TRAF2 deficient mice, CD40-medated activation of JNK is severely impaired in B cells.[66] In mice lacking CD40 binding motif for TRAF6, CD40-mediated isotype switching, proliferation and activation of p38 and JNK are severely impaired in B cells.[67] In WEHI 231 B cells, CD40-induced reactive oxygen species production requires TRAF3 recruitment and activation of PI3K.[68] In A20 B cells, CD40-TRAF6 engagement activates the PI3K/AKT pathway and rescues cells from CD95-induced apoptosis.[69] In MΦ, a cell-permeable peptide that inhibits CD40-TRAF6 interaction impairs activation of Src/ERK1/2 and inflammatory cytokines production.[70] In human DCs, CD40-TRAF2/3 engagement trigger Lyn-dependent ERK1/2 activation and Lyn-dependent/independent p38 activation, leading to IL-1α/β, IL-1Ra and IL12p40 expression, respectively.[71] In ECs, CD40-TRAF6 engagement activates the PI3K/AKT pathway and promotes proliferation, survival and migration, as well as vessel-like structure formation.[72] In lung fibroblasts, CD40-TRAF6 engagement activates the canonical NF-κB and JNK pathways, leading to IL-8 and IL-6 production.[73] In 293 cell line, both Ras-dependent and independent activation of ERK1/2 pathway involve CD40-TRAF6 engagement.[74] In malignant human urothelial cells, mCD40L but not sCD40L, triggers cell apoptosis via recruitment of TRAF3, followed by activation of JNK and translocation of AP1, as well as induction of the caspase-9/3-associated intrinsic apoptotic pathway.[75]

In addition to TRAF-mediated signaling, CD40 can also activate the Janus kinase 3 (JAK3)/ signal transducer and activator of transcription (STAT) pathway. In mouse primary B cells, CD40-induced proliferation and production of antibodies occur even though all TRAF binding sites are disrupted.[58] In human MCs, phosphorylation of JAK3 is associated with CD40 upon ligation and leads to STAT5 nuclear translocation.[76] In mouse B cells, JAK3 binds to PTNKAPHP motif in the membrane-proximal region of CD40 cytoplasmic domain and activates STAT3, which is essential for CD40-induced expression of CD23, intercellular adhesion molecule-1 (ICAM-1) and lymphotoxin-α.[77]

Effector function of CD40/CD40L interaction

Over the past decades, emerging evidence demonstrates that CD40/CD40L interaction regulates many biological processes including humoral immunity, cellular immunity, tissue inflammation, thrombosis, hematopoiesis and tumor cells fate. We list the details of CD40/ C40L interaction and function in Table 2 and present models to illustrate CD40/CD40L effector function in different cell types in Figure 4.

CD40/CD40L in B cell activation —

B cells constitutively express high levels of CD40. The engagement of CD40L with CD40 is essential for B cell proliferation, differentiation, high-affinity antibody production, isotype switching, memory response and costimulatory activity (Figure 4A).

Mature B cells have three major populations: follicular (FO) B cells, marginal zone (MZ) B cells and B1 cells.[78] FO B cells are the dominant population which present in lymphoid follicles and in the periphery. Upon encountering with antigen and interaction with follicular DCs and Tfh cells, FO B cells undergo proliferation and differentiate into germinal center (GC) B cells, accompanied by immunoglobin (Ig) class-switch recombination (CSR) and somatic hypermutation (SHM) which result in the production of IgG/E/A antibodies with high affinity to specific antigens. Furthermore, GC B cells with high-affinity BCR exit the GC and differentiate into long-lived memory B cells or plasma cells. The most known effector function of CD40/CD40L checkpoint is to regulate FO B cell division, survival, differentiation. CSR and SHM in the GC response. Individuals with CD40 or CD40L deficiency (hyper-IgM syndrome, HIGM) and $CD40^{-/-}$ or $CD40L^{-/-}$ transgenic mice lack GC in lymphoid organ follicles and have abnormally low levels of circulating IgG/E/A and memory B cells.[79–81] $CD4^+$ Tfh cells are identified as the primary CD40L-bearing cells that regulate FO B cell differentiation in the GC.[82] Furthermore, $CD4^+$ Tfh cells exist as discrete subsets to fine-tune GC response, and progressively differentiate with sequential IL-21 and IL-4 production and enhanced CD40L expression.[83] A strong CD40/CD40L signal leads to differentiation of plasma cells. CD40 promotes the differentiation of $Bcl6^{lo}CD69^{hi}$ GC B cells, which have a more stable contact with $CD4^+$ Tfh cells and favor a plasma cell fate.[84] A relatively strong CD40 signal provided by $CD4^+$ Tfh cells results in differentiation of $CD80^{hi}$ memory B cells toward plasma cells.[85] A population of $CD8^+$ Tfh cells was recently identified to regulate GC B cell response and autoantibody production through CD40/CD40L interaction.[86] Mast cells, which are often co-localized with B cells in lymphoid organs, can drive B cell proliferation and differentiation toward IgA-secreting plasma cells.[49] MZ B and B1 cells are innate-like B cells, which are mainly located in marginal sinus of the spleen and serous cavities, respectively. They are highly responsive to T cell-independent antigens. Upon encountering with antigens, MZ B cells and B1 cells rapidly differentiate into short-lived plasmablasts that are the dominant source of early protective antibodies. This response may undergo Ig CSR and exhibit reduced SHM. Compared with GC B cells, the role of CD40/CD40L checkpoint in MZ B cells and B1 cells is poorly understood. ILCs, neutrophils and NK cells have been found to activate MZ B cells via CD40/CD40L interaction and induce antibody production.[51, 87–88] In cultured B1 cells, CD40L induces proliferation and CSR.[89]

CD40/CD40L interaction between T cells and B cells also promotes the costimulatory activity of B cells which then act as competent antigen presenting cells (APCs) to prime T cells (Figure 4A). Expression of inducible T cell costimulator ligand on GC B cells is enhanced by CD40 activation which may be involved in maintenance of the Tfh cell phenotype.[90] In cultured B cells, the addition of CD40L-expressed T cells results in the upregulation of costimulatory molecules B7-1/2 which could be blocked by anti-CD40L.[91]

The costimulatory activity of B cells is reduced upon interaction with T cells from CD40L$^{-/-}$ mice.[92]

CD40/CD40L in myeloid cell activation —

Among the myeloid cells, CD40/CD40L checkpoint molecules are often related to the activation of MCs, MΦ, DCs and neutrophils. It is well-recognized that CD40/CD40L interaction regulates many aspects of MC/MΦ/DC/neutrophil biology including maturation, survival, cytokine production, costimulatory and anti-microbial activities (Figure 4B).

CD40/CD40L interaction between MCs and T cells/platelets is important in promoting MC differentiation into DCs,[93–94] stimulating expression of costimulatory molecules, such as CD86, inflammatory cytokines (such as IL-1β, TNF-α, IL-6 and IL-8),[95] MMPs,[96] pro-coagulate factors (such as collagenase, stromelysin and tissue factor),[97] and preventing apoptosis.[98] We recently found that serum concentration of sCD40L and peripheral levels of CD40$^+$ MC are significantly elevated in chronic kidney disease (CKD) patients.[33] As CD40$^+$ MC population is positively correlated with the severity of CKD in human, we proposed CD40$^+$ MC as a biomarker for CKD severity. Uremic toxin, homocysteine and sCD40L induce human peripheral mononuclear cells differentiation into CD40$^+$ MCs. Compared with the traditional human proinflammatory intermediate MCs (CD14^{++}CD16$^+$). CD40$^+$ MCs express higher levels of inflammatory markers. We proposed that CD40$^+$ MC represent stronger proinflammatory features than CD14^{++}CD16$^+$ intermediate MCs in human.

MΦ from CD40L deficient HIGM patients exhibits defective function in fungicidal killing and reduced oxidative burst.[99] CD40L produced by activated CD4$^+$ T cells is important for MΦ activation which leads to IFN-γ and nitrite production.[100] Consistently, MΦ stimulated with CD4$^+$ T cells from CD40L$^{-/-}$ mice produce less TNF-α and reactive nitrogen intermediates.[101] Engagement of CD40 reroutes *Toxoplasma gondii*, an intracellular pathogen, to the lysosomal compartment in MΦ and mediates an autophagy-dependent anti-microbial activity.[102]

In general, CD40 has been considered as a marker of maturation in DCs. MC-derived DCs from HIGM patients with CD40 deficiency exhibit low expression of HLA-DR and IL-12, as well as reduced costimulatory capacity to activate allogeneic T cells.[103] MC-derived DCs from X-HIGM patients with CD40L deficiency show reduced expression of CD80/CD86/HLA-DR and defective capacity to induce IFN-γ production in T cells, as well as impaired immune responses.[104] Plasmacytoid DCs from HIGM patients with CD40 deficiency show decreased IFN-α secretion in response to *herpes simplex virus 1* infection.[103] In a murine model of pancreatic cancer, CD40 agonist restores conventional DC1(cDC1) abundance, inhibits cDC1 apoptosis, and promotes cDC1 maturation.[105] In SLE patients, activated platelets enhance IFN-α secretion by plasmacytoid DCs through CD40/CD40L interaction.[106]

CD40/CD40L interaction is important for anti-microbial activity of neutrophils by regulating oxidative burst. CD40L deficient neutrophils from X-HIGM patients exhibit defective oxidative burst in response to *P brasiliensis*. Neutrophils from CD40L$^{-/-}$ mice also display

reduced oxidative burst and inability to control bacteria proliferation after peritoneal cavity infection.[107] Ligation of CD40 by sCD40L strongly stimulates oxidative burst in cultured neutrophils.[108]

CD40/CD40L in T cell activation —

As proposed in our recent publications,[109–110] CD40/CD40L interaction between T cells and APCs triggers two-way signaling including forward and reverse signal. The reverse signal leads to activation and differentiation of APCs, and the forward signal results in T cell/B cell activation and differentiation. CD40/CD40L interaction regulates Th1 differentiation. CD8+ cytotoxic T lymphocyte (CTL) activation and memory CTL maintenance through a bi-directional dialogue between T cells and APCs, providing an amplification loop in the immune response (Figure 4B).

CD40/CD40L ligation induces APCs to secret IL-12, which promotes Th1 polarization.[111] CD40/CD40L deficient HIGM patients are susceptible to opportunistic infections caused by various bacteria, whose clearance depend on effective pathogen-specific Th1 responses. [112–113] In response to *Leishmania major* challenge, CD40L$^{-/-}$ mice develop uncontrolled infection and fail to mount a vigorous Th1-like response.[114] Immunization with T-cell dependent antigen KLH induces a defective Th1 response in CD40L$^{-/-}$ mice.[115] In Th1 cell-mediated colitis,[116] blocking CD40/CD40L inhibits pathogenic Th1 response and attenuates tissue inflammation.

CD40/CD40L ligation is required for CD8+ CTL activation and memory CTL maintenance. [117] CD40L$^{-/-}$ mice produce impaired primary CD8+ CTL response to *vesicular stomatitis virus*, and fail to mount an efficient memory CD8+ CTL response to *lymphocytic choriomeningitis*[118] Intra-tumoral administration of an adenovirus encoding a chimeric m40L induces tumor-specific CD8+ CTL response and suppresses tumor growth.[119] It has been demonstrated that CD40L-bearing CD4+ T cells licenses APCs via CD40 signaling which in turn activate CD8+ T cells.[120] CD40L-bearing CD4+ T cells can also directly activate CD40-bearing CD8+ T cells.[121] Furthermore, it has been reported that 30-50% of stimulated effector CD8+ T cells express surface CD40L and could directly license DCs, which in turn promote proliferation and differentiation of CD8+ T cells.[122] In line with this finding, 20-30% memory CD8+ T cells were found to express CD40L after viral infection, and have the capacity to activate APCs.[123] These studies raise the possibility that CD40L-bearing CD8+ T cells and CD40-bearing APCs directly cooperate to maximize CD8+ T cell responses, and support the theory of two-way signaling of immune checkpoint, by which the reverse signal leading to activation and differentiation of APCs, and the forward signal results in T cell/B cell activation and differentiation.[109–110]

CD40/CD40L in platelet activation —

Activated platelets express high levels of CD40 and CD40L. Over 90% circulating sCD40L derive from activated platelets.[124] CD40/CD40L checkpoint molecules have been well elucidated in regulating platelet activation and platelet-leukocyte aggregation (Figure 4C).

CD40/CD40L interaction regulates platelet activation. A wide range of platelet activators such as thrombin, collagen and ADP induce formation of platelet aggregates and promote

rapid and transient expression of mCD40L on the cell surface and release of sCD40L.[45] Ligation of CD40 on the platelet by sCD40L further enhances platelet activation as evidenced by increased expression of CD62P, release of α-granule and dense granule, and classical morphological changes.[125–126] CD40L-bearing activated T cells, which are often co-localized with platelets in microcirculation,[127] induce platelet activation through a CD40-dependent pathway, leading to enhanced expression of CD62P and release of RANTES.[128] In addition, it has been suggested that CD40L regulates platelet activation through its non-classic receptor integrin αIIbβ3 rather than CD40. Exposure of platelets to sCD40L but not sCD40L containing a mutated αIIbβ3-binding domain, results in platelet activation.[129] In FeCl$_3$-induced injury of mesenteric arterioles, CD40L$^{-/-}$ApoE$^{-/-}$ mice but not CD40$^{-/-}$ApoE$^{-/-}$ mice, show instability of arterial thrombi which is reversed by infusion of sCD40L rather than sCD40L lacking the integrin αIIbβ3-recognition sequence.[130]

CD40/CD40L interaction is involved in the formation of platelet-leukocyte aggregates (PLAs), especially platelet-MC and platelet-neutrophil aggregates, which are linked to several inflammatory and thrombotic conditions such as cardiovascular diseases, rheumatoid arthritis and inflammatory bowel disease.[131] Reduced numbers of PLAs were detected with both CD40L$^{-/-}$ platelets and CD40$^{-/-}$ leukocytes.[132–133] sCD40L promotes formation of platelet-MC and platelet-neutrophil aggregates, and this action is abrogated by the addition of anti-CD40.[126] At the site of inflammation, intravascularly adherent platelets capture leukocytes through CD40/CD40L interaction, leading to leukocyte infiltration.[134]

CD40/CD40L in conditional innate immune cell activation —

Conditional innate immune cells refer to tissue parenchymal cells which can be transformed to innate immune-like cells under inflammatory stimuli. These include ECs, VSMCs, fibroblasts, epithelia, keratinocytes, etc.[135] CD40/CD40L interaction promotes conditional innate immune cells toward a proinflammatory phenotype (Figure 4C).

CD40L-bearing Jurkat T cells induce ICAM-1, vascular cell adhesion molecule-1 (VCAM-1) and E-selectin expression in human umbilical vein endothelial cells (HUVECs). [136] CD40L-bearing activated T cells induce the expression of MMP1/2/3/9 in human VSMCs by CD40 ligation.[137] sCD40L stimulates lung fibroblast activation, as evidenced by production of IL-6 and IL-8.[138] Ligation of CD40 on human intestinal fibroblasts by CD40L-bearing T cells or sCD40L, increases their T-cell binding capacity and promotes T cell migration through endothelial monolayers by upregulating ICAM-1 and VCAM-1 expression as well as interleukin-8 and RANTES production.[139] CD40 ligation by CD40L enhances ICAM-1 and IL-8 expression in IFN-γ stimulated keratinocytes.[140] In addition. CD40L induces TNF-α secretion in keratinocytes.[141] CD40L, in combination with IL-17, synergistically enhances production of IL-6, IL-8 and RANTES in human tubular epithelia. [142] CD40L-transfected fibroblasts induce human tubular epithelia to secrete IL-15, which could be blocked by anti-CD40L.[143]

Similarly, CD40L-bearing platelets induce ICAM-1, VCAM-1 and E-selectin expression, and IL-8 and MC chemotactic protein-1 secretion in HUVECs,[45] VSMC proliferation and MC-VSMC adhesion.[144] Collectively, CD40L-bearing T cells and platelets induce

inflammatory features in tissue parenchymal cells and their transformation to conditional innate immune cells.

CD40/CD40L in myelopoiesis —

CD40/CD40L interaction supports the proliferation and differentiation of CD34$^+$ hematopoietic progenitor cells (HPCs) toward the myeloid lineages (Figure 4D). In a murine model of bone marrow (BM) transplantation. sCD40L treatment promotes the recovery of hematopoietic precursors, primarily affecting myeloid lineages.[145] In response to TNF-α stimulation. CD34$^+$CD40$^+$ HPCs are highly enriched in monocytic, dendritic, granulocytic precursors, whereas CD34$^+$ CD40$^-$ HPCs contain granulocytic and erythroid precursors.[146] Supportively, CD40 ligation induces proliferation of human cord blood CD34$^+$ HPCs and their differentiation into functional DCs.[147] In addition, CD40/CD40L interaction has been suggested to indirectly promote myelopoiesis including megakaryocytopoiesis by inducing Flt3-ligand and thrombopoietin in BM stromal cells.[148]

CD40/CD40L in tumor cells —

CD40 is constitutively expressed in several types of malignant cells. CD40/CD40L interaction is associated with diverse functional changes in tumor cells, such as survival and proliferation, and sometimes apoptosis and growth arrest, based on tumor cell types and immune microenvironment (Figure 4E).

CD40 is highly expressed in B-cell malignancies including non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukemia (CLL) and multiple myeloma (MM). As in normal B cells, engagement of CD40 and CD40L promotes proliferation and inhibits apoptosis in malignant B cells.[149–151] CD40/CD40L interaction upregulates pro-inflammatory cytokines such as TNF-α and IL-6, and costimulatory molecules B7-1/2 in CLL cells, which in turn potentiate priming of T cells.[150,152]

Paradoxically, engagement of CD40 and CD40L has also been reported to induce growth arrest and apoptosis in malignant B cells.[153–155] CD40-mediated apoptosis in malignant B cells may be regulated by p53, which is mutated in over 50% of human malignancies.[156] Loss of p53 function in normal urothelial cells renders them more susceptible to CD40-mediated apoptosis.[157] In addition, CD40/CD40L interaction induces malignant B cells to express death receptors CD95, DR5 and TNF-R1, which in turn confer susceptibility to apoptosis in inflammatory microenvironment.[158–159] CD40/CD40L interaction has also been reported to promote apoptosis in other malignancies such as breast cancer[160], renal cell carcinoma, etc.[161]

Strategies and mechanisms of CD40/CD40L-targeted therapy

To date, several agents targeting CD40/CD40L interaction have been developed and evaluated in early-phase clinical trials. Strategies of CD40/CD40L agonist therapy include agonistic CD40 monoclonal antibody (mAb), adenovirus or plasmid or mRNA electroporation-mediated CD40/CD40L expression (Ad/plasmid/mRNA-CD40/CD40L), and trimeric sCD40L (Figure 5A). The agents of CD40/CD40L agonist therapy have been tested in various types of hematopoietic and solid tumor malignancies.

The primary mechanism underlying anti-tumor action of these agents is enhancing CD40/CD40L signaling. The constant fragment (Fc) of agonistic CD40 mAb can bind to Fc γ receptor (FcγRIIb) on immune cells (DCs, MΦ, T cell, B cells and NK cells). This leads to mAb accumulation, CD40 superclustering and a subsequent strong CD40 signaling.[162–163] In the cases of Ad/plasmid/mRNA-CD40/CD40L and trimeric sCD40L, the engagement of trimeric CD40L and mCD40 is enhanced, leading to enhancement of CD40 signaling as well. Enhanced CD40 signaling further results in immune cell activation and CD40-expressing tumor cell apoptosis. Other mechanisms underlying anti-tumor action of CD40 agonistic CD40 mAb involve complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC). CDC is induced by binding of complement 1q to the Fc region of CD40 IgG1 mAb, which initiates complement cascade and formation of membrane attack complex on mAb-coated tumor cells. ADCC is induced by the binding of CD40 IgG1 mAb to FcγRI/IIa/III on immune cells, which causes immune cell to release lysis granules (e.g. granzyme and perforin) and lyse mAb-coated tumor cells.[164]

In contrast to agonist therapy, CD40/CD40L antagonist therapy blocks CD40/CD40L interaction using antagonistic CD40/CD40L mAb and CD40L antagonist strategies (Figure 5B). Antagonistic CD40/CD40L mAb and CD40L antagonist switch off immune responses by inhibiting CD40 signaling and inactivating immune cells, which are beneficial for autoimmune diseases and allograft transplantation. Antagonistic CD40/CD40L mAb is either human IgG4 or engineered human IgG1 with minimal FcγR affinity to abrogate Fc/FcγR-mediated immune activation. As discussed above, CD40 signaling promotes growth and survival in malignant B cells.[150–152] Therefore, antagonistic CD40 IgG1 mAb is used to treat B cell malignancies because of its ability to abrogate CD40 signaling-mediated tumor growth and induce Fc-dependent CDC or ADCC.

Clinical trials of CD40-targeted agonist therapy —

We summarize clinical trials for CD40-targeted agonist therapy in Table 3A. All agents were well tolerated.

CP-870,893 is the first human IgG2 mAb with strong agonistic activity. Fc of IgG2 binds to complement or FcR with lower infinity than other isotypes. Thus, CP-870,893 induces minimal CDC or ADCC.[165] However, the hinge region of human IgG2 (h2) could adopt a h2B conformation, which promotes the clustering of mCD40 and imparts the strong agonistic property to CP-870,893.[166] In multiple phase 1 trials, CP-870,893 promoted activation of DCs, MΦ, B cells and T cells and showed single-agent anti-tumor efficacy[167] or synergic effects in combination with other anti-tumor drugs (gemcitabine, cisplatin, pemetrexed and tremelimumab), as demonstrated by partial or complete tumor remission in patients with metastatic melanoma, advanced pancreatic ductal adenocarcinoma and malignant pleural mesothelioma.[168–170] However, in a phase 1 trial with 27 advanced solid tumor patients where melanoma was also represented, weekly CP-870,893 failed to induce tumor remission.[171] The most common adverse events of CP-870,893 was transient grade 1 to 2 cytokine response syndrome (CRS).

Dacetuzumab is a humanized IgG1 mAb with weak agonistic activity. It induces growth inhibition, apoptosis and ADCC in human malignant B cell lines.[172] In phase 1 trials,

tumor remission was not observed with dacetuzumab monotherapy in CLL[173] and MM.[174] Dacetuzumab induced tumor remission as monotherapy in patients of NHL in a phase 1 trial[175] or had synergic anti-tumor activity in combination with rituximab and gemcitabine in patients with relapsed diffuse large B cell lymphoma (DLBCL) in a phase 2 trial.[176] In a phase 2b trial targeting patients with relapsed DLBCL after R-CHOP treatment (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone), dacetuzumab plus R-ICE (rituximab, ifosfamide, carboplatin and etoposide) led to more frequent cytopenias, cough and infection, but failed to demonstrate higher tumor remission rates when compared with placebo plus R-ICE.[177] Adverse events often related to dacetuzumab included grade 1 to 2 CRS and increased hepatic enzymes.

ChiLob7/4 is a chimeric IgG1 mAb with modest agonistic activity. ChiLob7/4 causes growth inhibition, CDC and ADCC in a variety of CD40-expressing human malignant cell lines,[178] and induces activation of DCs, B cells and NK cells.[179] In a phase 1 trial with patients of CD40-expressing solid tumors and DLBCL, ChiLob7/4 did not induce tumor remission.[180]

ADC-1013 is a human IgG1 mAb with strong agonistic activity. It induces ADCC and DC activation which in turn promotes tumor-specific T-cell proliferation. ADC-1013 demonstrated significant antitumor effects and induced anti-tumor T cell response in humanized bladder cancer model.[181] In a phase 1 trial conducted in advanced solid malignancies, intra-tumoral administration of ADC-1013 induced B cell activation and was well-tolerated in superficial lesions.[182]

Currently, several clinical trials with CD40 agonistic mAbs are ongoing. The ongoing trials show progresses as following: 1) combinatory therapy with new drugs such as antiangiogenic mAbs (NCT02665416) or anti-PDL1 (NCT03892525); 2) next generation mAbs with enhanced anti-tumor activity after Fab (CDX-1140: NCT04364230/ NCT03329950) or Fc engineering (APX005M: NCT02482168/NCT03123783/ NCT04130854/ NCT03719430/NCT04337931/NCT02706353/NCT03389802, SEA-CD40: NCT02376699 and 2141-V11: NCT04059588); and 3) expansion in targeted diseases including non-small cell lung cancer, urothelial carcinoma, head and neck cancer, rectal adenocarcinoma, soft tissue sarcoma, pediatric central nervous system tumors, etc. A clinical trial utilizing intra-tumoral injected adenoviral vector expressing CD40 agonistic mAb is also ongoing (NCT03852511), which may circumvent detrimental effects caused by systemic immune activation.

BPX101 is a novel strategy using autologous DCs expressing CD40 and tumor antigen to treat metastatic castration-resistant prostate cancer in a phase 1 trail.[183] To manufacture BPX101, autologous DCs are transduced with adenoviral vector Ad5f35 encoding inducible human CD40, incubated with human prostate-specific membrane antigen and then pre-activated with rimiducid and LPS to enhance maturation and antigen presentation. Enhancing CD40 signaling in tumor-antigen loaded DCs could license them as competent APCs, which in turn induce tumor-specific T cell response to eliminate tumors. BPX101 activated T cells and showed anti-tumor activity as demonstrated by prostate specific antigen decline and objective tumor remission.

Clinical trials of CD40-targeted antagonist therapy —

We summarize clinical trials for CD40-targeted agonist therapy in Table 3B. All agents were well tolerated.

Ch5D12 is a chimeric IgG4 mAb with antagonistic activity against CD40. It reduces B cell activation and proliferation and GC formation in non-human primates.[184–185] In a phase 1/2a trial with moderate to severe Crohn's disease patients, ch5D12 reduced mucosal inflammation and induced disease remission.[186]

Bleselumab is a human IgG4 mAb. It inhibits sCD40L stimulated proliferation of human peripheral blood mononuclear cells, and does not induce CDC and ADCC or destabilize platelet thrombi.[187] In a phase 1 trial with moderate-severe psoriasis patients, bleselumab failed to demonstrate clinical efficacy.[188] In a phase lb trial with kidney transplantation patients, bleselumab displayed dose-dependent prolonged B cell CD40 occupancy.[189] Recently, in a phase 2 trial with kidney transplantation patients, bleselumab plus tacrolimus demonstrated noninferiority to standard of care and had a favorable benefit-risk ratio.[190]

BI 655064 is humanized IgG1 mAb with mutated Fc. It blocks CD40L-induced proliferation of B cells and activation of ECs and DCs.[191] In two phase 1 trials with healthy subjects, BI 655064 induced dose-dependent CD40 occupancy.[192–193] In a phase 2a trial with active rheumatoid arthritis patients, BI 655064 reduced B cell activation, rheumatoid factor concentration and inflammatory markers levels, but did not demonstrate clinical efficacy.[194]

Iscalimab is human IgG1 mAb with a mutated Fc region. It inhibits CD40L-induced activation of cultured human leukocytes but does not lead to ADCC or CDC. It also blocks T cell-dependent antibody response and abrogates GC formation in nonhuman primates.[195] In a phase 1 trial with healthy subjects and rheumatoid patients, iscalimab showed antagonistic activity by inhibiting T-cell dependent antibody response and CD40L-induced B cell activation.[196] In a phase 2 trial, 47% of Grave's disease patients showed clinical response and decreased serum concentrations of autoantibodies.[197] Currently, multiple phase 1 to 2 trails with iscalimab are ongoing in various diseases including kidney transplantation (NCT02217410/NCT03663335), liver transplantation (NCT03781414), lupus nephritis (NCT03610516), systemic lupus erythematosus (SLE, NCT03656562) and Sjögren syndrome (NCT03905525).

Lucatumumab is a human IgG1 antagonistic mAb. It inhibits CD40L-induced proliferation and survival of CLL cells and mediates ADCC.[150] In a phase 1 trial with patients of relapsed CLL, lucatumumab demonstrated minimal anti-tumor activity.[198] In a phase 1 trial with patients of relapsed MM, lucatumumab showed modest anti-tumor activity.[199] In a phase la/2 trial with patients of advanced lymphoma, lucatumumab demonstrated modest anti-tumor activity, especially in patients with follicular lymphoma and marginal zone lymphoma of mucosa-associated lymphatic tissue.[200]

Clinical trials of CD40L-targeted agonist therapy —

We summarize current CD40L-targeted agonist therapy in Table 4A. All agents were well tolerated.

Avrend is a trimeric recombinant protein of human sCD40L. In preclinical studies, it activated anti-tumor immune responses by enhancing antigen presentation in APCs and triggering T cell response, which led to tumor remission in human breast cancer and lymphoma-bearing immune-deficient SCID mice.[201–202] In a phase 1 trial with patients of advanced solid tumors or NHL, Avrend showed anti-tumor efficacy.[203]

In a phase 1 trial with CLL patients, infusion of autologous CLL cells transduced with adenovirus encoding murine mCD40L, led to induction of tumor-specific T cells and antibody response, as well as tumor remission.[204] However, some patients developed antibodies against murine CD40L. To mitigate the problem, the same investigators further conducted a phase 1 trial with CLL cells transduced with adenovirus encoding ISF35 (humanized mCD40L).[205] ISF35-transduced CLL cells demonstrated anti-tumor activity in CLL patients. In two trails with metastatic melanoma patients, adenovirus carrying CD40L gene (Ad-CD40L) induced T cell activation correlated to prolonged survival.[206–207] In a trail with bladder cancer patients, Ad-CD40L promoted T cell infiltration into bladder and showed antitumor activity.[208]

In addition. CD40L co-expression with other immunostimulatory molecules by plasmid transfection or mRNA electroporation in bystander cells or autologous DCs were used to induce a tumor-specific immune response. For example, CD40L co-expressed with GM-CSF in bystander cells, and with toll-like receptor 4 (TLR4) /CD70 or TLR4/CD70/tumor antigen in autologous DCs were used to treat advanced lung adenocarcinoma and advanced melanoma. In phase 1 to 2 trials, these agents promoted lymphocytes infiltration in tumors. [209–212]

Clinical trials of CD40L-targeted antagonist therapy —

Current CD40L-targeted antagonist therapy is summarized in Table 4B. The majority of these agents were relatively well tolerated, although trials of some CD40L antagonistic mAbs were discontinued because of thrombosis.

BG9588 is a humanized CD40L antagonistic IgG1 mAb. Although BG9588 showed potential efficacy by inhibiting abnormal B cell response and production of autoantibodies in patients with lupus nephritis and SLE, two phase 1 trials with this agent were terminated prematurely due to increased thrombotic events (TEs).[213–214] IDEC-131 is also a humanized CD40L antagonistic IgG1 mAb and has been evaluated in several phase 1 to 2 trails with patients of SLE, thrombocytopenic purpura, multiple sclerosis and Crolm's disease.[215–218] In these trials, no definite TEs were reported in patients with SLE and thrombocytopenic purpura, but trials regarding multiple sclerosis and Crolm's disease were stopped because of TEs.[219] The TEs in these trails may be caused by Fc fragment of mAb crosslinking to FcγRIIa on platelets, leading to subsequent platelet activation and aggregation.[220]

Dapirolizumab is the Fab fragment of a humanized, monovalent pegylated CD40L antagonistic mAb. In a phase 1 trial with patients of SLE, Dapirolizumab showed potential efficacy in patients with high disease activity.[221]

Currently, phase 1 to 2 trials with Fc-engineered CD40L antagonistic mAbs (AT-1501 and BMS-986004), are ongoing in patients with amyotrophic lateral sclerosis (NCT04322149) or acute graft-versus-host disease (NCT03605927).

VIB4920 is an engineered human tenascin C protein which binds to human CD40L and has antagonistic activity.[222] In a phase 1 trial with healthy volunteers, VIB4920 suppressed T cell-dependent antibody response and B cell activation/memory response. In addition. VIB4920 reduces serum levels of rheumatoid factor autoantibody and showed efficacy in patients with rheumatoid arthritis. [223]

Summary of clinical efficacy of CD40/CD40L-targeted therapy —

So far, clinical evaluation of CD40/CD40L-targeted therapy only involved early-stage clinical trials enrolling limited numbers of patients. In general, the current completed trials of CD40/CD40L-targeted therapy demonstrated clinical safety and favorable immune responses as single-agent or combinatory therapy in malignancies and autoimmune diseases. In CD40/CD40L anti-tumor therapy, clinical efficacy is relatively low with complete response rate ranging from 2% to 20% and partial response rate from 4% to 28.6%. In contrast, CD40/CD40L anti-autoimmune disease therapy is promising with clinical response rate ranging from 46% to 75% (Table 5). Improvement in clinical efficacy is expected from the ongoing trails with advanced CD40/CD40L anti-tumor therapy because of the following: 1) CD40 agonistic mAbs have enhanced agonistic capacity (e.g. APX005M) and Fc-dependent ant-tumor activity (e.g. CDX-1140, SEA-CD40 and 2141-V11); 2) combinatory therapy with antiangiogenic mAbs or anti-PDL1 is syngenetic. Furthermore, recent preclinical studies have shown even greater promise in tumor control if CD40 agonists were co-administered with other immunostimulatory therapies, such as Flt3 ligand which promotes DC maturation and survival,[224] with anti-VEGFA/Ang2 mAbs which induce regression of the tumor microvasculature and APC activation,[225] and with anti-PD-1 and anti-CTLA4 mAbs which work together with CD40 agonist to enhance anti-tumor responses.[226]

Conclusion and future perspectives

In the past decades, mounting studies have been made in elucidating the molecular basis of CD40/CD40 immune checkpoint. Both CD40 and CD40L have a membrane form and a soluble form generated by proteolytic cleavage or alternative splicing. Initiation of CD40/CD40L signaling requires trimerization or multimerization of the receptor. CD40 are widely expressed in various types of immune cells and non-immune cells. B cells, MCs, MΦ and DCs are the primary cells constitutively expressing high levels of CD40 which can be further enhanced by "danger" signals including PAMP, DAMP and MADS. CD40+ MCs represent a population of MCs with strong inflammatory features in human. CD40/CD40L interaction plays indispensable roles in humoral and cellular immunity by regulating B cell, T cell and APC activation and immune memory. CD40/CD40L molecular pair mediates a two-way

signaling between T cells and APCs, by which the reverse signal leads to activation and differentiation of APCs and the forward signal results in T cell/B cell activation and differentiation. The role of CD40 expression in most immune cell subsets has not been tested and will require future experimental investigation.

Recent advances from recently accomplished early phase CD40/CD40L-targeted clinical trials demonstrate promising benefits in the treatments of malignancies, autoimmunity and allograft rejection. Considering its critical roles in the regulation of platelet and conditional innate immune cell activation, CD40/CD40L-targeted strategy could be beneficial for chronical disease involving abnormal activation of these cells, such as cardiovascular diseases and chronic kidney disease. Further investigation in molecular mechanisms underlying CD40/CD40L regulation in these cells would provide important insight and novel therapeutic targets.

Acknowledgments

Abbreviations

ADMA	a disintegrin and metalloprotease
ADP	adenosine di-phosphate
AKT	protein kinase B
AP1	activator protein 1
ApoE	apolipoprotein E
ADCC	antibody-dependent cell-mediated cytotoxicity
APC	antigen presenting cell
BM	bone marrow
BCR	B cell receptor
CRD	cysteine-rich domain
CD40L	CD40 ligand
CSR	class-switch recombination
CKD	chronic kidney disease
CDC	complement-dependent cytotoxicity
cDC1	conventional DC1
CTL	cytotoxic T lymphocyte

CRS	cytokine response syndrome
CLL	chronic lymphocytic leukemia
DC	dendritic cell
DLBCL	diffuse large B cell lymphoma
ERK	extracellular-signal-regulated kinase
EC	endothelial cell
FO B cell	follicular B cell
Fc	constant fragment
FcγR	Fc γ receptor
GC	germinal center
GM-CSF	granulocyte/macrophage colony-stimulating factor
HIGM	hyper-IgM syndrome
HLA-DR	leukocyte antigen-DR
HUVEC	human umbilical vein endothelial cell
HPC	hematopoietic progenitor cell
ICAM-1	intercellular adhesion molecule-1
Ig	immunoglobin
IKK	inhibitor of NF-κB kinase
IκB	NF-κB inhibitor
IL	interleukin
ILC	innate lymphoid cell
IFN	interferon
JNK	JUN N-terminal kinase
JAK3	janus kinase 3
mCD40	membrane CD40
mCD40L	membrane CD40 ligand
MC	monocytes
MΦ	macrophage
MMP	matrix metalloproteinase

MAPK	mitogen-activated protein kinase
MZ B cell	marginal zone B cell
MM	multiple myeloma
mAb	monoclonal antibody
NHL	non-Hodgkin's lymphoma
NF-κB	nuclear factor kappa-B
NK cell	natural killer cell
PI3K	phosphoinositide 3-kinase
PD1	programmed cell death protein 1
PDL1	prograimned cell death ligand 1
PLA	platelet-leukocyte aggregate
RANTES	regulated on activation, normal T cell expressed and secreted
sCD40	soluble CD40
sCD40L	soluble CD40 ligand
STAT	signal transducer and activator of transcription
SHM	somatic hypermutation
SLE	systemic lupus erythematosus
TLR4	toll-like receptor 4
TEs	thrombotic events
TCR	T cell receptor
Tfh cell	follicular helper T cell
Th cell	T helper cell
TNFRSF	tumor necrosis factor receptor superfamily
TNFSF	tumor necrosis factor superfamily
TRAF	TNF receptor associated factor
VCAM-1	vascular cell adhesion molecule-1
VSMC	vascular smooth muscle cells

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A. CD40/CD40L checkpoint molecules processing and interaction
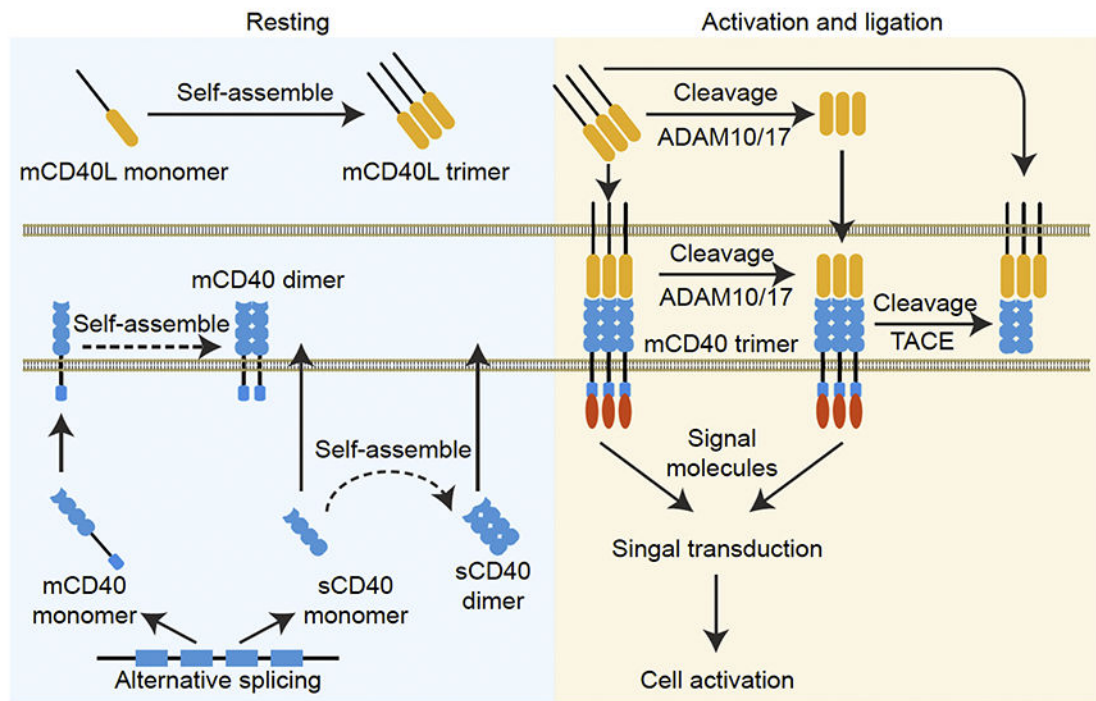
B. Structure, interaction and function upon ligation of CD40/CD40L checkpoint molecules

Molecules	Production	Structure		Function upon ligation	PMID
		Without ligation	With ligation		
mCD40L		Trimer	Trimer	Agonize mCD40	8995381
sCD40L	Cleavage of mCD40L	Trimer	Trimer	Agonize mCD40	7615003 8626375 20230421 24189063
mCD40		Monomer Dimer	Dimer Trimer	Cell activation	23463508 10411888 10433725
sCD40	Alternative splicing Cleavage of mCD40	Monomer Dimer	Dimer Trimer	Stabilize mCD40L Antagonize mCD40	11172023 12810728 1378075

Figure 1. CD40/CD40L checkpoint molecules processing and interaction.
A. CD40/CD40L checkpoint molecules processing and interaction. In resting condition, CD40L is expressed as a membrane monomer (mCD40L) which self-assembles into noncovalent trimers and is stored intracellularly. CD40 is expressed as a membrane monomer (mCD40) and a soluble monomer (sCD40) by alternative splicing. A small portion of mCD40 and sCD40 self-assemble into noncovalent dimers. After cell activation, mCD40 translocates to the cell surface and undergoes proteolytic cleavage by ADAMs to generate trimeric sCD40L. Binding of both mCD40L and sCD40L to CD40 induces mCD40

trimerization, recruitment of signal molecules and subsequent signal transduction, leading to cell activation. After ligation, mCD40 can be cleaved into sCD40 by TACE on the cell surface. sCD40 antagonizes mCD40 by shedding CD40/CD40L interaction. **B.** Structure, interaction and function upon ligation of CD40/CD40L checkpoint molecules.

Abbreviations: ADAM, a disintegrin and metalloprotease; CD40L, CD40 ligand; mCD40, membrane CD40; mCD40L, membrane CD40L; sCD40, soluble CD40; sCD40L, soluble CD40L; TACE, tumor necrosis factor-α converting enzyme.
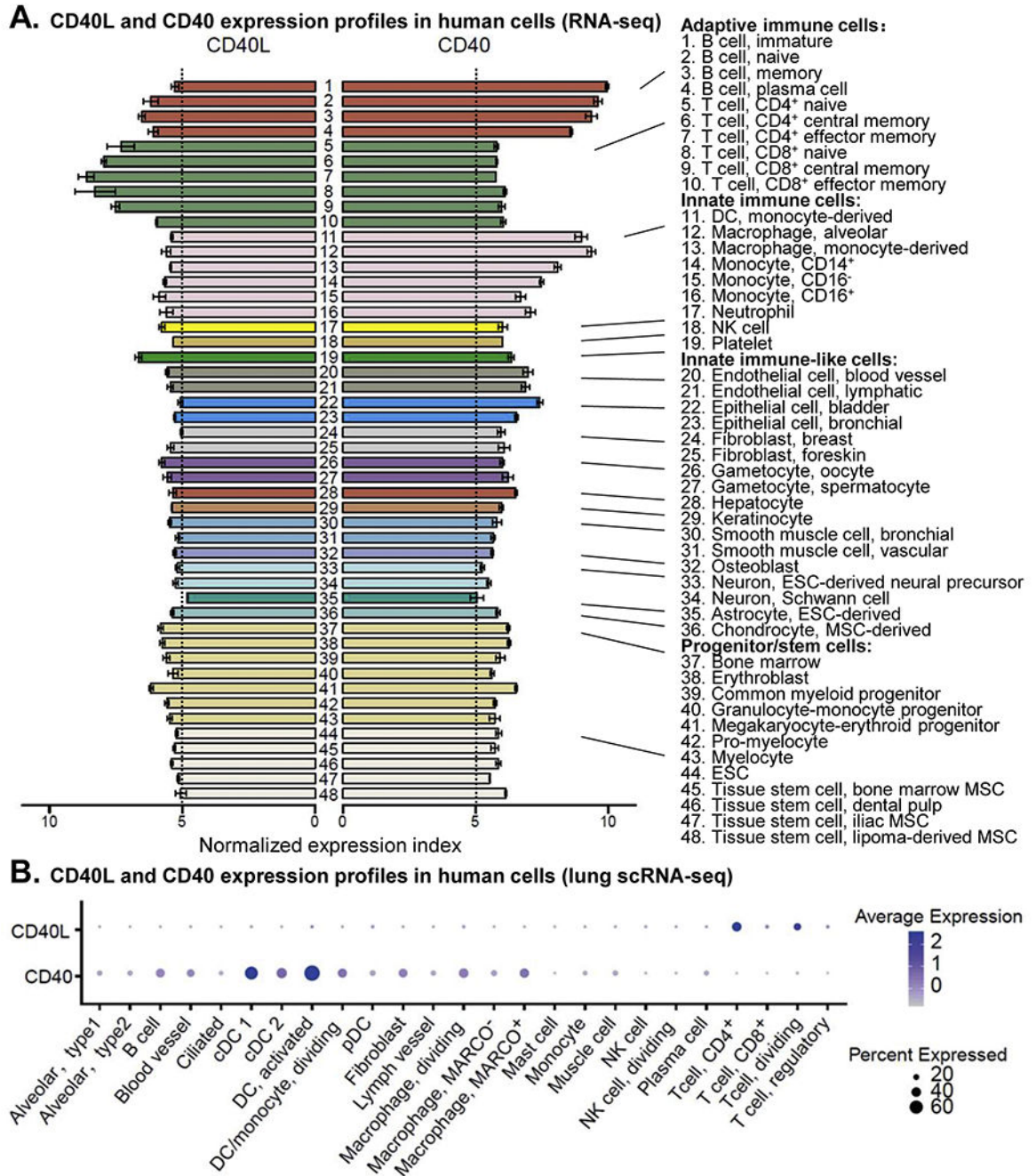
A. CD40L and CD40 expression profiles in human cells (RNA-seq)

Adaptive immune cells:
1. B cell, immature
2. B cell, naive
3. B cell, memory
4. B cell, plasma cell
5. T cell, CD4+ naive
6. T cell, CD4+ central memory
7. T cell, CD4+ effector memory
8. T cell, CD8+ naive
9. T cell, CD8+ central memory
10. T cell, CD8+ effector memory

Innate immune cells:
11. DC, monocyte-derived
12. Macrophage, alveolar
13. Macrophage, monocyte-derived
14. Monocyte, CD14+
15. Monocyte, CD16-
16. Monocyte, CD16+
17. Neutrophil
18. NK cell
19. Platelet

Innate immune-like cells:
20. Endothelial cell, blood vessel
21. Endothelial cell, lymphatic
22. Epithelial cell, bladder
23. Epithelial cell, bronchial
24. Fibroblast, breast
25. Fibroblast, foreskin
26. Gametocyte, oocyte
27. Gametocyte, spermatocyte
28. Hepatocyte
29. Keratinocyte
30. Smooth muscle cell, bronchial
31. Smooth muscle cell, vascular
32. Osteoblast
33. Neuron, ESC-derived neural precursor
34. Neuron, Schwann cell
35. Astrocyte, ESC-derived
36. Chondrocyte, MSC-derived

Progenitor/stem cells:
37. Bone marrow
38. Erythroblast
39. Common myeloid progenitor
40. Granulocyte-monocyte progenitor
41. Megakaryocyte-erythroid progenitor
42. Pro-myelocyte
43. Myelocyte
44. ESC
45. Tissue stem cell, bone marrow MSC
46. Tissue stem cell, dental pulp
47. Tissue stem cell, iliac MSC
48. Tissue stem cell, lipoma-derived MSC

B. CD40L and CD40 expression profiles in human cells (lung scRNA-seq)

Figure 2. CD40 and CD40L expression profiles in primary human cells.
A. CD40 and CD40L expression profiles in human cells (RNA-seq). Expression levels of CD40 and CD40 genes in various types of primary human cells, without risk factor challenge, were extracted from a normalized transcriptional dataset gathering 714 RNA-seq studies (PMID 24053356). Both CD40 and CD40L are expressed in all primary human cells presented in this dataset. CD40L gene is expressed relatively higher in T cells and platelets, whereas CD40 gene is higher in B cells and myeloid cell lineages. **B.** CD40 and CD40L expression profiles in human cells (lung scRNA-seq). Expression levels of CD40 and

CD40L genes in human lung across various cell types were extracted from a scRNA-seq datasets (PMID 31892341). **Abbreviations:** CD40L, CD40 ligand; cDC, conventional DC; DC, dendritic cell; ESC, embryonic stem cell; MSC, mesenchymal stem cell; NK cell, natural killer cell; pDC, plasmacytoid DC.
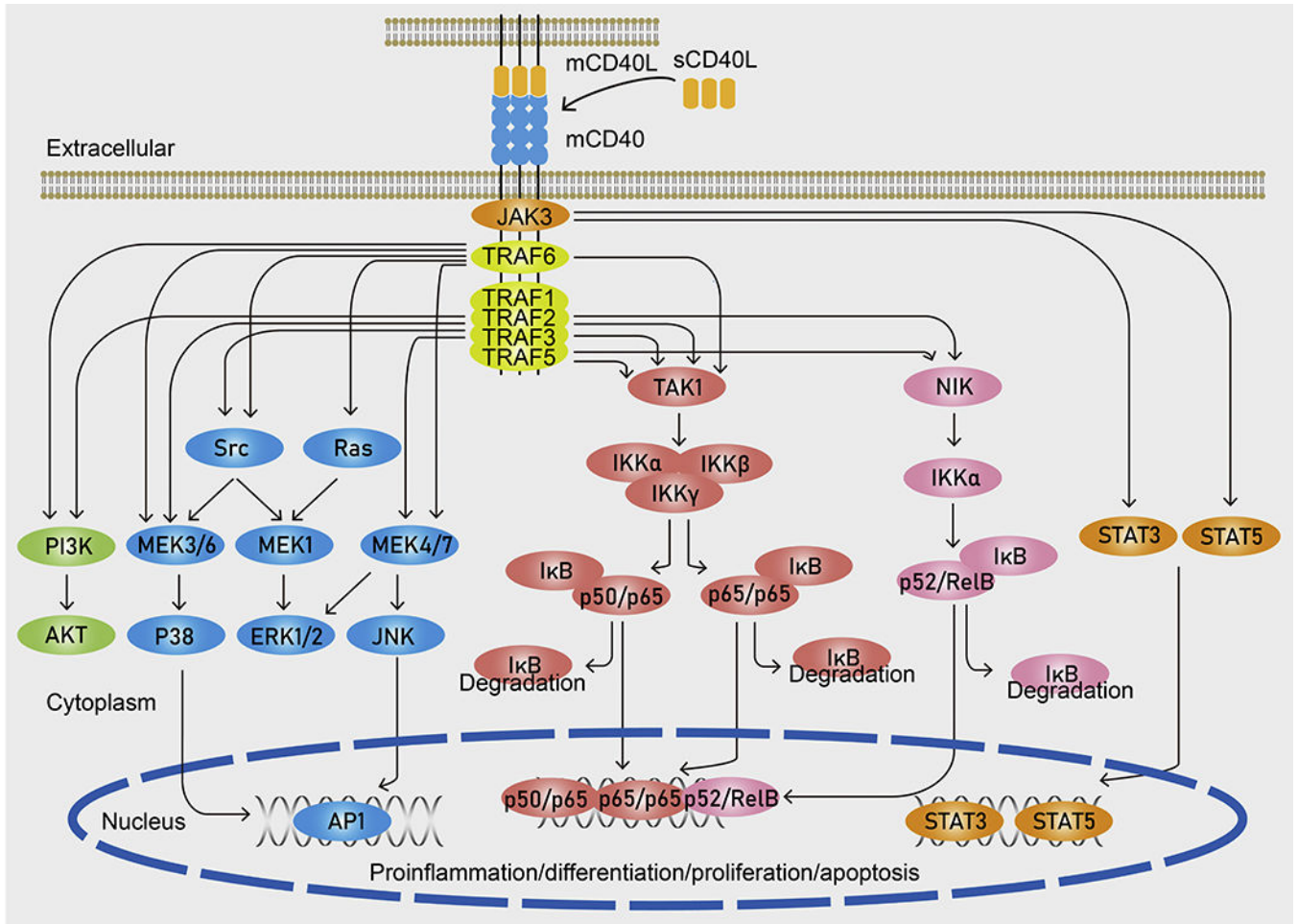
Figure 3. CD40/CD40L signal pathways.
The ligation of trimeric mCD40 with mCD40L or sCD40L results in the recruitment of signal molecules TRAFs to CD40 cytoplasmic domain. CD40 cytoplasmic domain contains two distinct TRAF binding sites. TRAF1, TRAF2, TRAF3 and TRAF5 bind competitively to one site and TRAF6 binds to another. CD40/CD40L ligation activates the canonical NF-κB pathway (IKKα, IKKβ and IKKγ dependent) and non-canonical NF-κB pathway (IKKα dependent), leading to nuclear translocation of p50/p65, p65/p65 and p52/RelB dimers and their DNA binding. Other well-documented downstream pathways of CD40-TRAF interaction include the three MAPK pathways (p38, ERK1/2 and JNK) and PI3K/AKT pathway, leading to AP1 nuclear translocation and DNA binding. In addition to TRAF-mediated signaling, CD40 can activate the JAK3/STATs pathways. **Abbreviations:** Ab, antibody; AKT, protein kinase B; AP1, activator protein 1; CD40L, CD40 ligand; ERK1/2, extracellular signal-regulated kinases 1/2; IKK, inhibitor of NF-κB kinase; IκB, NF-κB inhibitor; JNK, c-Jun N-terminal kinase; MEK1, MAPK/ERK kinase; NF-κB, nuclear factor kappa-B; NIK, NF-κB kinase inducing kinase; JAK3, janus kinase 3; PI3K, phosphoinositide-3-kinase; STAT, signal transducer and activator of transcription; TAK1, TGFβ-activated kinase 1; TRAF, tumor necrosis factor receptor-associated factor.
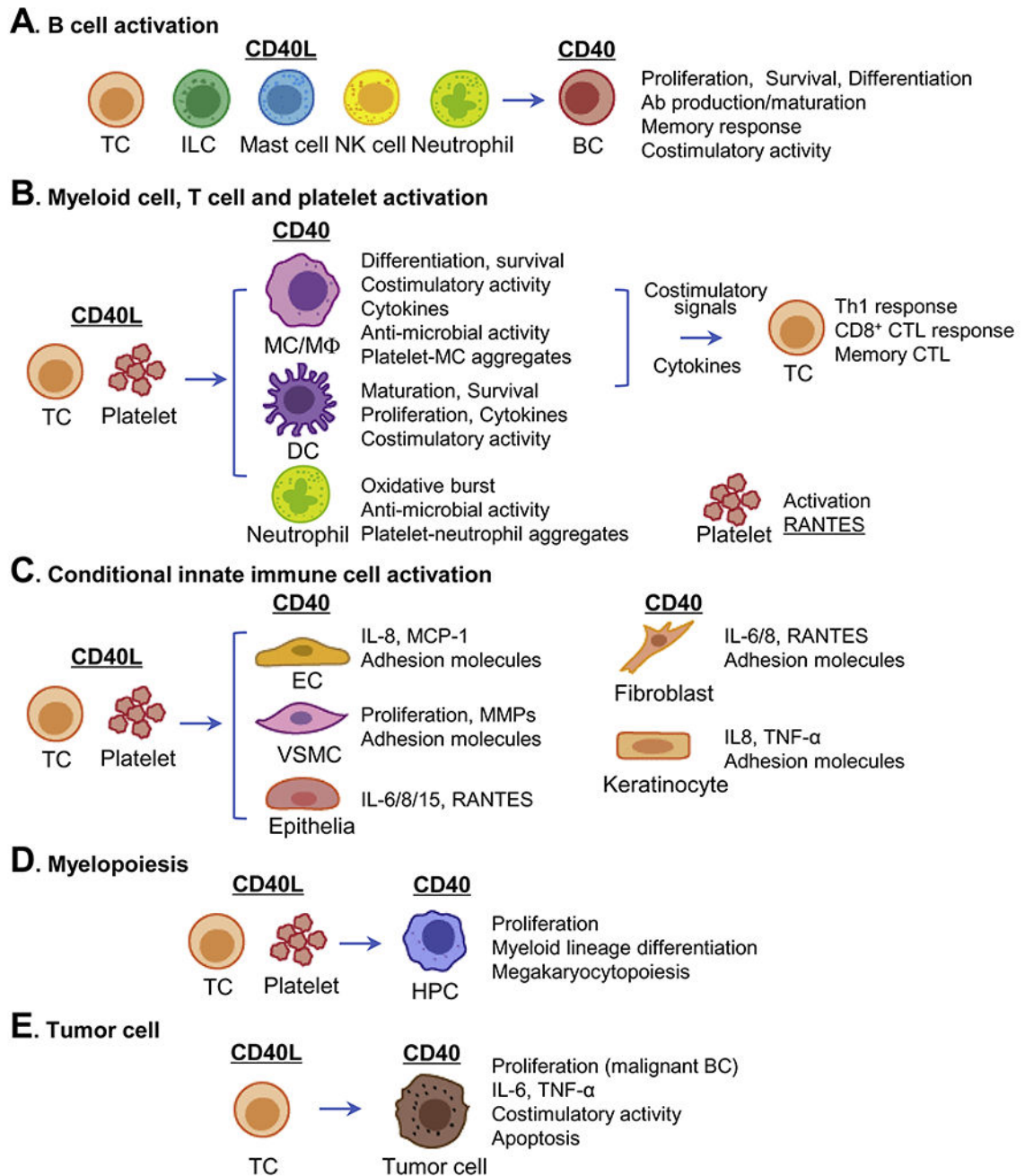
Figure 4. Effector function of CD40/CD40L interaction.

CD40L, expressed by several types of immune cells, binds to its receptor CD40 which is expressed by both immune cells and non-immune cells, participating in B cell activation (**A**), myeloid cell, T cell and platelet activation (**B**), conditional innate immune cell activation (**C**), myelopoiesis (**D**) and regulation of tumor cells (**E**). **Abbreviations:** Ab, antibody; BC, B cell; CD40L, CD40 ligand; CTL, cytotoxic T lymphocyte; DC, dendritic cell; EC, endothelial cell; HPC, hemopoietic progenitor cell; IL, interleukin; ILC, innate lymphoid cell; MC, monocyte; MCP-1, monocyte chemotactic protein-1; MΦ, macrophage; MMP,

matrix metalloprotease; NK cell, natural killer cell; RANTES, regulated upon activation normal T cell expressed and secreted factor; TC, T cell; Th1, T helper 1; TNF-α, tumor necrosis factor-α; VSMC, vascular smooth muscle cell.
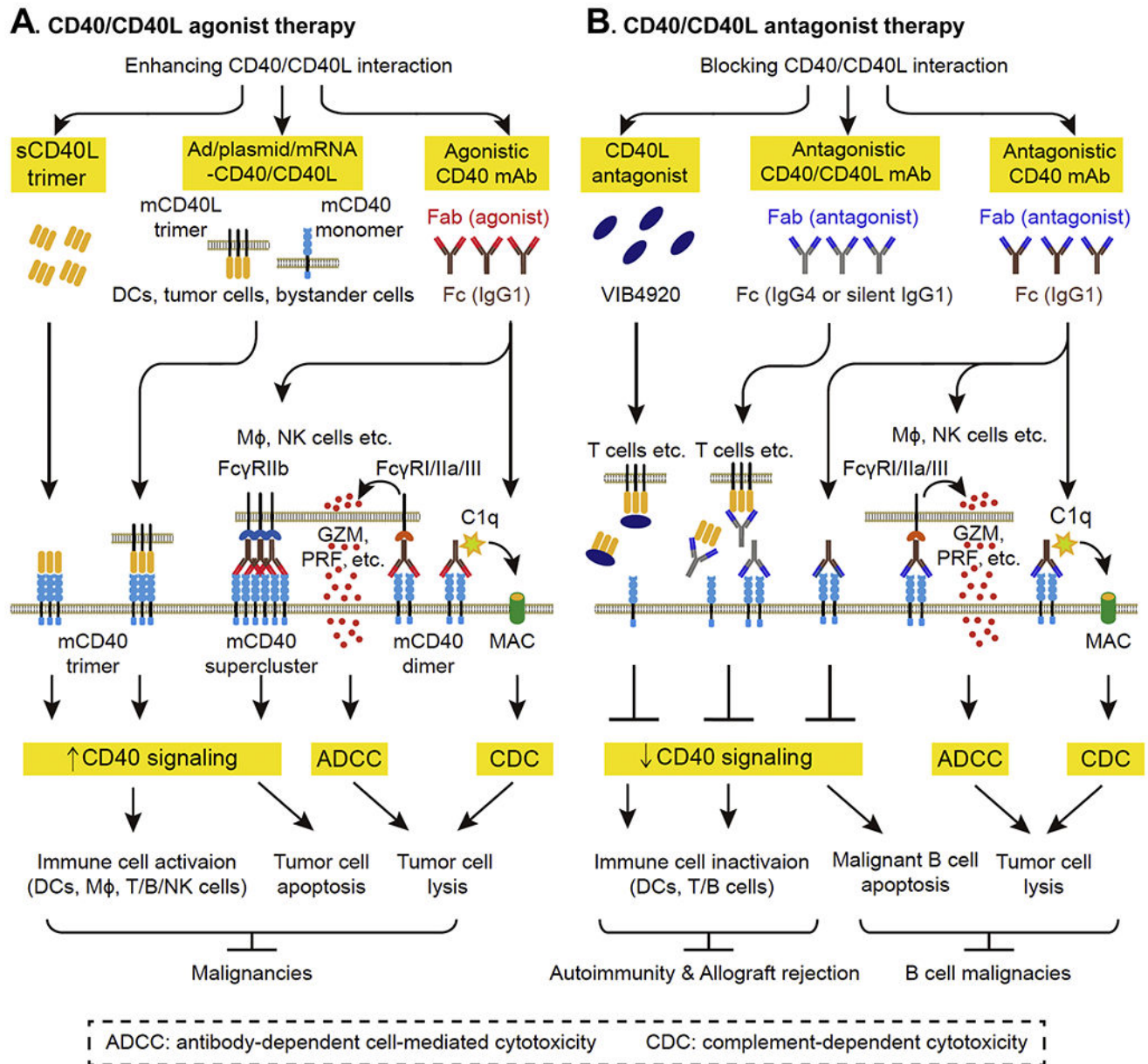
Figure 5. Strategies and mechanisms of CD40/CD40L-targeted therapy.
A. CD40/CD40L agonist therapy enhancing CD40/CD40L interaction by sCD40L trimer, adenovirus or plasmid or mRNA electroporation-mediated CD40/CD40L expression (Adv/plasmid/mRNA-CD40/CD40L) and agonistic CD40 mAb has been investigated in malignancies. **B.** CD40/CD40L antagonist therapy blocking CD40/CD40L interaction by CD40L antagonist and antagonistic CD40/CD40L mAb has been evaluated in autoimmune diseases, allograft rejection and B-cell malignancies. **Abbreviations:** CD40L, CD40 ligand; DC, dendritic cell; mAb, monoclonal antibody; Fab, antigen-binding fragment; Fc, constant fragment; FcγR, Fc γ receptor; GZM, granzyme; MAC: membrane attack complex;

mCD40, membrane CD40; mCD40L, membrane CD40L; MΦ, macrophage; NK cell, natural killer cell; PRF, perforin; sCD40L, soluble CD40L.

Table 1.

CD40/CD40L signal pathways in cellular immune response

Cell	Signal pathway	PMID
B cell	TRAF2↑ → canonical NF-κB (p65:p65)↑ & noncanonical NF-κB (p52:Rc1B)↓ → cell proliferation & activation↑	15539150
B cell	TRAF2↑ → MEKK1↑ → JNK & p38↑ → AP1↑ → cell proliferation & Ab production↑	17143273
B cell	TRAF6↑ → JNK & p38↑ → cell proliferation, activation & Ab isotype switching↑	12354380
WEHI 231 B cell	TRAF3↑ → PI3K↑ → NADPH oxidase↑ → ROS production↑	14688330
A20 B cell	TRAF6↑ → PI3K↑ → AKT↑ → apoptosis↓	12637493
B cell	JAK3↑ → STAT3↑ → CD23, ICAM-1 & lymphotoxin-α↑	9133417
DC	TRAF2 & 3↑ → Lyn↑ → ERK1/2↑ → IL-1α/β & IL-1Ra↑ TRAF2 & 3↑ → Lyn↑ → p38↑ → IL12p40↑ TRAF2 & 3↑ → p38↑ → IL12p40↑	10880443
MC/MΦ	TRAF6↑ → Src↑ → ERK1/2↑ → TNF-α IL-6 & IL-1β↑	15634933
MC	JAK3↑ → STAT5↑	10395671
Endothelial cell	TRAF6↑ → c-Cb1↑ → PI3K↑ → AKT ↑ → proliferation, motility, angiogenesis↑ & apoptosis↓	12637493
Lung fibroblast	TRAF6↑ → canonical NF-κB (p65) & JNK↑ → IL-8 & IL-6↑	16143987
Airway epithelial cell	TRAF3↑ → canonical NF-κB (p65:p65)↑ → RANTES↑	12122011
293T cell	TRAF6↑ → canonical NF-κB (p50:p65)↑ TRAF2 & 5↑ → canonical NF-κB (p50:p65)↑ TRAF2 & 5 noncanonical NF-κB (p52:RelB)↑	15708970
293T cell	Cytoplasmic tail-N → TRAF2, 3 & 5 ↑; Cytoplasmic tail-C → TRAF6 ↑	9990007
Spodoptera frugiperda (Sf21) cell	PVQET sequence of CD40 cytoplasmic domain → TRAF1, 2 & 3↑ QEPQEINF sequence of CD40 cytoplasmic domain → TRAF6↑	9718306
HEK 293 cell	Affinity to trimeric CD40: trimeric TRAF2 > TRAF3 >> TRAF1 & 6	10433725
HEK 293 cell	Affinity to CD40: TRAF2 & 3 > TRAF5 & 6	9990039
HEK 293 cell	TRAF6↑ → Ras↑ → Raf↑ → MEK1↑ → ERK1/2↑; TRAF6↑ → ERK1/2↑	9432981
Malignant urothelial cell	TRAF3↑ → JNK↑ → API↑ → caspase-9↑ → caspase-3↑ → apoptosis ↑	16429118

Abbreviations: Ab, antibody; AKT, protein kinase B; AP1, activator protein 1; CD40L, CD40 ligand; DC, dendritic cell; ERK1/2, extracellular signal-regulated kinases 1/2; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; JAK3, janus kinase 3; JNK, c-Jun N-terminal kinase; Lyn, LYN proto-oncogene, Src family tyrosine kinase; MC, monocyte; MΦ, macrophage; MEK1, MAPK/ERK kinase1; MEKK1, MAPK kinase kinase 1; NF-κB, nuclear factor kappa-B; PI3K, phosphoinositide-3-kinase; RANTES, regulated upon activation normal T cell expressed and secreted factor; ROS, reactive oxygen species; STAT, signal transducer and activator of transcription; TNF-α, tumor necrosis factor-α; TRAF, tumor necrosis factor receptor-associated factor.

Table 2.

CD40/CD40L checkpoint molecules interaction, effector function and immunological process

CD40L source	CD40 source	Effector function	Immunological process	PMID
TC	BC	BC proliferation, survival, differentiation, Ab production, IgD/M to IgA/G/E switching, memory response & costimulatory activity ↑	TC-dependent humoral immunity; Cellular immunity	27573866 29669250 7533092
Mast cell ILC Neutrophil NK cell	BC	BC proliferation, differentiation, Ab production, IgD/M to IgA/G/E switching & memory response↑	TC-independent humoral immunity	20101023 24562309 22197976 17630353
TC Platelet	MC/MΦ	MC/MΦ differentiation, costimulatory activity, cytokine production & anti-microbial activity ↑; MC apoptosis↓; TC Th1 response, CD8$^+$ CTL response & memory CTL maintenance↑	Cellular immunity	15100268, 7594496 7843250, 8929557 27554817,8627184 10810999
TC Platelet	DC	DC maturation, proliferation, costimulatory activity & cytokine production↑; DC apoptosis↓; TC Th1 response, CD8$^+$ CTL response & memory CTL maintenance↑	Cellular immunity	12893749 22154528 32453421 20811042
TC Platelet	Neutrophil	Oxidative burst & anti-microbial activity ↑	Cellular immunity	29518426 23785403
TC	Platelet	Platelet activation & release of RANTES↑	Thrombosis	14764664
TC Platelet	EC	EC IL-8, MCP-1 & adhesion molecules↑	Tissue inflammation	7500031 9468137
TC Platelet	VSMC	VSMC proliferation, MMPs & adhesion molecules↑	Tissue inflammation	9285647 28717419
TC	Fibroblast	Fibroblast IL6/8, RANTES & adhesion molecules↑; TC extravasation↑	Tissue inflammation	9144479
TC	KC	KC IL8, TNF-α & adhesion molecules↑	Tissue inflammation	8898941 8977185
TC	Epithelia	Epithelia IL6/8/15 & RANTES↑	Tissue inflammation	11053480, 11134253
TC Platelet	HPC	HPC proliferation, myeloid lineage differentiation & megakaryocytopoiesis↑	Myelopoiesis	9022082 9016882
Platelet	Platelet	Platelet activation↑	Thrombosis	12676820
Platelet	Leukocyte	Platelet-MC & platelet-neutrophil aggregates↑ Leukocyte extravasation↑	Thrombosis Tissue inflammation	12676820 27152726
TC	Tumor cell	Proliferation (malignant BC), IL-6, TNF-α costimulatory activity & apoptosis↑	Tumor cell fate	18497318,10068672 12070030, 9182676

Abbreviations: Ab, antibody; BC, B cell; CD40L, CD40 ligand; CTL, cytotoxic T lymphocyte; DC, dendritic cell; EC, endothelial cell; HPC, hemopoietic progenitor cell; IL, interleukin; ILC, innate lymphoid cell; MC, monocyte; MCP-1, monocyte chemotactic protein-1; MΦ, macrophage; MMP, matrix metalloprotease; NK cell, natural killer cell; RANTES, regulated upon activation normal T cell expressed and secreted factor; TC, T cell; Th1, T helper1; TNF-α tumor necrosis factor-α; VSMC, vascular smooth muscle cell.

Table 3A.

Clinical trials of CD40 agonist therapy

Drug	Feature	Phase	Disease	Outcome	PMID/Identifier
CP-870,893	Human mAb	1	Solid tumors	Safe; Effective	17327609
		1	Advanced solid tumor	Safe; Little efficiency	20855968
		1	Advanced pancreatic ductal adenocarcinoma	Safe [a]; Effective	23983255 21436454
		1b	Malignant pleural mesothelioma	Safe [b]	26386124
		1	Metastatic melanoma	Safe [c]; Effective	30288340
Dacetuzumab	Humanized mAb	1	Refractory NHL	Safe; Effective	19636010
		1	CLL	Safe; Little efficiency	20038235
		1	Advanced MM	Safe; Little efficiency	20133895
			Relapsed DLBCL	Safe [d]; Effective	22775314
		2	Relapsed DLBCL	Safe; Effective	24919462
		2b	Relapsed DLBCL	Safe [e]; Not effective	25651427
ChiLob 7/4	Chimeric mAb	1	CD40-expressing solid tumors and DLBCL	Safe; Little efficiency	25589626
ADC-1013	Human mAb	1	Advanced solid tumors	Safe	30664811
Selicrelumab	Human mAb	1	Solid tumors	Unpublished	NCT02665416
		1	Refractory B-cell NHL	Ongoing	NCT03892525
APX005M	Humanized mAb	1	Solid tumors	Not reported	NCT02482168
		1/2	NSCLC& melanoma	Ongoing	NCT03123783
		2	Rectal adenocarcinoma	Ongoing	NCT04130854
		2	Soft tissue sarcoma	Ongoing	NCT03719430
		2	Melanoma	Ongoing	NCT04337931 NCT02706353
		1	Pediatric central nervous system tumors	Ongoing	NCT03389802
SEA-CD40	Humanized mAb	1	Carcinoma	Ongoing	NCT02376699
CDX-1140	Human mAb	1	Carcinoma	Ongoing	NCT04364230
		1	Advanced malignancies	Ongoing	NCT03329950
2141-V11	Fc-engineered human mAb	1	Solid tumor & cancer of skin	Ongoing	NCT04059588
NG-350A	Ad-human CD40 mAb	1	Metastatic cancer & epithelial Tumor	Ongoing	NCT03852511
BPX101	Autologous DC transduced with Ad-human CD40	1	Metastatic castration-resistant prostate cancer	Safe; Effective	28608115

Abbreviations: Ad-human mAb, adenovirus carrying agonistic human CD40 mAb gene; Ad-human CD40, adenovirus carrying human CD40 gene; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; Fc, constant fragment; mAb, monoclonal antibody; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NSCLC, non-small cell lung cancer.

[a] in combination with gemcitabine;

[b] in combination with cisplatin & pemetrexed;

[c] in combination with tremelimumab;

^d in combination with rituximab & gemcitabine;

^e in combination with R-ICE (rituximab, ifosfamide, carboplatin and etoposide).

Table 3B.

Clinical trials of CD40 antagonist therapy

Drug	Feature	Phase	Disease	Outcome	PMID/Identifier
Ch5D12	Chimeric mAb	1/2a	Crohn's disease	Safe; Effective	16011669
Bleselumab	Human mAb	1	Moderate-severe psoriasis	Safe; Not effective	29679478
		1b, 2	Kidney Transplantation	Safe; Noninferiority	31509331 31397943
BI 655064	Humanized mAb	I	Healthy subject	Safe	29127458 30113724
		2a	Rheumatoid arthritis	Safe; Not effective	30902820
Iscalimab	Human mAb	1	Rheumatoid arthritis Healthy subject	Safe	31647605
		2	Grave's disease	Safe; Effective	31512728
		1/2	Kidney Transplantation	Unpublished	NCT02217410
		2	Kidney transplantation Liver transplantation	Ongoing	NCT03663335 NCT03781414
		2	Lupus nephritis	Ongoing	NCT03610516
		2	Systemic lupus erythematosus	Ongoing	NCT03656562
		2	Sjögren syndrome	Ongoing	NCT03905525
Lucatumumab	Human mAb	1	CLL	Safe; Minimal efficiency	22475052
		1	Relapsed MM	Safe; Moderate efficiency	22861192
		1a/2	Advanced lymphoma	Safe; Moderate efficiency	24219359

Abbreviations: CLL, chronic lymphocytic leukemia; mAb: monoclonal antibody; MM, multiple myeloma.

Table 4A.

Clinical trials of CD40L agonist therapy

Drug	Feature	Phase	Disease	Outcome	PMID/Identifier
Avrend	Trimeric soluble human CD40L	1	Advanced solid tumors and NHL	Safe; Effective	11432896
Ad-CD40L	Adenovirus carrying human CD40L gene	1, 2	Melanoma	Safe; Effective	27031851 28427434
		1/2	Bladder cancer	Safe; Effective	20448220
Ad-CD40L-CLL cells	Autologous CLL cells transduced with Ad-murine or humanized CD40L	1	CLL	Safe; Effective	11049967 20882050
GM.CD40L	Bystander cells expressing liuman GM-CSF/CD40L	2	Refectory lung adenocarcinoma	Safe	23994887
		1/2	Advanced lung adenocarcinoma	Safe	30209589
TriMixDC	Autologous DC electroporated with human CD40L/TLR4/ CD70 mRNA	1	Advanced melanoma	Safe	21577140
TriMixDC-MEL	Autologous DC electroporated with human CD40L/TLR4/ CD70/tumor antigen mRNA	1	Advanced melanoma	Safe	23904461
		2	Melanoma	Unpublished	NCT00101166

Abbreviations: CD40L, CD40L ligand; CLL, chronic lymphocytic leukemia; DC, dendritic cell; GM-CSF, granulocyte-macrophage colony-stimulating factor; NHL, non-Hodgkin lymphoma; TLR4, toll like receptor 4.

Table 4B.

Clinical trials of CD40L antagonist therapy

Drug	Feature	Phase	Disease	Outcome	PMID/Identifier
BG9588	Humanized mAb	1	Proliferative lupus nephritis	Thromboembolic events; Effective	12632425
		1	Systemic lupus erythematosus	Thromboembolic events; Effective	14617752
IDEC-131	Humanized mAb	1, 2	Systemic lupus erythematosus	Safe; Noneffective	11196549 12483729
		1	Refractory immune thrombocytopenic purpura	Safe; Effective	14551140 18341638
Dapirolizumab	Humanized mAb Fab	1	Systemic lupus erythematosus	Safe; Effective	28780512
AT-1501	Fc-engineered humanized mAb	2a	Amyotrophic lateral sclerosis	Unpublished	NCT04322149
BMS-986004	Fc-engineered humanized mAb	1/2	Acute graft-versus-host disease	Unpublished	NCT03605927
VIB4920	Engineered human protein	1	Rheumatoid arthritis	Safe; Effective	31019027

Abbreviations: CD40L, CD40L ligand; Fab, antigen-binding fragment; Fc, constant fragment; mAb: monoclonal antibody.

Table 5.

Summary of clinical efficacy of CD40/CD40L-targeted therapy

Drug	Feature	Disease	Efficacy, n (%)	PMID
Malignancy (single agent)				
CP-870,893	CD40 agonistic human mAb	Solid tumors	PR: 4 (27%) in melanoma	17327609
Dacetuzumab	CD40 agonistic humanized mAb	Refractory NHL	CR: 1 (2%); PR: 5 (10%); SD:13 (26%)	19636010
		Relapsed DLBCL	CR: 2 (4%); PR: 2 (4%); SD:13 (28%)	24919462
Avrend	Trimeric soluble human CD40L	Advanced solid tumors and NHL	CR: 1 (3%); PR: 1 (3%); SD: 4 (12%)	11432896
Lucatumumab	CD40 antagonistic human mAb	Relapsed MM	PR: 1 (4%); SD: 12 (43%)	22861192
		Advanced lymphoma	FL: CR: 1 (4.8%); PR: 6 (28.6%); SD:11 (52.4%); DLBCL: CR: 2 (5.9%); PR: 2 (5.9%); SD:11 (32.4%); MALT: CR: 1 (14.3%); PR: 2 (28.6%); SD:1 (14.3%); MCL: SD: 5 (41.7%); HL: PR: 5 (13.5%); SD:12 (32.4%)	24219359
Malignancy (combinatory therapy)				
CP-870,893+gemcitabine	+chemotherapy	Advanced pancreatic ductal adenocarcinoma	PR: 4 (18.2%)	23983255
CP-870,893+gemcitabine	+chemotherapy	Advanced pancreatic ductal adenocarcinoma	PR: 4 (19%); SD: 11 (52.4%)	2143645
CP-870,893+tremelimumab	+CTLA4 antagonistic mAb	Metastatic melanoma	CR: 2 (9.1%); PR: 4 (18.2)	30288340
Dacetuzumab+rituximab +gemcitabine	+ CD20 antagonistic mAb+chemotherapy	Relapsed DLBCL	CR: 6 (20%); PR: 8 (27%); SD:13 (28%)	22775314
Autoimmunity				
ch5D12	CD40 antagonistic chimeric mAb	Crohn's disease	CRR: 13 (72%)	16011669
Iscalimab	CD40 antagonistic human mAb	Grave's disease	CRR: 7 (47%)	31512728
Dapirolizumab	CD40L antagonistic humanized mAb Fab	Systemic lupus erythematosus	CRR: 11 (46%)	28780512
VIB4920	CD40L antagonist human protein	Rheumatoid arthritis	CRR: 8 (75%) in the 1500-mg group and 6 (50%) in 1000-mg	31019027

Abbreviations: CD40L, CD40L ligand; CR: complete response; CRR: clinical response rate; CTLA4: cytotoxic T-Lymphocyte associated protein 4; DLBCL: diffuse large B-cell lymphoma; Fab, antigen-binding fragment; FL: follicular lymphoma; HL: Hodgkin lymphoma; mAb: monoclonal antibody; MALT: mucosa associated lymphatic tissue lymphoma; MCL: mantle cell lymphoma; PR: partial response; SD: stable disease.