JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 9, NO. 6, 2024

STATE-OF-THE-ART REVIEW

Costimulatory and Coinhibitory Immune Checkpoints in Atherosclerosis

Therapeutic Targets in Atherosclerosis?

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HIGHLIGHTS

- **Immunotherapeutic strategies to combat cardiovascular disease are on the rise.**
- **Immune checkpoints are potent therapeutic targets in oncology and autoimmune diseases.**
- **Immune checkpoint-based therapeutics have great potential in cardiovascular disease.**

SUMMARY

The benefits of current state-of-the-art treatments to combat atherosclerotic cardiovascular disease (ASCVD) have stagnated. Treatments are mostly based on controlling cardiovascular risk factors, especially hyperlipidemia. Although the most recent advances with PCSK-9 inhibitors support the hyperlipidemia aspect of ASCVD, several lines of experimental evidence have outlined that atherosclerosis is also driven by inflammation. In the past years, phase 1, 2, and 3 clinical trials targeting inflammation to combat ASCVD have revealed that patients do tolerate such immune therapies, show decreases in inflammatory markers, and/or have reductions in cardiovascular endpoints. However, the search for the optimal anti-inflammatory or immune-modulating strategy and the stratification of patients who would benefit from such treatments and appropriate treatment regimens to combat ASCVD is only just beginning. In this review, we focus on immune checkpoint–based therapeutics (costimulation and coinhibition), many of which are already approved by the U.S. Food and Drug Administration for the treatment of cancer or autoimmune diseases, and discuss their use as a novel immunotherapeutic strategy to treat ASCVD. (J Am Coll Cardiol Basic Trans Science 2024;9:827–843) © 2024 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Although significant reductions in cardiovascular mortality and morbidity have been achieved using low-density lipoprotein (LDL) cholesterol-lowering therapies, antihypertensive agents, and the treatment of atherosclerotic cardiovascular disease (ASCVD)-aggravating diseases, including diabetes mellitus and obesity, ASCVD still poses a huge global health burden.[1-3] Consequently, over the past 5 years, the gold standard of treating patients with ASCVD has been shifting, and novel therapeutic strategies, including anti-inflammatory and immunotherapeutic approaches, are being introduced.[4]

Besides hyperlipidemia, inflammation is an important aspect in the pathogenesis of

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

Manuscript received March 7, 2023; revised manuscript received December 7, 2023, accepted December 11, 2023.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2023.12.007

ABBREVIATIONS AND ACRONYMS

APC = antigen-presenting cell

ASCVD = atherosclerotic cardiovascular disease

CAD = coronary artery disease

ICI = immune checkpoint inhibitor

LDL = low-density lipoprotein

SLE = systemic lupus erythematosus

SMI = small-molecule inhibitor

Th1 = T-helper cell type 1

T_{reg} = regulatory T

atherosclerosis. In the past 35 years, we have learned that immune cells are common inhabitants of both the nondiseased arterial wall and atherosclerotic plaque and that a large variability of immune cell types and subsets populate nondiseased and atherosclerotic arteries. Furthermore, numerous experimental and preclinical studies have proved a key role for both the innate and adaptive immune systems in atherosclerosis.[5,6]

CURRENT IMMUNOTHERAPIES FOR ASCVD

The first proof that targeting the innate immune system improves ASCVD outcomes in humans came several years ago from CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study) in which patients with histories of myocardial infarction and high-sensitivity C-reactive protein levels >2 mg/dL were randomized to the interleukin (IL)-1β antagonistic antibody canakinumab or placebo.[7] The LoDoCo (Low-Dose Colchicine),[8] LoDoCo2,[9] and COLCOT (Colchicine Cardiovascular Outcomes Trial)[10] trials showed that the anti-inflammatory agent colchicine reduced ASCVD risk in patients with coronary artery disease (CAD) or recent myocardial infarction. Phase 1 and 2 trials studying the effects of IL-6 blockade on ASCVD parameters using tocilizumab[11,12] and ziltivekimab[13] showed promising results, and results of IL-6 blockade in larger patient cohorts (ZEUS [Ziltivekimab Cardiovascular Outcomes Study]; NCT05021835) are awaited.[14] Also, inhibition of the antiphagocytic molecule CD47 by magrolimab in patients with cancer has been noted to reduce vascular inflammation in the carotid artery, as measured by ^{18}F fluorodeoxyglucose uptake.[15] However, not all anti-inflammatory treatments improved ASCVD outcomes. In the CIRT (Cardiovascular Inflammation Reduction Trial), patients with previous myocardial infarction or 3-vessel disease and diabetes or the metabolic syndrome were treated with weekly low-dose methotrexate, which failed to improve ASCVD endpoints and was associated with elevations in liver enzymes, lower leukocyte counts, and an increased level of basal cell carcinoma.[16] Thus, it might be speculated that a more targeted mechanistic and anti-inflammatory therapy might be necessary for the treatment of patients with atherosclerosis.

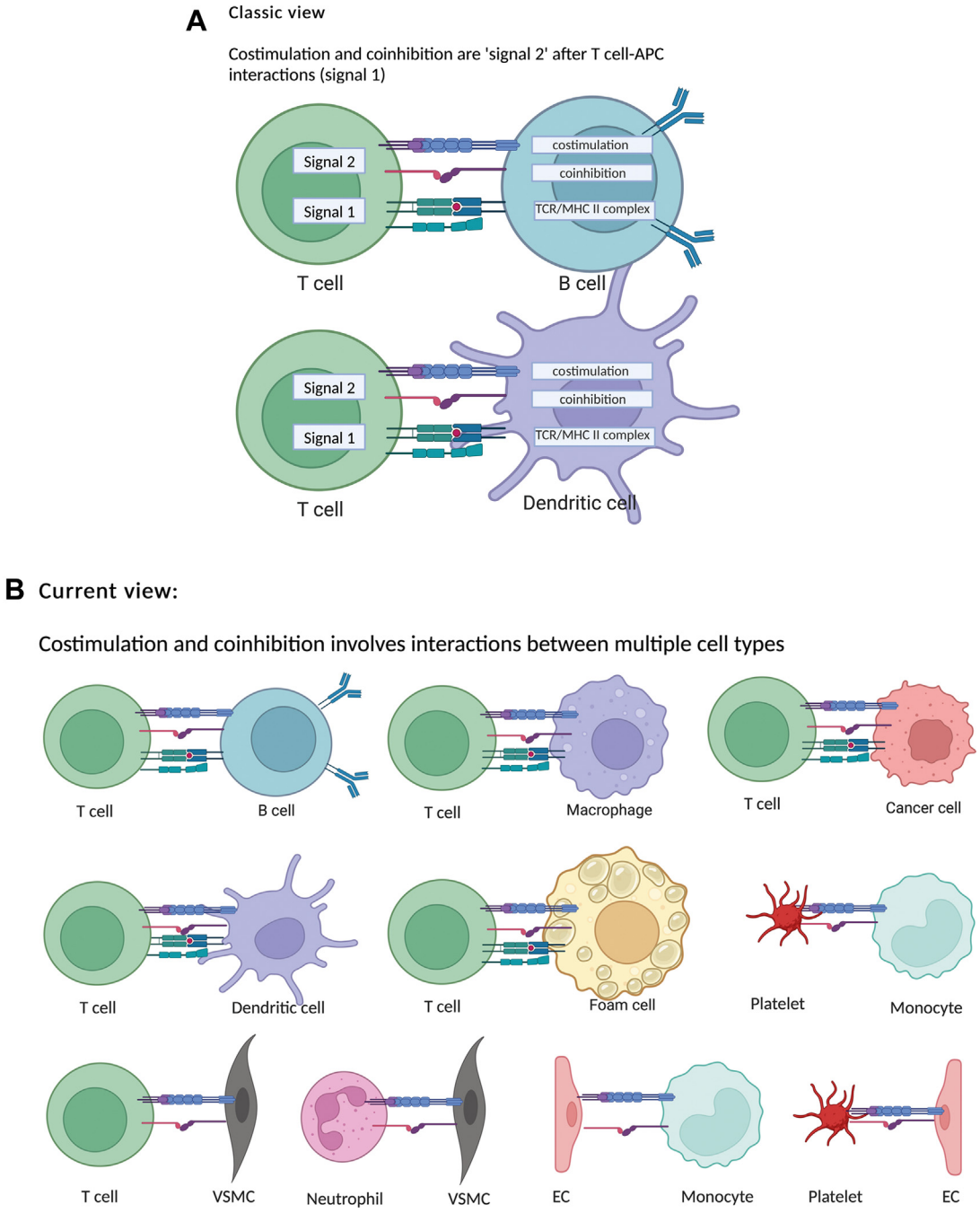
Targeting the adaptive immune system in patients with ASCVD also has great potential. In a safety study, mature B cells were depleted in patients with acute ST-segment elevation myocardial infarction using the anti-CD20 antibody rituximab (RITA-MI [Rituximab in Patients With Acute ST-Elevation Myocardial Infarction]), which was deemed safe and effective.[17] Another therapeutic strategy that targets the adaptive immune system is currently being tested in the IVORY (Low-Dose Interleukin 2 for the Reduction of Vascular Inflammation in Acute Coronary Syndromes; NCT04241601) trial.[18] In this strategy, low-dose IL-2 treatment is given to patients with acute coronary syndromes to reduce vascular inflammation. It follows the LILACS (Low Dose IL-2 in Patients With Stable Ischemic Heart Disease and Acute Coronary Syndromes) trial as the first proof-of-concept safety and biological efficacy study of low-dose IL-2 treatment. LILACS demonstrated significant expansion of regulatory T (T_{reg}) cells without major adverse events in patients with ischemic heart disease;[19] preclinical studies confirm an antiatherosclerotic effect of IL-2-based approaches.

Although the first trials showed that immunotherapeutic agents are a promising and novel strategy to lower ASCVD burden, this field is still under development, and novel and better immunotherapeutic approaches designed to combat ASCVD are eagerly awaited.[20] A very potent class of immunotherapeutic agents that have proved their efficacy in cancer and autoimmune diseases are Costimulatory and Coinhibitory immune checkpoint modulators.[21] In this review, we discuss the potential of immune checkpoint modulators to combat ASCVD.

IMMUNE CHECKPOINTS

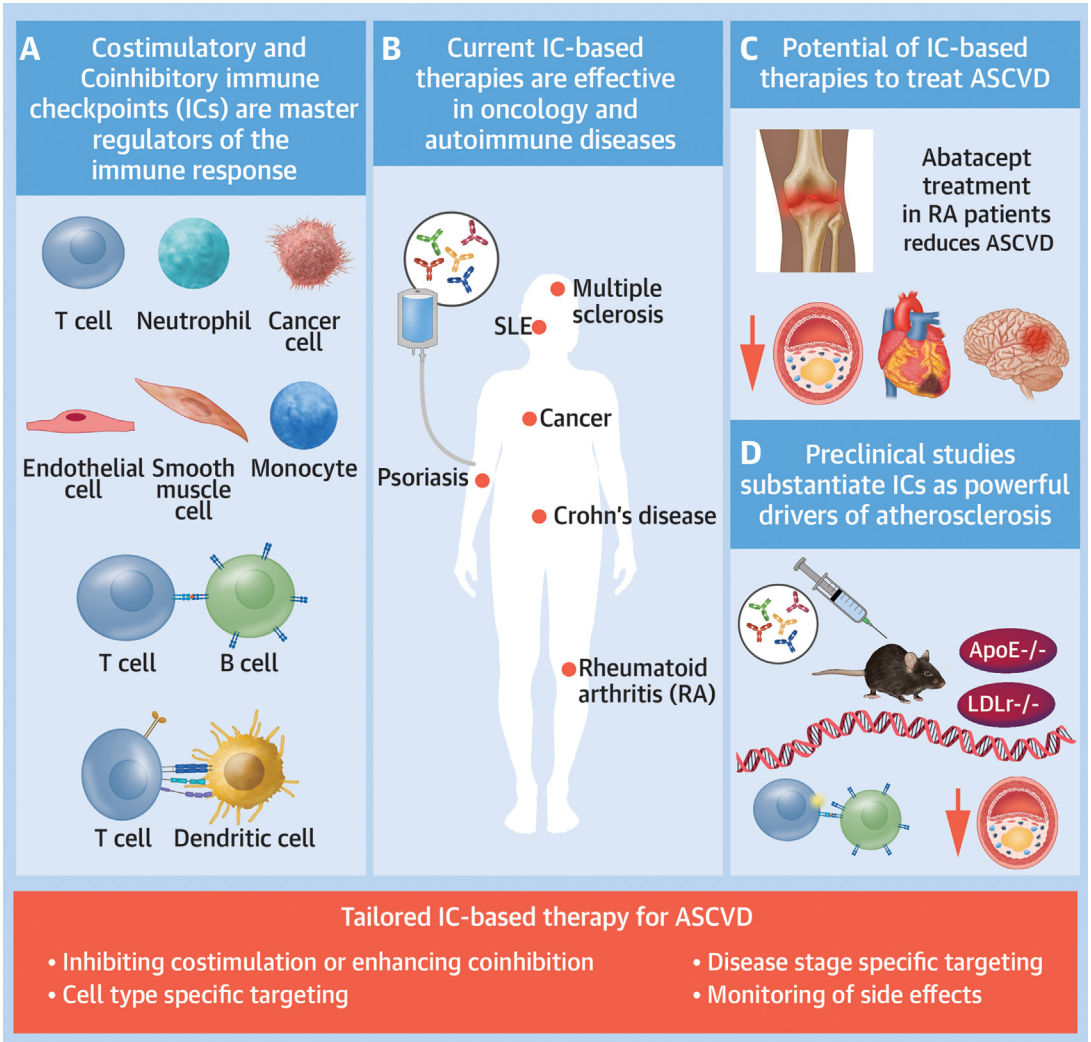
Immune checkpoint proteins are Costimulatory or Coinhibitory molecules that are master regulators of a wide range of immune reactions. Classically, Costimulatory molecules are known as “signal 2”: following the recognition of an antigen by the T cell receptor, costimulation is required to boost (and in some cases dampen) T cell proliferation, activation, and polarization, whereas Coinhibitory molecules counteract these responses[22,23] (**Figure 1**). Nowadays, it is known that immune checkpoints not only mediate interactions between antigen-presenting cells (APCs) and T cells but can provide (Costimulatory and Coinhibitory) signals that facilitate cell-cell communication and drive or dampen inflammation to a plethora of innate and adaptive immune cells and nonimmune cells, including endothelial cells, fibroblasts, vascular smooth muscle

FIGURE 1 **The Changing View of the Functions of Costimulatory and Coinhibitory Immune Checkpoints**



(A) Classic view: Costimulation and coinhibition are "signal 2" after interactions between T cells and antigen-presenting cells (signal 1). (B) Current view: Costimulation and coinhibition involve interactions among multiple cell types. APC = antigen-presenting cell; EC = endothelial cells; TCR = T cell receptor; VSMC = vascular smooth muscle cell.

CENTRAL ILLUSTRATION **Immune Checkpoints and Current and Immune Checkpoint-Based Therapies in Cancer and Autoimmune Diseases and Potential Applications for ASCVD**



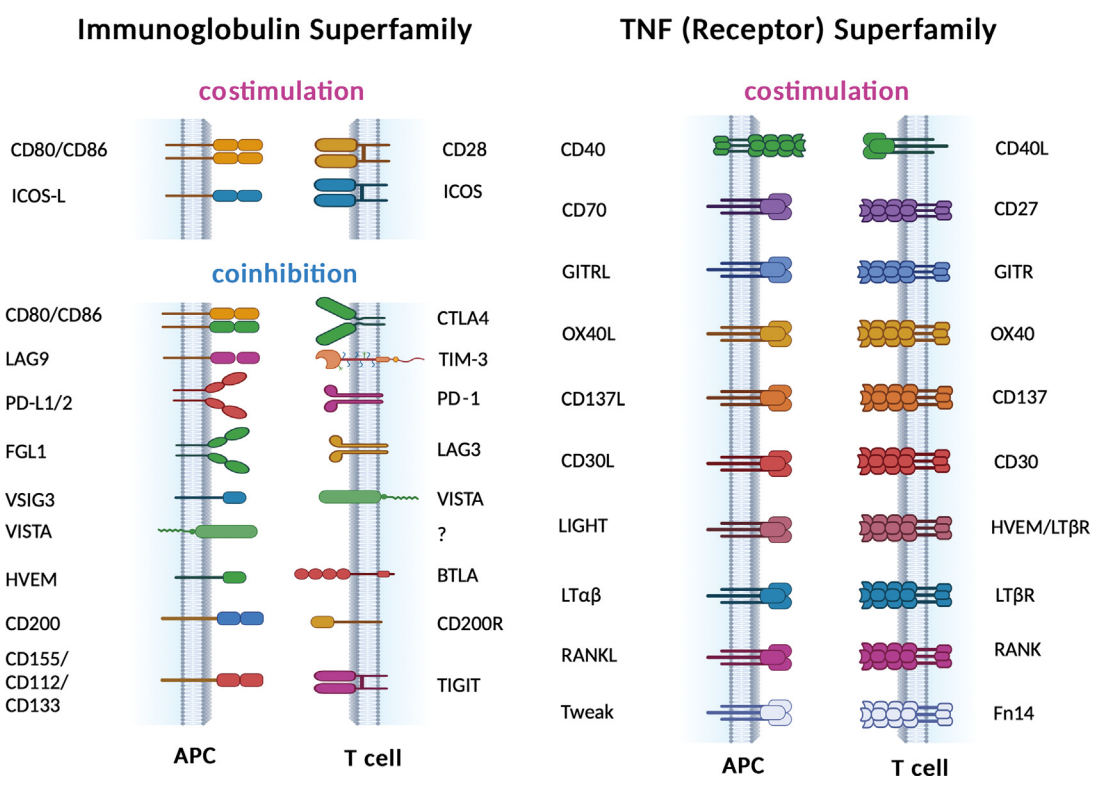
Nitz K, et al. J Am Coll Cardiol Basic Trans Science. 2024;9(6):827–843.

(A) Immune checkpoints (ICs) are master regulators of immune responses and cell-cell communication of non-immune cells. ICs exert costimulatory and/ or coinhibitory signals. (B) IC-based therapies are a current treatment for variety of cancer patients to enhance the tumor-killing capacity of immune cells, as well as for autoimmune diseases to reduce inflammatory response in diseases such systemic lupus erythematosus (SLE), multiple sclerosis, psoriasis, Crohn's disease or rheumatoid arthritis (RA). (C) Abatacept treatment (CTLA-4 ligand fusion protein that blocks the costimulatory CD28-CD80/CD86 axis) in RA patients led to decrease of ASCVD events in these patients. (D) Preclinical studies in atherosclerotic mouse models (ApoE-/ LDLr-deficient mice) have proven ICs as critical drivers in atherosclerosis. IC-based therapy in ASCVD to inhibit costimulation and/ or activate coinhibition are a promising tool. Celltype-specific and disease stage-specific treatment is pivotal for successful ASCVD therapy. Critical monitoring and assessment of side-effects in clinical trials is required before potential applications in ASCVD patients. ApoE = Apolipoprotein E; ASCVD = atherosclerotic cardiovascular disease; IC = immune checkpoint; LDLr = low-density lipoprotein receptor; RA = rheumatoid arthritis; SLE = systemic lupus erythematosus.

cells, epithelial cells, and cancer cells[24] (Figure 1, Central Illustration A).

Immune checkpoints belong to 2 major families: the immunoglobulin superfamily, which houses both Costimulatory and Coinhibitory immune checkpoint dyads, and the tumor necrosis factor (TNF) receptor superfamily, which houses Costimulatory immune checkpoint proteins, although some of them have Coinhibitory functions (Figure 2).[25] The immunoglobulin superfamily contains members such as

FIGURE 2 **Overview of the Most Important Members of the Immunoglobulin and TNF(R) Superfamilies of Costimulatory and Coinhibitory Immune Checkpoints**



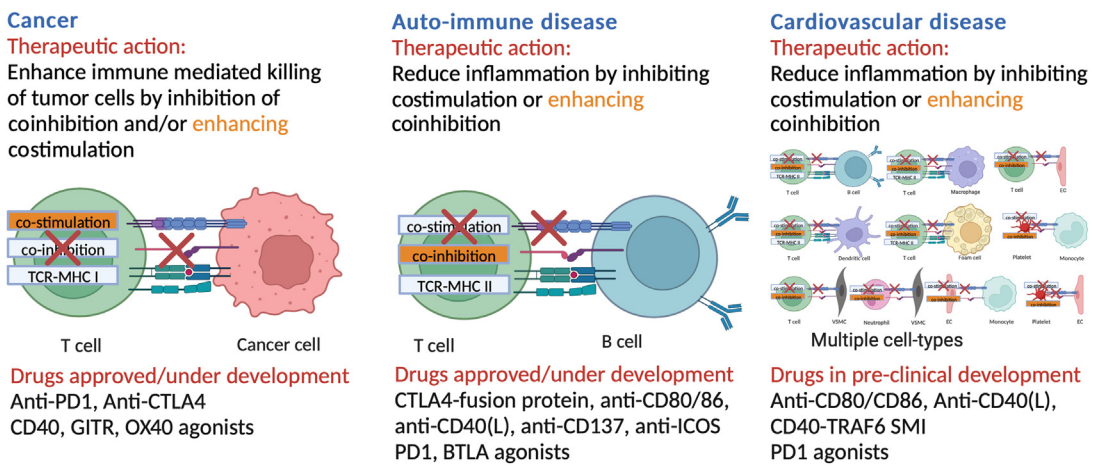
The Immunoglobulin superfamily consists of both costimulatory and coinhibitory proteins, of which some have a dual costimulatory and coinhibitory function. The tumor necrosis-factor (receptor) (TNF(R)) family only consists of costimulatory immune checkpoints, although may also exhibit immunomodulatory, coinhibitory-like functions. Note that many of the receptor-ligand pairs depicted in this figure are not limited to expression by T-cells and APCs but can be expressed by many cell-types. APC = antigen-presenting cell.

CD28-CD80/CD86, CTLA4-CD80/86, PD-L1/2-PD-1, LAG3 (lymphocyte activation gene 3), ICOS-ICOSL, BTLA, CD200, CD155, TIM3, and VISTA. The most well-known members of the TNF receptor superfamily are CD40L-CD40, OX40-OX40L, CD27-CD70, GITR-GITRL, CD137-CD137L, CD30-CD30L, LTβR-LTα/β, RANK-RANKL, Fn14-TWEAK, and LIGHT-HVEM/LTβR (**Figure 2**). Although each immune checkpoint receptor-ligand pair has a unique function within the immune system that is often cell type specific, as a rule of thumb, Costimulatory immune checkpoints mostly aggravate inflammation, whereas Coinhibitory checkpoints dampen inflammation.

IMMUNE CHECKPOINT–BASED THERAPIES IN ONCOLOGY

Immune checkpoint-based therapies have revolutionized the treatment of patients with a large variety of cancers. Inhibition of Coinhibitory immune checkpoints, activation of Costimulatory checkpoints, or a combination of both efficiently activates cytotoxic T cell responses to effectively kill tumor cells (**Figure 3**). The concept of immune checkpoint-based therapies for the treatment of cancer was pioneered by Dr James Allison, who received the Nobel Prize for this work in 2018, showing that blocking the Coinhibitory immune checkpoint CTLA4 can regress tumors in mice.[26] Later, it was shown that blocking the Coinhibitory immune checkpoint PD-1 or PD-L1 had similar effects.[27] CTLA4 antibodies (ipilimumab) were shown to extend survival among patients with metastatic melanoma,[28,29] and ipilimumab was approved for this disease in 2011, followed by approval for anti-PD-1 therapy (pembrolizumab and nivolumab) in 2014.[30] Since then, the use of immune checkpoint-based immunotherapies has been approved for >50 indications and is now being used in different types of primary and metastasized cancers, including melanoma, non-small cell lung

FIGURE 3 Different Pathologies Require Divergent Immune Checkpoint–Based Immunotherapeutic Strategies



EC = endothelial cell; SMI = small-molecule inhibitor; TCR = T cell receptor; VSMC = vascular smooth muscle cell.

cancer, advanced renal cell carcinoma, gastric and esophageal cancers, urothelial carcinoma, pleural mesothelioma, hepatocellular carcinoma, therapy-resistant colorectal carcinomas, and head and neck squamous cell carcinoma. However, inhibition of the inhibitory immune checkpoint also has its limitations. About 50% of patients are resistant to current treatments, because of "tumor escape" pathways, mostly linked to tumor-associated immune-suppressive mechanisms.[31] Immune checkpoint inhibitor (ICI) therapies are increasingly combined with other treatment modalities, such as chemotherapy, radiotherapy, and targeted therapies by small-molecule inhibitors (SMIs), with the addition of Costimulatory immune checkpoint agonists, thereby enhancing treatment efficacy.

Costimulatory immune checkpoint activation enhances the level of immunologic responses to malignant cells. Agonists against the Costimulatory immune checkpoints OX40, CD137, CD27, ICOS, and GITR, in combination with coinhibitory immune checkpoint antagonists (ie, as bispecific antibodies),[32,33] chemotherapy, or irradiation, have reduced tumor load in experimental models[34,35] and/or have entered the clinical trial arena.[36,37] Recently, it was reported that in patients with advanced pancreatic duct adenocarcinoma, combination treatment with an allogeneic tumor lysate vaccine plus a CD40 agonist enhanced tumor-specific immune responses and was safe.[38] When agonistic CD40 antibodies were combined with chemotherapy, this treatment regimen did not improve survival in patients with pancreatic duct adenocarcinoma.[39] However, a plethora of clinical trials testing the potential of Costimulatory immune checkpoint agonist as part of an immunotherapeutic regimen to treat a diversity of cancers are still ongoing, and the results are eagerly awaited.

IMMUNE CHECKPOINT–BASED THERAPIES IN AUTOIMMUNE DISEASES

Costimulatory and Coinhibitory immune checkpoints are key mediators of chronic inflammatory diseases, including systemic lupus erythematosus (SLE), lupus nephritis, multiple sclerosis, Crohn's disease, Sjögren syndrome, and rheumatoid arthritis, and are considered potent immunotherapeutic targets for this subclass of diseases[21,40] (**Figure 3**, **Central Illustration B**). Abatacept, the first and most administered U.S. Food and Drug Administration–approved drug blocking costimulation, blocks CD80/CD86-mediated costimulation and is an IgG1-CTLA4 fusion protein with a high affinity for CD80/CD86 that prevents CD28-CD80/86 interactions. Abatacept is indicated for the treatment of adult moderate to severe rheumatoid arthritis and acute psoriatic arthritis and for the prevention of graft vs host disease, and it is particularly effective as therapy for patients with rheumatoid arthritis with inadequate response to methotrexate or anti-TNFα treatment.[41] Another Costimulatory dyad that plays a major role in the pathogenesis of autoimmune diseases is CD40-CD40L. CD40-CD40L interactions are crucial for T cell activation, antibody production, and immune globulin (Ig) isotype switching.[42] Treatment with the

anti-CD40L antibody ruplizumab has shown promising results in ameliorating lupus glomerulonephritis,[43] but the trial was halted prematurely because of thromboembolic complications. A different anti-CD40L antibody, dapirolizumab pegol, improved clinical features of SLE, was well tolerated, did not cause thromboembolic complications,[44] and is currently being tested in patients with SLE in a phase 3 trial (NCT04294667). Other anti-CD40L antibodies, including VIB4920, a CD40L-binding protein, and SAR441344, a CD40L monoclonal antibody, have passed their respective phase 1 trials and are currently being tested in phase 2 trials in subjects with rheumatoid arthritis (NCT04163991) and Sjögren syndrome (NCT04572841). Also, antibodies targeting CD40 have entered the clinical trial arena and have shown promising preliminary results in the treatment of patients with psoriasis,[45] rheumatoid arthritis,[46] Graves's disease,[47] and Crohn's disease.[48] Other therapeutics antagonizing Costimulatory immune checkpoints (such as CD28, CD137, ICOS, APRIL, CD40, and CD40L) or antagonizing Coinhibitory immune checkpoints (such as PD-1 and BTLA) have been developed and are currently being tested in phase 1, 2, and 3 trials to evaluate their efficacy in the treatment of autoimmune diseases.[49,50]

IMMUNE CHECKPOINTS IN ATHEROSCLEROSIS

Although immune checkpoint-based therapies have been rapidly integrated in oncology and are increasingly being used in autoimmune diseases, especially rheumatoid diseases, their exploitation in ASCVD is still in a preclinical stage (**Figure 3**). However, numerous preclinical studies have proved that modulation of immune checkpoint pathways has the potential to become a beneficial therapeutic strategy for the treatment of patients with atherosclerosis.[21] An overview of the most important preclinical results of Costimulatory and Coinhibitory immune checkpoint inhibition or activation can be found in **Tables 1 to 3** and **Central Illustration D**. Here, we review the results of the Costimulatory and Coinhibitory immune checkpoints that have shown relevance in human disease and/or have potential to become immunotherapeutic targets to combat ASCVD in humans.

CD28/CTLA4-CD80/86. The CD28/CTLA4-CD80/CD86 Costimulatory and Coinhibitory immune checkpoint pathway, part of the immunoglobulin superfamily, has been studied extensively for many years. Both CD80 and CD86 are expressed on APCs, particularly B cells, dendritic cells, and macrophages, and the expression of CD28 and CTLA4 is confined to T cells. Upon interaction with CD80/CD86, CD28 propagates T cell responses and promotes survival and memory cell formation of $CD4^+$ and $CD8^+$ T cells, whereas CTLA4 limits T cell activation and promotes T_{reg} cell expansion and suppression.[51] In atherosclerotic mouse models, deficiency of CD80/86 reduces atherosclerosis because of diminished T effector cell responses.[52] Interestingly, chimeric $Cd80/Cd86^{-/-}LDLr^{-/-}$ mice show aggravated atherosclerosis because of a reduction in T_{reg} cell numbers.[53] These contradictory data emphasize the power of the CD28/CTLA4-CD80/CD86 pathway to modulate immune responses and determine disease outcomes. Overexpression of Coinhibitory CTLA4 reduced atherosclerotic burden and plaque inflammation as well as aneurysm formation in $Apoe^{-/-}$ mice because of a decrease in effector T cells.[54,55] Antibody-mediated inhibition of CTLA4 accelerated atherosclerosis progression and resulted in T cell-rich plaques with increased necrotic cores.[56] In human atherosclerotic plaques, expression of CD80/CD86 is correlated with plaque vulnerability,[57] and a recent bioinformatics study identified CD86 as 1 of 6 genes driving immune cell activation in human atherosclerosis.[58] Patients with CAD and those at risk for stroke displayed an increased expression of CD80 and CD86 on monocyte-derived dendritic cells and B cells.[59] Moreover, ex vivo inhibition of CD80 with the preclinical anti-CD86 compound RhuDex reduced cytokine production in human atherosclerotic plaques.[60] Experimental studies have shown that abatacept successfully decreases atherosclerosis burden in $Ldlr^{-/-}$ mice and neointima formation in C57Bl/6J mice. The plaques in these mice contained fewer $MHCII^+$ cells, fewer smooth muscle cells, and less collagen, suggesting that CD80/86 inhibition halts the progression of atherosclerosis.[61] Abatacept has also been shown to block age-related heart failure in mice and to ameliorate systolic and diastolic heart function.[62] Although only limited data are available on the effects of abatacept on cardiovascular disease in humans, data obtained from rheumatoid arthritis trials mostly show a beneficial impact of abatacept on ASCVD outcomes (**Central Illustration C**).[63-65] Abatacept treatment resulted in a significant reduction of ASCVD (HR: 0.50; 95% CI: 0.30-0.83) in a study comparing biologic agents with synthetic disease-modifying antirheumatic drugs,[63] and abatacept reduced ASCVD risk in TNFα nonresponders in a 2-year follow-up for myocardial infarction, major adverse cardiovascular events, stroke, and heart failure compared with rituximab.[64] Similar results were found in a study in which patients with rheumatoid arthritis using TNFα inhibitors were compared with

TABLE 1 Experimental Atherosclerosis Studies on Costimulatory and Coinhibitory Immune Checkpoint Members of the Immunoglobulin Superfamily

Immune Checkpoint Dyad	Atherosclerosis Model (Genetic Model/ICI/SMI)	Atherosclerotic Plaque Phenotype	Intraplaque Immune Cell Phenotype	First Author (Year)
Costimulation				
ICOS-ICOSL	Icos$^{-/-}$ Ldlr$^{-/-}$ BM chimera (8-wk atherogenic diet)	AR: ↑ lesion size, ↑ collagen, ↑ αSMA	↑ macrophages, ↑ CD4^{+} T cells	Gotsman et al (2006)[131]
CD28-CD80/CD86	B7-1/B7-2$^{-/-}$ (CD80/CD86) Ldlr$^{-/-}$ (8- and 20-wk atherogenic diet)	AR: ↑ lesion size, ↓ αSMA, ↓ collagen	↓ macrophages (8 wk)	Buono et al (2004)[52]
	Cd80/Cd86$^{-/-}$ Ldlr$^{-/-}$ BM and Cd28$^{-/-}$ Ldlr$^{-/-}$ BM chimera (20-wk atherogenic diet)	AR: ↑ lesion size	ND	Ait-Oufella et al (2006)[53]
Coinhibition				
CTLA4-CD80/CD86	Abatacept (CTLA4 ligand fusion protein) in ApoE3*Leiden; cuffed FA	FA: ↓ intimal thickening, ↓ lumen stenosis, ↑ αSMA	↓ macrophages and CD3^{+} T cells	Ewing et al (2013)[61]
	Anti-CTLA4, ApoE3*Leiden; cuffed FA	FA: ↑ intimal thickening, ↑ lumen stenosis	↑ CD3^{+} T cells	Ewing et al (2013)[61]
	Ctla4Tg ApoE$^{-/-}$ (16-wk NCD)	AR: ↓ lesion size	↓ macrophages, ↓ CD4^{+} T cells	Matsumoto et al (2016)[54]
	Anti-CTLA4 (9D9) in Ldlr$^{-/-}$ (6-wk atherogenic diet)	AR: no effect on lesion size, ↑ PIT and FCA, ↑ necrotic core	↑ CD4^{+} T cells	Poels et al (2020)[56]
PD-1-PD-L1/2	Pd-l1/l2$^{-/-}$ Ldlr$^{-/-}$ (6-wk and 16-wk atherogenic diet)	AR: ↑ lesion size, ↑ collagen, ↑ αSMA	↑ macrophages, ↑ CD4^{+}/CD8^{+} T cells	Gotsman et al (2007)[102]
	Pd-1$^{-/-}$Ldlr$^{-/-}$ (5-wk and 10-wk atherogenic diet)	AR: ↑ lesion size (10 wk)	↑ macrophages, ↑ CD4^{+}/CD8^{+} T cells	Bu et al (2011)[103]
	Anti-PD-1 in Ldlr$^{-/-}$ (5-wk atherogenic diet)	No effect on lesion size	↑ CD4^{+}/CD8^{+} T cells	Bu et al (2011)[103]
	Pd-1$^{-/-}$Ldlr$^{-/-}$ (9-wk atherogenic diet)	AR: ↑ lesion size	↑ macrophages, ↑ CD4^{+}/CD8^{+} T cells, ↑ T_{reg} cells	Cochain et al (2014)[132]
	Anti-PD-1 (BE0146) and anti-CTLA4 (BE0164) in Ldlr$^{-/-}$ (6-wk atherogenic diet)	AA/AR: no effect on lesion size, ↓ IX, ↑ PIT, ↑ necrotic core	↓ macrophages, ↑ CD3^{+} T cells	Poels et al (2020)[104]
	PD-1 agonist (PIM-2) in Ldlr$^{-/-}$ (6-wk atherogenic diet)	AR: ↓ lesion size	↓ CD4^{+} T cells	Grievink et al (2021)[105]
TIGIT-CD155/CD112/CD113	Anti-TIGIT agonist in Ldlr$^{-/-}$ (4- and 8-wk atherogenic diet)	AR: no effect on lesion size	No effect	Foks et al (2013)[133]
BTLA-HVEM	Anti-BTLA agonist (3C10) in Ldlr$^{-/-}$ (6- and 10-wk atherogenic diet)	AR: ↓ lesion size (6 wk), ↑ collagen (10 wk)	↓ CD4^{+} T cells	Douna et al (2020)[134]
CD200R-CD200	Cd200$^{-/-}$ ApoE$^{-/-}$ (27-wk NCD)	AR: ↑ lesion size, ↑ necrotic core, ↓ αSMA	↑ macrophages	Kassiteridi et al (2021)[124]
LAG3	PCSK9 (rAAV2/8 mediated) in Lag3$^{-/-}$; anti-LAG3 (C9B7W) in Ldlr$^{-/-}$; anti-LAG3 (C9B7W)/anti-PD-1 (29F.1A12) in Ldlr$^{-/-}$; Lag3$^{-/-}$ Ldlr$^{-/-}$ BM chimera (all 6- to 10-wk atherogenic diet)	AR: no effect on lesion size	↑ CD4^{+} T cells	Mulholland et al (2022)[116]
TIM-3-galectin 9	Anti-TIM-3 (RMT3-23) in Ldlr$^{-/-}$ (8-wk atherogenic diet)[a]	AA/AR: ↑ lesion size	↑ macrophages	Foks et al (2013)[132]
Costimulation/coinhibition				
TIM-1-TIM-4	Anti-TIM-1 (3D10) and anti-TIM-4 (21H12) in Ldlr$^{-/-}$ (4-wk atherogenic diet)	AR: ↑ lesion size	↑ CD4^{+} T cells (only anti-TIM-1)	Foks et al (2016)[135]
	Tim-1Δmucin with PCSK9 (rAAV2/8-D377Y-mPCSK9) (13-wk atherogenic diet)	AR: ↑ lesion size	↑ macrophages, ↓ CD8^{+} T cells	Douna et al (2022)[136]

Up arrows indicate increases, and down arrows indicate decreases. [a]No isotype control included.

AA = aortic arch; αSMA = alpha smooth muscle actin; ApoE = apolipoprotein E; AR = aortic root; BM = bone marrow; BTLA = B- and T-lymphocyte attenuator; CTLA4 = cytotoxic T-lymphocyte-associated protein 4 (also known as CD152); FA = femoral artery; FCA = fibrous cap atheroma; HVEM = herpes virus entry mediator; ICI = immune checkpoint inhibitor; ICOS(L) = inducible T cell costimulator (ligand); IX = intimal xanthoma; LAG3 = lymphocyte-activating gene 3; Ldlr = low-density lipoprotein receptor; NCD = normal chow diet; ND = not determined; PD-1 = programmed cell death protein 1; PD-L1 = programmed death ligand 1; PD-L2 = programmed cell death 1 ligand 2; PIT = pathological intimal thickening; rAAV2/8-D377Y-mPCSK9 = recombinant adeno-associated virus vector serotype 2/8-D377Y overexpression of murine proprotein convertase subtilisin/kexin type 9; SMI = small-molecule inhibitor; Tg = transgenic–knock-in to induce overexpression; TIGIT = T cell immune receptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains; Tim-1Δmucin =Tim-1 mucin-domain deficiency; TIM-1/3/4 = transmembrane immunoglobulin and mucin domain-1/3/4; T_{regs} = regulatory T.

TABLE 2 Experimental Atherosclerosis Studies on Costimulatory Immune Checkpoint Members of the TNF(R) Superfamily

Immune Checkpoint Dyad	Atherosclerosis Model (Genetic Model/ICI/SMI)	Atherosclerotic Plaques Phenotype	Atherosclerotic Plaques Phenotype	Intraplaque Immune Cell Phenotype	First Author (Year)
CD40-CD40L	CD40L	Anti-CD40L in Ldlr$^{-/-}$ (12-wk atherogenic diet)	AA: ↓ wall area, ↓ wall thickness, ↓ lipid content	↓ macrophages and CD3^{+} T cells, ↓ V-CAM	Mach et al (1998)[82]
		Cd40l$^{-/-}$ApoE$^{-/-}$ (23-wk NCD)	AA: ↓ lesion size, ↑ collagen	↓ macrophages and CD3^{+} T cells	Lutgens et al (1999)[79]
		Anti-CD40L in ApoE$^{-/-}$: 5 wk old (early stage), and 17 wk old (late stage) (12-wk NCD)	AA: no effect on lesion size, ↑ collagen, ↑ αSMA (early and late); ↑ fibrous cap, ↓ lipid content (late)	↓ CD3^{+} T cells (early); ↓ macrophages, ↓ CD3^{+} T cells (late)	Lutgens et al (2000)[80]
		Anti-CD40L in Ldlr$^{-/-}$ (13-wk atherogenic diet)	AA: ↓ wall area and thickness, ↓ collagen, ↑ αSMA, ↓ lipid content	↓ macrophages	Schonbeck et al (2000)[81]
		Cd40l$^{-/-}$Ldlr$^{-/-}$ (16-wk atherogenic diet)	AA/AR: ↓ lesion size, ↑ collagen, ↑ αSMA, ↓ lipid content	↓ macrophages	Bavendiek et al (2005)[137]
		Cd40l$^{-/-}$Ldlr$^{-/-}$ BM chimera (16-wk atherogenic diet)	AA/AR: no effect on lesion size	No effect	Bavendiek et al (2005)[137]
		Cd40l$^{-/-}$Ldlr$^{-/-}$ BM chimera (20-wk atherogenic diet)	AR: no effect on lesion size	No effect	Smook et al (2005)[138]
		Adoptive transfer of thrombin-activated Cd40l$^{-/-}$ platelets in ApoE$^{-/-}$ (29-wk NCD) and in collar-induced model (6-wk atherogenic diet)	AA/AR: ↓ lesion size, ↓ collagen (collar induced) CA: ↓ lesion size, ↓ αSMA	Collar induced: ↓ macrophages	Lievens et al (2010)[72]
		cM7 peptide in Ldlr$^{-/-}$ (20-wk atherogenic diet)	AR: ↓ lesion size, ↑ collagen, ↓ lipid content	↓ macrophages	Wolf et al (2011)[98]
		Cd4-CreTgCd40lfl/fl ApoE$^{-/-}$ (28-wk NCD)	AR: ↓ lesion size, ↓PIT, ↓ FCA, ↑ IX, ↑ fibrous cap, ↑ αSMA, ↓ necrotic core	↑ macrophages, ↓ CD4^{+} T cells	Lacy et al (2021)[83]
		Pf4-CreTg Cd40lfl/fl ApoE-/; WI model (28-wk NCD)	Only with WI: ↓ lesion size (↓ atherothrombosis)	ND	Lacy et al (2021)[83]
	CD40	Cd40$^{-/-}$Ldlr$^{-/-}$ (16-wk atherogenic diet)	AA: no effect on lesion size, ↑ lipid content	↑ macrophages	Zirlik et al (2007)[139]
		Cd40$^{-/-}$ApoE$^{-/-}$ (26-wk NCD)	AA: ↓ lesion size, ↑ collagen, ↑ αSMA	↓ macrophages, ↓ T cells	Lutgens et al (2010)[73]
		Adoptive transfer of thrombin-activated Cd40$^{-/-}$ platelets in ApoE$^{-/-}$ (29-wk NCD)	AA: ↓ lesion size, ↓ collagen, ↑ FCA and PIT, ↓ lipid content	↓ macrophages	Gerdes et al (2016)[140]
		TRAF-6/SMI 6877002 and 6860766 in ApoE$^{-/-}$ (18-wk [early] and 28-wk [advanced] NCD)	AA: ↓ lesion size, ↓ FCA, ↑ IX (early); ↓ lesion size, ↑ collagen, ↓ necrotic core (advanced)	↓ macrophages, ↓ CD3^{+} cells (early); ↓ macrophages (advanced)	Seijkens et al (2018)[96]
		TRAF-6i-HDL with SMI6877002 in ApoE$^{-/-}$ (12-wk atherogenic diet)	ND	↓ macrophages and monocyte in aorta	Lameijer et al (2018)[97]
		Bmx-CreERT2/CD40fl/fl ApoE$^{-/-}$ (25-wk NCD)	AR: no effect on lesion size, ↓ lipid content, ↑ collagen, ↑ αSMA	↓ macrophages	Gissler et al (2021)[84]
		Cd11c-CreTg/Cd40fl/fl ApoE$^{-/-}$ (28-wk NCD)	AR: ↓ lesion size, ↑ αSMA	↓ CD4^{+} T cells	Lacy et al (2021)[83]
		LysM-CreTgCd40fl/fl-ApoE$^{-/-}$ (14-wk atherogenic diet)	AA: ↓ lesion size, ↓ necrotic core AR: ↓ lipid content	↓ macrophages	Bosmans et al (2023)[74]
		AdipoQ-CreTgCd40fl/fl-ApoE$^{-/-}$ (11-wk atherogenic diet)	AR: ↓ lesion size, ↑ necrotic core	↓ CD3^{+} T cells	Reiche et al (2023)[85]

Up arrows indicate increases, and down arrows indicate decreases.

AdipoQ = adiponectin; Bmx-CreERT2 = tamoxifen-inducible bone marrow × Cre recombinase; CA = carotid artery; Cre = Cre recombinase expression under tissue-specific promoter; fl/fl = "floxed" gene: flanked by loxP sites to be recognized by Cre recombinase; HDL = high-density lipoprotein; LysM = lysozyme M; Pf4 = platelet factor 4; TRAF = tumor necrosis factor receptor-associated factor; V-CAM = vascular cell adhesion molecule; WI = wire injury; other abbreviations as in Table 1.

abatacept users using the Medicare and MarketScan databases. Abatacept was associated with a 20% reduced risk for major adverse cardiovascular events, and this reduction was particularly true for patients with diabetes.[65] However, when abatacept was compared with tocilizumab in patients with anti-TNFα treatment-resistant rheumatoid arthritis, no beneficial effects on ASCVD outcomes were found.[66] Well-designed clinical trials testing the effects of abatacept in an ASCVD setting are still awaited.

TABLE 3 Experimental Atherosclerosis Studies on Costimulatory Immune Checkpoint Members of the TNF(R) Family

Immune Checkpoint Dyad	Atherosclerosis Model (Genetic Model/ICI/SMI)	Atherosclerotic Plaques Phenotype	Intraplaque Immune Cell Phenotype	First Author (Year)
OX40/OX40L	Anti-OX40L (RM134) in $Ldlr^{-/-}$ 2-wk atherogenic diet; CA collar placement	CA/AR: ↓ lesion size	No effect on macrophages	Wanrooij et al (2007)[141]
	$Ox40l^{-/-}ApoE^{-/-}$ (8-wk atherogenic diet)	AR: ↓ lesion size	No effect on macrophages	Nakano et al (2010)[142]
	$Ox40l^{-/-}ApoE^{-/-}$ BM chimera (8-wk atherogenic diet)	No effect on lesion size	ND	Nakano et al (2010)[142]
	Anti-OX40L (MGP34) in $ApoE^{-/-}$ (16-wk atherogenic diet)	AR: ↓ lesion size	No effect on macrophages	Nakano et al (2010)[142]
	Anti-OX40L (RM134) in $Ldlr^{-/-}$ (10-wk atherogenic diet)	AA/AR: ↓ lesion size	↓ macrophages, ↓ mast cells	Foks et al (2013)[143]
CD137/CD137L	CD137 agonist (2A) in $ApoE^{-/-}$ (16-wk NCD)	Lesion size: ND	↑ MHC-II+ cells, ↑ $CD8^+$ T cells	Olofson et al (2008)[144]
	$Cd137^{-/-}ApoE^{-/-}$ (66-wk NCD) and $Cd137^{-/-}Ldlr^{-/-}$ (18-wk atherogenic diet)	AR: ↓ lesion size	↓ TNFα and ↓ MCP-1	Jeon et al (2010)[145]
GITR/GITRL	$GitrlTg^{-/-}Ldlr^{-/-}$ BM chimera (11-wk atherogenic diet)	AR: ↓ lesion size	↑ $CD3^+$/$FoxP3^+$ T cells (T_{reg} cells)	Meiler et al (2016)[122]
	$Gitr^{-/-}ApoE^{-/-}$ (28-wk NCD)	AR: ↓ lesion size, ↓ necrotic core, ↓ vulnerability index	↓ macrophages	Shami et al (2020)[123]
CD27/CD70	$Cd27^{-/-}ApoE^{-/-}$ BM chimera (18- and 28-wk NCD)	AR: ↑ lesion size, ↑ necrotic core, ↑ FCA	↑ macrophages, ↓ FoxP3+ T cells (T_{reg} cells)	Winkels et al (2017)[146]
	$Cd70^{-/-}ApoE^{-/-}$ BM chimera (7-wk atherogenic diet)	AR: ↑ lesion size, ↑ necrotic core, ↑ FCA	↑ macrophages	Winkels et al (2017)[147]
CD30/CD30L	Anti-CD30L in $Ldlr^{-/-}$ (8-wk atherogenic diet)	AR: ↓ lesion size	No effect on macrophages	Foks et al (2012)[148]

Up arrows indicate increases, and down arrows indicate decreases.

FoxP3 = forkhead box protein P3; GITR(L) = glucocorticoid-induced tumor necrosis factor receptor-related protein (also known as CD357, TNFRSF18 [tumor necrosis factor receptor superfamily, member 18]) (ligand); MCP-1 = monocyte chemoattractant protein-1, CC chemokine ligand CCL2; MHC-II = major histocompatibility complex II; OX40(L) = tumor necrosis factor receptor superfamily, member 4, CD134 (ligand); TNF-α/R = tumor necrosis factor-alpha/-receptor; other abbreviations as in Tables 1 and 2.

Although these studies demonstrate that the CD28/CTLA4-CD80/86 axis is involved in atherogenesis and that targeting the CD28/CTLA4-CD80/86 axis can reduce atherosclerosis and ASCVD, insights into potential side effects are lacking. The CD28/CTLA4-CD80/86 pathway is tightly regulated and involves more cell types and functions than just T cell-APC interactions. CD80/CD86 is present on many cell types, including B cells, dendritic cells, and macrophages,[25] but also endothelial cells[67,68] and even T cells.[69] Whereas dendritic cell CD80/86 affects T cell activation and proliferation, macrophage CD80/86 mediates macrophage activation and cytokine production.[23] B cell CD80/86 facilitates antibody production, and endothelial cell CD80/86 mediates T cell adhesion, activation, and migration.[25,67,68] Moreover, CD86 is involved in the maintenance and homeostasis of T_{reg} cells, and absence of CD80/CD86 strongly decreases the presence of T_{reg} cells and causes inflammation.[53,70] The same is true for CTLA4. Although CTLA4's main function seems to be the coinhibition of T cells,[25] CTLA4 expression was also found on many myeloid and lymphoid lineages, and its exact functions remain to be determined.[71] CD28 seems to be expressed only on T cells, but its effects on atherosclerosis have not been described in detail.[53] As the CD28/CTLA4-CD80/86 pathway is tightly regulated, inhibiting one of its components will cause adaptations of its other components or the involvement of other Costimulatory or Coinhibitory systems to maintain tissue homeostasis.

The tight regulation of the CD28/CTLA4-CD80/CD86 pathway, the wide variety of cell types that can express these molecules, and the diversity of its functions warrant a more detailed investigation of the role of this pathway in atherosclerotic disease. Until now, most studies have been performed in global knockout mouse models or bone marrow chimeras. Using cell type-specific gain- and loss-of-function models, more detailed insights into the cell type-specific functions and regulation of the CD28/CTLA4-CD80/CD86 pathway in atherosclerosis will be obtained. Better systemwide analyses of the effects of targeting CD28, CTLA4, CD80, or CD86, especially effects on systemic immune cell composition and activation, as well as composition of the atherosclerotic plaque in these models, will further pinpoint the cell type-specific roles of CD28/CTLA4-CD80/CD86 interactions, as well as its regulation in atherosclerosis. This knowledge will enable us to use cell-

targeted immunotherapeutic strategies to reduce atherosclerosis, while preventing immune dysregulation after targeting CD28/CTLA4-CD80/CD86 pathway components.

CD40L-CD40. CD40L-CD40 is a potent Costimulatory immune checkpoint dyad of the TNF-TNFR family. CD40L is expressed predominantly on T cells and platelets but can also be found on other cell types, including endothelial cells, vascular smooth muscle cells, mast cells, and natural killer cells. CD40, its receptor, is expressed by the typical APCs (B cells, dendritic cells, and macrophages) but also by neutrophils, platelets, endothelial cells, vascular smooth muscle cells, fibroblasts, adipocytes, and epithelial cells. During T cell-APC interactions, binding of CD40L to CD40 results in an effector T cell response, maturation of dendritic cells, and antibody production and Ig isotype switching in B cells.[42] CD40L-CD40 interactions between other cell types can result in platelet-leukocyte aggregation,[72] leukocyte adhesion to the endothelium,[73] and macrophage activation.[74]

CD40L-CD40 interactions play a key role in atherosclerosis. In humans, soluble (s)CD40L and/or sCD40 levels are correlated to ASCVD, including recurrent myocardial infarction and/or stroke,[75,76] and several single-nucleotide polymorphisms in CD40 have been associated with (features of) ASCVD.[77] Oxidized LDL induces up-regulation of the CD40/CD40L signaling pathway in human coronary endothelial cells, through its receptor LOX-1, an induces endothelial cell activation.[78] Genetic and/or antibody-mediated inhibition of CD40L or CD40, even when inhibited in established atherosclerosis, is highly effective in reducing atherosclerosis and generates plaques that are rich in collagen and contain a limited number of immune cells, the murine equivalent of a stable, clinically safe, atherosclerotic plaque.[73,79-82] Both CD40L and CD40 exert cell type-specific actions during atherogenesis. T cell CD40L drives atherosclerosis, and its deficiency results in a reduced T-helper cell type 1 (Th1) response, accompanied by reduced IFNγ levels.[83] In contrast, deficiency of platelet CD40L does not affect atherogenesis but does ameliorate atherothrombosis-induced plaque growth.[83] Absence of platelet CD40 or CD40L prevents the acceleration of atherosclerosis induced by thrombin induced platelet activation.[72] Deficiency of CD40 on dendritic cells mimics the results of T cell CD40L deficiency, with a reduction in atherosclerosis due to a deficient Th1 response.[83] Deficiency of macrophage CD40 also reduces atherosclerosis and reduces necrotic core formation. Absence of macrophage CD40 does not affect Th1 responses but reduces macrophage activation and enhances efferocytosis[74] and has no effects on the plaque's extracellular matrix content.[74] Deficiency of endothelial cell CD40 increases atherosclerotic plaque stability,[84] and deficiency of adipocyte CD40 decreased atherosclerotic plaques size but increased the number of lymphoid and myeloid progenitors in the bone marrow and resulted in an increase in T cell numbers in the plaques and increased necrotic core sizes.[85] These data highlight the cell-divergent function of CD40L-CD40 interactions in atherosclerosis.

Although these global and conditional experimental mouse models provided great insights into the (cell-divergent) role of CD40 and CD40L in atherosclerosis, and the potential for blocking this pathway as a therapy for atherosclerosis, these data should still be interpreted with care. Global CD40(L)-knockout mice have no CD40(L) during development or life, and compensatory pathways overcoming the effects of CD40(L) deficiency may have developed. Moreover, the Cre drivers that were used to determine cell type-specific effects of $Cd40^{fl/fl}ApoE^{-/-}$ and $Cd40l^{fl/fl}$ $ApoE^{-/-}$ mice are not 100% depleting or 100% cell type-specific, and the majority of the Cre drivers that were used depleted CD40(L) early in development. For example, $CD4^{cre}$ depletes all T cell lineages of CD40L, including T-helper cells, cytotoxic T cells, and T_{reg} cells, which makes detailing the role of CD40L in the different T cell subsets impossible.[86] The $LysM^{cre}$ driver, used to deplete CD40 in macrophages, will also deplete CD40 in neutrophils, and its depletion efficacy is only about 70%,[87] which will mask the potential immunosuppressive effects that lack of macrophage CD40 could cause. The $CD11c^{cre}$ driver has >90% depletion but in addition to dendritic cells will also deplete CD40 on $CD11c^{+}$ macrophages.[88] The $PF4^{cre}$ driver, used to deplete platelets of CD40L, has also been reported to induce gene depletion in macrophages,[89] although this will be of limited impact in our study, as macrophage CD40L expression is very low to absent. Better, more cell type-specific and inducible Cre drivers should be used to study the role of CD40(L) in future studies, as well as cell-targeted therapeutics with higher cell type specificity (cell-targeted nanoparticles, bispecific antibodies) than the current cell-lineage cre drivers.

The cell-divergent function of the CD40-CD40L dyad is also reflected in its signaling pathways. B cells rely mostly on CD40-TNF receptor-associated factor 2 (TRAF2)[90] and CD40-TRAF3[91] interactions, which can have counteracting effects. In endothelial cells, CD40 signaling via TRAF1, TRAF3, and TRAF6 reduce inflammation, whereas CD40-TRAF2 and CD40-TRAF5 signaling aggravates inflammation.[92] Monocytes and macrophages predominantly exhibit

CD40-TRAF6 signaling for their activation.[93] In atherosclerosis, blocking CD40-TRAF6 but not CD40-TRAF2/3/5 signaling in MHCII$^+$ cells results in a significant reduction in plaque size, which was due mostly to reductions in monocyte recruitment and macrophage activation.[73] The administration of an SMI that was designed to block CD40-TRAF6 signaling[94,95] to ApoE$^{-/-}$ mice was able to reduce the initiation and progression of atherosclerosis, even when given to mice with established atherosclerosis.[96] This immunotherapy was safe and selective to macrophage function, as treated ApoE$^{-/-}$ mice did not show any immune-suppressive side effects: antibody production and Ig isotype switching were still intact, and antigen-dependent T cell proliferation was not affected.[96] This SMI's specific delivery to macrophages using lipoprotein-based nanobiologic agents stabilized atherosclerotic plaques and is safe in nonhuman primates.[97]

Besides classical CD40L-CD40 interactions, the nonclassical interaction between CD40L and Mac-1 on monocytes plays a significant role in leukocyte recruitment in atherosclerosis. Therefore, a peptide that specifically blocks CD40L at its Mac-1 interaction site was designed (cM7) and was shown to reduce leukocyte recruitment in peritonitis and atherosclerosis.[98] In a follow-up study, Wolf et al[99] designed a monoclonal antibody against the CD40L interaction site at Mac-1 (anti-M7), which was highly effective in reducing myeloid cell recruitment in a murine model of sterile sepsis.

Although outcomes of preclinical studies have shown high potential of the CD40-CD40L dyad as therapeutic target in atherosclerosis, clinical studies blocking CD40(L) in ASCVD are still being awaited, despite the many ongoing phase 1 and 2 studies that target CD40(L) in classical autoimmune diseases. One of the major reasons is the chronicity and complexity of atherosclerosis. Atherosclerosis develops over decades, and is a continuous inflammatory process, but remains asymptomatic until a plaque erosion or rupture results in occlusion of the artery, so defining a treatment window is difficult.[100] We also have not yet fully grasped the actions of the CD40L-CD40 dyad in atherogenesis, and on the basis of preclinical data, cell-targeted anti-CD40(L) therapies, therapies targeting CD40-signaling intermediates, or therapies targeting nonclassical CD40L interactions may be preferred to complete blockade of CD40(L) to circumvent immune-suppressive side effects. As many trials are currently testing the clinical effects of blocking CD40L or CD40 in human autoimmune diseases, cardiovascular readouts of these trials will become available. Meanwhile, the (pre)clinical development of targeted CD40(L)-based immunotherapeutic agents for ASCVD, including anti-M7 antibodies[99] and CD40-TRAF6 SMIs,[96,97] specifically designed for treatment of human ASCVD, are being awaited.

PD-1-PD-L1. As described earlier, PD-1 is a Coinhibitory immune checkpoint of the immunoglobulin superfamily that is expressed predominantly on T cells, whereas its ligands, PD-L1 and PD-L2, can be found on a plethora of cell types, including APCs, endothelial cells, and tumor cells.[101] Although PD-1-PD-L1/2 interactions have been described predominantly in the context of cancer, this receptor-ligand dyad also plays a significant role in atherosclerosis. In preclinical mouse models, genetic deficiency of PD-L1/2 and antibody treatment with PD-1 antagonists increase atherosclerosis and results in an increased amount of CD4$^+$ and CD8$^+$ T cells into the arterial wall.[102,103] Short-term antibody-mediated blockade of PD-1 and CTLA4 in LDLr$^{-/-}$ mice, mimicking a combination therapy often used for the treatment of patients with melanoma, resulted in a vulnerable, proinflammatory atherosclerotic plaque phenotype. This phenotype was caused by an excess of effector T cells that resulted in endothelial activation and increased recruitment of CD8$^+$ T cells within the atherosclerotic lesion.[104] Treatment of LDLr$^{-/-}$ mice with an agonistic PD-1 antibody resulted in a reduction of atherosclerosis due to a decrease in the number of IFNγ-producing CD4$^+$ effector cells and cytotoxic CD8$^+$ T cells, an increase in immunomodulatory IL-10$^+$ CD4 T cells, as well as an increase in regulatory B cells, supporting the potential of stimulating PD-1 as a strategy to reduce atherosclerosis.[105]

In humans with cardiovascular disease, the expression of PD-1 and PD-L1 was reduced on T cells and dendritic cells compared with healthy control subjects and was associated with increased T cell activity.[106] In recent reports that unraveled the composition of advanced human carotid plaques using single-cell RNA sequencing, high levels of PD-1 were observed on plaque T cells, which was associated with T cell exhaustion pathways, occurring after T cell activation.[107] soluble PD-L1 levels are increased in patients with CAD and reflect its severity.[108,109] The relevance of PD-1-PD-L1 interactions in human atherosclerosis became clear from the impact of PD-1/PD-L1/2 inhibition in patients treated for malignancies. Coinhibitory immune checkpoint inhibition was associated with an increased risk for cardiovascular events in oncology patients. In a systematic review analyzing results from 10,106 patients treated with anti-CTLA4 or anti-PD-1 antibodies or both, the incidence of arterial thrombotic events (myocardial

infarction or stroke) was found to be 1.1%.[110] In another study involving 2,842 patients with cancer, the incidence of atherosclerotic cardiovascular events, defined as a composite of myocardial infarction, coronary revascularization, or ischemic stroke, increased 4.7-fold in patients who were treated with coinhibitory ICIs.[111] Moreover, anti-PD-1 treatment increased the risk for venous and arterial thromboembolism, suggesting that PD-1 blockade results in a prothrombotic state, although mechanisms are still unknown.[112,113] These studies not only increase awareness to the fact that inhibiting Coinhibitory immune checkpoints can increase the risk for cardiovascular events in patients with cancer[114] but also suggest the importance of coinhibition, and PD-1-PD-L1 interactions in particular, in keeping atherosclerotic plaque inflammation under control.[115] However, additional preclinical studies detailing the regulation of PD-1-PD-L1/2 interactions in atherosclerosis, the PD(L1/2)-expressing cell types, and their signaling pathways involved are needed before being able to apply and design PD(L1/2)-based immunotherapeutic agents in ASCVD.

LAG3. LAG3 (CD223) is a Coinhibitory molecule that is expressed on several immune cell subsets but is predominantly known for its effects on T cells. LAG3 interacts with MHCII and galectin 3 and negatively modulates T cell activation and proliferation.[71] Absence or inhibition of LAG3 does not affect atherosclerosis burden in mice but does result in an enhanced Th1 response and an increase in T cell infiltration in the atherosclerotic lesions, suggesting a potent role of LAG3 in reducing T cell activation in atherosclerosis.[116] In oncology, the most recent Food and Drug Administration approved ICI is relatlimab, which blocks the activation of LAG3. Anti-LAG3 antibody (relatlimab) treatment in combination with anti-PD-1 antibody (nivolumab) treatment was superior to nivolumab treatment alone in patients with advanced melanoma,[117,118] and relatlimab/nivolumab combination therapy has been approved for this indication. Human data on the effects of LAG3 treatment on ASCVD are not yet available, but as LAG3 and PD-1 blockade results in increased atherosclerotic plaque inflammation, cardiovascular health in these patients should be monitored carefully.

SUMMARY AND OUTLOOK

The realization that inflammation is a pathogenic driver of human ASCVD, together with the availability of more detailed single-cell data on the types and function of immune cells in arteries and atherosclerotic plaques,[119] has spiked the search for novel immunotherapeutic targets and the development of novel drugs that are suitable for targeted therapeutic approaches for ASCVD beyond the classic risk factors.[5] In this review, we discussed the potential and pitfalls of applying immune checkpoint-based therapeutics, especially those targeting CD28/CTLA4-CD80/CD86, CD40L-CD40, and PD-1-PD-L1/2, for the treatment of ASCVD.

Besides the receptor-ligand dyads mentioned herein, additional immune checkpoints may have great potential as therapeutic targets for patients with ASCVD. However, until now, only limited experience has been obtained with human treatment with their (ant)agonists specifically for this purpose. For example, GITR, a Costimulatory immune checkpoint of the TNFR family, is well known for its costimulatory functions to enhance anti-inflammatory functions in T_{reg} cells.[120,121] Overexpression of GITR on T cells, induced by GITRL overexpression on B cells, resulted in an increase in T_{reg} cells and a reduction of early stages of atherosclerosis.[122] In atherosclerotic plaques, GITR was also expressed on macrophages, and deficiency of GITR in advanced atherosclerosis reduced monocyte recruitment to the arterial wall and prevented macrophage activation and necrotic core formation, thereby reducing atherosclerosis.[123] Expression of GITR in human carotid atherosclerotic plaques was associated with cerebrovascular events, and levels of sGITR, which we found to be predominantly shed by macrophages, were significantly elevated in patients with ASCVD, showing relevance for GITR signaling in human ASCVD.[123] The CD200-CD200R pathway protects against atherosclerosis and can limit monopoiesis, monocyte recruitment, and macrophage activation in experimental atherosclerosis. CD200R on classical monocytes of patients with ASCVD is associated with a lower burden of CAD and a more stable plaque phenotype.[124]

Although members of both the Costimulatory and Coinhibitory immune checkpoints, such as CTLA4, PD1, PD-L1, CD80/86, CD40, and CD40L, have proved to be efficient therapies for a plethora and autoimmune diseases, their translation into the cardiovascular disease arena lags behind that of other indications. The diversity and complexity of the immune response in atherosclerosis, together with the intricate network, cellular diversity, and alternative signaling pathways of Costimulatory and Coinhibitory immune checkpoints, make their implementation as therapeutics for ASCVD challenging. Moreover, an important phenomenon is the changing immune checkpoint landscape during ASCVD. One

example is that patients with CAD had defective viral clearance by their macrophages because of upregulation of the Coinhibitory immune checkpoint CD155,[125] which was induced by LDL. To unlock the full translational potential of targeting immune checkpoints, additional knowledge is needed. As highlighted herein, many studies were performed in global knockout mice or conditional knockout mice with Cre drivers that target multiple cell types. Moreover, the cell type-specific expression patterns, their signal transduction pathways used, and their receptor-ligand interactions throughout the different stages of atherosclerosis are still unknown. The mouse models that were used in these studies represent human atherosclerotic disease but do not reach 100% similarity with human atherosclerosis.[5] Major differences include the time span over which the disease develops, the absence of atherosclerosis-related cardiovascular disease events such as myocardial infarction and stroke, as well as differences in immunology (as reviewed by Shay et al).[126] Furthermore, an integrated understanding of cell type-specific immune responses of immune checkpoint proteins in human atherosclerosis is still lacking.[127] Filling these knowledge gaps, together with the implementation of current cell-targeted therapeutic modalities such as nanomedicinal strategies,[128] bispecific antibodies,[129] and chimeric antigen receptor T cells[130] will hopefully contribute to the development of feasible, successful strategies enabling immune checkpoint-based immunotherapies to treat patients with ASCVD.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

Dr Lutgens is the U.S. coordinator of the 2022 LeDucq Network of Excellence CHECKPOINT ATHERO. Drs Nitz and Lerman are members of the 2022 LeDucq Network of Excellence CHECKPOINT ATHERO. The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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REFERENCES

1. Timmis A, Townsend N, Gale CP, et al. for the Atlas Writing Group. European Society of Cardiology: cardiovascular disease statistics 2019 (executive summary). *Eur Heart J Qual Care Clin Outcomes*. 2020;6:7–9.

2. Arnett DK, Blumenthal RS, Albert MA, et al. 2019 ACC/AHA guideline on the primary prevention of cardiovascular disease: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol*. 2019;74(10):e177–e232.

3. Knuuti J, Wijns W, Saraste A, et al. 2019 ESC guidelines for the diagnosis and management of chronic coronary syndromes. *Eur Heart J*. 2020;41:407–477.

4. Soehnlein O, Libby P. Targeting inflammation in atherosclerosis—from experimental insights to the clinic. *Nat Rev Drug Discov*. 2021;20:589–610.

5. Engelen SE, Robinson AJB, Zurke YX, Monaco C. Therapeutic strategies targeting inflammation and immunity in atherosclerosis: how to proceed? *Nat Rev Cardiol*. 2022;19(8):522–542.

6. Mallat Z, Binder CJ. The why and how of adaptive immune responses in ischemic cardiovascular disease. *Nat Cardiovasc Res*. 2022;1:431–444.

7. Ridker PM, Everett BM, Thuren T, et al. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med*. 2017;377:1119–1131.

8. Nidorf SM, Eikelboom JW, Budgeon CA, Thompson PL. Low-dose colchicine for secondary prevention of cardiovascular disease. *J Am Coll Cardiol*. 2013;61:404–410.

9. Nidorf SM, Fiolet ATL, Mosterd A, et al. Colchicine in patients with chronic coronary disease. *N Engl J Med*. 2020;383:1838–1847.

10. Tardif JC, Kouz S, Waters DD, et al. Efficacy and safety of low-dose colchicine after myocardial infarction. *N Engl J Med*. 2019;381:2497–2505.

11. Broch K, Anstensrud AK, Woxholt S, et al. Randomized trial of interleukin-6 receptor inhibition in patients with acute ST-segment elevation myocardial infarction. *J Am Coll Cardiol*. 2021;77:1845–1855.

12. Kleveland O, Kunszt G, Bratlie M, et al. Effect of a single dose of the interleukin-6 receptor antagonist tocilizumab on inflammation and troponin T release in patients with non-ST-elevation myocardial infarction: a double-blind, randomized, placebo-controlled phase 2 trial. *Eur Heart J*. 2016;37:2406–2413.

13. Ridker PM, Devalaraja M, Baeres FMM, et al. IL-6 inhibition with ziltivekimab in patients at high atherosclerotic risk (RESCUE): a double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet*. 2021;397:2060–2069.

14. Ridker PM. From RESCUE to ZEUS: will interleukin-6 inhibition with ziltivekimab prove effective for cardiovascular event reduction? *Cardiovasc Res*. 2021;117:e138–e140.

15. Jarr KU, Nakamoto R, Doan BH, et al. Effect of CD47 blockade on vascular inflammation. *N Engl J Med*. 2021;384:382–383.

16. Ridker PM, Everett BM, Pradhan A, et al. Low-dose methotrexate for the prevention of atherosclerotic events. *N Engl J Med*. 2019;380:752–762.

17. Zhao TX, Aetesam-Ur-Rahman M, Sage AP, et al. Rituximab in patients with acute ST-elevation myocardial infarction: an experimental medicine safety study. *Cardiovasc Res*. 2022;118:872–882.

18. Sriranjan R, Zhao TX, Tarkin J, et al. Low-dose interleukin 2 for the reduction of vascular inflammation in acute coronary syndromes (IVORY): protocol and study rationale for a randomised, double-blind, placebo-controlled, phase II clinical trial. *BMJ Open*. 2022;12:e062602.

19. Zhao TX, Kostapanos M, Griffiths C, et al. Low-dose interleukin-2 in patients with stable ischaemic heart disease and acute coronary syndromes (LILACS): protocol and study rationale for a randomised, double-blind, placebo-controlled, phase I/II clinical trial. *BMJ Open*. 2018;8:e022452.

20. Zhao TX, Mallat Z. Targeting the immune system in atherosclerosis: *JACC* state-of-the-art review. *J Am Coll Cardiol*. 2019;73:1691–1706.

21. Kusters PJH, Lutgens E, Seijkens TTP. Exploring immune checkpoints as potential therapeutic targets in atherosclerosis. *Cardiovasc Res*. 2018;114:368–377.

22. Croft M. The role of TNF superfamily members in T-cell function and diseases. *Nat Rev Immunol*. 2009;9:271–285.

23. ElTanbouly MA, Noelle RJ. Rethinking peripheral T cell tolerance: checkpoints across a T cell's journey. *Nat Rev Immunol*. 2021;21:257–267.

24. Rouwet E, Lutgens E. 2016 Jeffrey M. Hoeg Award lecture. *Arterioscler Thromb Vasc Biol*. 2018;38:1678–1688.

25. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat Rev Immunol*. 2013;13:227–242.

26. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science*. 1996;271:1734–1736.

27. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proc Natl Acad Sci U S A*. 2002;99:12293–12297.

28. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010;363:711–723.

29. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med*. 2011;364:2517–2526.

30. Robert C, Schachter J, Long GV, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N Engl J Med*. 2015;372:2521–2532.

31. Huang AC, Zappasodi R. A decade of checkpoint blockade immunotherapy in melanoma: understanding the molecular basis for immune sensitivity and resistance. *Nat Immunol*. 2022;23:660–670.

32. Chan S, Belmar N, Ho S, et al. An anti-PD-1-GITR-L bispecific agonist induces GITR clustering-mediated T cell activation for cancer immunotherapy. *Nat Cancer*. 2022;3:337–354.

33. Fromm G, de Silva S, Johannes K, Patel A, Hornblower JC, Schreiber TH. Agonist redirected checkpoint, PD1-Fc-OX40L, for cancer immunotherapy. *J Immunother Cancer*. 2018;6:149.

34. Compte M, Harwood SL, Munoz IG, et al. A tumor-targeted trimeric 4-1BB-agonistic antibody induces potent anti-tumor immunity without systemic toxicity. *Nat Commun*. 2018;9:4809.

35. Qi X, Li F, Wu Y, et al. Optimization of 4-1BB antibody for cancer immunotherapy by balancing agonistic strength with FcgammaR affinity. *Nat Commun*. 2019;10:2141.

36. Pourakbari R, Hajizadeh F, Parhizkar F, Aghebati-Maleki A, Mansouri S, Aghebati-Maleki L. Costimulatory agonists: An insight into the immunotherapy of cancer. *EXCLI J*. 2021;20:1055–1085.

37. Muller D. Targeting Costimulatory receptors of the TNF superfamily for cancer immunotherapy. *BioDrugs*. 2023;37:21–33.

38. Lau SP, van 't Land FR, van der Burg SH, et al. Safety and tumour-specific immunological responses of combined dendritic cell vaccination and anti-CD40 agonistic antibody treatment for patients with metastatic pancreatic cancer: protocol for a phase I, open-label, single-arm, dose-escalation study (REACtiVe-2 trial). *BMJ Open*. 2022;12:e060431.

39. Padron LJ, Maurer DM, O'Hara MH, et al. Sotigalimab and/or nivolumab with chemotherapy in first-line metastatic pancreatic cancer: clinical and immunologic analyses from the randomized phase 2 PRINCE trial. *Nat Med*. 2022;28:1167–1177.

40. Croft M, Siegel RM. Beyond TNF: TNF superfamily cytokines as targets for the treatment of rheumatic diseases. *Nat Rev Rheumatol*. 2017;13:217–233.

41. Genovese MC, Becker JC, Schiff M, et al. Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. *N Engl J Med*. 2005;353:1114–1123.

42. Elgueta R, Benson MJ, de Vries VC, Wasiuk A, Guo Y, Noelle RJ. Molecular mechanism and function of CD40/CD40L engagement in the immune system. *Immunol Rev*. 2009;229:152–172.

43. Boumpas DT, Furie R, Manzi S, et al. A short course of BG9588 (anti-CD40 ligand antibody) improves serologic activity and decreases hematuria in patients with proliferative lupus glomerulonephritis. *Arthritis Rheum*. 2003;48:719–727.

44. Chamberlain C, Colman PJ, Ranger AM, et al. Repeated administration of dapirolizumab pegol in a randomised phase I study is well tolerated and accompanied by improvements in several composite measures of systemic lupus erythematosus disease activity and changes in whole blood transcriptomic profiles. *Ann Rheum Dis*. 2017;76:1837–1844.

45. Anil Kumar MS, Papp K, Tainaka R, et al. Randomized, controlled study of bleselumab (ASKP1240) pharmacokinetics and safety in patients with moderate-to-severe plaque psoriasis. *Biopharm Drug Dispos*. 2018;39:245–255.

46. Visvanathan S, Daniluk S, Ptaszynski R, et al. Effects of BI 655064, an antagonistic anti-CD40 antibody, on clinical and biomarker variables in patients with active rheumatoid arthritis: a randomised, double-blind, placebo-controlled, phase IIa study. *Ann Rheum Dis*. 2019;78:754–760.

47. Kahaly GJ, Stan MN, Frommer L, et al. A novel anti-CD40 monoclonal antibody, iscalimab, for control of graves hyperthyroidism-a proof-of-concept trial. *J Clin Endocrinol Metab*. 2020;105:dgz013.

48. Kasran A, Boon L, Wortel CH, et al. Safety and tolerability of antagonist anti-human CD40 Mab ch5D12 in patients with moderate to severe Crohn's disease. *Aliment Pharmacol Ther*. 2005;22:111–122.

49. Felten R, Scherlinger M, Mertz P, Chasset F, Arnaud L. New biologics and targeted therapies in systemic lupus: from new molecular targets to new indications. A systematic review. *Joint Bone Spine*. 2023;90:105523.

50. Huang C, Zhu HX, Yao Y, et al. Immune checkpoint molecules. Possible future therapeutic implications in autoimmune diseases. *J Autoimmun*. 2019;104:102333.

51. Brzostek J, Gascoigne NR, Rybakin V. Cell type-specific regulation of immunological synapse dynamics by B7 ligand recognition. *Front Immunol*. 2016;7:24.

52. Buono C, Pang H, Uchida Y, Libby P, Sharpe AH, Lichtman AH. B7-1/B7-2 costimulation regulates plaque antigen-specific T-cell responses and atherogenesis in low-density lipoprotein receptor-deficient mice. *Circulation*. 2004;109:2009–2015.

53. Ait-Oufella H, Salomon BL, Potteaux S, et al. Natural regulatory T cells control the development of atherosclerosis in mice. *Nat Med*. 2006;12:178–180.

54. Matsumoto T, Sasaki N, Yamashita T, et al. Overexpression of cytotoxic T-lymphocyte-associated antigen-4 prevents atherosclerosis in mice. *Arterioscler Thromb Vasc Biol*. 2016;36:1141–1151.

55. Amin HZ, Sasaki N, Yamashita T, et al. CTLA-4 protects against angiotensin II-induced abdominal aortic aneurysm formation in mice. *Sci Rep*. 2019;9:8065.

56. Poels K, van Leent MMT, Reiche ME, et al. Antibody-mediated inhibition of CTLA4 aggravates atherosclerotic plaque inflammation and progression in hyperlipidemic mice. *Cells*. 2020;9:1987.

57. de Boer OJ, Hirsch F, van der Wal AC, van der Loos CM, Das PK, Becker AE. Costimulatory molecules in human atherosclerotic plaques: an indication of antigen specific T lymphocyte activation. *Atherosclerosis*. 1997;133:227–234.

58. Zhao B, Wang D, Liu Y, et al. Six-gene signature associated with immune cells in the progression of atherosclerosis discovered by comprehensive bioinformatics analyses. *Cardiovasc Ther*. 2020;2020:1230513.

59. Dopheide JF, Sester U, Schlitt A, et al. Monocyte-derived dendritic cells of patients with coronary artery disease show an increased expression of costimulatory molecules CD40, CD80 and CD86 in vitro. *Coron Artery Dis*. 2007;18:523–531.

60. Doesch AO, Zhao L, Gleissner CA, et al. Inhibition of B7-1 (CD80) by RhuDex® reduces lipopolysaccharide-mediated inflammation in human atherosclerotic lesions. *Drug Des Devel Ther*. 2014;8:447–457.

61. Ewing MM, Karper JC, Abdul S, et al. T-cell co-stimulation by CD28-CD80/86 and its negative regulator CTLA-4 strongly influence accelerated atherosclerosis development. *Int J Cardiol*. 2013;168:1965–1974.

62. Martini E, Cremonesi M, Panico C, et al. T cell costimulation blockade blunts age-related heart failure. *Circ Res*. 2020;127:1115–1117.

63. Ozen G, Pedro S, Michaud K. The risk of cardiovascular events associated with disease-modifying antirheumatic drugs in rheumatoid arthritis. *J Rheumatol*. 2021;48:648–655.

64. Hsieh MJ, Lee CH, Tsai ML, et al. Biologic agents reduce cardiovascular events in rheumatoid arthritis not responsive to tumour necrosis factor inhibitors: a national cohort study. *Can J Cardiol*. 2020;36:1739–1746.

65. Kang EH, Jin Y, Brill G, et al. Comparative cardiovascular risk of abatacept and tumor necrosis factor inhibitors in patients with rheumatoid arthritis with and without diabetes mellitus: a multidatabase cohort study. *J Am Heart Assoc*. 2018;7:e007393.

66. Kim SC, Solomon DH, Rogers JR, et al. No difference in cardiovascular risk of tocilizumab versus abatacept for rheumatoid arthritis: a multi-

database cohort study. *Semin Arthritis Rheum*. 2018;48:399–405.

67. Seino K, Azuma M, Bashuda H, Fukao K, Yagita H, Okumura K. CD86 (B70/B7-2) on endothelial cells co-stimulates allogeneic $CD4^+$ T cells. *Int Immunol*. 1995;7:1331–1337.

68. Amersfoort J, Eelen G, Carmeliet P. Immunomodulation by endothelial cells—partnering up with the immune system? *Nat Rev Immunol*. 2022;22(9):576–588.

69. Popma SH, Szeto WY, Moore JS, Krupnick AS, Kreisel D, Rosengard BR. Human T cells express CD86 and acquire APC function after stimulation with human or porcine APC. *J Heart Lung Transplant*. 2001;20:215.

70. Halliday N, Williams C, Kennedy A, et al. CD86 is a selective CD28 ligand supporting $FoxP3^+$ regulatory T cell homeostasis in the presence of high levels of CTLA-4. *Front Immunol*. 2020;11:600000.

71. Andrews LP, Marciscano AE, Drake CG, Vignali DA. LAG3 (CD223) as a cancer immunotherapy target. *Immunol Rev*. 2017;276:80–96.

72. Lievens D, Zernecke A, Seijkens T, et al. Platelet CD40L mediates thrombotic and inflammatory processes in atherosclerosis. *Blood*. 2010;116:4317–4327.

73. Lutgens E, Lievens D, Beckers L, et al. Deficient CD40-TRAF6 signaling in leukocytes prevents atherosclerosis by skewing the immune response toward an antiinflammatory profile. *J Exp Med*. 2010;207:391–404.

74. Bosmans LA, van Tiel CM, Aarts S, et al. Myeloid CD40 deficiency reduces atherosclerosis by impairing macrophages' transition into a proinflammatory state. *Cardiovasc Res*. 2023;119(5):1146–1160.

75. Shami A, Edsfeldt A, Bengtsson E, et al. Soluble CD40 levels in plasma are associated with cardiovascular disease and in carotid plaques with a vulnerable phenotype. *J Stroke*. 2021;23:367–376.

76. Schonbeck U, Varo N, Libby P, Buring J, Ridker PM. Soluble CD40L and cardiovascular risk in women. *Circulation*. 2001;104:2266–2268.

77. Sultan CS, Weitnauer M, Turinsky M, et al. Functional association of a CD40 gene single-nucleotide polymorphism with the pathogenesis of coronary heart disease. *Cardiovasc Res*. 2020;116:1214–1225.

78. Li D, Liu L, Chen H, Sawamura T, Mehta JL. LOX-1, an oxidized LDL endothelial receptor, induces CD40/CD40L signaling in human coronary artery endothelial cells. *Arterioscler Thromb Vasc Biol*. 2003;23:816–821.

79. Lutgens E, Gorelik L, Daemen MJ, et al. Requirement for CD154 in the progression of atherosclerosis. *Nat Med*. 1999;5:1313–1316.

80. Lutgens E, Cleutjens KB, Heeneman S, Koteliansky VE, Burkly LC, Daemen MJ. Both early and delayed anti-CD40L antibody treatment induces a stable plaque phenotype. *Proc Natl Acad Sci U S A*. 2000;97:7464–7469.

81. Schonbeck U, Sukhova GK, Shimizu K, Mach F, Libby P. Inhibition of CD40 signaling limits evolution of established atherosclerosis in mice. *Proc Natl Acad Sci U S A*. 2000;97:7458–7463.

82. Mach F, Schonbeck U, Sukhova GK, Atkinson E, Libby P. Reduction of atherosclerosis in mice by inhibition of CD40 signalling. *Nature*. 1998;394:200–203.

83. Lacy M, Burger C, Shami A, et al. Cell-specific and divergent roles of the CD40L-CD40 axis in atherosclerotic vascular disease. *Nat Commun*. 2021;12:3754.

84. Gissler MC, Scherrer P, Anto-Michel N, et al. Deficiency of endothelial CD40 induces a stable plaque phenotype and limits inflammatory cell recruitment to atherosclerotic lesions in mice. *Thromb Haemost*. 2021;121:1530–1540.

85. Reiche ME, Poels K, Bosmans LA, et al. Adipocytes control haematopoiesis and inflammation through CD40 signaling. *Haematologica*. 2023;108(7):1873–1885.

86. Lee PP, Fitzpatrick DR, Beard C, et al. A critical role for Dnmt1 and DNA methylation in T cell development, function, and survival. *Immunity*. 2001;15:763–774.

87. Clausen BE, Burkhardt C, Reith W, Renkawitz R, Forster I. Conditional gene targeting in macrophages and granulocytes using LysMcre mice. *Transgenic Res*. 1999;8:265–277.

88. Caton ML, Smith-Raska MR, Reizis B. Notch-RBP-J signaling controls the homeostasis of CD8-dendritic cells in the spleen. *J Exp Med*. 2007;204:1653–1664.

89. Pertuy F, Aguilar A, Strassel C, et al. Broader expression of the mouse platelet factor 4-cre transgene beyond the megakaryocyte lineage. *J Thromb Haemost*. 2015;13:115–125.

90. Woolaver RA, Wang X, Dollin Y, Xie P, Wang JH, Chen Z. TRAF2 deficiency in B cells impairs CD40-induced isotype switching that can be rescued by restoring NF-kappaB1 activation. *J Immunol*. 2018;201:3421–3430.

91. Bishop GA, Stunz LL, Hostager BS. TRAF3 as a multifaceted regulator of B lymphocyte survival and activation. *Front Immunol*. 2018;9:2161.

92. Zirlik A, Bavendiek U, Libby P, et al. TRAF-1, -2, -3, -5, and -6 are induced in atherosclerotic plaques and differentially mediate proinflammatory functions of CD40L in endothelial cells. *Arterioscler Thromb Vasc Biol*. 2007;27:1101–1107.

93. Mukundan L, Bishop GA, Head KZ, Zhang L, Wahl LM, Suttles J. TNF receptor-associated factor 6 is an essential mediator of CD40-activated proinflammatory pathways in monocytes and macrophages. *J Immunol*. 2005;174:1081–1090.

94. Zarzycka B, Seijkens T, Nabuurs SB, et al. Discovery of small molecule CD40-TRAF6 inhibitors. *J Chem Inf Model*. 2015;55:294–307.

95. Chatzigeorgiou A, Seijkens T, Zarzycka B, et al. Blocking CD40-TRAF6 signaling is a therapeutic target in obesity-associated insulin resistance. *Proc Natl Acad Sci U S A*. 2014;111:2686–2691.

96. Seijkens TTP, van Tiel CM, Kusters PJH, et al. Targeting CD40-induced TRAF6 signaling in macrophages reduces atherosclerosis. *J Am Coll Cardiol*. 2018;71:527–542.

97. Lameijer M, Binderup T, van Leent MMT, et al. Efficacy and safety assessment of a TRAF6-targeted nanoimmunotherapy in atherosclerotic mice and non-human primates. *Nat Biomed Eng*. 2018;2:279–292.

98. Wolf D, Hohmann JD, Wiedemann A, et al. Binding of CD40L to Mac-1's I-domain involves the EQLKKSKTL motif and mediates leukocyte recruitment and atherosclerosis—but does not affect immunity and thrombosis in mice. *Circ Res*. 2011;109:1269–1279.

99. Wolf D, Anto-Michel N, Blankenbach H, et al. A ligand-specific blockade of the integrin Mac-1 selectively targets pathologic inflammation while maintaining protective host-defense. *Nat Commun*. 2018;9:525.

100. Libby P, Buring JE, Badimon L, et al. Atherosclerosis. *Nat Rev Dis Primers*. 2019;5:56.

101. Bardhan K, Anagnostou T, Boussiotis VA. The PD1:PD-L1/2 pathway from discovery to clinical implementation. *Front Immunol*. 2016;7:550.

102. Gotsman I, Grabie N, Dacosta R, Sukhova G, Sharpe A, Lichtman AH. Proatherogenic immune responses are regulated by the PD-1/PD-L pathway in mice. *J Clin Invest*. 2007;117:2974–2982.

103. Bu DX, Tarrio M, Maganto-Garcia E, et al. Impairment of the programmed cell death-1 pathway increases atherosclerotic lesion development and inflammation. *Arterioscler Thromb Vasc Biol*. 2011;31:1100–1107.

104. Poels K, van Leent MMT, Boutros C, et al. Immune checkpoint inhibitor therapy aggravates T cell-driven plaque inflammation in atherosclerosis. *J Am Coll Cardiol CardioOnc*. 2020;2:599–610.

105. Grievink HW, Smit V, Verwilligen RAF, et al. Stimulation of the PD-1 pathway decreases atherosclerotic lesion development in Ldlr deficient mice. *Front Cardiovasc Med*. 2021;8:740531.

106. Lee J, Zhuang Y, Wei X, et al. Contributions of PD-1/PD-L1 pathway to interactions of myeloid DCs with T cells in atherosclerosis. *J Mol Cell Cardiol*. 2009;46:169–176.

107. Fernandez DM, Rahman AH, Fernandez NF, et al. Single-cell immune landscape of human atherosclerotic plaques. *Nat Med*. 2019;25:1576–1588.

108. Li S, Yi L, Wei X, et al. Soluble programmed cell death-ligand 1 as a new potential biomarker associated with acute coronary syndrome. *Front Cardiovasc Med*. 2022;9:971414.

109. Fujisue K, Yamamoto E, Sueta D, et al. Increased soluble programed cell death-ligand 1 is associated with acute coronary syndrome. *Int J Cardiol*. 2022;349:1–6.

110. Solinas C, Saba L, Sganzerla P, Petrelli F. Venous and arterial thromboembolic events with immune checkpoint inhibitors: a systematic review. *Thromb Res*. 2020;196:444–453.

111. Drobni ZD, Alvi RM, Taron J, et al. Association between immune checkpoint inhibitors with cardiovascular events and atherosclerotic plaque. *Circulation*. 2020;142:2299–2311.

112. Roopkumar J, Swaidani S, Kim AS, et al. Increased incidence of venous thromboembolism

with cancer immunotherapy. *Med*. 2021;2:423–434.

113. Sheng IY, Gupta S, Reddy CA, et al. Thromboembolism in patients with metastatic urothelial cancer treated with immune checkpoint inhibitors. *Target Oncol*. 2022;17:563–569.

114. Josan K, Neilan TG. Immune checkpoint inhibitors: acute and chronic cardiovascular complications. *Nat Rev Cardiol*. 2023;20:73–74.

115. Poels K, Neppelenbroek SIM, Kersten MJ, Antoni ML, Lutgens E, Seijkens TTP. Immune checkpoint inhibitor treatment and atherosclerotic cardiovascular disease: an emerging clinical problem. *J Immunother Cancer*. 2021;9:e002916.

116. Mulholland M, Kritikou E, Katra P, et al. LAG3 regulates T cell activation and plaque infiltration in atherosclerotic mice. *J Am Coll Cardiol CardioOnc*. 2022;4:635–645.

117. Tawbi HA, Schadendorf D, Lipson EJ, et al. Relatlimab and nivolumab versus nivolumab in untreated advanced melanoma. *N Engl J Med*. 2022;386:24–34.

118. Amaria RN, Postow M, Burton EM, et al. Neoadjuvant relatlimab and nivolumab in resectable melanoma. *Nature*. 2022;611:155–160.

119. de Winther MPJ, Back M, Evans P, et al. Translational opportunities of single-cell biology in atherosclerosis. *Eur Heart J*. 2023;44(14):1216–1230.

120. Tian J, Zhang B, Rui K, Wang S. The role of GITR/GITRL interaction in autoimmune diseases. *Front Immunol*. 2020;11:588682.

121. Shimizu J, Yamazaki S, Takahashi T, Ishida Y, Sakaguchi S. Stimulation of $CD25^{+}CD4^{+}$ regulatory T cells through GITR breaks immunological self-tolerance. *Nat Immunol*. 2002;3:135–142.

122. Meiler S, Smeets E, Winkels H, et al. Constitutive GITR activation reduces atherosclerosis by promoting regulatory $CD4^{+}$ T-cell responses—brief report. *Arterioscler Thromb Vasc Biol*. 2016;36:1748–1752.

123. Shami A, Atzler D, Bosmans LA, et al. Glucocorticoid-induced tumour necrosis factor receptor family-related protein (GITR) drives atherosclerosis in mice and is associated with an unstable plaque phenotype and cerebrovascular events in humans. *Eur Heart J*. 2020;41:2938–2948.

124. Kassiteridi C, Cole JE, Griseri T, et al. CD200 limits monopoiesis and monocyte recruitment in atherosclerosis. *Circ Res*. 2021;129:280–295.

125. Inhibitory immune checkpoints cause exhaustion of viral immunity in coronary artery disease. *Nat Cardiovasc Res*. 2022;1:607–608.

126. Shay T, Jojic V, Zuk O, et al. Conservation and divergence in the transcriptional programs of the human and mouse immune systems. *Proc Natl Acad Sci U S A*. 2013;110:2946–2951.

127. Lutgens E, Mulder WJM, for the CHECKPOINT ATHERO Consortium. CHECKPOINT ATHERO: developing immune checkpoint-based therapeutics for atherosclerosis. *Eur Heart J*. 2023;44(12):1010–1012.

128. van Leent MMT, Meerwaldt AE, Berchouchi A, et al. A modular approach toward producing nanotherapeutics targeting the innate immune system. *Sci Adv*. 2021;7:eabe7853.

129. Segues A, Huang S, Sijts A, Berraondo P, Zaiss DM. Opportunities and challenges of bispecific antibodies. *Int Rev Cell Mol Biol*. 2022;369:45–70.

130. Uscanga-Palomeque AC, Chavez-Escamilla AK, Alvizo-Baez CA, et al. CAR-T cell therapy: from the shop to cancer therapy. *Int J Mol Sci*. 2023;24:15688.

131. Gotsman I, Grabie N, Gupta R, et al. Impaired regulatory T-cell response and enhanced atherosclerosis in the absence of inducible costimulatory molecule. *Circulation*. 2006;114:2047–2055.

132. Cochain C, Chaudhari SM, Koch M, Wiendl H, Eckstein HH, Zernecke A. Programmed cell death-1 deficiency exacerbates T cell activation and atherogenesis despite expansion of regulatory T cells in atherosclerosis-prone mice. *PLoS One*. 2014;9:e93280.

133. Foks AC, Ran IA, Frodermann V, et al. Agonistic anti-TIGIT treatment inhibits T cell responses in LDLr deficient mice without affecting atherosclerotic lesion development. *PLoS One*. 2013;8:e83134.

134. Douna H, Amersfoort J, Schaftenaar FH, et al. B- and T-lymphocyte attenuator stimulation protects against atherosclerosis by regulating follicular B cells. *Cardiovasc Res*. 2020;116:295–305.

135. Foks AC, Engelbertsen D, Kuperwaser F, et al. Blockade of Tim-1 and Tim-4 enhances atherosclerosis in low-density lipoprotein receptor-deficient mice. *Arterioscler Thromb Vasc Biol*. 2016;36:456–465.

136. Douna H, Smit V, van Puijvelde GHM, et al. Tim-1 mucin domain-mutant mice display exacerbated atherosclerosis. *Atherosclerosis*. 2022;352:1–9.

137. Bavendiek U, Zirlik A, LaClair S, MacFarlane L, Libby P, Schonbeck U. Atherogenesis in mice does not require CD40 ligand from bone marrow-derived cells. *Arterioscler Thromb Vasc Biol*. 2005;25:1244–1249.

138. Smook ML, Heeringa P, Damoiseaux JG, et al. Leukocyte CD40L deficiency affects the $CD25^{+}$ CD4 T cell population but does not affect atherosclerosis. *Atherosclerosis*. 2005;183:275–282.

139. Zirlik A, Maier C, Gerdes N, et al. CD40 ligand mediates inflammation independently of CD40 by interaction with Mac-1. *Circulation*. 2007;115:1571–1580.

140. Gerdes N, Seijkens T, Lievens D, et al. Platelet CD40 exacerbates atherosclerosis by transcellular activation of endothelial cells and leukocytes. *Arterioscler Thromb Vasc Biol*. 2016;36:482–490.

141. van Wanrooij EJ, van Puijvelde GH, de Vos P, Yagita H, van Berkel TJ, Kuiper J. Interruption of the Tnfrsf4/Tnfsf4 (OX40/OX40L) pathway attenuates atherogenesis in low-density lipoprotein receptor-deficient mice. *Arterioscler Thromb Vasc Biol*. 2007;27:204–210.

142. Nakano M, Fukumoto Y, Satoh K, et al. OX40 ligand plays an important role in the development of atherosclerosis through vasa vasorum neovascularization. *Cardiovasc Res*. 2010;88:539–546.

143. Foks AC, van Puijvelde GH, Bot I, et al. Interruption of the OX40-OX40 ligand pathway in LDL receptor-deficient mice causes regression of atherosclerosis. *J Immunol*. 2013;191:4573–4580.

144. Olofsson PS, Soderstrom LA, Wagsater D, et al. CD137 is expressed in human atherosclerosis and promotes development of plaque inflammation in hypercholesterolemic mice. *Circulation*. 2008;117:1292–1301.

145. Jeon HJ, Choi JH, Jung IH, et al. CD137 (4-1BB) deficiency reduces atherosclerosis in hyperlipidemic mice. *Circulation*. 2010;121:1124–1133.

146. Winkels H, Meiler S, Lievens D, et al. CD27 co-stimulation increases the abundance of regulatory T cells and reduces atherosclerosis in hyperlipidaemic mice. *Eur Heart J*. 2017;38:3590–3599.

147. Winkels H, Meiler S, Smeets E, et al. CD70 limits atherosclerosis and promotes macrophage function. *Thromb Haemost*. 2017;117:164–175.

148. Foks AC, Bot I, Frodermann V, et al. Interference of the CD30-CD30L pathway reduces atherosclerosis development. *Arterioscler Thromb Vasc Biol*. 2012;32:2862–2868.

KEY WORDS atherosclerosis, coinhibition, costimulation, immune system, inflammation