The role of the innate and adaptive immune system on vascular remodeling

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Chapter 3

T cell co-stimulation and co-inhibition in cardiovascular disease, a double edged sword

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Nature Reviews Cardiology. 2019, February 15.

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Abstract

It is generally accepted that cardiovascular diseases (CVD) are (partially) driven by inflammation. Immune cells, including T cells, are influenced by inflammatory signals and play an important role in the onset and progression of CVD. T cells activation is modulated by T cell co-stimulation and inhibition pathways. The introduction of immune checkpoint inhibitors (ICIs) targeting the inhibition pathways revolutionized cancer treatments and improved cancer survival. However, ICIs may induce cardiovascular toxicity via T cell re-invigoration, since detrimental cardiovascular effects relate to the activation of T cells. With the rising use of ICIs for cancer treatment, a timely overview of the role of co-stimulation and inhibition molecules in CVD is desirable. In this review, the importance of these molecules in the pathogenesis of CVD will be highlighted in (pre-) clinical studies including vein graft disease, myocarditis, graft arterial disease, post-ischemic neovascularization and atherosclerosis. The review will discuss the potential use of targeting co-stimulation and inhibition pathways as a treatment for CVD, as well as the cardiovascular benefits and adverse events after treatment. Finally, we emphasize cardiovascular monitoring of patients treated with ICIs, since there is a close relation between treating cancer with ICIs and the risk of cardiovascular toxicity.

Introduction

The inflammatory nature of several cardiovascular diseases (CVDs) is well known. Recently the inflammation and immune modulation in CVD regained more attention with the presentation of an effective anti-inflammatory drug for atherosclerosis [1]. In addition, T cells of the adaptive immune system and their activation pathways also received growing interest because of their differential functions in CVD. After stimulation naïve T cells differentiate into effector T cells that migrate to the affected area. Memory cells that subsequently are formed have acquired the ability to respond more rapidly if a second infection occurs. Circulating memory T cells consist of central memory cells located mainly in the secondary lymphoid organs, and effector memory cells, which are mainly located in tissues. Non-circulating memory T cells, termed tissue-resident, have an activated phenotype similar to effector memory cells and can respond rapid to infection and tissue damage. Differential functions of CD8+ s and CD4+ T cell subtypes such as Th1, Th2, Treg and Th17 T cells are described in CVD (box 1). The modulating function of co-stimulation and inhibition pathways in CVD are however still being discovered. With the rising use of immune checkpoint inhibitors (ICIs) targeting these pathways in cancer therapy, a timely overview of the role of co-stimulation and inhibition pathways in CVD is desirable.

The initiation of co-stimulation popularity was founded in 1970 when Bretscher and Cohn introduced the two-signal model[2]. Lafferty and Cunningham extended this model specifically for T cell activation and discovered that naive T cells require two signals to become highly activated[3], otherwise T cell anergy appears. The first signal is provided by antigenic stimulation which occurs via the T cell receptor (TCR) upon binding to cognate peptide-MHC complexes on antigen presenting cells (APC) e.g. macrophages, vascular smooth muscle cells (VSMC) and especially dendritic cells (DC)[4, 5]. The second signal is provided via co-stimulation receptors expressed on T cells upon binding to co-stimulation ligands expressed on APCs. Co-stimulation signals influence cell proliferation, differentiation, survival and cell-cell cooperation[6]. Cytokines like IL-12 and type I interferons (IFNs) can provide a third signal for CD8+ T cell activation[7]. In fact, in absence of their cognate antigen and co-stimulation, cytokine alone can also activate CD8+ T cells leading to production of IFN gamma (IFNγ)[8-10].

After the discovery of CD28[11-13], numerous other co-stimulation molecules were discovered and assigned to either the immunoglobulin (Ig)-superfamily (IgSF) or the tumor necrosis factor (TNF) receptor superfamily (TNFSF)[14-17] (FIG 1). Interestingly, with the discovery of co-inhibition molecules cytotoxic T cell-associated protein 4 (CTLA-4), an inhibitory CD28 homolog, and programmed cell death protein 1 (PD-1) the two-

signal model of Lafferty and Cunningham was extended[18, 19]. It became apparent that co-stimulation was essential for the formation of T cell responses, while co-inhibition appeared to be crucial to counter-balance T cell activation to safeguard tolerance and induce immune homeostasis[20]. Co-inhibition pathways became, along with the co-stimulation pathways, the key mediators in immune responses to infections[21] and of T cell exhaustion that progresses during cancer[20, 22].

In recent years, modulation of the immune response against malignant cells has been successfully introduced in cancer treatment and showed beneficial effects[20, 23]. Several T cell inhibition molecules are inducible expressed on T cells such as CTLA- 4 and PD-1[24]. Cancer cells express a large quantity of programmed cell death ligand 1 (PD-L1), which binds to PD-1 and prevents immunological clearance by T cells[25]. By blocking CTLA-4 or the PD-1 pathway with monoclonal antibodies (called ICIs), however, cancer cell lysis by T cells can be enhanced[26, 27]. Monoclonal antibodies that trigger co-stimulation receptors are also potent in activating T cells leading to clinical benefit. For certain types of cancer, the introduction of ICIs revolutionized cancer treatment and improved survival. ICIs are altering the balance between T cell activation and inhibition mediated by co-stimulation and inhibition molecules via reduction of the inhibitory signals and/or enhancing co-stimulation signals resulting in enhanced cancer cell clearance. However, reduction of inhibitory signals can also activate T cells located in non-tumor bearing tissues leading to immunopathology including auto-immunity. Since it has been shown that activating some types of T cells may be detrimental for CVD, as excellently reviewed by Varricchi et al. and others[28-30], it should be realized that ICI treatment could be harmful for CVD patients.

Nowadays, several ICIs are already in use in clinical settings and even more ICIs are tested in clinical trials for several cancer types, despite the unknown magnitude of possible vascular effects and risks. This review will highlight, and place into perspective, the current knowledge on co-stimulation and co-inhibition pathways and their role in a number of CVDs, e.g. graft arterial disease, vein graft disease, remodeling, post ischemic neovascularization, myocarditis, dilated cardiomyopathy and atherosclerosis. All CVD that will be discussed have at least a partial inflammatory pathophysiology. However for the development of individual CVD, acute and chronic inflammation should be distinguished. The development of e.g. atherosclerosis is a chronic process compared to acute inflammation that occurs in myocarditis or post interventional remodeling. When clinical symptoms occur, treatment is indicated. However, the initiation of treatment can differ in several CVDs. Hence, also with co-stimulation or co-inhibition targeted therapy, it should be taken into consideration, whether to treat the patient shortly after intervention or disease onset, or lifelong.

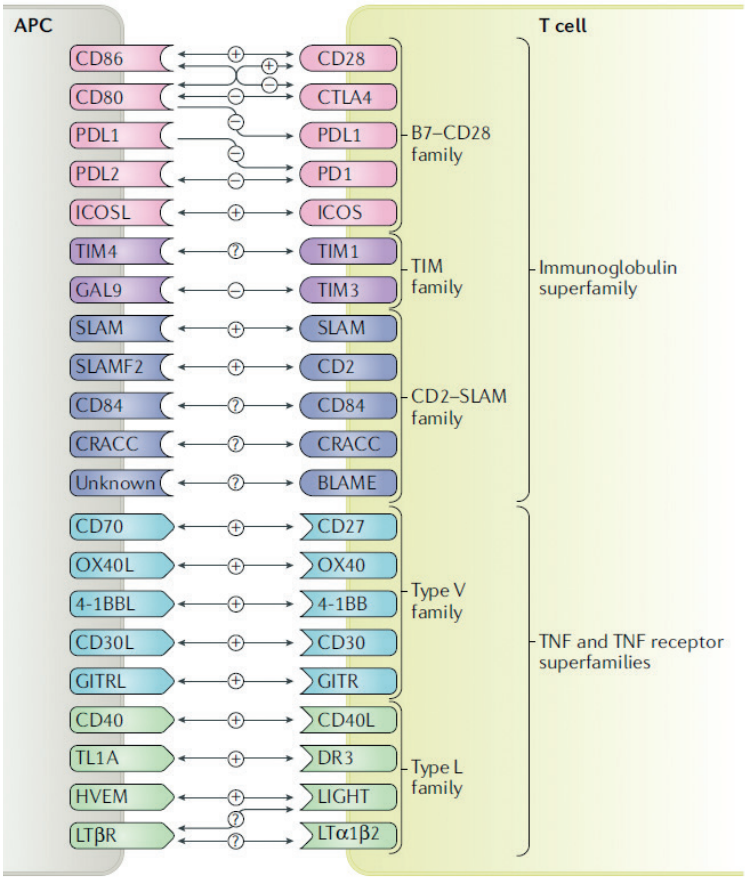

Figure 1. Co-stimulation and co-inhibition pathways.
Co-stimulation and co-inhibition molecules that are described, are shown. On top, members of the immunoglobin superfamily (IgSF) are shown. The members of the B7/CD28 family are shown in red. CD80-CD86 on antigen presenting cells (left) can bind to CD28 leading to T cell (right) activation or CTLA-4 inducting T cell anergy. CD80 can also bind to the inhibition molecule PD-L1. PD-L1 and PD-L2 also bind to PD-1. The B7/CD28 family member ICOS, binds to ICOSL. The T cell immunoglobulin and mucin (TIM) family shown in purple consist of 3 discovered members in human. TIM4 expressed on APC can bind to TIM1 on T cells, whereas TIM3 expressed on T cells binds to galectin-9 (Ga-9). In light blue the CD2/Signaling lymphocyte activation molecule (SLAM) family is shown. Several SLAM members expressed on APC binding to their namesake receptors on T cells; SLAM binding SLAM, CD84 binding CD84 and CRACC binding CRACC. Only CD48 binds CD2, and BLAME expressed on T cells binds to an unknown receptor on APCs. Below, members of the tumor necrosis factor (TNF) receptor superfamily (TNFSF) are shown. In dark blue member of the type V family are shown. CD70, expressed on APCs binds to CD27, stimulates T cell survival. OX40L binding to OX40 and 4-BBL binding to 4-1BB are interesting targets to inhibit co-stimulation. CD30L binding to CD30 and GITRL binding to GITR are less well described. The most well described co-stimulation molecule of the Type-L family, shown in orange, is CD40L binding to CD40L. CD40 is a popular target for the treatment of atherosclerosis and in mainly involved on monocyte recruitment. The function of other members, TL1A binding DR3, HVEM binding LIGHT and LTβR binding both LIGHT and $LT\alpha_1\beta_2$, are less investigated.

Box 1. CD4+ and CD8+ T cell (subtypes) in cardiovascular disease e.g. atherosclerosis.

T cells are identified in the vessel wall of several CVDs and can act either directly via their effector molecules such as inflammatory cytokines, or indirectly via the activation of B cells, producing antibodies. However, T cell subsets produce either pro-or anti-inflammatory cytokines, which make T cell subset functions in CVD differential. To highlight the differential functions of the CD4+ and CD8+ T cells (subsets), we here show their roles in atherosclerosis.

Naïve CD4+ T cells can be polarized into either Th1, Th2, Treg or Th17 cells. Th1 cells showed pro-atherogenic effects via production of IFNγ and TNFα, while Tregs showed mainly anti-atherosclerotic effects via secretion of IL-10 and TGFβ[50, 186-189]. Furthermore, IL-10 secreted by Tregs was shown to mitigate abdominal aortic aneurysms progression[190] and post-angioplasty restenosis[191, 192]. Th2 cells secrete IL-4, IL-5 and IL-13. It was shown that Th2 cells and IL-4 may be associated with advanced atherosclerosis in *ApoE*$^{-/-}$ mice[193]. In contrast, also anti-atherosclerotic effects were observed in *Il4*$^{-/-}$*Ldlr*$^{-/-}$ mice and in *ApoE*$^{-/-}$ mice[194, 195]. This is similar to the inconsistent and undefined functions of Th17 in atherosclerosis. Th17 cells secrete e.g. IL-17A and IL-17F. Inhibition or blockade of IL-17 in *ApoE*$^{-/-}$ mice resulted in pro-atherogenic effects[196-198]. However, *Il-17A*$^{-/-}$*ApoE*$^{-/-}$ mice accelerated unstable atherosclerotic lesions formation[199].

Besides CD4+ T cells and their subsets, CD8+ T cells are also involved in atherosclerosis. CD8+ T cells can secrete cytotoxins e.g., perforin and granzymes. It was shown that cytotoxins can induce apoptosis of macrophages, smooth muscle cells, and endothelial cells and thereby promote the development of vulnerable atherosclerotic lesions[200]. Moreover, mice treated with CD137 agonist showed increased numbers of CD8+ T cells and enhanced atherosclerotic lesions [201]. In contrast, also immunosuppressive CD8+ Tregs were discovered[202]. So both protective and pathogenic roles of CD8+ T cells are shown in atherosclerosis[203].

The differential functions and effects of co-stimulation and inhibition pathways of the **IgSF** e.g. CD80, CD86, CD28, CTLA4, PD-1, PD-L1, PD-L2, ICOS, ICOSL, TIM1, TIM3, TIM4, CD2 and SLAM, and the **TNFSF** e.g. CD27, CD70, OX40, 4-1BB, CD40, HVEM and DR3, in several CVD will be highlighted initially in pre-clinical trials and subsequently in clinical trials. The opposing functions of co-stimulation and inhibition pathways

in several CVD will be discussed in more detail to clarify the complex functions of these pathways. The therapeutic options of blocking co-stimulation to dampen the T cell response and concerns regarding ICIs that enhance T cell activity (e.g. abatacept, pembrolizumab and nivolumab) on vascular diseases will be outlined all in order to provide better insights and to direct future (co) treatment.

Immunoglobulin Superfamily (IgSF)

B7/CD28 family; CD80, CD86, CD28 and CTLA4

A major group of IgSF co-stimulation receptors are molecules of the B7/CD28 family, with CD80 (or B7.1) and CD86 (or B7.2) as the best characterized co-stimulation molecules. CD80 and CD86 are transiently expressed on APCs after activation[16, 31, 32]. Both CD80 and CD86 can bind to the co-stimulation receptor CD28, expressed constitutively on T cells, and to the co-inhibition receptor CTLA-4 (or CD152), expressed by activated T cells. CTLA-4 binds the CD80/86 molecules with a much higher affinity leading to regulation of the response.

The role of the B7/CD28 family in CVD has been differentially described. CD80/86 was observed to be involved in CD8+ T cell activation in **vein grafts**. Deficiency of both CD80/86 and CD70 resulted in a decrease in intimal hyperplasia formation in a vein graft disease model (Simons VGD, manuscript in preparation). This effect was independent of the TCR but involved by bystander cytokines in synergy with co-stimulation signals. Reduced intimal lesion development in CD80/CD86 deficient mice was also observed in a femoral artery cuff mouse model for **post-interventional remodeling**. In addition, systemic treatment with abatacept, a CTLA-4Ig which blocks CD80/86 co-stimulation and thereby inducing non-activated T cells, prevented intimal thickening by almost 60% [33].

Besides remodeling in vein grafts, CD80/86 co-stimulation also plays a role in **graft arterial disease (GAD)** after heart transplantation. GAD is characterized by chronic remodeling of allograft arteries [34]. Especially Th1 cytokines, such as IFNγ, influence vascular remodeling causing reduced luminal flow and eventually GAD[35]. Co-stimulation was thought to be involved in GAD since blocking co-stimulation resulted in non-reactive T cells or T cells that underwent programmed cells death[36, 37]. It has been shown that blockade of CD80/86 with CTLA-4Ig early after transplantation resulted in improved long-term graft survival and mitigated the development of graft arteriosclerosis via a decrease in T cell and macrophage activation[38]. Kim et al. determined the different effects on GAD between early and late post-transplant co-stimulation blockade treatment since GAD may also occur in a late phase. Late blockade of CD80/86 or selective blockade of CD80 alone attenuated the

development of GAD, most likely due to a decrease in T cell activation. Early graft loss was not prevented by blockade of CD80 alone, in contrast to CD80/86 blockade [39]. Along this line, a decrease in GAD was observed in MHC class II-mismatched allograft hearts that were transplanted in CD80 and CD80/86 deficient mice, but not in CD86 deficient mice [40], both highlighting the important and differential functions of CD80 and CD86 in vivo.

In addition, it was also demonstrated that CTLA-4Ig abatacept decreased the progression and severity of cardiac dysfunction in a mouse **heart failure** model[41]. This was realized via a reduction of cardiac infiltration and activation of T cells and macrophages, leading to a decrease in cardiomyocyte death.

In **atherosclerosis**, the role of co-stimulation can be differential. With the discovery of CD80, CD86 on macrophages in atherosclerotic lesions in human [42] and mouse[43], and on monocyte-derived DCs of coronary artery disease patients [44], a clear indication of a role for co-stimulation in atherosclerosis was provided. High expression of CD80 and CD86 was associated with vulnerable atherosclerotic lesions, shown in human carotid arteries[45]. However, it was suggested that the expression of co-stimulation might vary depending on the cell type, the presence of stimulating ligands e.g. toll like receptor ligands, or on the level of hypercholesterolemia [46]. Buono et al. showed that CD80 and CD86 regulate the development of atherosclerosis by priming T cells [47]. They showed reduced atherosclerotic lesions in *Cd80/86$^{-/-}$Ldlr$^{-/-}$* mice compared to control *Ldlr$^{-/-}$* mice on a high cholesterol diet. In addition, HSP60 stimulated CD4+ T cells of *Cd80/86$^{-/-}$Ldlr$^{-/-}$* mice subsequently secreted lower levels of IFNγ *in vitro*, indicating a role for CD80/86 on CD4+ T cell activation, next to the previously discussed role for CD80/86 on CD8+ T cells in vein graft disease (Simons et al., 2018 VGD manuscript in preparation). An important role for CD80/CD86 co-stimulation and CTLA-4 was also shown in **accelerated atherosclerosis** development. Hypercholesterolemic ApoE3*Leiden mice treated with CTLA-4Ig abatacept showed a reduction of almost 80% in accelerated atherosclerosis development, with decreased activation of CD4+ T cell. In contrast, CTLA-4 blocking antibodies increased vascular lesion size by almost 70% [33]. CTLA-4-Ig treatment also resulted in reduced homocysteine-accelerated atherosclerosis [48] and reduced atherosclerosis in mice with constitutive expression of CTLA-4 on T cells [49]. All pre-clinical studies described above show a beneficial role of co-stimulation blockade, which inhibits T cell activation, in atherosclerosis.

Interestingly, Ait-Oufella showed opposing results with increased atherosclerotic lesions in *Cd80/86$^{-/-}$Ldlr$^{-/-}$* bone marrow chimeras and *Cd28$^{-/-}$Ldlr$^{-/-}$* chimeras compared to control mice due to lack of Tregs. Pre-clinical studies described above did not

distinguish between T cell subtypes, but CTLA-4 is also expressed on Tregs and may contribute to an anti-atherogenic function[50-52]. This is in contrast to the effects of CD80/86 deficiency on atherosclerosis shown before, and might be due to the delicate balance between the pro-atherogenic T cells and the anti-atherogenic Tregs. As already pointed out by Lichtman et al. CD80, CD86 are partially essential in the thymus to develop Tregs[34]. The lack of CD80 and CD86 in the *Cd80/86*$^{-/-}$*Ldlr*$^{-/-}$ bone marrow chimeras used by Ait-Oufella, can result in a Treg deficiency.

In conclusion, in vein graft disease, GAD and post interventional remodeling, blocking CD80/86 and thereby decreasing T cell activation showed beneficial effects. The opposing effects of CD80/86 on atherosclerosis may relate to putative targeting of Tregs, and make the design of therapeutic strategies challenging. Blocking co-inhibition with ICIs would cause increased T cell activation, and based on these pre-clinical studies, might result in disadvantageous cardiovascular effects. Alternatively, blocking co-stimulation, instead of co-inhibition leading to a decrease in T cell activation, could be therapeutically interesting and feasible.

B7/CD28 family; PD-1, PD-L1 and PD-L2

The B7/CD28 family also includes the co-inhibition molecule PD-1 (or CD279), which can bind to PD-L1 (or B7-H1 or CD274) or PD-L2 (or B7-DC or CD273). In high-level chronic virus infection and in cancer, the activation of the PD-1/PD-L1 axis can lead to T cell exhaustion. PD-L1 can also bind to CD80 executing a similar inhibition function[25, 53]. The role of PD-1 in CVD has mainly been described by blocking PD-1 or PD-L1/PD-L2.

It has been shown that PD-1 and PD-L1 mediate tissue damage in a **cardiac injury model**. Ischemic-re-perfused cardiac cells expressed high levels of PD-1 and PD-L1. Besides an inhibition of T cell proliferation, an increase in cardiomyocyte death through a paracrine mode of action was observed in cardiac cells expressing high levels of PD-1 and PD-L1 [54].

In a mouse model for **dilated cardiomyopathy**, it was shown that two-third of mice deficient of PD-1 died within 30 weeks compared to 0 mice with PD-1. Mice deficient of PD-1 are not able to inhibit T cell activation and express high levels of activated T cells. Histological examination showed that the hearts of PD-1 deficient mice had dilated ventricles with thin right ventricle walls and a severely decreased contraction function [55]. Subsequently, it was discovered that the cardiomyopathy in PD-1 deficient mice was caused by autoantibodies against cardiac troponin I, which led to chronic stimulation of Ca2+ influx in cardiomyocytes [56]. T cell-mediated **myocarditis**

can chronically lead to dilated cardiomyopathy. Tarrio et al. showed in two distinct models of myocarditis that PD-1 protects against inflammation and cardiomyocyte damage [57], most likely via the function of PD-1 to inhibit inflammatory T cell activation. Murphy Roths Large (MRL) mice are genetically predisposed to systemic autoimmunity and were used to demonstrate a role for PD-1 and PD-L1 respectively in the development of myocarditis [58, 59]. Both *Pd-1*$^{-/-}$*-MRL* mice and *Pd-L1*$^{-/-}$*-MRL* mice developed fatal myocarditis, indicating that blocking PD-1 or PD-L1 with ICIs might be lethal. Juchem et al. also raised their concerns on blocking PD-L1, since in the absence of PD ligands, both effector memory T cells and naive T cells induced late-onset myocarditis in a graft versus host disease model [60].

Similarly as described before for the CD80/86 deficiency, blocking PD-1 can promote pro-atherogenic T cell activation and promote anti-atherogenic Treg responses. Increased **atherosclerotic** lesions were observed in *Pd-1*$^{-/-}$*Ldlr*$^{-/-}$ *mice in the aortic sinus and aorta, indicating that blocking PD-1 can exacerbate atherosclerosis*[61, 62. *The effect of* pro-atherogenic CD4+ T cell was stronger than the anti-atherogenic Treg responses[61], which neutralized the anti-atherogenic Treg response. Deficiency of PD-1 ligands, also showed increased atherosclerotic lesions in the aorta of *Pd-L1/2*$^{-/-}$*Ldlr*$^{-/-}$ mice compared to control mice. In the atherosclerotic lesion increased numbers of CD4+ T cells and CD8+ T cells were observed, with increased numbers of activated CD4+ T cells [63]. This indicates that PD-1 interactions with its ligands PD-L1 and PD-L2 have an important role in protecting atherosclerotic lesion development.

In conclusion, all the pre-clinical research described, showed a protective role of PD-1, PD-L1 and PD-L2 in CVD and blocking these molecules might result in disadvantageous cardiovascular effects. Thus, in addition to the concerns raised by Juchem et al. on blocking PD-L1, we raise our concerns on blocking PD-1, PD-L1 and PD-L2 and with regard to cardiovascular outcomes.

B7/CD28 family; ICOS and ICOSL

Another member of the B7/CD28 family is inducible co-stimulation molecule ICOS (or CD278) that binds to ligand ICOSL (or CD275). ICOS is expressed on activated CD4+ T cells and CD8+ T cells and Tregs, [64]. The role of ICOS in CVD remains partially elusive due to the homology between ICOS, co-stimulation molecule CD28 and co-inhibition molecule CTLA-4. Yet, only pre-clinical research is performed to show the role of ICOS and ICOSL in **atherosclerosis**.

In murine atherosclerotic lesions, ICOS and ICOSL are abundantly expressed [42, 65]. Increased aortic lesion size of almost 40% was observed in *ApoE*$^{-/-}$ mice fed a high

fat diet, after blocking ICOS with anti-ICOS antibodies compared to control *ApoE*$^{-/-}$ mice fed a high fat diet[66]. Gotsman et al. supported this by showing that lethally irradiated *Ldlr*$^{-/-}$ mice, injected with bone marrow cells of *Icos*$^{-/-}$ donor mice, developed enlarged atherosclerotic lesions compared to control mice. In the iliac lymph nodes and the spleen the number of Tregs was reduced in mice transplanted with *Icos*$^{-/-}$ cells compared with control mice, however, the reduced number of Tregs could not be shown in the atherosclerotic lesions[67]. Interestingly, ICOS deficiency resulted in increased atherosclerotic lesions, opposed to results shown with other co-stimulation members of the B7/CD28 family previously. As described before, this might be due to the bone marrow chimeras used, since there can be a deficiency in Treg development in this model. Subsequently, it was concluded that ICOS is essential for the development and function of anti-atherogenic Treg and promotion of Treg proliferation, survival and maintenance[68]. Thus a deficiency of ICOS will lead to a decrease in anti-atherogenic Tregs, causing an increase in atherosclerotic lesion development. Thus, stimulating ICOS might be beneficial in CVD via stimulation of Tregs. However, agonistic ICOS antibodies cannot (yet) be used since this will also influence other T cells than Tregs, which may limit its clinical effect.

T cell immunoglobulin and mucin (TIM) family; TIM1, TIM3 and TIM4.

Eight members of T cell immunoglobulin and mucin domain (TIM), also known as Hepatitis A virus cellular receptor 1 (HAVCR1), of the TIM family are discovered in mice (TIM1-8), while only three TIM genes (TIM1, TIM3 and TIM4) have been identified in humans. Therefore in this review we will mainly focus on TIM1, TIM3 and TIM4. Both co-stimulation and co-inhibition functions are described for the TIM family[69], but only limited studies describe the role of the TIM family in CVD.

In the Han Chinese population it was shown that TIM1 polymorphisms are associated with **dilated cardiomyopathy** susceptibility and prognosis [70]. Lind et al. showed that there was also an association between TIM1 and **atherosclerotic** lesions occurrence in carotid arteries[71]. TIM1 can be expressed on activated T cells and regulatory B cells and binds to TIM4 mainly expressed on APCs. A recent genome-wide association study showed that TIM4 genetic variants were associated with the risk of **coronary heart disease** and **ischemic stroke** [72]. In pre-clinical studies, TIM1 together with TIM4 showed involvement in the development of **atherosclerosis**. Enhanced atherosclerotic lesions were observed in *Ldlr*$^{-/-}$ mice treated with antibodies against TIM1 or TIM4 compared to untreated mice, caused by impaired efferocytosis and an increased amount of CD4+ T cells in the lesions. TIM1 effects were mainly based on the Th1/Th2 balance and regulation of Tregs [73]. TIM3 is highly expressed on Th1 cells and in low levels expressed on monocytes, NK, and NK T cells[74]. Blocking TIM3 increased

myocarditis abundantly, in a mouse model for **myocarditis**. This was partly due to a reduced expression of CD80 on macrophages, resulting in a decreased amount of anti-inflammatory Tregs in the heart [75]. In addition, less TIM3 was expressed on mast cells, macrophages and CD4+ T cells in the inflamed heart, following coxsackievirus B3 infection, in male compared to female mice [76].

All preclinical studies on TIM1, TIM3 and TIM4 describe unfavourable cardiovascular effects when blocking TIM. This might imply that TIM has more co-inhibitory functions than stimulatory. However, since the function of TIM is not completely clear yet, the best possible way to exploit TIMs as a treatment to prevent CVD requires further investigation.

CD2/Signaling lymphocyte activation molecule (SLAM) family; CD2, CD48 and SLAM

The CD2/SLAM is a broad family that consists of SLAM (CD150 or Slamf1), CD48 (or Slamf2), 2B4 (CD244 or Slamf4), Ly9 (CD229 or Slamf3), CD84 (or Slamf5), NK-T-B-antigen (NTB-A, or CD352 or Slamf6), CD2-like receptor activating cytotoxic cells (CRACC, also known as CD319 or Slamf7), B lymphocyte activator macrophage expressed (BLAME or Slamf8), and SF2001 (CD84H or Slamf9). Despite the number of family members, the experimental studies performed on the CD2/SLAM family is limited[77]. In human carotid endarterectomy samples the expression of CD2/SLAM family genes was compared between stable and unstable atherosclerotic segments within one sample. The expression of BLAME and CRACC was >5-fold up regulated in areas of stable compared to unstable atherosclerotic lesion segments[78]. In contradiction, Levula et al. showed an up regulation of BLAME in human advanced atherosclerotic (AHA type V-VI) lesions, using gene set enrichment analysis **79. This indicates that there might be an association between SLAM genes and atherosclerosis.** However, similar to the TIM family members, the exact role of the CD2/SLAM family members has yet to be determined with regard to CVD.

Butyrophilin (BTN) family; BTNL1 and BTNL2

Family members of the Butyrophilin (BTN) family such as butyrophilin/B7-like molecule 1 and 2 (BTNL1 and BTNL2) are the latest members of the IgSF [80]. Despite the structural resemblance to the B7/CD28 family, the function remains to be determined. It has been suggested that BTNL1 and BTNL2 are co-inhibition molecules [81, 82]. However, BTNL2 is mainly expressed in the digestive tract tissue, which questions their role in CVD[82]. Due to lack of experimental studies, the BTN family can be added to the list of sparsely investigated molecules in CVD, together with the TIM and SLAM family.

Tumor Necrosis Factor (Receptor) Super Family (TNFRSF)

The TNF super family consist of several subfamilies of which the type V and type L family members are co-stimulation and co-inhibition molecules that functions as T cell (de)activators. The interaction of members of the TNFSF and their receptors belonging to the TNFRSF, induce diverse biological responses implicated in CVD. TNF receptor activation induces the expression of pro-inflammatory cytokines, adhesion molecules, tissue factor and matrix metalloproteinases. Some members of the TNFSF, such as CD27, OX40, 4-1BB, CD40, CD30, DR3 and lymphotoxin β Receptor (LTβR), are co-stimulation molecules, inducing T cell activation and survival. Upon ligation to their receptors located in the plasma membrane, TNF receptor-associated factors (TRAFs) are recruited to the cytoplasmic tail. TRAFs enhance pro-inflammatory signaling by mitogen-activated protein kinases and nuclear factor kappa B[83].

Type V; CD27 and CD70

CD27 and CD70 (CD27L) are the best described co-stimulation members of the TNFSF/TNFRSF, type V family in CVD. CD70 is expressed on activated CD4+ and CD8+ T cells, B cells, macrophages and DCs upon stimulation by pattern recognition receptors or antigens via the TCR[32]. CD27, the receptor for CD70, is expressed on naïve and mature T cells, activated B cells and NK cells and mainly induces cell survival. The function of CD27 and CD70 in CVD has been described in several pre-clinical studies.

Sardella et al. observed that CD27 expressed on Tregs is associated with ST-elevated myocardial infarctions (**STEMI).** The percentages of both CD27+ Tregs and CD27- Tregs were significantly lower in these patients in comparison with controls. However, the Treg ratio was skewed toward the CD27- population [84]. It was suggested that CD27 deficiency could lead to the dysfunction of T cell immunity to limit the ongoing inflammation, and that coronary revascularization was associated with partial reconstitution of the peripheral Treg pool.

In vivo, the CD27-CD70 T cell co-stimulation pathway appears to be the most important co-stimulation pathway in pre-existing collateral formation and post-ischemic blood flow recovery, by induction of arteriogenesis and angiogenesis [85]. This has been described in a hindlimb ischemia mouse model; *Cd70*$^{-/-}$ mice and *Cd70*$^{-/-}$*Cd80*$^{-/-}$*Cd86*$^{-/-}$ mice showed a hampered blood flow restoration in the paw after ligation of the femoral artery.

In addition to post-ischemic blood flow recovery and STEMI, CD27-CD70 also appeared to play a role in **atherosclerosis**. A putative protective role for CD70 has been observed in humans. Higher CD70 expression was detected in stable plaques

compared to ruptured plaques in the carotid arteries of atherosclerotic patients. These atherosclerotic plaques with a distinct lipid core showed a gradual decrease in the expression of CD70, CD80, and CD86 towards the atheroma. Macrophages immediately adjacent to the atheroma did not express CD70, CD80 and CD86. Interestingly, a high number of CD27+ T cells were observed in the atherosclerotic plaques suggesting the clearance of pro-inflammatory monocytes [42].

Furthermore, in hyperlipidemic *ApoE*$^{-/-}$ mice, histological analysis revealed that CD70 was highly expressed on macrophages located in **atherosclerotic** plaques and the expression was most evident in the superficial layers of the fibrous cap [86]. It was shown that *Cd70*$^{-/-}$ mice displayed larger atherosclerotic plaques characterized by lower cellularity and a more advanced plaque phenotype compared to control *ApoE*$^{-/-}$ mice. It was suggested that a chronic CD27 stimulation by CD70 overexpression ameliorates atherosclerosis by enhanced apoptosis of circulating, pro-inflammatory Ly6C^{hi} monocytes. In addition, van Olffen et al. reported that overexpression of CD70 is atheroprotective in CD70 transgenic ApoE*3-Leiden mice [87]. Macrophages of CD70 transgenic ApoE*3-Leiden mice had no defects in phagocytosis or in TNF production, but monocytes were more prone to apoptosis. This reduced viability of monocytes suggests that CD70 signaling promotes macrophage survival and thus CD70 overexpression has been stated as atheroprotective.

In conclusion, CD27 and CD70 are likely protective in vascular remodeling due to their pro-survival and stimulating effects on T cells. CD27/CD70 may also contribute to post-ischemic blood flow recovery. Therapeutic stimulation of the CD27-CD70 co-stimulation pathway might thus be interesting for the treatment of CVD.

Type V; OX40 and OX40L

Another co-stimulation member of the type V family is OX40 (or CD134), binding to OX40 ligand (OX40L or CD252). OX40 is able to potentiate T cell receptor signaling of CD4+ and CD8+ T cells leading to their activation in response to their specific antigen. Binding of OX40 to its ligand OX40L, which is mainly expressed by APC and ECs[88], increases the proliferation, differentiation and cytokine production by T cells[89].

In an experimental mouse model of aortic banding induced **cardiac hypertrophy,** it was demonstrated that OX40 regulates pressure overload-induced cardiac hypertrophy and remodeling via CD4+ T cells[90]. The extent of perivascular and interstitial cardiac fibrosis in response to aortic banding in *Ox40*$^{-/-}$ mice was remarkably decreased, compared to control mice. OX40$^{-/-}$CD4+ T cells were less proliferative and produced decreased levels of inflammatory cytokines TNFα, IL-1β

and IL2 and enhanced levels of anti-inflammatory cytokine IL-10 after activation with agonistic antibody CD3 and CD28. Also, the mRNA expression levels of TNFα, IL-1β, and IL-6 as well as CD4+ and CD8+ T cell influx were significantly decreased in OX40$^{-/-}$ mice hearts compared with control mice hearts. From the other perspective, *Ox40*$^{-/-}$ mice that received reconstituted CD4+ T cells presented excessive perivascular and interstitial fibrosis compared to control mice[90]. This indicated that OX40 contribute to the progression of cardiac remodeling via CD4+ T cells.

In **coronary heart disease** (CHD) patients, OX40 and OX40L mRNA levels were increased and correlated with clinical pathological features of CHD [91]. OX40 and OX40L mRNA levels also correlated with known risk factors for CHD, such as gender. It was demonstrated that patients with an **acute coronary syndrome** or **stable angina pectoris** have increased OX40L blood levels [92]. In addition, it was observed that soluble OX40 ligand (sOX40L) measured in the circulation correlated with troponin I and the increase in sOX40L (>40ng/ml) was associated with a higher risk for **acute myocardial infarction (ACS), sudden death** or **recurrent angina pectoris** [93, 94]. Kotani et al. observed an increase in the chemokine CCL5 expression measured by flow cytometry in ECs treated with sOX40L in vitro. [95]. The chemokine CCL5 is able to regulate T cell adhesion to EC and subsequently T cell extravasation. This soluble form of OX40L might play a role in mediating a systemic pro-inflammatory state in which T cells extravasate and drive cardiovascular diseases.

Dumitriu et al. demonstrated that patients with ACS, but not patients with stable angina pectoris, have an expansion of highly inflammatory CD4+ CD28- T cells. These T cells lack CD28, needed for optimal T cell function[96]. CD4+CD28- T cells from ACS patients express higher levels of the alternative co-stimulation receptors OX40 and 4-1BB compared to classical CD4+CD28+ T cells. CD4+CD28- T cells were present in all atherosclerotic plaques studied and constituted up to 23% of all CD4+ T cells resident in the plaque. Importantly, these CD4+CD28- T cells expressed OX40 and 4-1BB co-stimulation receptors, which regulated perforin and granzyme B release. Ligands to OX40 and 4-1BB are available in the microenvironment of atherosclerotic lesions that results in the activation of these CD4+ CD28- T cells [97]. These CD4+CD28- T cell subset, activated via OX40 and 4-1BB, might be a contributing factor to plaque rupture in atherosclerotic patients.

In addition, Foks et al. demonstrated that blockade of the OX40-OX40L pathway with anti-OX40L antibodies reduced the number of T cells in **atherosclerotic** plaques, reduced plaque size and reduced the Th2 responses in *Ldlr*$^{-/-}$ mice. An increase in oxLDL specific IgM antibodies was observed in these anti-OX40L treated mice due to

the production of IL-5 by Th2 cells [88]. This implicated that the blockade of the OX40-OX40L pathway reduces the development of atherosclerotic plaques in vivo.

Genetic deletion of the OX40-OX40L signaling pathway suppresses the development of vasa vasorum neovascularization in the arterial wall and inhibits the development of **atherosclerosis** in vivo[98]. The vasa vasorum serves as a mediator of transport of cellular and non-cellular pro-inflammatory components into the vessel wall by the expression of adhesion molecules [99] and may be the origin of plaque infiltrating angiogenic vessels[100, 101]. Nakano et al., observed similar results as Moreno et al. since blockade of OX40L by anti-OX40L antibodies in *ApoE*$^{-/-}$ mice also showed reduced aortic atherosclerotic plaque formation compared to untreated *ApoE*$^{-/-}$ mice because of the OX40/OX40L dependent neovascularization in the vasa vasorum[102].

In conclusion, *Ox40*$^{-/-}$ mice and mice treated with anti-OX40L antibodies showed beneficial outcomes on CVD in pre-clinical studies. OX40 mRNA expression levels or soluble OX40 was demonstrated to be elevated in several CVDs. Thus, OX40 seems to be a promising therapeutic target for CVD, and future research on pre-clinical and clinical trials blocking OX40 should be encouraged.

Type V; 4-1BB (CD137) and 4-1BBL

The third co-stimulation member of the type V family is 4-1BB (or CD137), which binds uniquely to 4-1BBL (or CD137L). Activated CD4+ T cells, DCs, and NK cells express 4-1BB, while 4-IBBL, is expressed on B cells, DCs, and ECs. Activation results in expansion of T cells and pro-inflammatory cytokine expression[103].

4-1BB expression was detected in human atherosclerotic plaques on T cells and induced by pro-inflammatory cytokines in ECs and SMC. Elevated levels of soluble 4-1BB were measured in blood and peripheral monocytes from patients with **ACS** [104, 105]. An increase in soluble 4-1BB was demonstrated to be associated with an increased risk of ACS and suggested to be a potential prognostic marker for ACS.

Furthermore, human genetic evidence for the involvement of 4-1BB in **atherosclerosis** has been identified in the IMPROVE cohort consisting of 3418 patients. The minor T allele in the 4-1BB gene was associated with increased intima-media thickness in the common carotid artery independently of the subject vascular risk factors [106]. However, this allele was not associated with an increased risk for CAD or MI in the PROCARDIS/WTCCC case-control cohort [107].

As described before, Dumitriu et al demonstrated that patients with **ACS** have an expansion of highly inflammatory CD4+ CD28- T cells, which express higher levels of 4-1BB [96]. Although there is an association between 4-1BB, atherosclerosis, and ACS; pre-clinical trials demonstrated adverse effects on experimental **atherosclerosis** with the activation of 4-1BB. Treatment of *ApoE*$^{-/-}$ mice with a 4-1BB agonist induced an increase in vascular inflammation; T cell infiltration, expression of pro-inflammatory cytokines and MHC class II molecule up regulation in **atherosclerotic** lesions. Activation of the 4-1BB pathway resulted in EC and SMC proliferation[108].

ApoE$^{-/-}$ *4-1BB*$^{-/-}$ and *Ldlr*$^{-/-}$ *4-1BB*$^{-/-}$ mice had smaller atherosclerotic plaques compared to control mice, which was attributed to a down-regulation of IFNγ, MCP1, VCAM, ICAM, and IL-6. Stimulation of 4-1BB signaling induced the production of pro-inflammatory cytokines by macrophages in the atherosclerotic vessels in vivo[109]. In perspective, 4-1BB is able to regulate T cell activation as a co-stimulation receptor but also mediates atherosclerosis via effects on ECs and macrophages because 4-1BBL is expressed in other cell types, including DCs and activated B cells.

In conclusion, deficiency of 4-1BB in vivo leads to reduced atherosclerosis and blocking of the 4-1BB - 4-1BBL co-stimulation pathway may be a valuable target to reduce the development of atherosclerosis. However, pre-clinical trials should point out whether this is also effective in other CVD than atherosclerosis.

Type V; CD30 and CD30L

CD30 (or tnfsf8) is expressed by activated CD4+ and CD8+ T and B cells, while its ligand CD30L (or CD153) is also expressed on macrophages and DCs. Signaling via CD30 is complex as it can lead to either proliferation or apoptosis. Interaction via CD30L induces pro-inflammatory cytokine release from CD30+ CD4+ T cells resulting in a potent helper activity for B cell antibody production [110].

Human alloreactive T cells reside within the CD30+ population indicating a regulatory role of T cell immune responses via CD30L. Strong expression of CD30L, CD70, 4-1BBL and weak to moderate expression of OX40L was found in the cardiac myocytes of patients with **acute myocarditis**. Moderate expression of CD30L, CD27L, 4-1BBL and weak expression of OX40L was found on the cardiac myocytes of patients with **dilated cardiomyopathy** [111].

CD30 was regarded as a regulator of **atherosclerosis**. Treatment of *Ldlr*$^{-/-}$ mice with anti-CD30L antibodies resulted in a reduction of atherosclerotic plaques in the aortic roots compared to control mice. Also, a reduced amount of CD3+ T cells in spleens

and in the adventitial layers were observed, while no differences in the collagen of macrophage content were identified. This indicates that the function of the CD30-CD30L pathway is largely inhibiting T cell responses [112].

Thus, CD30 is involved in CVD and anti-CD30 treatment is able to modulate atherosclerosis formation and could be used to inhibit atherosclerosis plaque development and, although not investigated yet, may also prevent other CVDs.

Type V; GITR and GITRL

Glucocorticoid-induced tumor necrosis factor receptor (GITR, also known as CD357), a multifaceted regulator of immunity belonging to the TNF(R)SF, is expressed on activated CD4+ T cells and binds to GITR ligand (GITRL). The mechanism of GITR signaling is thoroughly reviewed elsewhere [113].

GITR and GITRL are considered pro-atherogenic since they were observed to be mainly expressed in **atherosclerotic** plaques rich in macrophages. GITR signaling in macrophages induced the expression of TNFα, and MMP9 via NF-κB[114]. In addition, it was shown that *Ldlr*$^{-/-}$ mice constituted with bone marrow from B cell-specific GITRL transgenic mice showed significantly smaller atherosclerotic lesions than in control mice [115]. Although the role of GITR and GITRL in cardiovascular disease is only characterized in a limited amount of pre-clinical studies, it is described more comprehensively in other diseases. A murine model of cortical infarction showed that GITR triggering on CD4+ T cells increased post-stroke inflammation and decreases the number of neural stem/ progenitor cells induced by ischemia [116]. Depletion of the GITR high cells induced autoimmune myocarditis at a high incidence in BALB/c mice in addition to other organ-specific autoimmune diseases, such as gastritis and thyroiditis, which are commonly produced by depletion of CD25+ Tregs [117].

Type L; CD40 and CD40L

The type-L family CD40 (or tnfsf5) is expressed by APCs and binds to CD40L (or CD154) that is expressed by activated (follicular) T helper cells, macrophages, B cells and ECs. In the latter cells, activation results in the induction of ICAM, VCAM, and E-selectins and may promote the development of atherosclerosis. CD40L is also expressed by activated thrombocytes and induces recruitment of monocytes to the inflamed endothelium. This is important for platelet-leukocyte aggregate formation, which can contribute to the progression of atherosclerosis [118].

Increased levels of CD40L were observed in patients with **unstable angina**, asymptomatic hypercholesterolemia and more importantly, **acute myocardial infarction** [119, 120]. Lutgens et al. showed that the CD40-CD40L co-stimulation pathway is an important pathway in **atherosclerosis** development. *ApoE*$^{-/-}$ mice treated with agonistic CD40 antibodies resulted in smaller atherosclerotic plaques compared to untreated *ApoE*$^{-/-}$ mice. These atherosclerotic plaques contained low numbers of inflammatory cells with considerable fibrosis indicating a stable phenotype [121]. In addition, Mach et al. showed that anti-CD40L antibodies were able to stabilize atherosclerotic plaques because the CD40 pathway induces inflammation via stimulation of pro-inflammatory cytokine release from macrophages[122]. Activation of the CD40 pathway induced the production of matrix metalloproteinase, aggravating plaque destabilization, plaque rupture and arterial occlusion.

ApoE$^{-/-}$*Cd40*$^{-/-}$ mice with a deficiency in the CD40-TRAF2/3/5 binding showed no differences in atherosclerotic plaque development compared to control mice. In contrast, deficiency in the CD40-TRAF6 abrogated atherosclerosis as measured by a reduced recruitment of Ly6C^{+} monocytes in the arterial wall and reduced blood counts of Ly6C^{+} monocytes. Besides monocytes, macrophages were also identified in the arterial wall but these macrophages were polarized towards the anti-inflammatory M2 phenotype [123]. This could be due to the anatomic difference since TRAF1/2/3/5 is recruited to the proximal cytoplasmic domain of CD40 while TRAF6 is recruited to the distal end.

Since CD40-TRAF6 inhibition showed promising pre-clinical results, strategies were developed to target this pathway. Seijkens et al. successfully used nanotherapy to target the CD40-TRAF pathway in an atherosclerotic mice model leading to a decrease in atherosclerotic plaque development[124]. In the future, it would be interesting to translate this approach to other CVDs.

Type L; DR3 and TL1A

A second molecule of the type-L family is DR3, expressed on lymphocytes, NK cells, endothelial cells, and macrophages. Its known ligand, TL1A, is expressed on macrophages and DCs and can drive inflammatory disease progression [125, 126]. TL1A promotes foam cell formation in human macrophages in vitro by increasing both acetylated and oxidized low-density lipoprotein uptake, via enhancing intracellular total and esterified cholesterol levels and reducing cholesterol efflux [127]. Signaling through DR3 induces both apoptosis and activation of NF-kB [128]. This indicated that DR3 and TL1A may contribute to atherosclerosis development.

Table 1. Co-stimulation and inhibition molecules and their pre-clinical effects in cardiovascular diseases.

Super-family	Family	Molecule on APC	Receptor on T cell	Effect in cardiovascular disease
IgSF	B7/CD28	CD80, CD86	CD28	*Cd80/86/70-/-* mice ↓intimal hyperplasia in **VGD** mouse model (Simons et al.) *Cd80/CD86*$^{-/-}$ mice ↓intimal lesion in femoral artery cuff mouse model for **post-interventional remodeling**[33] *Cd80-/- and Cd80/86-/-* mice ↓ **GAD** after MHC class II-mismatched allograft heart transplantation[40] *Cd80/86*$^{-/-}$*Ldlr*$^{-/-}$ mice ↓ **atherosclerotic** lesions in aortic arch and descending aorta[47] *Cd80/86*$^{-/-}$*Ldlr*$^{-/-}$ bone marrow chimeras and *Cd28*$^{-/-}$*Ldlr*$^{-/-}$ chimeras ↑ atherosclerotic lesions due to ↓ Tregs[50]
IgSF	B7/CD28	CD80, CD86	CTLA4	CTLA-4Ig blocking CD80/86 treatment ↓intimal lesion in femoral artery cuff mouse model for **post-interventional remodeling** and ↓ **accelerated atherosclerosis** in a model of accelerated atherosclerosis in hypercholesterolemic ApoE3*Leiden mice[33] CTLA-4Ig blocking CD80/86 treatment ↓ **GAD** in a Lewis (LEW) to Fisher (F344) rat cardiac transplant model[38] CTLA-4Ig blocking CD80/86 treatment or CD80 blockade with mutant fusion protein Y100F ↓ **GAD** in a LEW to F344 rat cardiac transplant model[39] CTLA-4Ig blocking CD80/86 treatment ↓ cardiac dysfunction in a mouse **heart failure** model[41]. CTLA-4Ig blocking CD80/86 treatment ↓ homocysteine-accelerated atherosclerosis in *ApoE3*$^{-/-}$ mice[48] CTLA-4Ig blocking CD80/86 treatment ↓ **atherosclerosis** in mice with constitutive expression of CTLA-4 on T cells[49]
IgSF	B7/CD28	PD-L1, PD-L2	PD-1	Ischemic-reperfused cardiac cells expressed high levels of PD-1 and PD-L1 in a cardiac injury mouse model[54] *Pd-1*$^{-/-}$ mice ↑ dilated ventricles and ↓ contraction function In a spontaneous model of **dilated cardiomyopathy**[55] *Pd-1*$^{-/-}$ mice ↑ cardiomyopathy caused by autoantibodies against cardiac troponin I[56] *Pd-1*$^{-/-}$ mice CD8+ T cells cause ↑ myocarditis in a disease CD8+ T cell-mediated adoptive transfer model for myocarditis and *Pd-1*$^{-/-}$ mice ↑ myocarditis in an experimental autoimmune myocarditis model[57] *Pd-L1*$^{-/-}$*-MRL* mice ↑ development of fetal autoimmune myocarditis in Murphy Roths Large (MRL) mice[59] *Pd-1*$^{-/-}$*-MRL* mice in the development of fetal autoimmune myocarditis in Murphy Roths Large (MRL) mice[58] *Pd-L1/2*$^{-/-}$ mice, both effector memory T cells and naive T cells induced late-onset myocarditis in a GVHD model[60] Ldlr$^{-/-}$Pd-1$^{-/-}$ mice ↑ **atherosclerotic** lesion size in the aortic sinus and aorta[61, 62] *Pd-L1/2*$^{-/-}$*Ldlr*$^{-/-}$ mice ↑ **atherosclerotic** lesions in the aortic sinus and arch[63]
IgSF	B7/CD28	ICOSL	ICOS	ICOS and ICOSL are abundantly expressed in murine **atherosclerotic** leions[42, 66] *Ldlr*$^{-/-}$ mice transplanted with bone marrow of *ICOS*$^{-/-}$ mice ↑ lesion size in aortic sinus[67]
IgSF	TIM	TIM4	TIM1	Ldlr$^{-/-}$ mice treated with antibodies against TIM1 or TIM4 ↑ atherosclerotic lesions[73] TIM4 variants are associated with the **risk coronary heart disease and ischemic stroke**[72]
IgSF	TIM	Unknown	TIM3	In a mouse model for **myocarditis**, blocking TIM3 increased **myocarditis** abundantly in BALB/c mice[75]
IgSF	CD2/SLAM	CD48	CD2	Unknown
IgSF	CD2/SLAM	CRACC	CRACC	The expression of CRACC was ↑ in areas of stable compared to unstable **atherosclerotic** lesion segments[78]
IgSF	CD2/SLAM	Unknown	BLAME	The expression of BLAME was ↑ in areas of stable compared to unstable **atherosclerotic** lesion segments[78] ↑ expression of BLAME in human advanced **atherosclerotic** (AHA type V-VI) lesions[79]
IgSF	BTN	BTNL1, BTNL2	Unknown	Unknown

Table 1. Continued

TNFSF	Type V	CD70	CD27	CD27+ T cells are present in atherosclerotic lesions[42] *ApoE*$^{-/-}$ mice reconstituted with *Cd70*$^{-/-}$ mice bone marrow displayed ↑ **atherosclerotic** lesions[86] Overexpression of CD70 in CD70 transgenic ApoE*3 Leiden mice↓**atherosclerotic** lesions[209] *Cd70*$^{-/-}$ mice ↓ post-ischemic blood flow recovery, ↓ vasculogenesis, ↓ angiogenesis, ↓ arteriogenesis in a hindlimb ischemia mouse model[85]
TNFSF	Type V	OX40L	OX40	Patients with an **acute coronary syndrome** or **stable angina pectoris** ↑ OX40L blood levels[92] ↑in (soluble) OX40L (>40ng/ml) is associated with ↑risk for **acute myocardial infarction, sudden death** and **recurrent angina pectoris**[93, 94] OX40/OX40L mRNA levels ↑ in **coronary heart disease** patients and correlated with some clinical pathological features of coronary heart disease [91] ↑ OX40 expression in **pressure overload-induced hypertrophic** murine hearts regulated via CD4+ T cells[90] *Ldlr*$^{-/-}$ *mice* treated with OX40L antagonist ↓ number of T cells in **atherosclerotic** lesions and ↓ atherosclerotic lesion size[88] ↑ levels of OX40 characterize CD4+CD28null T cells in patients with acute coronary syndrome[96] BALB/C mouse treated with anti-OX40L blockade ↓myocarditis progression in a **myocarditis mouse** model [149] In *ApoE*$^{-/-}$ treated with OX40L-specific neutralizing antibody and *ApoE*$^{-/-}$ *Ox40l*$^{-/-}$ ↓ extent of aortic atheroma[102]
TNFSF	Type V	4-1BBL	4-1BB	*ApoE*$^{-/-}$*CD137*$^{-/-}$ and *Ldlr*$^{-/-}$*CD137*$^{-/-}$ mice ↓ in atherosclerotic lesions in atherosclerosis mouse models[109] *ApoE*$^{-/-}$ mice treated with CD137 agonist ↑ atherosclerotic lesions[108]
TNFSF	Type V	CD30L	CD30	*Ldlr*$^{-/-}$ mice treated with CD30L blocking antibody ↓ atherosclerotic lesion formation in the **aortic arch**[112] Expression of CD30 was up regulated on T cells in patients with **acute myocarditis and dilated cardiomyopathy**[111]
TNFSF	Type V	GITRL	GITR	GITR and GITRL are expressed in macrophage-rich areas of human **atherosclerotic** lesions[114] Lethally irradiated *Ldlr*$^{-/-}$ mice reconstituted with bone marrow from B cell specific GITRL transgenic mice ↓ **atherosclerotic** lesions[115] BALB/c mice receiving cells depleted of GITR^{HIGH} ↑autoimmune **myocarditis** [117]
TNFSF	Type L	CD40L	CD40	↑ levels of soluble CD40L in the peripheral blood from patients with **unstable angina**[210] *ApoE*$^{-/-}$*Cd154*$^{-/-}$ mice ↓ advanced **atherosclerotic** lesions[211]. *ApoE*$^{-/-}$ mice treated with anti-CD40L antibody induces a stable **atherosclerotic** lesion phenotype[212] *Ldlr*$^{-/-}$ mice treated with antibody against CD40L ↓ aortic **atherosclerotic** lesions[213] Human **atherosclerotic** lesions showed expression of CD40L on EC, VSMC, and macrophages[214] Cd40-Traf6$^{-/-}$ApoE$^{-/-}$ ↓ development of **atherosclerosis**[123]
TNFSF	Type L	TL1A	DR3	TL1A and DR3 are expressed in carotid endoarterectomy **atherosclerotic** lesions. TL1A and DR3 are involved in atherosclerosis via the induction of pro-atherogenic cytokines and ↓ in atherosclerotic lesion stability by inducing extracellular matrix degrading enzymes[129]
TNFSF	Type L	HVEM	LIGHT, LTβR	In human plasma samples, atherosclerosis reproduced by the amount of **coronary calcium, aortic wall thickness** and **aortic atherosclerotic lesion size** correlated with the level of circulating LTβR[134] LTβR signaling has been linked to the formation of tertiary lymphoid organs in the aortic adventitia of *ApoE*$^{-/-}$ mice and protection against **atherosclerosis** via VSMC–LTβR signaling[135] *ApoE*$^{-/-}$*Ltβr*$^{-/-}$ mice ↓ aortic **atherosclerotic** lesion burden[136]

VGD = Vein graft disease, GVHD = Graft versus host disease, EC = Endothelial cell, VSMC = Vascular smooth muscle cell

In human **atherosclerotic** plaques, TNFα induced the expression of TL1A. Both TL1A and DR3 were identified in regions rich in foam cells in carotid endarterectomy tissues[129]. The expression of DR3 was induced by activated monocytes and macrophages by atherogenic stimuli. In addition, Kim et al. observed that the vascular inflammation is accelerated by TL1A and DR3 ligation resulting in the induction of MMP-9, TNFα, IL-8, and MCP-1 and subsequently, progression of atherosclerotic plaques[130].

Type L; LTβR and LTα$_1$β$_2$

The biologically active heterotrimer Lymphotoxin (LT)α$_1$β$_2$ is unique in the TNF superfamily[131]. LTα$_1$β$_2$ possesses two binding sites for the LTβ receptor (LTβR, also known as TNFRSF3) with distinct affinities. Dimerization of LTβR by LTα$_1$β$_2$ is necessary and sufficient for signal transduction via TRAF3[132]. Surface LTα$_1$β$_2$ is detected on subsets of activated T and B cells and NK cells[133]. LTβR is expressed on ECs, SMCs, and cells of the myeloid lineage. Communication via LTβR dictates interactions between immune cells and modifies correct trafficking of lymphoid cells.

In humans, plasma LTβR was measured in blood from 3215 patients enrolled in the Dallas Heart Study. Higher levels of LTβR were observed in patients with increased coronary calcium, aortic plaques and aortic wall thickness measured by computed tomography [134]. This indicated that LTβR might be associated with **atherosclerotic** plaque development, although a causal relation still needs to be established.

Hu et al. observed an increase in atherosclerotic lesions in 32-35 weeks old *ApoE$^{-/-}$LTβR$^{-/-}$* mice compared to *ApoE$^{-/-}$* mice [135]. Grandoch et al. obtained comparable results from the aortic root of 23 weeks old *ApoE$^{-/-}$LTβR$^{-/-}$* mice. Fewer macrophages were invading the aorta, whereas the relative distribution of M1 and M2 macrophages remained unchanged [136]. In contrast, with the use of aged 78 to 85 weeks old *ApoE$^{-/-}$LTβR$^{-/-}$* mice, Hu et al. revealed an acceleration of atherosclerosis compared to age-matched *ApoE$^{-/-}$* mice. In vivo, this indicates that LTβR signaling may be atheroprotective in a site-specific and age-dependent way.

Therapeutic targeting of co-stimulation

Anti-CD80/86

Most pre-clinical studies described above, show beneficial effects of blocking co-stimulation on CVD. Therefore, investigating co-stimulation blockade as a therapeutic approach to prevent CVD should be encouraged. Anti-CD80 was the first antibody to block co-stimulation, however, since both CD80 and CD86 can bind to the co-stimulation receptor CD28, blocking only one co-stimulation molecule was not

effective[137]. The second developed co-stimulation blocker was a CD28Ig, blocking the interaction between CD28 and CD80/86[138]. However, because of the low affinity of CD28 for CD80/86, CD28Ig was unsuccessful. Subsequently, based on the fact that CTLA-4 binds CD80/86 with a much higher affinity, CTLA4-Ig was developed as a suitable candidate to block CD80/86 co-stimulation. CTLA4-Ig is a fusion protein consisting of the extracellular domain of CTLA-4 linked to the Fc region of immunoglobulin G1 (IgG1), and effectively blocks CD80/86 thereby inhibiting T cell activation [139]. Two CTLA4-Igs, abatacept and belatacept, are the first US Food and Drug Administration (FDA) approved co-stimulation blockers (FIG 2a). CTLA4-Ig is a fusion protein consisting of the extracellular domain of CTLA-4 linked to the Fc region of immunoglobulin G1 (IgG1), blocking CD80/86 and thereby inhibiting T cell activation. Abatacept was FDA approved in 2005 for the treatment of rheumatoid arthritis (RA), juvenile idiopathic arthritis, psoriatic arthritis and systemic lupus erythematosus (SLE) (BOX 2). Belatacept was FDA approved in 2011 for adult patients receiving a kidney transplant. Subsequently, abatacept and belatacept made their appearance in the cardiovascular research field.

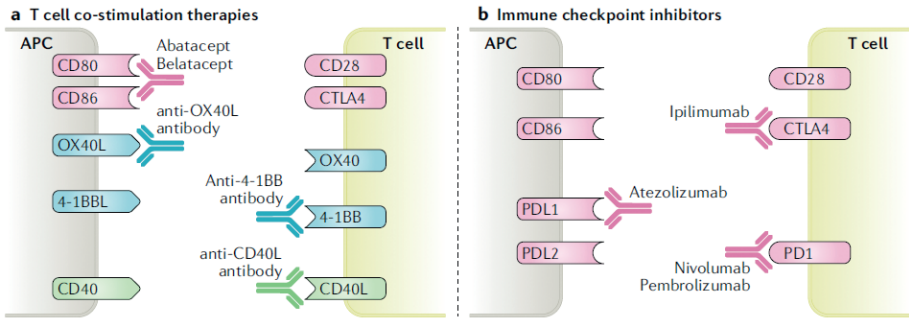

Figure 2. Therapeutic treatment options to target co-stimulation pathways and co-inhibition pathways. **a.** Therapeutic targeting of co-stimulation to block T cell activation can be via blocking co-stimulation molecules on antigen presenting cells (APCs) or T cells (antibodies shown in light blue). Abatacept and Belatacept are CTLA4-Ig fusion proteins consisting of the extracellular domain of CTLA-4 linked to the Fc region of immunoglobulin G1 (IgG1), blocking CD80/86 of the B7/CD28 family (in red) and thereby inhibiting T cell activation. Members of the type-L family of the TNFSF e.g., CD40 expressed on T cells can be blocked via a CD40-TRAF6 inhibitor. In addition anti-OX40 blocks the OX40-OX40L interaction which induces T cell anergy similar to anti-4-1BB antibodies blocking 4-1BB. **b.** In addition to the co-stimulation blockers abatacept and belatacept, several immune checkpoint inhibitors (ICIs) are already FDA approved. Blocking inhibitory pathways, leading to enhancement of T cell activation, can be accomplished via antibodies blocking inhibitory molecules on antigen presenting cells (APCs) or T cells (antibodies shown in yellow). The most well described ICIs block the co-inhibition pathways of CTLA-4 and PD-1 or PD-L1. Ipilimumab is a monoclonal antibody blocking CTLA-4 expressed on T cells, inducing T cell activation. Nivolumab and pembrolizumab block PD-1 expressed on T cells and atezolizumab blocks PD-L1 expressed on APCs, inducing T cell activation.

BOX 2 co-stimulation blockers in SLE and RA patients

Abatacept, a CTLA-4Ig fusion protein, selectively blocks CD80/CD86 co-stimulation, inhibiting T cell activation via CD28. Several autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) might benefit from abatacept due to their anti-inflammatory properties. Abatacept was FDA approved in 2005 for SLE and RA, and subsequently made their appearance in the cardiovascular research field

In RA patients, the effect of abatacept has been shown in several clinical trials. In the ADJUST study, abatacept reduced anti-CCP antibody levels in RA patients and maintained the inhibition of radiographic and MRI progression for more than 6 months after treatment in patients with very early RA[204]. In the AGREE trial, treatment with abatacept plus methotrexate (MTX) increased the proportions of patients achieving remission, even in the RA patient population at high risk of disease progression [205]. In the AVERT trial, patients treated with abatacept plus MTX achieved significantly higher rates of disease activity score defined remission, compared to MTX treatment alone. A small but significantly higher number of these patients achieved an absolute, drug-free, disease activity score defined remission following a withdrawal of all RA treatment[206]. Abatacept plus MTX was also able to inhibit radiographic disease progression in patients with early RA that are at high risk for disease progression[207].

In SLE patients, abatacept was able to prevent severe articular flares in comparison to placebo. In a subgroup of patients treated with a low dose of prednisone less than 7.5 mg/day, the proportion of patients who did not develop a new flare was higher in the abatacept group than in the control group. In addition, abatacept improved fatigue and sleep disturbance [208]. These clinical trials in patients with RA and SLE showed benefit of inhibiting co-stimulation in disease progression and this could be beneficial in other autoimmune diseases.

CTLA-4Ig has shown to reduce GAD, post interventional remodeling, heart failure and accelerated atherosclerosis in experimental models[33, 38, 41, 48, 140], but has not been tested yet in clinical trials for CVD. Interestingly, in addition to abatacept and belatacept, other co-stimulation blockers such as anti-CD40 (CD154) antagonists are being developed[141].

Anti-CD40

Although anti-CD40 showed promising results in pre-clinical studies, and may cause an even higher immune suppression compared to CTLA-4Ig[142, 143], in clinical trials severe side effects occurred in patients such as thromboembolic events. As described before, the CD40-TRAF6 signaling pathway is the preferred pathway. Nanotherapy was used as a therapeutic approach to target the CD40-TRAF pathways in macrophages. TRAF-STOP 6877002, incorporated into recombinant HDL nanoparticles, reduced atherosclerosis in *ApoE*$^{-/-}$ mice after 6 weeks[124] and was demonstrated as a safe and efficient target for atherosclerosis in both mice and non-human primates [144]. In addition, CD40-TRAF6 inhibitors were successfully used in the treatment of obesity-associated insulin resistance and other inflammatory diseases in experimental models[145-147]. This makes CD40-TRAF6 inhibitors potentially interesting for the treatment of CVD.

Anti-OX40

Another potentially interesting co-stimulation blocker is anti-OX40, which showed promising results in pre-clinical studies. OX40Ig blocks the OX40-OX40L interaction and induces T cell anergy. The use of OX40Ig resulted in beneficial outcomes in atherosclerosis [88] and other diseases such as acute renal allograft rejection [148]. In addition, blocking OX40 with an anti-OX40L antibody which also inhibits T cell activation, protected mice from myocarditis[149], atherosclerosis[150] and has been shown to inhibit graft rejection and graft-versus-host disease (GVHD)[151, 152]. Although the results of blocking OX40 in experimental models seems promising for CVD treatment, clinical trials are not on-going yet due to the limited amount of pre-clinical data. On the other hand, an anti-OX40 agonistic antibody GSK3174998, stimulating OX40 induced T cell activation, instead of the preferred T cell inhibition in CVD, is now being tested in a clinical trial for patients with selected advanced solid tumors[153]. Close supervision of the potential cardiovascular risks thus seems warranted.

Anti-4-1BB

Inhibition of T cell activation with anti-4-1BB-antibodies, showed promising pre-clinical cardiovascular results in atherosclerotic mouse models[109]. However, only agonistic 4-1BB antibodies, activating T cells, are currently being tested in cancer clinical trials. Utomilumab and urelumab target 4-1BB which stimulates clearance of cancer cells by T cell activation. A phase I study was successfully completed and showed that utomilumab was safe and efficient as an anti-cancer treatment[154]. Urelumab showed beneficial results in patients with locally advanced or metastatic solid tumors in a phase I study. However, an additional phase II study had to be terminated due to fatal hepatoxicity [155, 156]. Currently several clinical trials with utomilumab and urelumab

are ongoing for several cancer types alone or in combination with other ICIs such as pembrolizumab[157]. Although cardiovascular side effects are not reported in these studies, a pre-clinical study showed that agonistic 4-1BB antibodies promoted atherosclerosis in hypercholesterolemic mice[108] which is in line with the pre-clinical data of Jeon et al. who showed a decrease in atherosclerotic lesion development in 4-1BB deficient mice. This indicates that both utomilumab and urelumab might result in disadvantageous effects in CVD. Thus, the development of antagonistic 4-1BB antibodies, inhibiting T cell activation, should be encouraged and cardiovascular side effects of agonistic 4-1BB antibodies, activating T cells, should be monitored.

Conclusion therapeutic targeting of co-stimulation

Although targeting the IgSF and TNF(R)SF co-stimulation molecules seems promising in pre-clinical studies, clinical studies should learn us whether the cardiovascular outcomes are as promising as expected. Anti-CD80/86 treatment with CTLA4-Ig, such as the FDA approved abatacept and belatacept, and CD40-TRAF6 inhibitors are already well developed, but blocking of OX40 and anti-4-1BB co-stimulation could be interesting entrants. However, it should be taken into account that blocking co-stimulation can also lead to unwanted side effects. As previously described by Bluestone et al. [158], anergic T cells will no longer be able to clear viral infections, since the co-stimulation-mediated immune response is blocked. This might have detrimental long-term consequences. Furthermore, co-stimulation blockade can also lead to a decrease in activated Tregs, which can result in opposing cardiovascular outcomes. Thus, although promising, the effects on CVDs of therapeutic targeting of co-stimulation should be thoroughly clinically investigated.

Cardiovascular toxicity after immune checkpoint inhibitors treatment

In addition to co-stimulation blockers abatacept and belatacept, several co-inhibition blockers (ICIs) are already FDA approved and used as a cancer treatment (FIG 2b). The described ICIs block co-inhibition pathways involving of CTLA-4 and PD-1 or PD-L1. The first clinically FDA approved ICI was ipilimumab in 2011 for the treatment of unresectable or metastatic melanoma [159]. Ipilimumab is a monoclonal antibody blocking CTLA-4 and is currently undergoing several clinical trials for the treatment of other cancer types e.g. non-small cell lung carcinoma (NSCLC), small cell lung cancer (SCLC), bladder cancer and metastatic hormone-refractory prostate cancer. Other clinically FDA approved immune checkpoint inhibitors for several cancer types, are nivolumab and pembrolizumab blocking PD-1, and atezolizumab blocking PD-L1[160, 161]. Several other ICIs are currently in clinical or preclinical development such as an anti-TIM-3 antibody, dual anti-LAG-3/anti-PD-1 antibody, anti-lymphocyte-activated gene-3 (LAG-3, CD223) or anti-BTLA antibody [161].

The therapeutic potential of ICIs in several types of cancer is due to their anti-cancer response by blocking co-inhibition molecules expressed on tumor-reactive T cells. In physiological conditions, this prevents T cells from being activated by healthy cells in the human body, but in a cancer environment it causes a boost of (cytotoxic) T cell activation and cancer cell clearance[23]. Unfortunately, there are no clinical trials on CVD (safety) for these FDA approved drugs. Most pre-clinical, experimental studies mentioned above describe a protective role of CTLA-4, PD1 or PD-L1 and PD-L2 in CVD, and blocking these molecules may lead to severity of CVD. Boosting CD8+ T cell responses is beneficial in clearing cancer cells, but the accompanied increase in inflammation might be detrimental for cardiovascular outcomes in cancer patients treated with ICIs.

Since the proportional use of ICIs, several studies on the harmful cardiovascular effects of PD-1 blockers are reported. Lethal myocarditis was reported in a study of two patients diagnosed with a melanoma, treated with a combination of ipilimumab and nivolumab [29]. Post mortem analysis of the myocardium revealed an increase in PD-L1 expression on the myocardial epithelium. Interestingly, in a pre-clinical study Grabie et al. observed that PD-L1 up regulation on the myocardial endothelium was involved in the regulation of cardiac injury[162]. The cardiovascular toxicity in these patients was caused by the T cells activated by ipilimumab and nivolumab. It was observed that these T cells target an antigen shared by the tumor, skeletal muscle, and the heart that resulted in both tumor-and muscle (cardiac and skeletal) cell recognition and clearance[163]. However, in the safety databases of Bristol-Myers Squibb Corporate they observed that only 0.27% of the patients treated with a combination of ipilimumab and nivolumab developed myocarditis, with only 0.17% fatal events. Treatment with nivolumab alone resulted in significantly lower percentage of myocarditis (0.06%)[29]. Although combination treatment of ipilimumab and nivolumab has beneficial effects on tumor reduction, in the study of Postow et al. and Larkin et al., 52% and 36.4% respectively of the patients could not continue treatment of ipilimumab and nivolumab combined due to drug-related toxicity/side effects[164, 165].

Described in a case report, smoldering myocarditis was detected in a patient with an elevated troponin I level, after use of ipilimumab and nivolumab [166]. In this case, the myocarditis could be treated with high dose glucocorticoid therapy and troponin I levels rapidly decreased along the patients recovery. Interestingly, it was shown previously in a pre-clinical study that elevated cardiac troponin I was associated with cardiomyopathy in PD-1 deficient mice[56].

Furthermore, several other studies and case reports described fatal cardiac arrest[164], myocardial fibrosis [167], left ventricular dysfunction[168] and Takotsubo cardiomyopathy[169], after treatment of metastatic melanoma with ipilimumab. Also, several other reports on the induction of cardiovascular toxicity by ipilimumab are described [170].

In addition, cases of acute heart failure were reported after pembrolizumab treatment for metastatic melanoma[171] and NSCLC[172]. Zimmer et al. described atrial flutter, left ventricular systolic dysfunction and stable angina pectoris in cases of patients treated with pembrolizumab and sudden asystolia after treatment with nivolumab [30].

Acute myocarditis after treatment with nivolumab is described after treatment of metastatic melanoma and NSCLC [173-176]. Also combination therapy of both ipilimumab and nivolumab showed myocarditis as an adverse event in a patient treated for metastatic melanoma [177]. Here we reviewed all the 27 cases mentioned above and described that 10 patients died from drug-related toxicity (table 2).

Besides the fact that ICIs can cause cardiovascular toxicity, it is also suggested that ICIs aggravate atherosclerosis, by increasing effector T cell responses and reducing Treg functions[178], but this will probably only become more clear at prolonged survival/ follow up.

In conclusion, it can be stated that if cardiovascular adverse side effects of ICIs occur, they are often severe, fulminant and even can be lethal. Although these case reports and studies described events of cardiovascular toxicity after ICI treatment, the substantial incidence of cardiovascular toxicity after ICI treatment should be further examined in order to apply proper additional (counter) therapies.

Future perspectives

There is a close relation between treating cancer with ICIs and the risk of cardiac injury. Treating cancer with ICIs may be lifesaving, however, cardiovascular toxicity is an underlying complication that can be lethal. ICI related cardiovascular toxicity can occur since the ICI targets are not unique to cancer cells. Therefore, oncologists, cardiologists and immunologist should work closely together when patients are treated with ICIs. Several cardio-oncology societies such as the British Cardio-oncology society (BS-OS), Canadian Cardiac Oncology Network (CCON) and International Cardioncology Society, North America (ICOSNA) were already launched in the past years, which should act as an example for the rest of the world.

Table 2. Cardiovascular side effects of immune checkpoint inhibitors.

Case #	Disease	Treatment	Cardiovascular side effect	Outcome after treatment of side effects	Reference
1	Melanoma	Ipilimumab/nivolumab	Myocarditis	Deceased	Johnson 2016[29]
2	Melanoma	Ipilimumab/nivolumab	Myocarditis	Deceased	Johnson 2016[29]
3	Melanoma	Ipilimumab/nivolumab	Smoldering myocarditis	Respond to corticosteroid therapy	Norwood 2017[166]
4	Melanoma	Ipilimumab/nivolumab	Myocarditis and cardiomyopathy	Respond to steroid therapy	Heinzerling 2016[170]
5	Melanoma	Ipilimumab/nivolumab	Myocarditis	Deceased	Koelzer 2016[176]
6	Melanoma	Ipilimumab/nivolumab	Myocarditis	Respond to steroid therapy	Mehta 2016[177]
7	Melanoma	Ipilimumab/nivolumab	ventricular arrhythmia	Deceased	Postow 2015[165]
8	Melanoma	Ipilimumab	Cardiac arrest	Deceased	Larkin 2015[164]
9	Melanoma	Ipilimumab	Myocardial fibrosis	Deceased	Voskens 2013[167]
10	Melanoma	Ipilimumab	Left ventricular dysfunction	Respond to ACE inhibitor and beta-blocker treatment	Roth 2016[168]
11	Melanoma	Ipilimumab	Takotsubo cardiomyopathy	Respond to beta-blocker treatment	Geisler 2015[169]
12	Melanoma	Ipilimumab	Cardiomyopathy	Respond to diuresis treatment	Heinzerling 2016[170]
13	Melanoma	Ipilimumab	Myocardial fibrosis	Deceased	Heinzerling 2016[170]
14	Melanoma	Ipilimumab	Heart failure	Permanent ↓ EF despite diuresis treatment	Heinzerling 2016[170]
15	Melanoma	Ipilimumab	Myocarditis/CHF	Respond to steroid therapy, ACE inhibitor and beta blocker	Heinzerling 2016[170]
16	Melanoma	Ipilimumab	Myocarditis	Deceased	Heinzerling 2016[170]
17	Melanoma	Ipilimumab	Myocarditis	Deceased	Heinzerling 2016[170]
18	Melanoma	Pembrolizumab	Cardiac arrest	Respond to AED with defibrillation, catecholamines, steroids	Heinzerling 2016[170]
19	Melanoma	Pembrolizumab	Heart failure	Respond to prednisone, AT2-receptor blocker, beta-blocker, aldosterone-antagonist and diuretics treatment	Laubli 2015[171]
20	Melanoma	Pembrolizumab	Myocarditis	Deceased despite prednisolone treatment	Zimmer 2016[30]
21	Melanoma	Pembrolizumab	Sinus tachycardia	Respond to metoprolol succinate	Zimmer 2016[30]
22	Melanoma	Pembrolizumab	Stable angina pectoris	Respond to stop of pembrolizumab	Zimmer 2016[30]
23	Melanoma	Nivolumab	Sudden asystolia	Respond to resuscitation, prednisolone and stop of nivolumab.	Zimmer 2016[30]
24	Melanoma	Nivolumab	Myocarditis	Respond to prednisolone treatment	Tadokoro 2016[173]
25	NSCLC	Nivolumab	Myocarditis	Respond to glucocorticoids and amiodarone infusion	Gibson 2016[175]
26	NSCLC	Nivolumab	Myocarditis	Respond to prednisolone, ACE inhibitor, beta-blocker and diuretics treatment	Semper 2016[174]

CHF = congestive heart failure, EF = ejection fraction, AT2 = angiotensin II, ACE = angiotensin-converting enzyme, NSCLS = Non-small-cell lung cancer

Currently, colleagues are developing recommendations for the treatment of immune therapy related myocarditis since no guidelines are available yet [179-181]. Although this is highly appreciated, we also recommend focusing on preventing cardiovascular toxicity and clinical trials to test ICIs or co-stimulation therapy in CVD. Many clinical trials excluded patients with pre-existing CVD. Therefore, the incidence of cardiac toxicity after ICI treatment may be even higher than we now think. Interestingly, cardiovascular toxicity is usually reported shortly after the first ICI treatment. Future studies should also focus on late-onset cardiovascular toxicity and include patients with pre-existing CVD. Furthermore, a difference between short-and long-term CVD treatment should be taken into account. Cardiovascular interventions such as vein graft bypass surgery and percutaneous transluminal angioplasty should be treated shortly after intervention since VGD and restenosis can start to develop in the first weeks after intervention and can be probably limited in time. In contrast, atherosclerosis develops over the years and it is not clear when to start the treatment to prevent atherosclerosis. However, when clinical symptoms develop, long-term and even lifelong atherosclerosis treatment is indicated. So also with co-stimulation or co-inhibition targeted therapy, it should be taken into consideration, whether to treat the patients shortly after intervention, of life long with a good eye for the balance between benefit and risk.

With the expected increased use of ICIs in the future, several questions raised and should be answered. Since we know that combination treatment of anti-CTLA4 and anti-PD-1 drugs is associated with higher frequency of toxicities[182], it might be an option to only allow mono checkpoint inhibitor therapy instead of a combination of checkpoint inhibitors. To prevent systemic immune (de)activation, local ICI therapy may be a good solution[183]. Moreover, focusing on the stimulation of Tregs might have an important protective role in CVD[184] and can be therapeutically interesting.

As suggested by Brüstle et al., patients treated with ICI therapy should receive pre-therapeutic screening to assess cardiovascular risk factors. When treated, it would be good if they receive regular surveillance tests to check for signs of cardiovascular toxicity. Cardiac parameters and troponins levels are not routinely measured, but routine surveillance by cardiologist of patients treated with ICIs may be a solution to better monitor these patients and mitigate potential cardiac injury. The initiation of the EACVI/HFA Cardiac Oncology Toxicity registry, which registers all anti-cancer drug-related cardiovascular toxicity in breast cancer in Europe, is the first step towards excessive monitoring of cardiovascular toxicity[185]. However, since prevention is always better than cure, it is suggested that the focus of future (pre-) clinical trials should be on the development of co-stimulation blockers for CVD and not the treatment of cardiovascular toxicity after ICI use.

Conclusion

Blocking of co-stimulation pathways might be interesting therapeutic targets for CVD. On the other hand, inhibition of the co-inhibition pathways and stimulation of co-stimulation receptors might poses serious cardiovascular risks. Thus, although ICIs are revolutionary in cancer treatment, the risk of cardiovascular toxicity should be taken into consideration. Subsequently, focussing on the inhibition of co-stimulation pathways in the future could be world-shattering for the treatment of CVD.

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