Principles and Applications of Cardio-immunology in Heart Failure
Markousis-Mavrogenis, Georgios

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CHAPTER 2
Immunopharmacology and Immunomodulation in Heart Failure


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Abstract

The immune system is intimately involved in the pathophysiology of heart failure. However, it is currently underused as a therapeutic target in the clinical setting. Moreover, the development of novel immunomodulatory therapies and their investigation for the treatment of patients with heart failure are hampered by the fact that currently used, evidence-based treatments for heart failure exert multiple immunomodulatory effects. In this Review, we discuss current knowledge on how evidence-based treatments for heart failure affect the immune system in addition to their primary mechanism of action, both to inform practicing physicians about these pleiotropic actions and to create a framework for the development and application of future immunomodulatory therapies. We also delineate which subpopulations of patients with heart failure might benefit from immunomodulatory treatments. Furthermore, we summarize completed and ongoing clinical trials that assess immunomodulatory treatments in heart failure and present several therapeutic targets that could be investigated in the future. Lastly, we provide future directions to leverage the immunomodulatory potential of existing treatments and to foster the investigation of novel immunomodulatory therapeutics.

Key points

• Immune activation is intimately involved in the pathophysiology of heart failure.
• Immunomodulation is an underused therapeutic approach for the treatment of patients with heart failure.
• All current evidence-based treatments for heart failure can modulate immune activation in diverse ways, which has important clinical and therapeutic implications.
• The development of novel immunomodulatory interventions for the treatment of heart failure should take the immunomodulatory effects of existing treatments into account.
• Future developments in immunomodulation for heart failure should investigate both the optimization of immunomodulatory effects of current treatments and the potential benefits that can be derived by novel treatments.
Introduction

Heart failure (HF) is a heterogeneous clinical syndrome with increasing prevalence worldwide. This increase in prevalence, combined with increasing average life expectancy, constitutes a burgeoning healthcare problem. Additionally, hospitalizations for HF increase health-care expenditures, and robust epidemiological evidence indicates a 20–30% 1-year mortality among patients with HF. Therefore, concerted scientific efforts are necessary to optimize prevention and increase the number of available treatment modalities for prevalent or incident HF. The standard approach for the treatment of HF is focused on modulation of neurohormonal mechanisms, particularly of the sympathetic nervous system and renin–angiotensin–aldosterone system. Additional treatment options include the modification of fluid balance using diuretics and dietary fluid and salt restriction, as well as positive inotropic or mechanical support in selected individuals. Therapeutic options for HF have been extended beyond traditional neurohormonal modulation only in the past 3–4 years, with the advent of sodium–glucose cotransporter 2 (SGLT2) inhibitors.

Immunomodulation is a therapeutic avenue that remains underused in HF and could provide high-yield gains in both prevention and treatment. An extensive literature has documented that evidence-based HF treatments exert multiple beneficial immunomodulatory effects. Therefore, the search for novel immunomodulatory therapies in HF should be performed in the context of these effects. However, such approaches are hampered by the lack of literature collectively summarizing the immunomodulatory effects of each evidence-based treatment for HF. Additionally, lack of familiarity with the principles of immunology among cardiologists interferes with the widespread understanding of this important aspect of HF treatment. Therefore, the aims of this Review are fourfold: to provide clinicians with a summary of the pleiotropic immunomodulatory effects of evidence-based HF treatments; to present the mechanisms by which evidence-based HF treatments modulate the immune system; to delineate potential niches for the application of immunomodulation in HF; and to discuss research efforts in the field and promising therapeutic options for future investigation in the context of the immunomodulatory effects of current HF treatments.

To understand the immunomodulatory effects of HF treatments, we first need to consider how to study such effects. The most commonly used approach in the clinical setting is based on measurements of circulating markers of immune function (cytokines and immune cells), which do not constitute causal links. Most of the causal evidence demonstrating that immunomodulation is a mechanism of current HF therapeutics can be inferred from preclinical studies. The expression or levels of drug targets in cells with immune functions is also relevant in this context. In this Review, we present...
both clinical and experimental evidence regarding the immunomodulatory properties of evidence-based therapies for HF. Because of the high number of available studies, we only consider cardiovascular or immunology studies in humans or animals (in vivo or in vitro). We also consider studies of isolated immune cells from healthy animals, healthy individuals, or patients with cardiovascular disease. Fibrosis, which is closely related to immune function, has been reviewed previously and thus is not extensively discussed in this article. This Review also does not discuss HF caused by autoimmune cardiomyopathies because the pathophysiology of autoimmunity in the heart merits independent discussion.

Main principles of cardioimmunology

In this section, we summarize essential aspects of immunology and cardioimmunology for the benefit of researchers and clinicians who might not be familiar with some of the subjects discussed in this Review. A complete discussion of the involvement of the immune system in the pathophysiology of HF is beyond the scope of this Review and is discussed in greater detail elsewhere.

The immune system can be functionally subdivided into innate and adaptive immunity. Innate immune responses constitute the first responses at sites of inflammatory activation, whereas adaptive immune responses require more time to initiate but have a broader specificity for a large range of target antigens, as well as memory responses upon repeat encounter with the same antigen. Innate and adaptive immune responses are orchestrated by a complex interaction between immune and non-immune cells that leads to the generation of cellular, biochemical, and humoral responses.

Innate immune responses

Innate immune cells originate from pluripotent haematopoietic progenitors in the bone marrow or, in the case of tissue-resident cells, from self-renewing mesenchymal stem cell pools in their respective host tissues. The main innate immune cells are neutrophils and mononuclear phagocytes (monocytes and macrophages). Neutrophils are short-lived cells that arrive first at an inflammatory site and can phagocytose and kill microbes, degranulate to release antimicrobial enzymes, generate reactive oxygen species (ROS) and produce neutrophil extracellular traps (NETs) composed of strands of DNA, histones, and granule contents. Neutrophils can be detrimental in acute cardiac inflammation because they can promote the occurrence of ventricular tachycardia. Monocytes arrive at sites of injury from the blood later than neutrophils and differentiate into macrophages. Macrophages have traditionally been classified into two primary phenotypes: classically activated macrophages (M1), which promote
pro-inflammatory responses, and alternatively activated macrophages (M2), which promote wound healing, repair, and fibrosis. Macrophages can phagocytose microbes, apoptotic cells (including apoptotic infiltrating neutrophils) and, crucially in the context of HF, mitochondria and cellular debris ejected from stressed cardiomyocytes. These functions are essential to limit the pro-inflammatory effects of cellular debris from apoptotic or necrotic cells. Macrophages can produce ROS and nitric oxide (NO) in response to inflammatory activation. Other cells that participate in innate immune responses are basophils, eosinophils, mast cells and innate lymphoid cells, such as natural killer (NK) cells.

Innate immune responses are initiated by cellular sensors that recognize either pathogen-associated molecular patterns (PAMPs), which are microbial components that are not normally produced by the body (such as single-stranded or double-stranded RNA, bacterial lipoproteins and peptidoglycan), or damage-associated molecular patterns (DAMPs), which are components released by damaged or dying cells (such as extracellular ATP, mitochondrial DNA and heat-shock proteins) that act as indicators of cellular distress or damage. PAMPs and DAMPs are detected by germline-encoded pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), NOD-like receptors, retinoic acid-inducible gene I-like receptors, C-type lectin receptors and acute phase proteins. DAMPs can also be sensed by non-PRRs, such as the receptor for advanced glycation end products. In the context of this Review, TLR4 is particularly relevant because it binds to lipopolysaccharide (LPS), a bacterial product that is used as a leukocyte activation signal in vivo and in vitro studies.

Ligands binding to PRRs activate intracellular signalling cascades, including nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) signalling, that subsequently activate inflammatory gene programmes as well as inflammasomes. The NLRP3 inflammasome in particular has attracted considerable attention in HF research. The NLRP3 inflammasome is a complex of the NOD-like receptor NLRP3, an adaptor called ASC and the protease caspase 1 that is formed after binding of NLRP3 to various ligands. The activated NLRP3 inflammasome produces mature forms of IL-1β and IL-18. NLRP3 inflammasome activation requires two signals: a priming signal that leads to the upregulation of the expression of the genes encoding NLRP3, pro-IL-1β and pro-IL-18 (secondary to NF-κB activation) and a second signal provided by a plethora of PAMPs or DAMPs.

**Antigen-presenting cells**

Antigen-presenting cells (APCs) bridge innate and adaptive immune responses. The most characteristic APCs are dendritic cells, which are phagocytes that continuously sample their milieu, capture extracellular proteins from microbes and infected or
transformed cells, present peptides on major histocompatibility complex (MHC) class I or class II molecules, and migrate to draining lymph nodes where they can initiate adaptive immune responses by activating naive T cells. Macrophages and B cells can also function as APCs but are less efficient than dendritic cells. Cardiac fibroblasts have been found to express MHC class II molecules, suggesting that these cells can also potentially function as APCs.

Adaptive immune responses

Adaptive immune cells include B and T lymphocytes, both of which are produced in the bone marrow and mature either in the bone marrow (B cells) or the thymus (T cells). B cells are produced continuously throughout life in the bone marrow. However, the thymus ceases to produce new T cells by early adulthood, and the T cell population is maintained by division of mature T cells. T cells and B cells express structurally similar receptors, which are produced by somatic recombination of genes; this genetic recombination ensures a broad range of specificity for both microbial and non-microbial antigens. T cell receptors recognize peptides of intracellular origin presented on MHC class I molecules and peptides of extracellular origin presented on MHC class II molecules. Immature CD4⁺ and CD8⁺ T cells express T cell receptors with random specificities and are positively or negatively selected to eliminate autoreactive T cells or T cells that do not bind to MHC molecules. The remaining naive T cells differentiate into naïve CD8⁺ cytotoxic T cells, which can recognize MHC class I molecules, or naïve CD4⁺ T cells, which can recognize MHC class II molecules. Naïve CD4⁺ T cells can then be activated by APCs through peptide antigen presentation on MHC class II. A second co-stimulatory signal from specific surface receptors (such as CD40, CD70, CD80 and CD86) on APCs is required to prevent inappropriate T cell activation. A third signal derived from cytokines in the cellular milieu polarizes the CD4⁺ T cells towards specific effector subtypes, such as T helper (Tₜ₁), Tₜ₂, Tₜ₁₇ and regulatory T (Tₚₑ₉) cells. CD4⁺ T cells have various functions including the activation of phagocytes, B cells and CD8⁺ T cells by producing cytokines and co-stimulatory molecules. Naïve CD8⁺ T cells are activated by dendritic cells that present peptide fragments from infected or transformed cells on MHC class I molecules in a process called cross-presentation. CD8⁺ T cells normally kill infected cells or cancer cells by inducing apoptosis but have also been shown to exert cytotoxic activity against cardiomyocytes in the setting of myocardial infarction (MI). Both CD4⁺ and CD8⁺ T cells are also involved in various aspects of the pathophysiology of HF. Lastly, B cells can differentiate into antibody-producing plasma cells after binding of protein or non-protein antigens to B cell receptors. B cells have also been implicated in the pathophysiology of HF.
Cytokines as mediators of the immune response

Cytokines are a heterogeneous group of secreted proteins with many functions that are related not only to the immune system but also to other systems, most notably the nervous system. Cytokines are classified into superfamilies, including interleukins, chemokines, interferons, tumour necrosis factors, transforming growth factors and colony-stimulating factors, each with various members and distinct functions. Cytokines can also be classified according to their receptors. Among other functions, cytokines provide growth signals to immune cells and aid in their differentiation, activate effector functions of lymphocytes and phagocytes, and promote the migration of immune cells from the blood into target tissues. In chronic inflammatory states, cytokines can have detrimental effects, as observed in HF. However, cytokines such as IL-10 exert potent anti-inflammatory effects. A discussion of all cytokines and their functions is beyond the scope of this Review and has been published previously, including in the context of HF specifically.

Leukocyte recruitment to tissues

Immune cell migration is orchestrated by complex interplay between adhesion molecules and their ligands on endothelial cells and leukocytes, as well as directed migration (chemotaxis) induced by specific cytokines termed chemokines. Chemokines can be classified into CC, CXC, C and CX3C families based on the structure of their cysteine residues. Chemokines control the influx of immune cells into lymphoid tissues and sites of injury through a concentration gradient, whereby immune cells that express chemokine receptors for a specific chemokine follow the concentration gradient of the chemokine towards the tissue of origin. Notably, chemokine receptors can bind to more than one type of chemokine to promote migration. Immune cell migration also requires a bidirectional interaction between endothelial cells and immune cells, which involves adhesion molecules and their receptors on both cell types. The adhesion molecules E-selectin and P-selectin are expressed on endothelial cells that have been activated by inflammatory stimuli (such as tumour necrosis factor (TNF) and IL-1β) and bind to ligands present on circulating immune cells. The adhesion molecule L-selectin is expressed on leukocytes and binds to ligands on endothelial cells. Activated endothelial cells can capture circulating leukocytes in a process called selectin-mediated rolling. During this process, leukocytes are exposed to endothelial membrane-bound chemokines that activate other adhesion molecules on leukocytes called integrins (such as α4β1, αMβ2, αLβ2 and α4β7 integrins). These integrins bind to endothelial cell ligands that are also upregulated by inflammatory stimuli (such as intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1)). At this point, leukocytes firmly adhere to the endothelium and can subsequently transmigrate through the endothelial barrier into the inflamed tissue. Although this overall process is fundamentally the same for all immune cells, distinct combinations of selectins, integrins and chemokines can recruit specific cell types from the circulation depending on the nature of the underlying inflammatory response. The phases of immune activation in HF are presented in Figure 1.
Immunomodulatory effects of established HF therapies

The main immunomodulatory effects of established evidence-based treatments for HF are summarized in Box 1 and Figure 2 and described in detail in the following sections.

β-Adrenergic receptor antagonists

Physiological functions of adrenergic receptors in immunity
Adrenergic and noradrenergic signalling is involved in diverse immune processes. Both primary and secondary lymphoid organs are innervated by sympathetic nerves, and sympathetic activation halts lymphocyte mobility to and from these tissues. Additionally, most immune cells express α-adrenergic receptors and/or β-adrenergic receptors, including T cells, B cells, NK cells, monocytes, macrophages, neutrophils and dendritic cells. Under physiological conditions, adrenergic signalling has diverse immunosuppressive effects in all aforementioned cell types. However, sympathetic overactivation and subsequent adrenergic receptor desensitization are well-established pathophysiological phenomena in HF and constitute the basis for the therapeutic use of β-adrenergic receptor antagonists (also known as β-blockers).

Effects of β-adrenergic blockade on inflammation and the immune system
Therapy with β-blockers has been shown to reduce the levels of circulating inflammatory cytokines in patients with HF. In 32 patients with dilated cardiomyopathy (DCM), treatment with metoprolol or bisoprolol reduced the circulating levels of IL-1, TNF and soluble TNF receptor 2 (sTNFR2) compared with levels before treatment initiation. In 24 patients with DCM treated with a β-blocker carvedilol or metoprolol, treatment for >6 months reduced circulating IL-6 levels compared with baseline. A reduction in plasma IL-6 levels was also observed in a study in 21 patients with HF treated with metoprolol. Furthermore, in an additional study in patients with HF, patients taking β-blockers had lower plasma levels of IL-6, sTNFR1 and sTNFR2 than patients who had not received β-blocker therapy.

β-Blocker therapy also normalizes circulating leukocyte counts in patients with HF. In a study that examined patients attending a HF centre, patients with HF treated with β-blockers (n = 22) had nonsignificant increases in the number of circulating T cells and significantly lower interferon-γ (IFNγ) to IL-10 ratios compared with untreated patients (n = 12). In a subsequent better-powered study, total leukocyte counts were similar in patients with chronic HF (n = 75) and age-matched healthy controls (n = 20), but patients with HF had neutrophilia and lymphopenia. In addition, those who received β-blockers (n = 51) had increased circulating T cell and B cell numbers and decreased neutrophil numbers compared with untreated patients (n = 24).
Figure 1. Phases of immune activation in heart failure. (1) A cardiac insult (such as hypertension, diabetes mellitus, atherosclerosis, or valvular disease) causes cardiomyocyte stress or death. (2) Stressed or dying cells release mediators that act as damage-associated molecular patterns (DAMPs), including mitochondrial DNA (mtDNA), heat-shock proteins (HSPs) and reactive oxygen species (ROS), which activate pattern recognition receptors (PRRs) on cardiac-resident macrophages and dendritic cells. These cells produce cytokines and chemokines as a result of the activation of the NLRP3 inflammasome and pro-inflammatory signalling via nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK). (3) Cytokines and chemokines activate endothelial cells, which in turn start producing chemokines and expressing adhesion molecules, including selectins (E-selectin and
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P-selectin) and integrin ligands (intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1)). (4) Cytokines and chemokines also leak into the circulation and mobilize immune cells from reservoirs (spleen and bone marrow), which then migrate to the site of injury by following the chemokine concentration gradient. (5) In parallel, activated dendritic cells migrate from the injured myocardium to draining lymph nodes and initiate T helper (T\textsubscript{H}) cell activation and expansion by presenting cardiac antigens to the T cell receptor (TCR) (signal 1), by providing co-stimulatory signals such as CD40 and its ligand (CD40–CD40L) (signal 2) and by secreting cytokines (signal 3) that guide the differentiation of T\textsubscript{H} cells into specific subsets (T\textsubscript{H}1, T\textsubscript{H}2 and T\textsubscript{H}17 cells among others). Activated T\textsubscript{H} cells can in turn activate B cells. Dendritic cells also activate cytotoxic T cells specific for cardiac antigens. Activated T cells and antibody-producing plasma cells then enter the blood circulation at later stages. (6) At the site of injury, immune cells interact with endothelium-derived chemokines and adhesion molecules to exit the blood circulation and infiltrate the myocardium. Neutrophils are the first responders, followed by macrophages and, later on, T cells. Other immune cells can also infiltrate into the myocardium but are not shown in the figure. (7) Infiltrating immune cells perform their corresponding functions at the site of injury and produce cytokines, chemokines, and ROS. (8) The inflammatory response can resolve completely, secondary to immunoregulation by regulatory T (T\textsubscript{reg}) cell and reparative M2 macrophages, with subsequent release of (among others) IL-10 and transforming growth factor-β (TGF\textbeta). This resolution phase can lead to deposition of fibrous tissue and return to homeostasis or can develop into chronic inflammation. Generation of memory responses or trained immunity occurs in both scenarios. Unresolved inflammation or memory responses and trained immunity can propagate cardiac damage in a vicious cycle. ECM, extracellular matrix, MHC, major histocompatibility complex; TNF, tumour necrosis factor.

Studies in experimental models suggest that β2-adrenergic receptor blockade can interfere with leukocyte migration and that adrenergic receptors have a key role in immune cell mobilization from reservoirs to the injured heart. Mice with bone marrow-specific β2-adrenergic receptor deficiency and thus by extension β2-adrenergic receptor-deficient immune cells, die from myocardial rupture after induction of MI. 49 These animals showed increased splenic macrophage retention caused by increased VCAM1 expression in the spleen and macrophages. 49 VCAM1 expression was also reduced by β2-adrenergic receptor activation in both mouse and human macrophages in vitro. 49 In mice receiving chronic isoproterenol infusion, immune cell-specific deficiency in β2-adrenergic receptors reduced cardiac immune infiltrates (macrophages, CD4\textsuperscript{+} T cells and B cells) and ameliorated adverse cardiac remodelling and systolic dysfunction. 50 Wild-type mice receiving chronic treatment with the β2-selective β-blocker ICI-118551 or with clinically used β-blockers (carvedilol or low-dose or high-dose metoprolol) showed incremental increases in spleen size and splenic VCAM1 expression over time with all interventions (β2-selective and non-selective) except low-dose metoprolol (β1-selective). 51 These changes were coupled with increased accumulation of monocytes, macrophages, mast cells and neutrophils in the spleen and reduction in the expression of CC-motif chemokine receptor 2 (CCR2) in bone marrow and peripheral blood leukocytes and in CC-motif chemokine 2 (CCL2)-mediated migration. 51 Similarly, peripheral blood
leukocytes from patients receiving chronic treatment with β-blockers (n = 38; ~55% with HF) showed reduced CCR2 expression and reduced chemotaxis to CCL2 compared with leukocytes from patients who had not received β-blocker therapy. Taken together, these studies illustrate that in the context of myocardial ischaemia, β2-adrenergic receptors are required to maintain myocardial integrity after loss of cardiomyocytes. By contrast, in the context of chronic hyperstimulation of β-adrenergic receptors, as would occur in patients with established HF, β2-adrenergic receptor blockade has numerous beneficial immunomodulatory effects.

Non-class immunomodulatory effects of metoprolol and carvedilol

A non-class pharmacological effect is defined as an effect that is not generalizable to all drugs within the drug class. Interestingly, some β-blockers have non-class immunomodulatory effects. In mice with ischaemia–reperfusion injury, treatment with metoprolol (but not atenolol or propranolol) inhibited neutrophil migration and infiltration into the injured myocardium and reduced infarct size compared with vehicle treatment.

By contrast, in the COMET trial, metoprolol was less effective at improving outcomes than carvedilol in patients with established HF, even though this drug has also been shown to reduce neutrophil migration. Carvedilol is 4.5 times more selective for β2-adrenergic receptors than β1-adrenergic receptors, whereas metoprolol is 2.3 times more selective for β1-adrenergic receptors than β2-adrenergic receptors. Therefore, the different effects of each drug could be mediated by their differential receptor selectivity. Carvedilol also interferes with T cell activation and cytokine production (IFNγ, IL-2, IL-4 and IL-10) by downregulating NF-κB activity. Compared with untreated patients with HF and those treated with bisoprolol or nebivolol, patients treated with carvedilol had reduced expression of CD107 (a degranulation marker) on CD8+ T cells and HLA-DR expression on all lymphocytes. In addition, carvedilol inhibits the NLRP3 inflammasome by inducing autophagy in macrophages. These and other potential non-class effects remain to be investigated.
Box 1. Summary of immunomodulatory properties of established treatments for heart failure.

**β-Blockers**
- β-Blockers (also known as β-adrenergic receptor antagonists) modulate immune activation by reducing the levels of circulating cytokines and by normalizing neutrophil counts and increasing lymphocyte numbers in the blood. Blockade of the β2-adrenergic receptor on immune cells interferes with their mobilization from the spleen by upregulating retention factors in the spleen and immune cells, and by reducing chemokine generation. Metoprolol and carvedilol have non-class immunomodulatory effects. Both metoprolol and carvedilol inhibit neutrophil migration, whereas carvedilol interferes with multiple immune functions and inhibits the NLRP3 inflammasome. The observed differences in the effects of each drug might be related to their pharmacological properties.

**ACE inhibitors**
- Angiotensin-converting enzyme (ACE) inhibitors interfere with the immune system by modulating the levels of circulating immune mediators (decrease CCL2, IL-6 and C-reactive protein (CRP) and increase IL-10). ACE inhibitors also prevent immune cell mobilization from the spleen and bone marrow via upregulation of retention factors and downregulate the production of chemokines in damaged cardiac tissues and endothelium. Furthermore, ACE inhibitors blunt vascular inflammation and atherosclerosis and interfere with the activation, cellular signalling and cytokine production of monocytes, T cells, dendritic cells. They also inhibit T cell proliferation.
- In terms of use for immunomodulation, ACE inhibitors and angiotensin II receptor type 1 (AT1R) blockers (ARBs) are not completely interchangeable. Enalapril can inhibit the dimerization of AT1R, and lisinopril modulates dendritic cell activity and cardiac inflammation via upregulation of angiotensin II receptor type 2, which was not seen with candesartan. Any additional immune-specific effects of ACE inhibitors remain to be elucidated.

**MRAs**
- Mineralocorticoid receptor antagonists (MRAs) interfere with T cell migration by modulating the expression of adhesion molecules and chemokine receptors in these cells. MRAs also inhibit monocyte and lymphocyte functions through pleiotropic effects beyond antagonism of mineralocorticoid receptor signalling.
- Liposomal delivery of eplerenone to macrophages recapitulated many of the beneficial effects of systemic eplerenone in an animal model of ischaemic HF. MRAs also improved cardiac function in various animal models of HF with preserved ejection fraction through various mechanisms, including reduction of reactive oxygen species and downregulation of the expression of ACE, angiotensin II, cytokines, chemokines and adhesion molecules, leading to reduced monocyte and lymphocyte infiltration in the heart.
- MRAs also mediate neuroimmune interactions in HF and prevent the inflammatory activation of brain centres in animal models of HF.

**Dietary interventions**
- The Dietary Approaches to Stop Hypertension (DASH) diet (high fruit, vegetable, nut and legume intake, low-fat dairy content, and low salt intake) reduces HF incidence and plasma CRP levels.
- Salt restriction is often advocated, but clinical evidence to support this intervention is so far lacking. In experimental settings, sodium-rich environments favour T cell polarization into Th17 cells and promote pro-inflammatory macrophage activation.

**ARBS**
- Cardiac cells and various immune cells express AT1R and can produce angiotensin II. Angiotensin II has important roles in the function of macrophages, including reduction of reactive oxygen species and downregulation of the expression of ACE, angiotensin II, cytokines, chemokines and adhesion molecules, leading to reduced monocyte and lymphocyte infiltration in the heart.
- ARBs reduce the levels of circulating immune mediators (CCL2, CRP, IL-6, soluble intercellular adhesion molecule 1, soluble vascular cell adhesion molecule 1 and tumour necrosis factor), interfere with angiotensin II-mediated T cell co-stimulation and differentiation towards Th1 cells and Th17 cells and inhibit T cell activation. ARBs also inhibit reactive oxygen species production in polymorphonuclear and mononuclear cells and inhibit the inflammatory activation of monocytes, as well as dendritic cell function. ARBs also counteract vascular inflammation by inhibiting inflammatory activation and mediator production in vascular cells and by promoting T cell differentiation into Th2 cells and regulatory T (Treg) cells. Furthermore, ARBs interfere with angiotensin II-induced T cell and NK cell proliferation.
- ARBs mediate immune functions by directly antagonizing AT1R and by inhibiting the NLRP3 inflammasome and inducing the activation of peroxisome proliferator-activated receptor-γ and the expression of its target genes. The immunomodulatory effects of ARBs depend on the degree of expression of peroxisome proliferator-activated receptor-γ target genes. ARBs can also act as direct antagonists of CC-motif chemokine receptor 2 (CCR2).
**Figure 2.** Summary of the immunomodulatory properties of currently used therapies for heart failure. Systemic and organ-specific effects are shown on the left and immune cell-specific effects are shown on the right. Mechanisms are described for each drug class in detail in the corresponding text sections. Organ-specific effects include inhibition of leukocyte mobilization from the bone marrow and spleen, cardiac inflammation, vascular inflammation and hypothalamic inflammation, and modulation of circulating immune cells and mediators. ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor type 1 blocker; ARNI, angiotensin receptor–neprilysin inhibitor; DASH, Dietary Approaches to Stop Hypertension; MRA, mineralocorticoid receptor antagonist; NK cell, natural killer cell; ROS, reactive oxygen species; SGLT2, sodium–glucose cotransporter 2.
Angiotensin-converting enzyme inhibitors

Physiological functions of ACE in immunity
Angiotensin-converting enzyme (ACE) is a dicarboxypeptidase produced in various cells including myeloid cells. ACE converts angiotensin I (Ang I) into Ang II and also participates in innate and adaptive immunity.\textsuperscript{58} Differentiation of monocytes into macrophages or dendritic cells is associated with increased ACE production.\textsuperscript{59,60} Furthermore, mice overexpressing ACE in myeloid cells show resistance to tumour growth and improved bacterial clearance compared with wild-type mice.\textsuperscript{58} Neutrophils also increase ACE expression in response to infection and can more effectively eliminate bacteria if they overexpress ACE, owing to increased superoxide production, ROS generation and NET formation.\textsuperscript{58} Additionally, ACE trims peptides that are presented on MHC class I and class II molecules and is thus important for antigen presentation.\textsuperscript{58}

Effects of ACE inhibitors on inflammation and the immune system
ACE inhibitors modulate the levels of various circulating markers of immune activation. In a randomized study in patients with HF, therapy with a high dose of the ACE inhibitor enalapril (40 mg per day, \( n = 37 \)) reduced circulating IL-6 levels compared with low-dose enalapril (5 mg per day, \( n = 38 \)).\textsuperscript{61} Patients with coronary artery disease (CAD) and hypertension who received enalapril (20 mg per day, \( n = 27 \)) or the angiotensin II receptor type 1 (AT1R) blocker (ARB) irbesartan (300 mg per day, \( n = 21 \)) for 3 months had increased plasma IL-10 levels compared with baseline.\textsuperscript{62} Additionally, in 90 patients with stable CAD without hypertension, therapy with the ACE inhibitor perindopril (4 mg per day, \( n = 30 \)) progressively reduced the circulating levels of C-reactive protein (CRP) and CCL2 while increasing circulating IL-10 levels at 30 days and 90 days of treatment compared with baseline.\textsuperscript{63} Similar but less-pronounced effects were observed with enalapril (20 mg per day, \( n = 30 \)), but not for high-sensitivity CRP (hsCRP).\textsuperscript{63} In a follow-up study in 134 patients with stable CAD, both enalapril and perindopril reduced circulating hsCRP after 30 days and 90 days of treatment compared with baseline.\textsuperscript{64}

Numerous studies in mice with left anterior descending coronary artery (LAD) ligation have demonstrated that the ACE inhibitors enalapril, ramipril and lisinopril interfere with monocyte mobilization from the spleen and bone marrow. In mice with MI, early (3 days before MI) or late (7 days after MI) therapy with enalapril reduced the TSPO–PET signal (a marker of macrophage content\textsuperscript{65}) in the infarct zone, remote myocardium, and brain, compared with untreated animals.\textsuperscript{66} This finding was also observed in mice with established HF.\textsuperscript{66} Another study in mice confirmed that pretreatment with enalapril abrogated splenic monocyte recruitment to the heart after MI.\textsuperscript{67} Furthermore, enalapril inhibited AT1R dimerization and crosslinking in monocytes, a process that is augmented by MI.\textsuperscript{67} Treatment with enalapril after MI also impaired
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monocyte trafficking to the heart, but to a lower extent than enalapril pre-treatment.\textsuperscript{67} In addition, enalapril reduced the expression of the monocyte markers CD68 and LY6C, the expression of the pro-inflammatory mediators myeloperoxidase (MPO) and TNF, as well as the expression of the pro-fibrotic mediator transforming growth factor-β (TGFβ) in infarcted tissues.\textsuperscript{67} Similarly, treatment with ramipril in mice immediately after MI reduced cardiac IL-1β levels compared with control mice.\textsuperscript{68} Ramipril-treated mice also showed an accumulation of haematopoietic progenitors in the bone marrow niche, driven by the upregulation of retention factors (angiopoietin 1, CXC-motif chemokine 12 (CXCL12), KIT ligand and VCAM1).\textsuperscript{68} Interestingly, the effects of ACE inhibitors on leukocyte migration are not limited to monocytes. Treatment with lisinopril inhibited splenic dendritic cell maturation and mobilization to the heart in mice with permanent LAD ligation, via an Ang II receptor type 2 (AT2R)-dependent mechanism not observed with candesartan treatment.\textsuperscript{69}

ACE inhibitors can also blunt vascular inflammation. Treatment of diabetic mice with perindopril\textsuperscript{70} or high-fat-fed rabbits with fosinopril\textsuperscript{71} reduced monocyte infiltration to atherosclerotic plaques. In atherosclerosis-prone mice, therapy with captopril strongly reduced aortic atherosclerosis and the infiltration of CD8\textsuperscript{T} T cells and CCR9\textsuperscript{+} macrophages in the aortic wall compared with control mice.\textsuperscript{72} Captopril therapy also reduced the expression of CCL25, a chemokine that attracts CCR9\textsuperscript{+} macrophages, in aortic atherosclerotic plaques. Human vascular smooth muscle cells (VSMCs) increased IL-6 production in response to Ang I administration in vitro, which was inhibited by administration of captopril or ramiprilat.\textsuperscript{73} Lastly, in mice with ischaemic HF, treatment with ramipril reduced aortic myeloid cell infiltration, ROS production and TNF expression.\textsuperscript{68}

Additionally, ACE inhibitors have been shown to reduce inflammation in metabolic, hypertensive and pressure overload-induced HF. In mice with metabolic cardiomyopathy, therapy with captopril affected whole-heart transcriptional profiles by reducing the transcription of genes related to acute phase response signalling, T cell survival signals, dendritic cell maturation, NO and ROS production in macrophages, complement system and leukocyte extravasation signalling.\textsuperscript{74} Additionally, transient enalapril treatment in spontaneously hypertensive rats reduced the expression of the chemokines CCL2, CCL7, CXCL1 and CXCL16 as well as oxidative stress responses and cyclooxygenase 1 expression in cardiac fibroblasts.\textsuperscript{75} Lastly, in mice with transverse aortic constriction (TAC), treatment with captopril reduced circulating TNF and IL-6 levels and ameliorated the HF phenotype compared with untreated animals.\textsuperscript{76} Captopril also normalized myocardial WNT3A–β-catenin and JAK–STAT signalling in these mice. Reduction of WNT3A–β-catenin signalling has been associated with reparative M2-like macrophage
polarization, whereas JAK–STAT signalling activates the transcription of numerous genes in leukocytes in response to cytokine signalling.

ACE inhibitors can also interfere with diverse leukocyte functions. Enalapril dose-dependently inhibited the maximal proliferation of mouse splenocytes exposed to a T cell-activating stimulus. Furthermore, captopril reduced the expression of the activation markers CD25 and CD69 in activated mouse splenic CD4+ and CD8+ T cells and reduced their transmigration by downregulating expression of α4 and α5 integrins in these cells. In human peripheral blood mononuclear cells (PBMCs), pre-treatment with captopril or enalapril induced dose-dependent reductions in TNF and IL-1 secretion in response to either LPS or IL-1β stimulation. Cilazapril also reduced IL-1β-induced cytokine production in PBMCs at high concentrations. Activated PBMCs from healthy donors showed reduced IL-12 production in response to either captopril or lisinopril stimulation, whereas both medications reduced IFNγ production in activated T cells. In addition, lisinopril inhibited TNF and IL-6 production and the expression of T cell co-stimulatory molecules CD80 and CD86 in mouse bone marrow-derived dendritic cells, through an AT2R-dependent mechanism that was not observed with candesartan treatment. Similarly, captopril decreased IL-1α, IL-10, IL-12, IL-18 and TNF production in LPS-activated, monocyte-derived dendritic cells from healthy donors. Lastly, PBMCs from patients with HF receiving ACE inhibitors produced less TNF in response to T cell activation in vitro, and T cells from patients with CAD treated with enalapril or perindopril showed reduced TNF, IFNγ and IL-2 production in response to activation in vitro.

Angiotensin II receptor type 1 antagonists

Physiological functions of angiotensin II in immunity
Ang II is not only an important mediator in the renin–angiotensin–aldosterone system, but it also has important roles in immunity. The heart possesses its own renin–angiotensin system, which becomes activated in response to cardiac stress (such as volume and pressure overload). This activation is particularly important because Ang II functions as a chemokine for monocytes, dendritic cells, T cells and NK cells. Thus, under cardiac stress, leukocytes can follow the Ang II concentration gradient to the sites of production. Additionally, human monocytes, T cells and NK cells express AT1R, AT2R and Ang II. Furthermore, Ang II has a vital role in the activation and function of various immune cells, as detailed in the following sections.

Effects of ARBs on inflammation and the immune system
ARBs modulate the levels of circulating immune mediators. In patients with chronic HF, treatment with the ARB candesartan (n = 14) reduced the circulating levels of TNF, IL-6,
soluble ICAM1 and soluble VCAM1 compared with placebo (n = 9). Similarly, in 100 patients with hypertension, treatment with olmesartan induced progressive decreases in the circulating CRP, TNF, IL-6 and CCL2 levels compared with placebo. Lastly, valsartan reduced circulating CRP levels in healthy volunteers compared with baseline levels.

Furthermore, ARBs have been shown to inhibit leukocyte activation, function, and chemotaxis. Mouse splenic T cells and B cells express AT1R, and its expression is increased up to sevenfold in CD8 T cells and up to tenfold in all T cells after activation. In lymphocytes from healthy donors, in vitro administration of Ang II increased IFNγ production threefold to fivefold. Furthermore, Ang II administration augmented CD8 T cell and NK cell proliferation in vitro and functioned as a co-stimulatory molecule when the TCR was engaged; these effects were partly reduced by losartan. Losartan also reduced the expression of the activation markers CD25 and CD69 on activated mouse splenic T cells in vitro and interfered with their adhesion to and transmigration through an endothelial basal membrane. Lastly, mice treated with chronic Ang II infusion had increased splenic IFNγ-secreting T<sub>H1</sub> cells, which was completely reversed by treatment with Olmesartan.

The immunomodulatory effects of ARBs are not limited to lymphocytes. Valsartan reduced ROS production in vitro in polymorphonuclear and mononuclear cells from healthy individuals and reduced NF-κB activity and increased IκB expression in mononuclear cells. In activated monocytes from healthy donors, candesartan reduced TLR2 and TLR4 mRNA and protein expression, NF-κB activity and CCL2, IL-1β, IL-6 and TNF expression. Similarly, telmisartan dose-dependently decreased TNF, IL-1β and IL-6 mRNA and protein expression and CCL2, cyclooxygenase 2, ICAM1 and prostaglandin E2 (PGE2) expression in LPS-treated monocytes. Ang II administration activated the NLRP3 inflammasome in rat cardiac fibroblasts and mouse macrophages in vitro, which was inhibited by treatment with losartan. Furthermore, candesartan, losartan or irbesartan administration interfered with human monocyte differentiation to dendritic cells in vitro. Candesartan and losartan also reduced the endocytic and allostimulatory activity of human monocyte-derived dendritic cells. Similarly, mouse dendritic cells differentiated in vitro in the presence of losartan had reduced capacity to induce antibody responses in immunized mice. In addition, bone marrow from AT1R-deficient mice, cultured in vitro in the presence of granulocyte–monocyte colony-stimulating factor (GM-CSF) to generate myeloid dendritic cells, showed less efficient CD11c<sup>+</sup> dendritic cell generation than bone marrow from wild-type mice, and the dendritic cells generated from AT1R-deficient progenitors had reduced MHC class II expression, decreased allostimulatory activity and lower TNF production.
ARBs also exert vascular immunomodulatory effects. In human VSMCs, Ang II dose-dependently increased IL-6 production, which was inhibited by treatment with losartan. Rat VSMCs treated with Ang II in vitro showed increased TNF, TLR4 and matrix metalloproteinase 9 (MMP9) expression and reduced peroxisome proliferator-activated receptor-γ (PPARγ) activation. In atherosclerosis-prone mice, Ang II infusion for 8 weeks increased aortic plaque burden and instability compared with saline-treated animals. Ang II treatment shifted splenic T cell phenotypes from T_{H}2 and T_{reg} cells to T_{H}1 and T_{H}17 cells, which was mirrored by changes in the respective circulating cytokine profiles. The aortic wall of Ang II-treated mice also showed higher expression of transcription factors related to T_{H}1 and T_{H}17 cells (TBX21 and RORγt, respectively) and lower expression of T_{H}2 and T_{reg} cell transcription factors (GATA3 and FOXP3, respectively). All these effects were reversed by treatment with valsartan, whereas depletion of T_{H}2 cells nullified the effects of valsartan therapy. Lastly, in Apoe^{-/-} mice, AT1R co-localized with both CCR9 and CCL25 on plaque-resident cells and treatment with losartan reduced CCL25 levels in aortic plaques (similar to findings with the ACE inhibitor captopril). Losartan also reduced CCR9 expression in circulating mononuclear cells in these mice.

The mechanisms by which ARBs modulate immune responses are pleiotropic. A molecular modelling study predicted that the ARB telmisartan is a strong modulator of PPARγ, whereas losartan, irbesartan, olmesartan and valsartan were predicted to have comparatively less PPARγ modulatory activity. PPARγ is a transcription factor of the nuclear receptor superfamily that is expressed in adipose tissue, the intestines and in numerous types of leukocytes, including lymphocytes (T cells, B cells and NK cells), mononuclear cells (macrophages and dendritic cells) and polymorphonuclear cells (neutrophils, mast cells, basophils and eosinophils). PPARγ has a key role in tempering immune responses by antagonizing pro-inflammatory transcription factors and promoting eicosanoid degradation. In mouse adipocytes in vitro, treatment with irbesartan and losartan markedly induced PPARγ activity, independently of AT1R. In patients with hypertension, telmisartan, but not the calcium channel blocker amlodipine, decreased CCL2 and increased PPARγ expression in circulating monocytes.

In LPS-treated monocytes from healthy volunteers, the immunomodulatory effects of telmisartan (via MAPK, ERK and NF-κB inhibition) depended on the expression of PPARγ target genes (CD36 and ABCG1) and were abrogated by PPARγ silencing or antagonism. Lastly, a comparison of telmisartan, candesartan and losartan showed that their capacity to reduce TNF production in LPS-stimulated cells was proportional to their capacity to induce CD36 expression (telmisartan > candesartan > losartan).

The aforementioned molecular modelling study also determined that olmesartan and irbesartan had antagonistic activity against CCR2. Interestingly, AT1R can heterodimerize with the structurally similar CCR2, and the interaction of the two
receptors has been involved in the pathogenesis of renal disease.\textsuperscript{110,111} In patients with hypertension, treatment with telmisartan did not affect monocyte CCR2 expression.\textsuperscript{112} However, in patients with MI, 6 months of treatment with valsartan or losartan reduced monocyte CCR2 expression compared with baseline.\textsuperscript{113} Thus, more research is necessary to clarify whether CCR2 antagonism is an immunomodulatory property of ARBs.

**Mineralocorticoid receptor antagonists**

*Physiological functions of aldosterone in immunity*

Aldosterone is produced by the adrenal glands in response to Ang II, with some evidence of cardiac and central nervous system production as well.\textsuperscript{114} Aldosterone acts through the mineralocorticoid receptor and activates both innate (monocytes, macrophages, and dendritic cells) and adaptive (T cells) immune cells and promotes the activation of pro-inflammatory transcription factors (NF-κB and AP1) and the production of various cytokines, chemokines, and adhesion molecules in these cells.\textsuperscript{115} Furthermore, aldosterone is a potent activator of the NLRP3 inflammasome, promotes immune cell infiltration to the heart and kidneys, and has pro-fibrotic properties.\textsuperscript{115}

*Effects of MRAs on inflammation and the immune system*

Mineralocorticoid receptor antagonists (MRAs) have been shown to modulate leukocyte migration. In humans, circadian increases in the circulating levels of adrenal steroids promote naive T cell homing to lymph nodes during the early phase of sleep.\textsuperscript{116} MRAs can influence T cell migration by controlling the expression of adhesion molecules (CD62L) and chemokine receptors (CCR7 and CXCR4) in T cells.\textsuperscript{117} Additionally, mice with coxsackievirus B3-induced myocarditis receiving early treatment with the MRA eplerenone had reduced myocardial expression of ICAM1 and monocyte infiltration compared with untreated mice.\textsuperscript{118}

Second, MRAs have diverse immunomodulatory effects on PBMCs from healthy volunteers. In PBMCs stimulated with phytohaemagglutinin (a mitogen and activator of immune cells, particularly T cells) and LPS in vitro, spironolactone, but not its major metabolite canrenone, reduced the expression of approximately 700 genes including those encoding TNF, IL-1α, IL-1β, IL-1 receptor antagonist, IL-6, IL-10, IFNy, osteopontin, granulocyte colony-stimulating factor (GCSF) and GM-CSF.\textsuperscript{119} In another study in human PBMCs, a 4 h spironolactone treatment regimen altered the expression of 1,018 transcripts that were mainly related to apoptosis, but also included cytokines and chemokines controlled by NF-κB, CEBPβ and MYC.\textsuperscript{120} Spironolactone also suppressed the production of various inflammatory mediators at the protein level (GM-CSF, IL-1α, IL-1β, IL-2, IL-6, IL-10, IFNy, TGFβ and TNF) in human PBMCs.\textsuperscript{119–121} Longer exposures to spironolactone induced proportionally higher rates of apoptosis in human monocytes,
T cells and B cells in vitro. Interestingly, the findings from the RALES and EPHEUS trials, which demonstrated the effectiveness of MRAs for the treatment of HF, raise the question of whether MRAs exert pleiotropic effects beyond mineralocorticoid receptor antagonism, given that both studies used fairly low treatment doses and included patients with low plasma aldosterone levels. Indeed, two other studies have demonstrated that the addition of aldosterone to human blood or PBMCs in vitro at supraphysiological concentrations (>103-fold to 104-fold) did not significantly affect cytokine production or the capacity of spironolactone to modulate cytokine production after PBMC activation with LPS and phytohaemagglutinin.

Both aldosterone and MRAs modulate cytokine production in macrophages and have opposing effects on M1–M2 macrophage polarization. Aldosterone administration to mouse macrophages in vitro induced an increase in the transcription of Ccl5, Il12 and Tnf, which was reversed by treatment with eplerenone. Similarly, treatment of LPS-stimulated mouse macrophages with spironolactone reduced the expression of Il12, Il16, Ccl2 and Cccl5. Macrophages from mice with myeloid-specific mineralocorticoid receptor deficiency show alternative M2-like activation and reduced CCL2-directed migration compared with those from wild-type animals. Furthermore, in mice with obesity, spironolactone treatment induced a shift in aortic macrophages towards alternative activation (an increased IL-10 to CCL2 ratio). In a subsequent study using the same model, spironolactone decreased the cardiac expression of CCL2 and αMβ2 integrin and increased the expression of alternative activation markers (CD206) and the M2 to M1 ratio.

Dendritic cell and T cell functions might also be influenced by MRAs. Aldosterone dose-dependently increased mouse CD8+ T cell activation by mouse bone marrow-derived dendritic cells, which was inhibited by treatment with spironolactone or eplerenone. Dendritic cells, but not T cells, expressed mineralocorticoid receptor protein, and spironolactone reduced IL-2 production in the absence of aldosterone through dendritic cell-specific effects. Furthermore, aldosterone decreased the expression of programmed death ligand 1 (which inhibits CD8+ T cell activation) on dendritic cells and increased IL-6 and TGFβ levels, and these effects were reversed by spironolactone. Moreover, aldosterone promoted a Th17 phenotype, which was inhibited by treatment with spironolactone or eplerenone. Interestingly, rats with deoxycorticosterone acetate-induced or high salt-induced hypertension also showed Th17 polarization in the blood and spleen, and Th17-polarizing cytokines (IL-1β and IL-23p19) were upregulated in the heart and kidneys. These rats had reduced circulating Treg cell numbers, and the expression of the Treg cell-specific transcription factor FOXP3 was reduced in PBMCs, kidneys, heart, and spleen. Spironolactone treatment reversed all these effects and shifted CD4+ T cells from Th17 to Treg cells. In addition, mice with T cell-specific
mineralocorticoid receptor deficiency showed attenuated hypertensive responses to Ang II infusion and reduced monocyte and T cell infiltrates and inflammatory cytokines in the kidneys and aorta compared with littermate controls. The numbers of splenic CD4+ and CD8+ IFNγ-producing T cells were also reduced. Importantly, these results were reproduced with eplerenone. The mineralocorticoid receptor was shown to interact with the transcription factors NFAT1 and AP1 to induce IFNγ production in T cells, suggesting that mineralocorticoid receptor signalling promotes T<sub>H1</sub> polarization. Lastly, in mouse naive CD4+ and CD8+ T cells activated with anti-CD3 antibodies, spironolactone inhibited IL-2 expression, effector memory cell expansion and the upregulation of activation markers (CD25, CD44 and CD69).

MRAs have also been shown to modulate immune responses in experimental models of ischaemic HF. In mice with MI, myeloid-specific deletion of the mineralocorticoid receptor improved cardiac function and reduced left ventricular (LV) dilatation after MI. These animals showed greater neovascularization and scar maturation and reduced neutrophil numbers in the infarcted myocardium. Furthermore, macrophages in the infarct zone had an intermediate M1–M2 transcriptional profile that favoured tissue repair, wound healing, phagocytosis of apoptotic cells and downregulation of genes encoding inflammatory factors. In addition, infarct zone mineralocorticoid receptor-deficient macrophages showed more efficient efferocytosis of apoptotic neutrophils, as well as reduced superoxide production and upregulation of antioxidant enzymes compared with wild-type macrophages. Interestingly, targeted liposome-mediated delivery of eplerenone to macrophages mimicked the effects of myeloid-specific mineralocorticoid receptor deficiency. Lastly, in rats with MI, treatment with spironolactone or eplerenone promoted cardioprotective signalling via IL-33–soluble IL-1 receptor-like 1 (also known as ST2), by decreasing soluble ST2 levels in infarcted myocardium and increasing IL-33 expression in non-infarcted myocardium.

Additionally, MRAs can modulate cardiac inflammation in HF with preserved ejection fraction (HFrEF). In mice with TAC, both eplerenone and finerenone reduced cardiac CCL2 expression and ameliorated the HF phenotype. Similarly, eplerenone reduced myocardial ROS production, ICAM1 expression and monocytic infiltrates. In mice with abdominal aortic constriction, eplerenone inhibited cardiac hypertrophy and reduced cardiac T cell infiltrates. In these mice, T cell mineralocorticoid receptor deficiency ameliorated cardiac hypertrophy, dysfunction and fibrosis and reduced immune cell infiltrates (neutrophils, macrophages, and T cells), as well as cytokine and chemokine production (including IL-1β, CCL2 and CCL5). Lastly, in obese rats with HFrEF, treatment with spironolactone ameliorated the cardiac phenotype, reduced cardiac ROS production, and promoted M2 macrophage (CD163+) and T<sub>reg</sub> cell (CD4<sup>+</sup>FOXP3+) polarization.
Lastly, MRAs mediate neuroimmune interactions in HF. Circulating cytokines serve, among other functions, to inform the brain of ongoing inflammation. These signals are transduced through the blood–brain barrier by cyclooxygenase 2 upregulation, leading to PGE2 production, which in turn promotes corticotropin-releasing hormone (CRH) release by the hypothalamus.¹³⁹ In rats with ischaemic HF, treatment with eplerenone reduced cyclooxygenase 2 levels in hypothalamic blood vessels, and fewer neurons showed chronic activation and staining for TNF, IL-1β and CRH.¹³⁹ Eplerenone also reduced the HF-induced overexpression of cyclooxygenase 2 and CRH in the hypothalamus, as well as the circulating levels of TNF, IL-1β, IL-6 and noradrenaline.¹³⁹ The same investigators also showed that intracerebroventricular infusion of spironolactone normalized circulating TNF levels in rats with ischaemic HF.¹⁴⁰

**Dietary interventions and iron supplementation**

Two dietary interventions have gained attention in relation to HF. First, the Dietary Approaches to Stop Hypertension (DASH) diet — a diet rich in fruit, vegetables, nuts, legumes, seeds, and low-fat dairy products, combined with low salt consumption — has been shown to be associated with reduced HF incidence¹⁴¹ and decreased plasma CRP levels compared with usual diets.¹⁴² Second, salt restriction is advocated in patients with HF. Sodium-rich environments favour T_{H17} polarization, inhibit the suppressive functions of T_{reg} cells¹⁴³,¹⁴⁴ and promote pro-inflammatory activation of mouse macrophages.¹⁴⁴ Nevertheless, low salt intake (<1,500 mg per day) did not reduce cardiovascular events in patients with HF, despite improving symptoms and quality of life.¹⁴⁵ However, this study had several limitations, including being potentially underpowered owing to early termination and subsequent lower event rates, as well as relying on food recall to measure salt intake.

Intravenous iron supplementation is currently the only nutraceutical intervention used for the treatment of patients with HF and iron deficiency.² Despite the observed strong association between iron deficiency and inflammation,¹⁴⁶ intravenous ferric carboxymaltose did not affect plasma IL-6, IL-10 and CRP levels in patients with chronic kidney disease.¹⁴⁷–¹⁴⁹ In vitro evidence demonstrates that ferric carboxymaltose strongly reduces the phagocytic capacity of M1 macrophages and mildly reduces monocyte differentiation to dendritic cells.¹⁵⁰,¹⁵¹ Intravenous ferric carboxymaltose also induced modest reductions in inflammatory markers in the lungs of healthy rats.¹⁵²

**Immunomodulatory effects of novel HF treatments**

The main immunomodulatory effects of novel evidence-based treatments for HF are summarized in **Box 2** and **Figure 2** and are discussed in detail in the following sections.
Angiotensin receptor–neprilysin inhibitor

Physiological functions of neprilysin in immunity
The combined angiotensin receptor–neprilysin inhibitor (ARNI) sacubitril–valsartan is a novel HF therapy that integrates ARBs with inhibition of neprilysin, also known as neutral endopeptidase 24.11, CD10 or common acute lymphocytic leukaemia antigen. Neprilysin cleaves atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), Ang I, Ang II, bradykinins, enkephalins, substance P and other substrates. In the haematopoietic and immune systems, neprilysin is produced by mature neutrophils. Neprilysin cleaves atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), Ang I, Ang II, bradykinins, enkephalins, substance P and other substrates. In the haematopoietic and immune systems, neprilysin is produced by mature neutrophils. Numerous immune mediators control the expression and activity of neprilysin on neutrophils. Neutrophil incubation with N-formylmethionyl-leucyl-phenylalanine (fMLP), GM-CSF or TNF has been shown to increase neprilysin enzymatic activity. In neutrophils from healthy donors incubated with various cytokines, only GM-CSF caused a significant concentration-dependent increase in neprilysin activity, whereas GM-CSF and IL-1 acted synergistically. Neprilysin is also expressed by haematopoietic progenitors, transitional and immature B cells, and apoptotic lymphocytes. Neprilysin is involved in B cell development, the inactivation of pro-inflammatory peptides and the regulation of peptide-induced chemotaxis in neutrophils.

Effects of neprilysin inhibition on inflammation and the immune system
First, neprilysin inhibition modulates immune responses to cytokines and neuropeptides. The pivotal role of neprilysin in attenuating immune responses has been illustrated in neprilysin-deficient mice, which have greatly increased mortality in response to LPS or IL-1β–TNF administration compared with wild-type littermates. By contrast, intraperitoneal injection of neprilysin conferred resistance to LPS-induced shock in neprilysin-deficient mice. Although neprilysin inhibition with phosphoramidon does not affect TNF-induced activation of human peripheral blood granulocytes in vitro, neprilysin deactivates IL-1β by hydrolysis and interferes with IL-1β-mediated T cell proliferation. Additionally, phosphoramidon augmented the stimulatory effects of IL-1β on bone marrow cell proliferation tenfold. Neprilysin also degrades various neuropeptides with immunological functions. In granulocytes from healthy volunteers, phosphoramidon enhanced the inhibitory effects of α-melanocyte-stimulating hormone (αMSH) on TNF-induced activation. By contrast, the addition of met-enkephalin and substance P promoted TNF-induced granulocyte activation more strongly when combined with neprilysin inhibition with phosphoramidon or thiorphan. In met-enkephalin activated granulocytes, administration of phosphoramidon reversed the inhibitory effects of αMSH. Phosphoramidon-treated human polymorphonuclear cells required lower concentrations of metenkephalin by five orders of magnitude to become activated compared with untreated cells, and...
phosphoramidon reduced the minimal concentration of substance P necessary to induce changes in neutrophil size and shape.\textsuperscript{154} Similarly, thiorphan increased neutrophil chemotaxis towards substance P.\textsuperscript{154} Neutrophils treated with phosphoramidon or thiorphan and exposed to substance P showed augmented expression of the adhesion molecules CD11b and CD18 and reduced expression of L-selectin compared with neutrophils exposed to substance P only, which might facilitate extravasation.\textsuperscript{154}

Evidence also suggests that neprilysin modulates neutrophil activation and chemotaxis. Neprilysin cleaves fMLP,\textsuperscript{167} which is the principal chemotactic peptide and neutrophil activator produced by Gram-negative bacteria.\textsuperscript{154} Neprilysin inhibition with phosphoramidon inhibits fMLP-directed chemotaxis in human neutrophils in vitro, but does not affect random migration, degranulation, or aggregation.\textsuperscript{167,168} Although another study reported similar findings,\textsuperscript{169} other investigators observed the opposite effects. Namely, phosphoramidon reduced the concentration of fMLP needed to alter neutrophil size and shape by three orders of magnitude, whereas thiorphan increased fMLP-directed chemotaxis in neutrophils from healthy volunteers.\textsuperscript{154} These contrasting findings might be attributable to neprilysin saturation at higher substrate concentrations.\textsuperscript{154,169} Collectively, these data suggest that neutrophil neprilysin tempers responsiveness to inflammatory stimuli.

\textit{Immunomodulatory effects of ARNIs in HF}

In 44 patients with HF, treatment with sacubitril–valsartan increased the numbers of most circulating B cell subtypes, particularly total and CD10\textsuperscript{+} transitional B cell counts.\textsuperscript{170} Mice with MI treated with either enalapril or sacubitril–valsartan showed no differences in macrophage or neutrophil numbers, structure, function or TNF and CCL2 expression in infarcted myocardium.\textsuperscript{171} Sacubitril–valsartan-treated mice had lower Il1b and Il6 mRNA expression in infarcted myocardium and reduced MMP9 activity in cardiac macrophage infiltrates than enalapril-treated mice. In dogs with ischaemia–reperfusion injury, neprilysin inhibition with UK73967 after MI reduced MPO activity in the injured myocardium and neutrophil accumulation in the interstitium and vascular lumina.\textsuperscript{172} However, sacubitril–valsartan therapy showed neutral results compared with ramipril in patients with MI in the PARADISE-MI trial,\textsuperscript{173} potentially owing to late treatment initiation (>4 days after MI) or because most of the participants were already treated with β-blockers, which also modulate neutrophil migration.\textsuperscript{47,51,52}

The immunomodulatory effects of ARNI in HF are challenging to understand because their interpretation requires the attribution of effects to the ARB, to neprilysin inhibition or to their combination. Their interpretation is additionally hampered by the choice of ACE inhibition as a comparator treatment in most of the available basic and clinical studies. Indeed, although sacubitril–valsartan reduced Il1b and Il6 mRNA expression
in infarcted tissues in mice with MI, similar effects have been observed with ARBs in isolation in other animal studies. Furthermore, in rats with MI, both valsartan and sacubitril–valsartan comparably ameliorated cardiac dysfunction and reduced LV oxidative stress, TNF protein levels in the myocardium and plasma BNP levels. Similarly, no clinical benefits over valsartan were observed in patients with chronic advanced HF treated with sacubitril–valsartan in the LIFE trial. Collectively, these findings make it difficult to attribute the immunomodulatory effects of ARNI to the addition of neprilysin inhibition to an ARB.

Several studies have postulated that some of the beneficial effects of ARNI might be secondary to the increased half-life of ANP and BNP. Both ANP and BNP have been shown to have anti-inflammatory effects. For example, pre-conditioning of activated neutrophils with ANP dose-dependently reduced lysozyme and MMP9 release as well as neutrophil adhesion to endothelial cells under hypoxic conditions. In addition, supernatant from stimulated neutrophils induced apoptosis in cultured VSMCs, which was inhibited by ANP administration. These effects of ANP were potentiated by retrothiorphan, which inhibits neprilysin. Furthermore, pre-treatment of human activated neutrophils with ANP or BNP dose-dependently reduced cytotoxicity towards endothelial cells, neutrophil adhesion to endothelium, the expression of the adhesion molecule CD18 and elastase release. These effects were potentiated by neprilysin inhibition with UK73967 or phosphoramidon. However, other studies have reported pro-inflammatory effects of ANP and BNP. For example, in activated neutrophils, pre-treatment with BNP increased superoxide production, and ANP dose-dependently increased CD11b expression and peroxide production and the ANP fragments 1–28 and 7–28 dose-dependently increased respiratory bursts. Notably, the increase in respiratory bursts was not modified by phosphoramidon. ANP-1–28 also potentiated lysosomal enzyme release and increased neutrophil adhesiveness. Thus, whether neprilysin inhibition modulates neutrophil immune responses favourably remains unclear. ANP and BNP also exert immunomodulatory effects beyond neutrophil functions, which are discussed elsewhere.

In contrast to acute MI, chronic HF leads to systemic downregulation of neprilysin. Neutrophil neprilysin expression was not affected by ARNI therapy and was inversely associated with worse outcomes in patients with chronic HF. Nepriylsin deficiency in these patients could indicate the presence of emergency haematopoiesis. BNP can also inhibit neprilysin expression in HF. Nepriylsin inhibition could thus offer more potent immunomodulatory properties in acute HF than in chronic HF, although this aspect needs to be investigated further.
Box 2. Summary of immunomodulatory properties of novel treatments for heart failure.

**ARNIs**
- Angiotensin receptor–neprilysin inhibitors (ARNIs) combine an angiotensin II receptor type 1 blocker (valsartan) with neprilysin inhibition (sacubitril). Neprilysin is produced mainly in neutrophils among immune cells and cleaves various substrates including atrial natriuretic peptide, B-type natriuretic peptide (BNP), angiotensin I, angiotensin II, neuropeptides and IL-1β. Neprilysin inhibition increases mortality in animals treated with IL-1β, lipopolysaccharide or tumour necrosis factor and increases neutrophil responsiveness to various pro-inflammatory neuropeptides.
- There is conflicting evidence on whether neprilysin inhibition reduces or increases neutrophil migration in response to specific stimuli. Similarly, although neprilysin inhibition reduced the degradation of atrial natriuretic peptide and BNP by human neutrophils, this reduction did not lead to inhibition of neutrophil activation in all studies.
- The immunomodulatory effects of ARNI therapy in HF are difficult to understand because some studies used angiotensin-converting enzyme inhibitors as comparators. Sacubitril–valsartan does not seem to exert considerable additive immunomodulatory effects compared with valsartan alone except for some macrophage functions.
- Neprilysin is systemically downregulated in HF, possibly owing to emergency haematopoiesis or downregulation induced by BNP. Therefore, neprilysin inhibitor therapy might offer more benefits in the acute setting.

**SGLT2 inhibitors**
- Sodium–glucose cotransporter 2 (SGLT2) inhibitors exert multiple immunomodulatory effects by preventing the generation of reactive oxygen species, increasing AMPK activity and interfering with NLRP3 inflammasome activation and subsequent cytokine production in cardiac and immune cells. SGLT2 inhibitors also promote alternative macrophage activation by activating AMPK.
- Treatment with SGLT2 inhibitors reduces markers of systemic inflammation in patients with acute myocardial infarction with or without diabetes mellitus.
- SGLT2 inhibitors promote the differentiation of CD4+ T cells into regulatory T cells and interfere with T cell proliferation.
- SGLT2 inhibitors dampen inflammation caused by uraemia and hyperinsulinaemia.
- SGLT2 inhibitors protect the endothelium from glycocalyx disruption, reduce vascular inflammation and prevent immune cell adhesion to endothelial cells by reducing oxidative stress and the expression of vascular adhesion molecules. These drugs also directly inhibit inflammation and foam cell formation in atherosclerotic plaques.
- SGLT2 inhibitors exert sympatholytic effects by interfering with catecholamine biosynthesis and might reduce epicardial adipose tissue inflammation, but more research is required to characterize these effects.

**Sodium–glucose cotransporter 2 inhibitors**

**Physiological functions of SGLT2**
Inhibition of SGLT2 has beneficial effects in HF irrespective of LV ejection fraction (LVEF). SGLT2 is primarily expressed in the kidneys and its physiological function is related to the resorption of sodium and glucose from the glomerular ultrafiltrate. Although no intrinsic immune functions of SGLT2 have been described to date, the levels of SGLT2 mRNA and protein are increased in renal epithelial cells in vitro in response to IL-6 and TNF stimulation. In addition, SGLT2 expression in renal epithelial cells and its translocation to the cell surface is sensitive to noradrenaline release from renal nerves.

**Effects of SGLT2 inhibitors on inflammation and the immune system**
SGLT2 inhibitors have been shown to inhibit the NLRP3 inflammasome in cardiac cells. Studies in mice with TAC-induced HFRef, rats with HfpEF or leptin-deficient mice with type 2 diabetes mellitus (T2DM) have demonstrated that treatment with either empagliflozin or dapagliflozin reduces cardiac NLRP3 inflammasome priming, IL-1β, IL-6, IL-18 and TNF production, and macrophage infiltration. Furthermore, myocardial biopsy samples from 30 patients with HfpEF showed reduced TNF and IL-6 production after...
incubation with empagliflozin, which was also observed in hearts from empagliflozin-treated Zucker diabetic fatty rats.  

Studies of human LPS-activated cardiomyocytes, mouse atrial cardiomyocytes or mouse cardiac fibroblasts cultured with dapagliflozin or empagliflozin reproduced these findings and demonstrated that these effects depend on AMPK activation, reduced inducible nitric oxide synthase (iNOS) activity and changes in calcium handling. Additionally, both dapagliflozin and empagliflozin reduced cardiac oxidative stress in rats with MI, patients with HFpEF and rats with obesity and insulin resistance. Intracellular calcium and oxidative stress regulate AMPK activity and NLRP3 inflammasome activation. Thus, SGLT2 inhibitors interfere with NLRP3 inflammasome assembly and function by modulating calcium handling, reducing oxidative stress, and promoting AMPK activation.

SGLT2 inhibitors can also shift macrophages to alternative activation. Empagliflozin increased AMPK phosphorylation in resting and LPS-activated mouse macrophages in vitro and induced AMPK-dependent reductions in iNOS activity and TNF production in LPS-activated macrophages. Treatment with empagliflozin before LPS stimulation also induced an M2 phenotype in mouse macrophages (low CD40 expression and high CD206 expression). A subsequent study showed that various SGLT2 inhibitors reduced IL-6 and TNF production in response to LPS stimulation in RAW264.7 monocyte–macrophage-like cells. However, only the SGLT2 inhibitor canagliflozin decreased IL-1α production and also mediated greater reductions in IL-6 than the other SGLT2 inhibitors. Additionally, canagliflozin dose-dependently reduced ROS production and TNF, IL-1α and IL-6 mRNA and protein levels in RAW264.7 cells by inhibiting glycolysis and glucose uptake, increasing autophagy, and promoting lysosomal degradation of IL-1α. Canagliflozin also favoured alternative activation of bone marrow-derived macrophages from LPS-treated C57BL/6 mice in vitro. Moreover, in macrophages with M1 or M2 polarization induced in vitro, the addition of canagliflozin reduced M1 marker (IL-1β, TNF and iNOS) and increased M2 marker (ARG1 and CD206) expression. In LPS-stimulated M1 macrophages, empagliflozin attenuated the expression of inflammatory mediators (iNOS, TNF, IL-1β, IL-6 and IFNγ) and chemokines (CCL3, CCL4, CCL5 and CXCL10) by reducing NF-κB, JNK, STAT1 and STAT3 phosphorylation. Lastly, in rats with MI, dapagliflozin increased the amount of cardiac M2 macrophages 4.5-fold compared with vehicle-treated animals. Dapagliflozin-treated rats also showed decreased cardiac expression of M1 markers (iNOS, IL-1β and IL-6) and increased expression of M2 markers (IL-10 and CD206).

SGLT2 inhibitors have been shown to attenuate systemic inflammation in patients with MI with or without T2DM. In patients with MI who had undergone percutaneous coronary intervention (PCI), therapy with dapagliflozin alone or dapagliflozin plus metoprolol reduced circulating hsCRP, IL-6 levels and oxidative stress markers (malondialdehyde...
and ischaemia-modified albumin) and increased the plasma levels of the antioxidant enzyme superoxide dismutase compared with placebo.\textsuperscript{207} Dapagliflozin and metoprolol showed additive effects on hsCRP, superoxide dismutase and malondialdehyde levels. In mice with ischaemia–reperfusion injury, treatment with dapagliflozin improved cardiac function by increasing the number of bone marrow naive B cells ($\text{IgM}^{-}\text{IgD}^{+}$), thus suggesting the presence of additional bone marrow-specific immunomodulatory effects in the context of cardiac ischaemia.\textsuperscript{208} In a registry-based study in patients with T2DM and acute MI treated with PCI, SGLT2 inhibitor use was associated with lower neutrophil counts, higher lymphocyte counts, lower neutrophil-to-lymphocyte ratios and lower CRP levels in the blood, both at study inclusion and 24 h after hospital admission.\textsuperscript{209} SGLT2 inhibitors also prevented neutrophil count increases after 24 h of hospital admission and were associated with reduced plasma CRP levels at discharge. The reduction in neutrophil numbers and CRP levels implies that SGLT2 inhibitors directly modify leukocyte functions, thereby leading to sustained benefits in patients with MI. Indeed, isolated PBMCs from patients with T2DM who had received empagliflozin treatment for 30 days released less TNF and IL-1$\beta$ in response to activation in vitro than PBMCs isolated before the patients received empagliflozin treatment.\textsuperscript{210} CD4$^{+}$ T cells from patients with T2DM treated with empagliflozin for 6 months were shifted from T$_{h}$17 to T$_{reg}$ polarization compared with baseline measurements in cells from the same patients as well as with cells from untreated patients. Furthermore, in vitro T cell supernatants showed reduced IL-17 and increased IL-10 concentrations (the respective T$_{h}$17 and T$_{reg}$ cell representative cytokines) after activation, in the same comparisons.\textsuperscript{211} CD4$^{+}$ T cell proliferation was also inhibited in cells from empagliflozin-treated patients and could be inhibited in cells from untreated patients by adding empagliflozin to culture medium.

Another potential immunomodulatory mechanism of SGLT2 inhibitors might be related to their capacity to reduce circulating uric acid concentrations and systemic insulin resistance. Renal dysfunction and hyperuricaemia, as well as insulin resistance and hyperinsulinaemia, often complicate both HF and T2DM.\textsuperscript{212–215} Additionally, both hyperuricaemia and hyperinsulinaemia are potent pro-inflammatory stimuli.\textsuperscript{212,216,217} Compared with glucose-lowering medications, dapagliflozin, canagliflozin and empagliflozin reduced insulin, uric acid, and IL-6 levels in plasma in patients with T2DM.\textsuperscript{217} Furthermore, SGLT2 inhibitors have been shown to ameliorate muscle and systemic insulin resistance through a combination of mechanisms in both animal and human studies,\textsuperscript{218–220} and empagliflozin reduced blood uric acid levels in patients with HFrEF compared with placebo.\textsuperscript{213}

Evidence also suggests that SGLT2 inhibitors might reduce vascular inflammation and immune cell adhesion. Canagliflozin, but not dapagliflozin or empagliflozin, dose-dependently increased AMPK phosphorylation in human umbilical vein endothelial
cells, human aortic endothelial cells (HAECs) and human VSMCs in vitro.\textsuperscript{221} Furthermore, canagliflozin reduced CCL2 and IL-6 secretion in IL-1β-stimulated human umbilical vein endothelial cells and CCL2 expression in IL-1β-stimulated HAECs.\textsuperscript{221} These effects were suppressed by AMPKα1 inactivation. Canagliflozin also inhibited the adhesion of IL-1β-stimulated pro-monocytic cells to HAECs, without affecting ICAM1, VCAM1 or E-selectin expression in HAECs. SGLT2 inhibitors also ameliorated vascular inflammation caused by disruption of the glycocalyx, a layer of small molecules that separates the endothelium and the blood.\textsuperscript{222} Empagliflozin reduced NB4 promyelocytic leukaemia cell adhesion on TNF-stimulated HAECs in vitro, with or without sustained glycocalyx degradation, by attenuating endoplasmic reticulum and oxidative stress, independently of changes in ICAM1 or endothelial nitric oxide synthase expression.\textsuperscript{223,224} Additionally, empagliflozin dose-dependently reduced oxidative stress, NLRP3 expression and NLRP3 inflammasome function in IL-17A-treated human aortic VSMCs in vitro and reduced the proliferation and cellular migration of VSMCs in response to IL-17A.\textsuperscript{225} Lastly, myocardial biopsy samples from patients with HFpEF showed reduced expression of the adhesion molecules ICAM1 and VCAM1 after 60 min of incubation with empagliflozin, and the same was observed in hearts from empagliflozin-treated Zucker diabetic fatty rats.\textsuperscript{197}

In addition to reducing general vascular inflammation, SGLT2 inhibitors have also been shown to reduce atherosclerosis-associated inflammation. Canagliflozin ameliorated atherosclerotic progression and burden in atherosclerosis-prone mice by decreasing aortic VCAM1 and CCL2 expression.\textsuperscript{226} In canagliflozin-treated mice, the levels of CCL2 were reduced in aortic plaques, whereas the content of CD68\textsuperscript{+} macrophages was marginally reduced compared with untreated controls.\textsuperscript{226} In the same mouse model, empagliflozin reduced the atherosclerotic burden and dose-dependently reduced CD68\textsuperscript{+} monocyctic infiltrates and NF-κB, IL-1β and IL-6 levels in atheromatous plaques as well as circulating IL-1β and IL-6 levels.\textsuperscript{227} In RAW264.7 monocyctic cells activated using oxidized LDL, empagliflozin attenuated Ccl2, Il1b, Il6 and Tnf mRNA expression, reduced NF-κB protein levels and dose-dependently increased the phosphorylation of AMPK.\textsuperscript{227} The effects of empagliflozin on NF-κB were blunted by AMPK inhibition. Lastly, empagliflozin dose-dependently reduced oxidized LDL phagocytosis by RAW264.7 cells, a crucial process in foam cell formation.\textsuperscript{227}

Interestingly, SGLT2 inhibitors can also exert sympatholytic effects.\textsuperscript{228,229} In mice with neurogenic hypertension, dapagliflozin decreased renal and cardiac tyrosine hydroxylase expression (a marker of sympathetic innervation) as well as renal noradrenaline content, compared with untreated mice.\textsuperscript{230} Dapagliflozin also ameliorated the adverse cardiac phenotype in rats with isoproterenol-induced cardiomyopathy.\textsuperscript{231} Notably, dapagliflozin reduced cardiac inflammation in both models.\textsuperscript{230,231} These sympatholytic effects of SGLT2
inhibitors might be caused by increased ketone production and inhibition of adrenal catecholamine biosynthesis.\textsuperscript{232,233}

Lastly, SGLT2 inhibitors can have direct immunomodulatory effects on adipose tissue,\textsuperscript{234,235} and preliminary evidence from human tissues and pig studies suggests that SGLT2 inhibitors reduce oxidative stress, chemokine secretion (CCL2, CCL5, CXCL8) and macrophage infiltration in epicardial adipose tissue compared with untreated controls.\textsuperscript{236,237} However, more research is needed to identify the relevance of these observations, particularly given the increasing recognition of the importance of epicardial adipose tissue in the pathogenesis of HF.\textsuperscript{238}

**Direct or indirect immunomodulatory effects of HF treatments**

Findings from studies performed specifically in immune cells could be considered as evidence of direct effects on immune cells, and these are thus displayed separately in Figure 2. However, systemic, or organ-specific immunomodulatory effects can be more difficult to dissect because they can stem from direct immunomodulatory effects on leukocytes, from indirect amelioration of immune activation secondary to improvements in the HF phenotype, or a combination of the two. Depending on the study methodology, in some cases it is possible to determine whether the observed effect is direct or indirect. For example, in an aforementioned study in mice with MI, liposomal delivery of eplerenone to macrophages recapitulated the beneficial immunomodulatory effects of eplerenone,\textsuperscript{134} which indicates a direct effect of the drug. Similarly, direct effects of captopril, losartan and empagliflozin can be inferred from studies of vascular inflammation.\textsuperscript{72,101,223} We discuss such evidence in the corresponding sections. However, whether some of the reported systemic immunomodulatory effects are indeed direct or indirect remains to be determined.

**Clinical contexts and target groups for immunomodulation in HF**

Evidence-based treatments for HF are consistently prescribed at suboptimal therapeutic dosages or not prescribed, discontinued, or not adhered to, which can lead to worse outcomes in these patients.\textsuperscript{239–243} Adverse effects such as symptomatic hypotension, worsening renal function and hyperkalaemia are the most common medication-related causes of undertreatment in patients with HF.\textsuperscript{244} Patient education can also be a crucial factor in improving adherence to prescribed treatments.\textsuperscript{244} Notably, suboptimal treatment can diminish the favourable immunomodulatory effects of currently used
interventions, and patients in whom treatment up titration is difficult might benefit from bridging treatment with therapies developed for use as directed immunomodulators. Little evidence is available in this regard, and this strategy could be investigated in the future.

Delineation of distinct therapeutic algorithms according to HF acuity might also provide added clinical value. From a cardioimmunology standpoint, different immune mechanisms might be at play in the acute and chronic stages, or in patients with de novo or acutely decompensated HF. Acute inflammatory processes centred on innate immune responses might be more relevant targets in de novo HF, whereas adaptive immune responses or immune memory (including trained immunity) might be more relevant in chronic HF or acutely decompensated HF. These nuances should be considered when investigating potential immunomodulatory treatments. Similarly, the immunology-related pathophysiological differences between ischaemic and non-ischaemic heart disease merit additional investigation, particularly in humans. Namely, whether distinct immunomodulatory interventions are necessary to prevent the onset of HF is currently not known and should be researched further. Additionally, for those patients who do progress to HF, whether a one-size-fits-all approach is preferable to tailored treatment according to aetiology is poorly understood. However, evidence from our previous findings in animal models suggests that different HF aetiologies have both shared and distinct mechanisms. These differences should be taken into account when designing and studying future treatments.

Targeted immunomodulation might also be an attractive treatment approach in patients with established HFpEF. This notion is also supported by the current pathophysiological paradigm for HFpEF, which centres around cumulative inflammatory burden from a combination of comorbid diseases. Nevertheless, this should neither preclude nor interfere with the evaluation of immunomodulatory treatments in patients with heart failure with reduced ejection fraction (HFrEF). That being said, targeted immunomodulation might also be a promising treatment for selected subpopulations of patients with HF, such as patients with comorbid severe renal dysfunction, cardio renal syndrome and/or diuretic resistance, clonal haematopoiesis of indeterminate potential (CHIP), Chagas cardiomyopathy, DCM, or virus-negative inflammatory cardiomyopathy. Lastly, immunomodulation with pharmacological or dietary interventions can have a role in the prevention of HF in specific clinical contexts, such as in atherosclerosis and hypertension. The clinical contexts and target groups for which immunomodulation might be relevant as a treatment for HF are summarized in Figure 3 and discussed in the following sections.
Current status of immunomodulatory treatments for HF

At present, no immunomodulatory interventions are recommended for treating or preventing HF. Prospective randomized or 1:1 matched studies as well as randomized clinical trials that investigated immunomodulatory interventions in patients with HF, idiopathic or inflammatory DCM, or MI are summarized in Table 1 (pharmacological interventions) and Table 2 (device-based interventions). Meta-analyses that included such studies are summarized in Table 3. In patients with HF related to virus-negative inflammatory cardiomyopathy, various studies have shown the effectiveness of prednisone, azathioprine, rituximab, cyclosporine, mycophenolate mofetil and other immunomodulatory therapies. Many of the published studies in this patient population do not meet our criteria for inclusion in this Review, or did not exclusively examine patients with HF, but have been discussed previously in more detail.

A multitude of immunomodulatory interventions have been assessed for the treatment or prevention of HF (Tables 1, 2 and 3). However, the patient populations in these studies had limited uniformity, thus rendering the extrapolation of generalizations difficult. Interventions
that did not provide significant benefits in clinical studies include TNF inhibition with infliximab or etanercept,\textsuperscript{254,255} xanthine oxidase inhibition with allopurinol or oxypurinol\textsuperscript{256,257} and anti-inflammatory treatment with rosuvastatin.\textsuperscript{258,259} The established immunomodulating drugs methotrexate and thalidomide have shown some promising effects in the context of HF, including reducing circulating inflammatory markers and improving exercise capacity and quality of life for methotrexate,\textsuperscript{260} and improving LVEF and LV remodelling and reducing circulating neutrophil counts and TNF levels for thalidomide.\textsuperscript{261} Nevertheless, these studies were small and the findings were not consistently observed in other investigations.\textsuperscript{262,263} Therefore, these findings require further validation in larger clinical trials. Nonspecific, device-based immunomodulation using the Celacade system involves the intramuscular administration of autologous blood that has previously been subjected ex vivo to oxidative stress (ozone and ultraviolet light), with the purpose of activating anti-inflammatory mechanisms upon exposure to apoptotic blood cells.\textsuperscript{264} However, Celacade treatment in patients with HF did not lead to reductions in all-cause mortality or the risk of first cardiovascular rehospitalization compared with saline injection, although Celacade improved outcomes in pre-specified analyses of patients without a history of MI or in those with NYHA class II HF.\textsuperscript{264} Treatment with colchicine in patients with established HF reduced circulating CRP and IL-6 levels compared with placebo, but had no effect on mortality, hospitalizations for HF or exercise capacity.\textsuperscript{265} However, in the larger, placebo-controlled COLCOT trial,\textsuperscript{266} colchicine reduced the risk of a composite outcome including cardiovascular death in patients treated within 30 days after MI, although the study was not specifically powered to detect differences in the incidence of HF. By contrast, complement C5 inhibition with pexelizumab\textsuperscript{267–269} or anti-inflammatory treatment with cyclosporine A\textsuperscript{270–273} did not reduce the incidence of HF compared with placebo in a multitude of clinical trials in patients with MI.

Two interventions that seem to be promising for the treatment of HF, but that have not been investigated in large-scale clinical trials, are corticosteroids (dexamethasone and prednisone) and pentoxifylline. Corticosteroids, either in isolation or in a combined dexamethasone–prednisone scheme, have decongestive effects by increasing diuresis and natriuresis, and have also been shown to reduce serum creatinine and NT-proBNP levels as well as body weight and dyspnea.\textsuperscript{274–276} Additionally, they were shown to reduce cardiovascular mortality in one study.\textsuperscript{274} However, potential adverse effects of corticosteroids, including hypertension, hyperglycaemia and electrolyte abnormalities should be taken into account.\textsuperscript{277} Pentoxifylline was found to improve NYHA class, LVEF, exercise capacity and circulating inflammatory biomarkers in several small, placebo-controlled studies in patients with HF.\textsuperscript{278–282} Additionally, a meta-analysis that included 221 patients from six randomized, placebo-controlled trials of pentoxifylline in HF identified a reduction in all-cause mortality with pentoxifylline treatment.\textsuperscript{283} However, most of the evidence on pentoxifylline was derived from studies performed at a single centre; therefore, larger, multicentre studies are required to clarify the significance of these findings.
<table>
<thead>
<tr>
<th>Therapy</th>
<th>Study (year)</th>
<th>Patient population</th>
<th>Number of Patients</th>
<th>Median follow-up</th>
<th>Outcomes</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Autoantibody reduction</strong></td>
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<tr>
<td>Intravenous immunoglobulin</td>
<td>McNamara et al. (2001)</td>
<td>Recent-onset NYHA class I–IV DCM with LVEF ≤40%</td>
<td>62</td>
<td>12 months</td>
<td>Similar increase in LVEF in the intravenous immunoglobulin and placebo groups at 6 months A greater proportion of patients treated with intravenous immunoglobulin showed an increase in LVEF ≥10% from baseline</td>
<td>388</td>
</tr>
<tr>
<td></td>
<td>Gullestad et al. (2001)</td>
<td>NYHA class II–III HF with LVEF &lt;40% (ischaemic or idiopathic DCM)</td>
<td>40</td>
<td>6 months</td>
<td>Decrease in plasma NT-proANP levels, increase in anti-inflammatory markers (IL-10, IL-1RA and sTNFR) in plasma and LVEF (independent of aetiology)”</td>
<td>389</td>
</tr>
<tr>
<td><strong>Complement C5 inhibition</strong></td>
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<tr>
<td>Pexelizumab</td>
<td>COMMA (2003)</td>
<td>STEMI, enrolled within 6 h of symptom onset and treated with PCI</td>
<td>960</td>
<td>3 months</td>
<td>No effect on new or worsening HF</td>
<td>267</td>
</tr>
<tr>
<td></td>
<td>COMPLY (2003)</td>
<td>STEMI, enrolled within 6 h of symptom onset and treated with fibrinolysis</td>
<td>943</td>
<td>3 months</td>
<td>No effect on new or worsening HF</td>
<td>268</td>
</tr>
<tr>
<td></td>
<td>APEX-AMI (2007)</td>
<td>STEMI, enrolled within 6 h of symptom onset and with high-risk ECG findings and treated with PCI</td>
<td>5,745</td>
<td>3 months</td>
<td>No effect on the development of HF</td>
<td>269</td>
</tr>
<tr>
<td><strong>HMG-CoA reductase inhibition, pleiotropic</strong></td>
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<tr>
<td>Rosuvastatin</td>
<td>CORONA (2007)</td>
<td>NYHA class II–IV ischaemic HF with LVEF ≤40%</td>
<td>5,011</td>
<td>2.7 years</td>
<td>No reduction in cardiovascular death, non-fatal MI or non-fatal stroke, all-cause mortality or coronary events Decreased hospitalizations for cardiovascular causes”</td>
<td>258</td>
</tr>
<tr>
<td></td>
<td>GISSI-HF (2008)</td>
<td>NYHA class II–IV HF irrespective of cause and LVEF</td>
<td>4,574</td>
<td>3.9 years</td>
<td>No reduction in cardiovascular hospitalizations or mortality</td>
<td>259</td>
</tr>
</tbody>
</table>
## IL-1β receptor antagonism, IL-1β Inhibition

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Study Details</th>
<th>Cohort Characteristics</th>
<th>Duration</th>
<th>Outcome Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anakinra</td>
<td>ADHF, LVEF &lt;40%, plasma CRP ≥5 mg/l</td>
<td>30 days</td>
<td>Reduced plasma IL-6 and CRP levels&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>RedHart (2016)</td>
<td>Recently decompensated HF with LVEF &lt;50% and plasma CRP &gt;2 mg/l</td>
<td>3 months</td>
<td>Improved aerobic exercise capacity with 12-week treatment&lt;sup&gt;a&lt;/sup&gt; Non-significant trend towards reduced composite of all-cause mortality and hospitalization for HF with 12-week treatment</td>
<td></td>
</tr>
<tr>
<td>DharT2 (2018)</td>
<td>HFpEF with obesity and plasma CRP &gt;2 mg/l</td>
<td>3 months</td>
<td>Reduced plasma CRP and NT-proBNP levels&lt;sup&gt;a&lt;/sup&gt; No improvement in VO&lt;sub&gt;2&lt;/sub&gt; max and VE/VCO&lt;sub&gt;2&lt;/sub&gt; slope</td>
<td></td>
</tr>
<tr>
<td>VCUART Pooled Analysis (2022)</td>
<td>STEMI, enrolled within 12 h of reperfusion</td>
<td>12 months</td>
<td>Reduced all-cause mortality or new-onset HF, all-cause mortality or hospitalization for HF, and CRP area under the curve (from baseline to 14 days)&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Canakinumab</td>
<td>Previous MI and plasma CRP ≥2 mg/l</td>
<td>3.7 years</td>
<td>Reduced composite of non-fatal MI, non-fatal stroke or cardiovascular death&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>CANTOS HF substudy (2019)</td>
<td>History of HF, previous MI, and plasma CRP ≥2mg/l</td>
<td>3.7 years</td>
<td>Non-significant trend for lower HF hospitalizations or the composite of hospitalization for HF and HF-related mortality&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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</table>

## NF-κB inhibition

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Study Details</th>
<th>Cohort Characteristics</th>
<th>Duration</th>
<th>Outcome Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prednisone</td>
<td>NYHA class II–IV DCM with LVEF ≤45%</td>
<td>52 days</td>
<td>No improvement in survival or heart transplantation rates</td>
<td></td>
</tr>
<tr>
<td>Parillo et al. (1989)</td>
<td>Idiopathic DCM</td>
<td>6 months</td>
<td>Increased proportion of patients with LVEF improvement ≥5%; LVEF improved more often in patients with signs of cardiac or systemic inflammation&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Liu et al. (2006)</td>
<td>Stable HF without fluid retention</td>
<td>20 days</td>
<td>Reduced serum creatinine levels; increased diuresis and natriuresis&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Push-Path (2013)*</td>
<td>NYHA class III–IV HF with LVEF ≤40% and hyperuricaemia</td>
<td>34 days</td>
<td>Reduced serum uric acid level (similar to allopurinol); serum creatinine level, body weight and serum NT-proBNP level; increased diuresis and improved clinical status&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>
| Prednisone and azathioprine | Wojnicz et al. (2001) | NYHA class II–IV DCM with LVEF ≤40% and increased cardiac HLA expression | 84 | 2 years | No difference in the composite of death, heart transplantation or hospital readmission Improved LVEF, LV remodelling and NYHA class

Dexamethasone and prednisone | COPE-ADHF (2014) | De novo AHF or ADHF | 102 | 30 days | Reduced dyspnoea, serum creatinine levels and cardiovascular mortality; increased diuresis

**Pleiotropic**

| Colchicine | Deftereos et al. (2014) | Stable NYHA class I–III HF with LVEF ≤40% | 267 | 6 months | No reduction in death or hospitalization for HF; no increase in exercise capacity Reduced plasma CRP and IL-6 levels

| COLCOT (2019) | MI in the previous 30 days | 4,745 | 1.9 years | Reduced composite of cardiovascular mortality, resuscitated cardiac arrest, MI, stroke or urgent coronary revascularization Reduced stroke or urgent coronary revascularization as separate outcomes

| Cyclosporine A | Piot et al. (2008) | STEMI, enrolled within 12 h of symptom onset and treated with PCI | 58 | 48 h | Reduced incidence of HF

| Ghaffari et al. (2013) | STEMI and candidate for thrombolytic treatment | 101 | 6 months | No effect on the occurrence of HF

| Cung et al. (2015) | STEMI and undergoing PCI within 12 h of symptom onset | 395 | 12 months | No improvement in worsening HF, hospitalization for HF or adverse LV remodelling

| CYCLE (2016) | Large STEMI, enrolled within 6 h of symptom onset and treated with PCI | 410 | 6 months | No effect on hospitalization for HF or death due to HF

| Methotrexate | Gong et al. (2006) | NYHA class II–IV HF (ischaemic, hypertensive, or idiopathic DCM) with LVEF <45% | 62 | 4 months | No effect on LVEF, LV remodelling or adverse events (death, rehospitalization or worsening HF) Reduced TNF, IL-6, CCL2, siCAM1 and CRP levels and increased IL-10 and IL-1RA levels in plasma; improved NYHA class, 6-MWT distance and quality of life
<table>
<thead>
<tr>
<th>Study</th>
<th>Condition Description</th>
<th>Participants</th>
<th>Duration</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>METIS (2009)</td>
<td>NYHA class II–IV HF with LVEF &lt;45% and CAD</td>
<td>50</td>
<td>3 months</td>
<td>No reduction in a composite of all-cause death, MI, stroke, hospitalization for HF or coronary revascularization; no improvement in 6-MWT distance, NYHA class or SF-36 scores; no change in CRP levels</td>
</tr>
<tr>
<td>Pentoxifylline</td>
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<tr>
<td>Silwa et al. (1998)</td>
<td>NYHA class II–III idiopathic DCM with LVEF ≤40%</td>
<td>28</td>
<td>6 months</td>
<td>Improved NYHA class and LVEF; reduced plasma TNF</td>
</tr>
<tr>
<td>Skudicky et al. (2000)</td>
<td>NYHA class II–III idiopathic DCM with LVEF &lt;40%</td>
<td>49</td>
<td>6 months</td>
<td>Improved NYHA class and LVEF; reduced plasma TNF and FAS levels</td>
</tr>
<tr>
<td>Skudicky et al. (2001)</td>
<td>NYHA class II–III idiopathic DCM with LVEF &lt;40%</td>
<td>39</td>
<td>6 months</td>
<td>Improved NYHA class, 6-MWT distance and LVEF</td>
</tr>
<tr>
<td>Silwa et al. (2002)</td>
<td>NYHA class IV idiopathic DCM with LVEF ≤40% and requirement for intravenous inotropic agents</td>
<td>18</td>
<td>1 month</td>
<td>Reduced plasma TNF and FAS levels; increased LVEF</td>
</tr>
<tr>
<td>Bahrmann et al. (2004)</td>
<td>NYHA class II–III HF (ischaemic, hypertensive or idiopathic DCM) with LVEF &lt;40%</td>
<td>47</td>
<td>6 months</td>
<td>No improvement in LVEF or VO2max compared with placebo; no effect on plasma TNF, IL-6 or BNP levels, quality of life, NYHA class or exercise time</td>
</tr>
<tr>
<td>Silwa et al. (2004)</td>
<td>NYHA class II–III HF due to CAD with LVEF &lt;40%</td>
<td>38</td>
<td>6 months</td>
<td>Improved NYHA class, systolic blood pressure and LVEF; reduced plasma CRP, NT-proBNP, TNF and FAS levels</td>
</tr>
<tr>
<td>Thalidomide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gullestad et al. (2005)</td>
<td>Stable NYHA class II–III HF due to idiopathic DCM or CAD with LVEF &lt;40%</td>
<td>56</td>
<td>3 months</td>
<td>Improved LVEF (particularly in patients with idiopathic DCM) and LV remodelling; reduced heart rate and blood neutrophil counts; increased plasma TNF levels</td>
</tr>
<tr>
<td>Orea-Tejeda et al. (2007)</td>
<td>NYHA class II–III ischaemic or non-ischaemic HF with LVEF &lt;40%</td>
<td>80</td>
<td>6 months</td>
<td>Reduced plasma TNF levels; No effect on NYHA class, LVEF or LV remodelling</td>
</tr>
<tr>
<td><strong>TNF inhibition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etanercept</td>
<td>NYHA class III HF with LVEF &lt;35% and elevated plasma TNF levels</td>
<td>18</td>
<td>14 days</td>
<td>Decreased TNF bioactivity; increased quality of life, 6-MWT distance and LVEF</td>
</tr>
<tr>
<td><strong>Notes</strong>:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>* Denotes statistically significant changes compared to placebo or baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>* TNF inhibition results may vary depending on specific TNF inhibitor used</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Design (Year)</td>
<td>Population</td>
<td>N</td>
<td>Duration</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------</td>
<td>-----------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Bozkurt et al. (2001)</td>
<td></td>
<td>NYHA class III–IV HF with LVEF &lt;35%</td>
<td>47</td>
<td>3 months</td>
</tr>
<tr>
<td>Renewal (Recovery/ Renaissance) (2004)</td>
<td></td>
<td>NYHA class II–IV HF with LVEF ≤30%</td>
<td>2,048 (1,123/925)</td>
<td>5.7/12.9 months</td>
</tr>
<tr>
<td>Infliximab</td>
<td>ATTACH (2003)</td>
<td>NYHA class III–IV HF with LVEF ≤35%</td>
<td>150</td>
<td>7 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xanthine oxidase inhibition</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allopurinol</td>
<td>EXACT-HF (2015)</td>
<td>High-risk patients with NYHA class II–IV HF with LVEF ≤40% and hyperuricaemia</td>
<td>253</td>
<td>6 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxypurinol</td>
<td>OPT-CHF (2008)</td>
<td>NYHA class III–IV HF with LVEF ≤40%</td>
<td>405</td>
<td>6 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

For the studies in patients with myocardial infarction (MI), only those that examined heart failure (HF) outcomes are included. All studies were randomized controlled trials (RCTs) and placebo-controlled unless stated otherwise. 6-MWT, 6-minute walking test; ADHF, acutely decompensated heart failure; AHF, acute heart failure; BNP, B-type natriuretic peptide; CAD, coronary artery disease; CCL2, CC-motif chemokine 2; CRP, C-reactive protein; DCM, dilated cardiomyopathy; ECG, electrocardiogram; FAS, programmed cell death receptor; HFrEF, heart failure with preserved ejection fraction; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; IL-1RA, IL-1 receptor antagonist; LV, left ventricular; LVEF, left ventricular ejection fraction; NF-κB, nuclear factor-κB; NT-proANP, N-terminal pro-atrial natriuretic peptide; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PCI, percutaneous coronary intervention; SF-36, 36-item short-form survey; sICAM1, soluble intercellular adhesion molecule 1; STEMI, ST-segment elevation myocardial infarction; sTNFR, soluble tumour necrosis factor receptor; VE/VCO₂, minute ventilation/carbon dioxide production ratio; VO₂max, maximal oxygen consumption; TNF, tumour necrosis factor. *Study results showing promising beneficial outcomes. bRCT versus allopurinol.
### Table 2. Studies of device-based immunomodulatory interventions in patients with HF or idiopathic DCM.

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Study (year)</th>
<th>Study type</th>
<th>Patient population</th>
<th>Number of patients</th>
<th>Median follow-up</th>
<th>Outcomes</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Autoantibody reduction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immuno-adsorption</td>
<td>Felix et al. (2000)</td>
<td>RCT</td>
<td>NYHA class III–IV DCM with LVEF &lt;30%</td>
<td>18</td>
<td>3 months</td>
<td>Improved cardiac index, stroke volume index, LVEF and NYHA class; decreased systemic vascular resistance&lt;sup&gt;a&lt;/sup&gt;</td>
<td>354</td>
</tr>
<tr>
<td></td>
<td>Müller et al. (2000)</td>
<td>Matched case-control study</td>
<td>NYHA class II–IV idiopathic DCM, with LVEF &lt;29% and evidence of anti-β1-adrenergic receptor autoantibodies</td>
<td>34</td>
<td>12 months</td>
<td>Improved LVEF, LV remodelling and NYHA class&lt;sup&gt;a&lt;/sup&gt;</td>
<td>355</td>
</tr>
<tr>
<td></td>
<td>Staudt et al. (2001)</td>
<td>RCT</td>
<td>NYHA class III–IV DCM with LVEF &lt;30%, positive for anti-β1-adrenergic receptor autoantibodies and cardiac lymphocytic infiltrates</td>
<td>25</td>
<td>3 months</td>
<td>Improved LVEF; reduced total leukocyte, total lymphocyte, CD4&lt;sup&gt;+&lt;/sup&gt; T cell and CD8&lt;sup&gt;+&lt;/sup&gt; T cell counts and MHC-II expression in the heart&lt;sup&gt;a&lt;/sup&gt; No effect on cardiac fibrosis</td>
<td>396</td>
</tr>
<tr>
<td></td>
<td>Pokrovsky et al. (2013)</td>
<td>Randomized case-control study</td>
<td>NYHA class II–IV DCM with LVEF ≤35%</td>
<td>16</td>
<td>6 months</td>
<td>Improved LVEF, LV remodelling, 6-MWT distance and NYHA class&lt;sup&gt;a&lt;/sup&gt;</td>
<td>397</td>
</tr>
<tr>
<td></td>
<td>Yoshikawa et al. (2016)</td>
<td>RCT (versus delayed immuno-adsorption)</td>
<td>NYHA class III–IV DCM with LVEF &lt;30%</td>
<td>40</td>
<td>3 months</td>
<td>Improved LVEF, cardiothoracic ratio, NYHA class, quality of life, VO&lt;sub&gt;2&lt;/sub&gt; max and 6-MWT distance with early treatment (particularly pronounced in patients with higher autoantibody burden)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>398</td>
</tr>
<tr>
<td><strong>Non-specific immunomodulation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Celacade</td>
<td>ACCLAIM (2008)</td>
<td>RCT</td>
<td>NYHA class II–IV HF with LVEF ≤30%</td>
<td>2,426</td>
<td>10.2 months</td>
<td>No reduction in all-cause mortality or first cardiovascular hospitalization Reduction of all-cause mortality or first cardiovascular hospitalization in patients with no history of MI or NYHA class II&lt;sup&gt;a&lt;/sup&gt;</td>
<td>264</td>
</tr>
</tbody>
</table>
### Vagus nerve stimulation

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Patient population</th>
<th>Number of patients</th>
<th>Median follow-up (months)</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BioControl INOVATE-HF (2016)</td>
<td>NYHA class III HF with LVEF ≤40%</td>
<td>707</td>
<td>16</td>
<td>Improved quality of life, NYHA class and 6-MWT distance&lt;sup&gt;a&lt;/sup&gt; No reduction in all-cause mortality or first event for worsening HF; no change in LV end systolic volume index</td>
</tr>
<tr>
<td>Parasymp transcutaneous stimulator Stavrakis et al. (2022)</td>
<td>HfPEF with two of either obesity, diabetes or hypertension</td>
<td>52</td>
<td>3</td>
<td>Improved LV GLS and GCS, and quality of life; reduced plasma TNF and IL-8 levels&lt;sup&gt;a&lt;/sup&gt; No effect on E/e′, E/A, LVEF, LAVI, plasma BNP level or 6-MWT distance</td>
</tr>
<tr>
<td>Precision implantable pulse generator NECTAR-HF (2015)</td>
<td>NYHA class II–III HF with LVEF ≤35%</td>
<td>87</td>
<td>6</td>
<td>Improved quality of life and NYHA class&lt;sup&gt;a&lt;/sup&gt; No effect on cardiac remodelling, exercise capacity, VO2max or plasma NT-proBNP levels</td>
</tr>
</tbody>
</table>

All studies were randomized controlled trials (RCTs) and sham-controlled unless stated otherwise. 6-MWT, 6-minute walking test; BNP, B-type natriuretic peptide; DCM, dilated cardiomyopathy; E/A, early-to-late diastolic transmitral flow velocity ratio; E/e′, ratio of early diastolic transmitral flow velocity to early diastolic mitral annular tissue velocity; e′, early diastolic mitral annular tissue velocity; GCS, global circumferential strain; GLS, global longitudinal strain; HF, heart failure; HfPEF, heart failure with preserved ejection fraction; LAVI, left atrial volume index; LV, left ventricular; LVEF, left ventricular ejection fraction; MHC-II, major histocompatibility complex class II; MI, myocardial infarction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; VO2max, maximal oxygen consumption; TNF, tumour necrosis factor. *Study results showing promising beneficial outcomes.

### Table 3. Meta-analyses of pharmaceutical- or device-based immunomodulatory interventions in patients with HF, idiopathic DCM, or MI.  

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Therapy</th>
<th>Study (year)</th>
<th>Patient population</th>
<th>Number of patients</th>
<th>Median follow-up (months)</th>
<th>Outcomes</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody reduction</td>
<td>Intravenous immunoglobulin or immunoadsorption</td>
<td>Bian et al. (2021)</td>
<td>DCM</td>
<td>395 (12 RCTs)</td>
<td>4</td>
<td>Improvement in LVEF; left ventricular remodelling and NYHA class&lt;sup&gt;a&lt;/sup&gt;</td>
<td>251</td>
</tr>
<tr>
<td>Complement C5 inhibition</td>
<td>Pexelizumab</td>
<td>Testa et al. (2008)</td>
<td>Acute MI or patients undergoing CABG surgery</td>
<td>15,196 (7 RCTs)</td>
<td>3</td>
<td>No effect on the risk of HF</td>
<td>400</td>
</tr>
<tr>
<td>Pleiotropic</td>
<td>Cyclosporine A</td>
<td>Yingzhong et al. (2016)</td>
<td>MI</td>
<td>776 (5 RCTs)</td>
<td>6</td>
<td>No effect on the risk of HF</td>
<td>401</td>
</tr>
<tr>
<td>Pentoxylline</td>
<td></td>
<td>Champion et al. (2014)</td>
<td>DCM, ischaemic or hypertensive HF with LVEF ≤40%</td>
<td>221 (6 RCTs)</td>
<td>6</td>
<td>Reduced all-cause mortality*</td>
<td>283</td>
</tr>
<tr>
<td>Vagal nerve stimulation or baroreflex activation</td>
<td>BioControl CardioFit, precision implantable pulse generator, or Barostim neo system</td>
<td>Sant’Anna et al. (2021)</td>
<td>HF with LVEF ≤40%</td>
<td>1,263 (4 RCTs)</td>
<td>6</td>
<td>Improvement in NYHA class, 6-minute walking test, quality of life and plasma N-terminal pro-B-type natriuretic peptide levels’ No effect on mortality</td>
<td>402</td>
</tr>
</tbody>
</table>

CABG, coronary artery bypass grafting; DCM, dilated cardiomyopathy; HF, heart failure; LVEF, left ventricular ejection fraction; MI myocardial infarction; RCT, randomized control trial.
Potential new targets for immunomodulation

In this section, we discuss scientific evidence on the effects of directed immunomodulation for the treatment of HF. The section is organized by therapeutic target, each of which can potentially be modulated with various treatment strategies. A discussion of all possible therapeutic avenues would be too extensive, and thus, we discuss only therapeutic targets that have received sufficient scientific interest. Therefore, the evidence presented in this section is not exhaustive. In previous sections, however, we also present a synthesis of available evidence on the immunomodulatory properties of currently used treatments for HF. This analysis serves to inform the future development of potential immunomodulatory interventions. Namely, the development of new interventions should not proceed in isolation but should instead be carried out in the context of the aforementioned immunomodulatory mechanisms, as described in detail in the text and summarized in Boxes 1 and 2 and Figure 2.

The existing evidence base could be integrated into future scientific endeavours by classifying the therapeutic targets into targets that are modulated by currently available therapeutic approaches for HF; targets that are modulated by current therapeutic approaches that might be amenable to additional therapeutic targeting (which we term ‘incompletely studied targets’ in the next sections); and targets that are promising but whether they are modulated by current therapeutic approaches is unknown (termed ‘not yet studied targets’). This division is meaningful because it allows for the realization that some therapies might not show clinical benefit when used in combination with current approaches, because of diminishing returns caused by cumulative targeting of the same mechanism (Table 4). Nonetheless, additional research into targets already modulated by current HF therapies is not without merit, given that new treatments that modulate such targets could be used in cases in which immunomodulation by existing treatments is not possible owing to contraindications or patient intolerance.

Targets modulated by current therapies for HF

CCR2

CCR2 is an important chemokine receptor for macrophages and controls their migration to inflammatory sites. CCR2+ macrophage infiltration in the myocardium is associated with persistent LV systolic dysfunction in patients with advanced HF.\textsuperscript{284} CCR2 inhibition or CCR2+ monocyte depletion reduced T cell population expansion and attenuated adverse LV remodelling, dysfunction, and fibrosis in mice with HF.\textsuperscript{285} Numerous CCR2 antagonists are currently available and have been evaluated in clinical trials for use in various diseases, although so far none has been approved for use in patients owing to limited success in clinical trials.\textsuperscript{286} Furthermore, considering that various HF treatments
interfere with monocyte migration and responses to CCR2-directed chemotaxis, whether CCR2 inhibition could provide additive benefits for HF treatment remains to be investigated.

**Cardiosplenic axis**

The cardiosplenic axis is involved in the pathophysiology of HF, and splenic immune responses are modulated by various HF treatments. Interestingly, the vagus nerve dampens splenic inflammatory responses via the splenic nerve. However, in the INOVATE-HF trial, vagus nerve stimulation using the CardioFit stimulator did not improve outcomes in patients with chronic HF compared with continued medical therapy. Of note, the CardioFit system does not provide stimulation currents in patients with low heart rates for safety reasons, which might reduce the efficacy of this treatment, considering that pharmacologically induced or non-pharmacologically related bradycardia can often complicate HF. Non-invasive vagus nerve stimulation via the auricular nerve using the Parasym device exerted anti-inflammatory and cardioprotective effects in patients with HFP EF. In addition, splenic stimulation using pulsed ultrasound might be an interesting therapeutic approach for additional research in HF. However, as detailed in previous sections, both ACE inhibitors and β-blockers can affect leukocyte mobilization from the spleen, thus potentially causing diminishing returns if used in combination with vagus or splenic nerve stimulation.
Table 4. Classification of potential targets for immunomodulation and the corresponding therapies.

<table>
<thead>
<tr>
<th>Target classification*</th>
<th>Target name</th>
<th>Available treatment strategiesb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modulated by currently available therapies</td>
<td>CCR2</td>
<td>CCR2 inhibitors, anti-CCR2 monoclonal antibodies</td>
</tr>
<tr>
<td></td>
<td>CCR2 inhibitors, anti-CCR2 monoclonal antibodies</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cardiosplenic axis</td>
<td>Pulsed ultrasonography, vagus nerve stimulation</td>
</tr>
<tr>
<td></td>
<td>NLRP3 inflammasome</td>
<td>Colchicine, NLRP3 inhibitors, caspase 1 inhibitors</td>
</tr>
<tr>
<td>Incompletely studied</td>
<td>IL-1β</td>
<td>Canakinumab, anakinra</td>
</tr>
<tr>
<td></td>
<td>IL-6</td>
<td>Tocilizumab, ziltivekimab</td>
</tr>
<tr>
<td></td>
<td>IL-10</td>
<td>Recombinant human IL-10</td>
</tr>
<tr>
<td></td>
<td>IL-17</td>
<td>Ikekizumab, secukinumab</td>
</tr>
<tr>
<td>T cell co-stimulation</td>
<td>Abatacept</td>
<td></td>
</tr>
<tr>
<td>Regulatory T cell migration</td>
<td>Dorzagliatin</td>
<td></td>
</tr>
<tr>
<td>CHIP</td>
<td>Canakinumab, other treatments</td>
<td></td>
</tr>
<tr>
<td>Not yet studied</td>
<td>Myeloperoxidase</td>
<td>AZD4381</td>
</tr>
<tr>
<td></td>
<td>TLR4</td>
<td>Eritoran</td>
</tr>
<tr>
<td></td>
<td>GLP1R</td>
<td>Semaglutide, dulaglutide, liraglutide</td>
</tr>
<tr>
<td></td>
<td>NETosis</td>
<td>DNase 1, PAD4 inhibitors, myeloperoxidase inhibitors, elastase inhibitors</td>
</tr>
<tr>
<td></td>
<td>Autoantibodies</td>
<td>Rituximab, immunoabsorption</td>
</tr>
<tr>
<td></td>
<td>Dietary interventions</td>
<td>Mediterranean diet, foods containing angiotensin-converting enzyme-inhibiting peptides, potassium-rich salt</td>
</tr>
<tr>
<td></td>
<td>Micronutrient deficiencies</td>
<td>Selenium, zinc, magnesium</td>
</tr>
</tbody>
</table>

CCL2, CC-motif chemokine 2; CHIP, clonal haematopoiesis of indeterminate potential; GLP1R, glucagon-like peptide 1 receptor; PAD4, protein-arginine deiminase type 4; TLR4, Toll-like receptor 4. *Therapeutic targets are classified into those modulated by existing therapies, those modulated by existing therapies but that might be amenable to additional therapeutic targeting (incompletely studied) and those that might be promising but whether they are modulated by current therapeutic approaches is not known (not yet studied). bThe table is not an exhaustive categorization of all available treatments and simply provides some illustrative examples.

NLRP3 inflammasome

The NLRP3 inflammasome can be targeted with direct NLRP3 or caspase 1 inhibition, or indirectly by inhibiting upstream or downstream processes. NLRP3 inflammasome inhibition with various pharmacological inhibitors reduced infarct size and myocardial fibrosis and preserved cardiac function in various animal models of MI. A notable example of indirect NLRP3 inflammasome targeting is the use of colchicine, a nonspecific NLRP3 inflammasome inhibitor. Colchicine improved cardiac function in a mouse model of viral myocarditis and reduced the composite of death or hospital admission in community-treated patients with coronavirus disease 2019 (COVID-19) compared with placebo, suggesting potential benefits of this drug in individuals at risk of COVID-
19-related complications, including myocarditis. Administration of colchicine also prevented adverse cardiovascular events in patients with stable CAD, but did not improve outcomes in patients with HFrEF compared with placebo. However, the latter study was much smaller (n = 267) and might have been underpowered to detect differences in outcomes. Furthermore, patients were followed up only during the 6-month treatment period and long-term outcomes were not available. Notably, patients treated with colchicine showed larger reductions in circulating hsCRP and IL-6 levels than patients who received placebo, but the short duration of patient follow-up hampers the interpretability of these results. In addition, most patients were already treated with β-blockers, ACE inhibitors, ARB or MRAs. Given that carvedilol, ARBs and SGLT2 inhibitors can interfere with NLRP3 inflammasome assembly and functions, the combination with an NLRP3 inflammasome inhibitor can potentially cause diminishing returns in terms of therapeutic benefits. Colchicine is currently under investigation in the COLICA trial in patients with acutely decompensated HF.

Incompletely studied targets

Pro-inflammatory cytokines
Two cytokines have been extensively investigated in the context of HF, IL-1β and IL-6. Although current HF treatments reduce the levels of IL-1β and IL-6 in the circulation, in damaged myocardium and in activated leukocytes, residual elevated circulating levels of IL-1β and IL-6 are associated with adverse outcomes in this patient population. Thus, additional targeted inhibition of these cytokines might be beneficial.

IL-1β blockade improved LV function in mice after MI. Additionally, the IL-1 receptor antagonist anakinra improved cardiopulmonary performance in mice and patients with HF. In the REDHART study, patients with HF and LVEF <50% treated with anakinra for 12 weeks showed improved peak maximal oxygen consumption (VO2max) compared with those receiving placebo. In addition, anakinra use was associated with a nonsignificant trend for a lower incidence of the composite of all-cause mortality and hospitalization for HF compared with placebo. By contrast, in the DHART2 study, anakinra did not improve VO2max and minute ventilation/carbon dioxide production ratio (VE/VCO2) slopes compared with placebo in patients with HFpEF and obesity and elevated CRP levels, although anakinra treatment reduced plasma CRP and NT-proBNP levels. In the Anakinra ADHF study, 30 patients with acutely decompensated HF who were treated with anakinra showed significantly higher reductions in plasma CRP and IL-6 levels at 30 days of follow-up compared with placebo-treated patients. Lastly, a pooled analysis of the VCUART trials, which included 139 patients with ST-segment elevation myocardial infarction (STEMI) enrolled within 12 h of reperfusion, showed that treatment with anakinra reduced plasma CRP levels and the rate of new-onset HF.
and hospitalization for HF at 12 months of follow-up compared with placebo. Despite these promising findings on IL-1β blockade, large and well-designed clinical trials that clarify the advantages of anakinra use for the prevention and treatment of HF are currently lacking. In the landmark CANTOS trial, the anti-IL-1β monoclonal antibody canakinumab reduced recurrent cardiovascular events in patients with previous MI and elevated hsCRP, compared with placebo. Additionally, in the CANTOS HF substudy, which included 2,173 patients with a history of HF, previous MI and elevated plasma CRP levels, canakinumab was associated with a trend for lower rates of hospitalization for HF or the composite of HF-related death and hospitalization for HF. Nevertheless, tailored studies in patients with HF are necessary to evaluate the potential of canakinumab as an add-on immunomodulation in these patients. The ongoing REDHART and Virginia-ART trials are currently investigating the role of anakinra in patients with acutely decompensated HF and elevated hsCRP and patients with STEMI, respectively.

IL-6 has been shown to exert negative inotropic effects in animals and to worsen natriuresis by stimulating renal epithelial sodium channels. Furthermore, elevated urinary IL-6 levels are associated with diuretic resistance and worse renal function. The IL-6 inhibitor ziltivekimab was safe and effective in patients with chronic kidney disease in the RESCUE trial and will be further investigated in patients with HF in the HERMES trial.

**IL-10**

IL-10 is a cytokine with potent anti-inflammatory properties. Various HF treatments either increase IL-10 production or promote the function of IL-10-producing leukocytes (such as T<sub>reg</sub> cells). IL-10 supplementation attenuated cardiac remodelling and preserved cardiac function in mouse models of pressure overload-induced HF and ischaemia-induced HF. Additionally, lower plasma IL-10 levels have been associated with HF development after MI in patients. Beyond its immunomodulatory effects on leukocytes, IL-10 also inhibits TNF-induced cardiomyocyte apoptosis and acts on myofibroblasts to reduce cardiac fibrosis. Nevertheless, recombinant IL-10 administration for the treatment of HF has not been studied in humans. Therefore, additional research on the therapeutic applications of IL-10 is warranted.

**Clonal haematopoiesis of indeterminate potential**

CHIP is a pre-malignant condition characterized by clonal expansion of haematopoietic stem cells, most often caused by variants in TET2 or DNMT3A. Bone marrow-specific TET2 deficiency mimicking the presence of CHIP is associated with more severe cardiac dysfunction in mice with pressure overload-induced or ischaemia-induced HF, and CHIP driver variants in DNMT3A activate pro-inflammatory signalling in monocytes and T cells from patients with HF. The presence of CHIP driver variants is also associated
with increased mortality in patients with HFrEF. Interestingly, patients with previous MI and TET2-driven CHIP showed a reduced risk of major adverse cardiovascular events with canakinumab treatment, potentially suggesting that patients harbouring TET2 variants might respond better to canakinumab therapy than those without CHIP. However, these findings remain to be validated in larger studies. In addition, genetic variants that disrupt IL-6 signalling were associated with reduced mortality in patients with HFrEF, whereas genetic variants associated with increased circulating IL-18 concentrations and IL-6 signalling had the opposite effect. Therefore, a combined diagnostic and therapeutic approach that includes screening for CHIP, the identification of patients with specific genetic variants and therapeutic interventions that target IL-1β, IL-6 or IL-18 merits future investigation.

**T cell activation and effector functions**

T cell co-stimulation, an essential immune process related to T cell activation, is associated with increased mortality in HF and thus might constitute a promising therapeutic target. Abatacept, a humanized fusion protein that inhibits co-stimulatory CD80–CD86 signalling on APCs, delayed HF progression, ameliorated cardiac dysfunction and decreased immune cell infiltrates in mice with HF. The number of circulating T<sub>H</sub>17 cells and the plasma levels of IL-17 (a cytokine produced by T<sub>H</sub>17 cells) are elevated in patients with advanced HF. In addition, plasma IL-17 levels correlated with symptom severity, cardiac dysfunction and higher plasma NT-proBNP levels in elderly patients with HF. Furthermore, IL-17 promoted cardiac fibrosis in mice with diabetes or MI, apoptosis in mouse cardiomyocytes and disturbances in cardiac calcium handling. By contrast, IL-17 deficiency increased cardiomyocyte contractility and inhibited HF development in mice with TAC. Additionally, IL-17 deficiency in mice with MI decreased infarct size, myocardial fibrosis, and apoptosis, and improved cardiac function. Therefore, targeting IL-17 signalling or T<sub>H</sub>17 cells might be a promising therapeutic approach for the treatment of HF. Lastly, most existing HF treatments have been found to polarize CD4<sup>+</sup> T cells to anti-inflammatory T<sub>reg</sub> cells. Interestingly, glucokinase-activating drugs, such as dorzagliatin, which was originally developed for the treatment of T2DM, can selectively promote T<sub>reg</sub> cell migration to inflammatory sites. Therefore, this mechanism could potentiate the favourable effects of current HF treatments.

**Targets not yet studied**

**TLR4**

TLR4 is a PRR that initiates inflammatory cytokine production via NF-κB signalling and has an important role in myocardial inflammation. In animal models, TLR4 upregulation in cardiomyocytes worsens HF symptoms after MI, and TLR4 stimulation decreases
contractility via NF-κB signalling.\textsuperscript{340,341} By contrast, TLR4 deficiency ameliorated cardiac dysfunction, inflammation, oxidative stress, and cardiac apoptosis in a mouse model of doxorubicin-induced cardiomyopathy.\textsuperscript{342} TLR4 deficiency also improved survival and cardiac function and remodelling in mice after MI.\textsuperscript{343} Lastly, the TLR4 antagonist eritoran reduced cardiac hypertrophy and the cardiac expression of genes encoding natriuretic peptides in mice with TAC.\textsuperscript{344} Currently, whether TLR4 signalling is affected by current treatments for HF is unknown and might thus be a promising target for future interventions.

**NETosis**

NETosis is a function of neutrophils that involves the release of aggregates of DNA strands, histones and granule contents known as NETs. NETs have important roles in infectious and sterile immune responses and are involved in cardiac inflammation and myocardial damage in HF.\textsuperscript{345} Inhibition of NETosis by DNase 1 ameliorated cardiac fibrosis and function in mice with HF.\textsuperscript{346} Although various treatments for HF might interfere with neutrophil mobilization from immune cell reservoirs, whether these therapies affect NETosis is unknown. Therefore, additional research is needed to clarify the role of NETosis as a future therapeutic target in HF.

**Myeloperoxidase**

MPO is expressed in neutrophils, monocytes and macrophages and catalyses the formation of ROS and regulates vascular NO bioavailability.\textsuperscript{347} MPO-generated oxidants adversely affect LV remodelling and function in mice.\textsuperscript{348} Circulating MPO levels are elevated in patients with HF and are associated with disease severity and predict adverse outcomes.\textsuperscript{349} MPO is also involved in NET formation, and MPO inhibitors have been shown to inhibit NETosis.\textsuperscript{350} The efficacy and safety of the oral MPO inhibitor AZD4381 in patients with HF and LVEF >40% is currently under investigation in the ENDEAVOR trial.\textsuperscript{351}

**Autoantibodies**

Autoantibodies participate in the pathogenesis of severe HF in patients with idiopathic DCM and can be extracted by immunoadsorption.\textsuperscript{352,353} Immunoadsorption drastically improved cardiac function and reduced ventricular dimensions and systemic vascular resistance in patients with DCM compared with standard medical therapy.\textsuperscript{251,354,355} Immunoadsorption might also serve as a bridge to transplantation in patients with DCM, owing to the drastic and durable improvements in LVEF and LV remodelling that can last for up to 1 year after treatment.\textsuperscript{355} Lastly, in a case series of six patients with B cell-positive inflammatory DCM and insufficient responses to standard HF treatment, with or without steroid-based immunosuppression, B cell depletion with rituximab improved
Despite this evidence, immunoadsorption and immunomodulation in general are not routinely recommended in patients with DCM owing to lack of large-scale studies, and such interventions should be investigated in adequately powered clinical trials.

**GLP1R**

Glucagon-like peptide 1 receptor (GLP1R) is actively being investigated as a therapeutic target for T2DM using GLP1R agonists. GLP1R agonists modulate epicardial and visceral adipose tissue inflammation and exert multiple immunomodulatory effects on various leukocytes. Although GLP1R agonists improved LV diastolic function in patients with HFrEF compared with placebo, they did not improve outcomes in these patients. Nevertheless, GLP1R might still be an attractive target for the treatment of HFrEF. In a multi-hit HFrEF mouse model, liraglutide improved cardiac function, hypertrophy and fibrosis, and reduced lung congestion and cardiac expression of the gene encoding ANP. GLP1R agonists also improved diastolic function in patients with HFrEF compared with placebo, although large clinical trials in these patients are not yet available.

**Dietary interventions**

A Mediterranean diet intervention supplemented with extra-virgin olive oil led to reductions in the plasma levels of CRP, TNF, IL-1β, IL-5, IL-6, IL-7, IL-12, IL-18, IFNγ, CCL2, CCL4, GCSF and GM-CSF at 3 and 5 years after initiation compared with a low-fat diet in patients with high cardiovascular risk but without overt cardiovascular disease. Components of this diet also show immunomodulatory effects. Extra-virgin olive oil contains bioactive compounds that have been shown to downregulate NF-κB signalling and reduce the expression of adhesion molecules in both human and animal in vivo studies, and nut consumption reduced soluble ICAM1 production according to a meta-analysis of 23 placebo-controlled clinical trials. Increased fruit, vegetable and whole-grain consumption reduced plasma CRP, TNF and IL-6 levels in numerous studies of healthy participants or various patient cohorts, although results for whole-grain consumption did not always reach statistical significance in individual studies or in meta-analyses.

Several foods, including dairy milk, Greek yogurt, egg white, salmon and cod contain bioactive peptides with ACE inhibitory activities. Several plants (such as strawberry, pear tree, rose hip and apple tree) have SGLT2 inhibitory effects, especially those containing phlorizin. Notably, treatment with ACE inhibitors or SGLT2 inhibitors before admission to hospital is associated with reduced hospital mortality in patients with pneumonia and sepsis. Whether dietary intake of bioactive molecules with
ACE inhibitor and SGLT2 inhibitor actions could have a similar function in patients with HF remains to be investigated.

Lastly, experimental evidence demonstrates that immune cells are sensitive to ions in their environment. High extracellular concentrations of potassium promote $T_{reg}$ cell development\textsuperscript{375} and inhibit CD4$^+$ T cell polarization into effector cells in both in vitro and in vivo mouse studies.\textsuperscript{375} The SSaSS trial investigated a salt substitute consisting of 75% NaCl and 25% KCl in 20,995 participants with a history of stroke or aged $>$ 60 years with poorly controlled hypertension.\textsuperscript{376} This intervention reduced the risk of adverse cardiovascular events compared with participants assigned to regular salt (100% NaCl), particularly in women.\textsuperscript{376} In the EPIC-Norfolk cohort ($n = 24,963$), higher potassium intake was associated with lower cardiovascular risk in men and women.\textsuperscript{377} Thus, dietary salt substitution with potassium-rich substitutes could be an easily accessible immunomodulatory intervention.

**Micronutrient deficiencies**

Micronutrient deficiencies are common in patients with HF,\textsuperscript{378} and addressing these deficiencies might have immunomodulatory effects. Selenium is essential for the synthesis of 25 selenoproteins with important immunological functions,\textsuperscript{379} and it modulates T cell activation\textsuperscript{380} while promoting $T_{reg}$ cell differentiation.\textsuperscript{381} Similarly, zinc is essential for innate and adaptive immunity,\textsuperscript{382} and a meta-analysis of various studies in healthy individuals and patient populations found that zinc supplementation reduced circulating CRP levels and neutrophil counts and increased circulating CD4 levels.\textsuperscript{383} Additionally, magnesium deficiency has been linked to inflammation.\textsuperscript{384} However, two meta-analyses of magnesium supplementation in patients who were overweight or patients with T2DM showed conflicting results regarding the effects on CRP reduction.\textsuperscript{385,386} The absence of magnesium deficiency in the study populations of some of the examined studies might explain these conflicting findings, given that participants with sufficient magnesium might not derive any benefits from supplementation.\textsuperscript{385} Correction of micronutrient deficiency, particularly of selenium and zinc, might thus be a promising target for the treatment of HF.

**Future directions**

**Current treatments for HF**

In summary, although much is already known about the immunomodulatory properties of evidence-based treatments for HF, much remains to be elucidated. A particular point of attention should be the investigation of potential non-class immunomodulatory effects of HF therapies, which would permit the treatments to be tailored to individual
patients. For example, prescription of β-blockers according to disease acuity (such as using metoprolol early after MI and carvedilol at later stages) should be investigated further. Furthermore, targeting treatments specifically to immune cells might be of interest. This strategy would avoid potential systemic adverse effects and might allow for optimal dosing of medications even in patients with therapy contraindications or intolerance. For example, this specific targeting could be achieved with liposomal\textsuperscript{134} or nanoparticle-based\textsuperscript{137} drug delivery to macrophages.

Studies investigating the immunomodulatory properties of current treatments are fairly recent (Supplementary Figure 1), and immunophenotyping studies of HF animal models with the use of multi-omics and single-cell approaches are lacking. In addition, very few studies have investigated inflammatory activation in animals with chronic HF and how this can be modulated by current and novel treatments. The lack of such studies hampers scientific efforts to develop immunomodulatory treatments against chronic inflammatory activation, as observed in HF. Therefore, this lack of evidence should motivate additional targeted research to elucidate the underlying pathophysiological mechanisms of chronic inflammation in HF.

**Future potential targets**

Repurposing immunomodulatory drugs used for autoimmune diseases is an attractive option for the treatment of patients with HF, although some medications, such as infliximab,\textsuperscript{255} did not show beneficial effects for the treatment of HF in clinical studies. Nevertheless, treatments that have shown promising effects for HF treatment in small studies (such as corticosteroids, pentoxifylline and immunoadsorption) have not been investigated in large clinical trials. Additionally, therapies that modulate some of the aforementioned potential targets could be investigated in randomized clinical trials. Currently ongoing clinical trials investigating immunomodulatory interventions in HF are presented in Table 5.

**Conclusions**

In summary, most of the currently used treatments for HF modulate the immune system through various mechanisms. The development of interventions to temper disproportionate immune responses in HF should thus be performed on the basis of the immunomodulatory effects of current HF treatments. Nevertheless, regardless of future endeavours, the clinician should remain acutely aware of the need to optimize guideline-directed medical treatment of patients with HF. However, perhaps the most important realization is that the cardiologist is also, in fact, an immunologist.
<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Therapy</th>
<th>Study (start year)</th>
<th>Patient population</th>
<th>Number of patients</th>
<th>Follow-up</th>
<th>Trial registration number</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β receptor antagonism</td>
<td>Anakinra</td>
<td>REDHART2 (2019)</td>
<td>ADHF with plasma hsCRP &gt;2 mg/L</td>
<td>102</td>
<td>6 months</td>
<td>NCT03797001</td>
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<tr>
<td></td>
<td></td>
<td>Virgina-ART4 (2022)</td>
<td>STEMI</td>
<td>84</td>
<td>1.5 month</td>
<td>NCT05177822</td>
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<tr>
<td>IL-6 inhibition</td>
<td>Ziltivekimab</td>
<td>HERMES (2023)</td>
<td>HF with LVEF &gt;40% and plasma hsCRP &gt;2 mg/L</td>
<td>5,600</td>
<td>4 years</td>
<td>NCT05636176</td>
</tr>
<tr>
<td>MMP2 inhibition</td>
<td>Doxycycline</td>
<td>Doxycycline to Protect Heart Muscle After Heart Attacks (2020)</td>
<td>STEMI</td>
<td>170</td>
<td>12 months</td>
<td>NCT03508232</td>
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<tr>
<td>Myeloperoxidase inhibition</td>
<td>AZD4831</td>
<td>ENDEAVOR (2021)</td>
<td>HF with LVEF &gt;40%</td>
<td>1,480</td>
<td>12 months</td>
<td>NCT04986202</td>
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<tr>
<td>Pleiotropic</td>
<td>Colchicine</td>
<td>COLICA (2021)</td>
<td>ADHF</td>
<td>278</td>
<td>2 months</td>
<td>NCT04705987</td>
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<td></td>
<td></td>
<td>Colchicine plus spironolactone</td>
<td>CLEAR SYNERGY (2018)</td>
<td>7,063</td>
<td>2 years</td>
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<td>Vagus nerve stimulation</td>
<td>Low-level tragus stimulation</td>
<td>TREAT-HF (2016)</td>
<td>ADHF</td>
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<td></td>
<td>Neuromodulation of Inflammation and Endothelial Function (2022)</td>
<td>HF with LVEF &lt;40%</td>
<td>158</td>
<td>4 months</td>
<td>NCT05230732</td>
</tr>
</tbody>
</table>

For the studies in patients with myocardial infarction (MI), only those investigating the occurrence of heart failure (HF) are included. ADHF, acutely decompensated heart failure; hsCRP, high-sensitivity C-reactive protein; LVEF, left ventricular ejection fraction; MMP2, matrix metalloproteinase 2; STEMI, ST-segment elevation myocardial infarction.
Supplementary Figure 1. Publication timeline of studies investigating the immunomodulatory effects of currently used pharmacologic treatment for heart failure. Visualizations are based on studies included in this review.
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