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The role of the NLRP3 inflammasome and pyroptosis in cardiovascular diseases

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Abstract

An intense, stereotyped inflammatory response occurs in response to ischaemic and non-ischaemic injury to the myocardium. The NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome is a finely regulated macromolecular protein complex that senses the injury and triggers and amplifies the inflammatory response by activation of caspase 1, cleavage of pro-inflammatory cytokines, such as pro-IL-1 β and pro-IL-18 in their mature forms, and inducing inflammatory cell death (pyroptosis). Inhibitors of the NLRP3 inflammasome and blockers of IL-1 β and IL-18 activity have been shown to reduce injury to the myocardium and pericardium, favour resolution of the inflammation and preserve cardiac function. In this Review, we discuss the components of the NLRP3 inflammasome and how it is formed and activated in variety of ischaemic and non-ischaemic cardiac pathologies (acute myocardial infarction, cardiac dysfunction and remodelling, atherothrombosis, myocarditis and pericarditis, cardiotoxicity and cardiac sarcoidosis). We also summarize current preclinical and clinical evidence from studies of agents that target the NLRP3 inflammasome and related cytokines.

Introduction

An estimated 19 million deaths were attributed to cardiovascular diseases (CVD) globally in 2020, an increase of 18.7% from 2010¹. Ischaemic and non-ischaemic injury to the heart both induce acute cardiac dysfunction and can be life-threatening. A stereotyped inflammatory response follows cardiac injury, irrespective of its nature, promoting further injury and dysfunction and enhancing the risk of complications and death^{2,3}. The NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome has emerged as a central mediator of the inflammatory response to tissue injury and sterile inflammation^{3,4}, making this multiprotein complex an important therapeutic target.

In this Review, we discuss the components of the NLRP3 inflammasome and how it is formed and activated in variety of ischaemic and non-ischaemic cardiac pathologies (acute myocardial infarction (AMI), cardiac dysfunction and remodelling, atherothrombosis, myocarditis and pericarditis, cardiotoxicity and cardiac sarcoidosis). We also summarize the

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Competing interests

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evidence that identified the NLRP3 inflammasome and derived cytokines, IL-1 β and IL-18, and examine preclinical and clinical data from studies of drugs that target these molecules. We place emphasis on how our understanding of the mechanism and development of therapies has changed during the past 5 years^{3,4}. The role of non-NLRP3 inflammasomes, such as the NLRP1, NLR family CARD domain-containing protein 4 (NLRC4) and interferon-inducible protein (AIM2) inflammasomes, in CVD is not well established and is beyond the scope of this Review.

Inflammatory response to cardiac injury

AMI is the prototypical model of non-infectious, ischaemic injury ensuing sterile inflammation, which results in resorption of the wound and the formation of a scar. Early reperfusion in AMI reduces the extent of the injury. Inflammation was once considered necessary for the formation of infarct scar and to prevent wall rupture. However, it is now clear that overzealous inflammation promotes further injury, dysfunctional healing and loss of cardiac function^{5,6}. An intense inflammatory response also promotes wall rupture, however, such an event is now unlikely due to the reduced incidence of transmural infarction with reperfusion^{1,7-9}.

The inflammatory response to both AMI and non-ischaemic injury induces a paracrine effect on the myocardium in the infarct border zone, a portion of the tissue that was salvaged by reperfusion but remained in jeopardy. A systemic response also occurs, regulated by IL-1 β and downstream mediators¹⁰. Pro-inflammatory cytokines promote cardiac dysfunction and unfavourable remodelling, in a similar fashion to neurohormonal activation⁶. Sensing is essential to initiate inflammation. In the case of infection, sensing the pathogen allows for a targeted immune response. In sterile inflammation, cell debris provides the trigger and initiates the response^{3,10}.

The NLRP3 inflammasome as a sensor

Inflammasomes are macromolecular protein complexes that have evolved to activate inflammatory caspases (1, 4, 5 and 11)^{11,12}. Caspase 1 is the effector enzyme that, in humans as well as in mice, promotes the maturation and secretion of powerful pro-inflammatory cytokines, such as IL-1 β and IL-18, and a form of inflammatory, lytic cell death termed 'pyroptosis'^{11,12}. Caspases 4 and 5 in humans, and their murine ortholog caspase 11, mediate non-canonical inflammasome activation^{13,14}. The classic structure of the NLRP3 inflammasome sensing component is tripartite, with a central nucleotide oligomerization domain (defined as NACHT), a C-terminal leucine-rich repeats (LRR) domain and an N-terminal effector domain (pyrin domain, or PYD), which interacts with downstream signalling molecules^{11,12}. NLRP3 activation, due to microbial pathogen-derived molecules and extracellular or intracellular damage-associated molecular patterns (DAMPs), induces the formation of NLRP3 disk-like oligomers¹⁵. The ATPase activity of the NACHT domain is essential for NLRP3 activation, and NACHT and LRR domains participate in oligomer formation and conformational changes¹⁵. Active NLRP3 engages a scaffold protein, the apoptosis speck-like protein containing a caspase recruiting domain (ASC), facilitating the polymerization of ASC into a stable, long filament structure favouring the formation of multiple star-like caspase 1 filaments, thereby increasing caspase 1 density to

promote contact with substrates^{15,16}. Caspase 1, also known as IL-1 β -converting enzyme (ICE), cleaves and converts pro-IL-1 β and pro-IL-18 into active forms^{17,18}. In addition, caspase 1 cleaves gasdermin D (GSDMD), leading to multimerization of its N-terminal fragments (GSDMD-NT). GSDMD-NT form 21 nm pores on the plasma membrane, enabling extracellular release of IL-1 β and IL-18 and loss of membrane integrity, eventually leading to cell death^{19,20}. Mature IL-1 β and IL-18 are 17 and 18 KDa, respectively, and measure ~4 nm (for comparison, serum albumin is a protein of 66 KDa and 7.5 nm²¹). Pro-IL-1 α , another member of the IL-1 family, lacks a caspase 1 cleavage site, is already active in the pro-form and functions as an alarmin¹⁰. Cleavage of pro-IL-1 α by calpains in the cell (or neutrophil elastase, granzyme B and chimase outside the cell) increases the affinity of IL-1 α for the IL-1 receptor type I (IL-1RI)²². Although not responsible for its cleavage, caspase 1 activation facilitates pro-IL-1 α release²³. In the mouse, forced overexpression of caspase 1 in the heart leads to apoptosis without cytokine release²⁴. Signalling downstream of the IL-1 receptor is mediated by myeloid differentiation primary response protein MyD88 (MyD88) and interleukin-1 receptor-associated kinases (IRAKs) leading to nuclear factor κ B (NF- κ B) activation^{10,17}.

Pyroptosis

Inflammasome-dependent activation of caspases promotes pyroptosis, a GSDMD-dependent form of lytic cell death^{19,25}, which is associated with the active release of IL-1 β and IL-18 and exacerbates the inflammatory response. Caspase 1 is the main pyroptosis effector. Unlike apoptosis, a typical ‘silent’ form of programmed cell death, pyroptosis is associated with increased membrane permeability, mediated by GSDMD-NT, leading to cell swelling and ultimately, through the activity of ninjurin 1, to plasma membrane rupture^{25,26}. Other gasdermins (i.e. GSDMB in humans and GSDME in humans and mice) induce pyroptosis through inflammasome-independent mechanisms²⁷. GSDMD-NT have a high affinity for phosphatidylinositol phosphates and phosphatidylserine, which are mostly present on the intracellular-facing side of the plasma membrane, thus reducing damage to membranes of neighbouring cells¹⁹. Studies of GSDMD-NT pore structure have revealed that they favour the passage of neutral and positively charged proteins, and reduce the transit of negatively charged proteins¹⁹. This selectivity promotes the passage of mature IL-1 β and IL-18 and increases the cytoplasmic permanence of pro-IL-1 β and pro-IL-18, which have an acidic/negatively charged pro-domain. GSDMD-NT can also regulate the release of pro-IL-1 α and active IL-1 α ²⁸. At low concentrations, GSDMD-NT form non-lytic pores, thus favouring the release of pro-inflammatory cytokines rather than cell death¹⁹. Caspase 1 cleaves enzymes of the Krebs cycle, dramatically reducing energy production^{29,30}, as well as other substrates (e.g. actin, cytoplasmic 1 (also known as β -actin), calpastatin and E3 ubiquitin-protein ligase parkin). Caspases 4 and 5 in humans, or caspase 11 in mice, bind to cytosolic lipopolysaccharide leading to GSDMD cleavage and non-canonical NLRP3 inflammasome activation and pyroptosis³¹.

Activation of the NLRP3 inflammasome

Activation of the NLRP3 inflammasome in cardiomyocytes and other cardiac resident cells is a two-step process³². In the healthy, uninjured heart, the expression of components of the NLRP3 inflammasome is very low, insufficient to promote oligomerization

and polymerization³². Priming — transcriptional regulation through activation of NF- κ B — is the first necessary step in activation and is regulated by pro-inflammatory cytokines, neurohormones, cellular debris or microbial products, which function as DAMPs or pathogen-associated molecular patterns (PAMPs) and bind to the Toll-like receptors (TLRs)¹⁵ (Fig. 1). Nucleotide-binding oligomerization domain-containing protein 2 (NOD2), a member of the innate immune receptor family, contributes to inflammasome priming by inducing NF- κ B activation, and exacerbates myocardial injury in mice with AMI³³. NOD2 deletion reduces myocardial damage and the expression of pro-inflammatory markers, including IL-1 β ^{4,33}. Once a critical mass of NLRP3 inflammasome components is reached, activation signals (or triggers) converging on NLRP3, and leading to K⁺ efflux from the cell, catalyse the formation of the inflammasome. Extracellular ATP binding to the purinergic P2X receptor 7 (P2X7) is a classical triggering signal that leads to K⁺ efflux. In addition, lysosome destabilization, by cholesterol/uric acid crystals or pathogens, leads to intracellular cathepsin B leakage and K⁺ efflux^{34,35} (Fig. 1).

The process of NLRP3 inflammasome formation and activation in the heart is cell-type specific^{36–38}. Circulating monocytes constitutively express inflammasome components and require no, or minimal priming to produce large amounts of IL-1 β when triggered³⁹. Experimental activation of the NLRP3 inflammasome in resident macrophages, fibroblasts and endothelial cells, leads to the release of large amounts of IL-1 β , whereas it is minimal in cardiomyocytes^{36–38}. By contrast, cardiomyocytes with NLRP3 inflammasome formation have active caspase 1, secrete IL-18 and die of pyroptosis^{40–43}.

Important to note is that some inflammasome-independent functions of NLRP3 have been reported in colon and kidney cells, although their role in the heart has not been established^{44,45}. Deletion of NLRP3, but not ASC, inhibits ex vivo ischaemic preconditioning through an IL-1-independent effect⁴⁶.

Oxidative stress and kinase-mediated activation—Several cytoplasmic adaptor proteins and kinases participate in the process of NLRP3 inflammasome activation. A member of the never in mitosis A-related kinase family, serine/threonine-protein kinase NEK7, is highly expressed in the heart, where it senses K⁺ efflux, binds NLRP3 and induces its oligomerization⁴⁷. Tyrosine-protein kinase BTK (also known as Bruton kinase) is required for the activation of NLRP3 in vivo, and also interacts with ASC⁴⁸. BTK inhibition suppresses inflammasome activation in vivo, reduces infarct size after cerebral ischaemia and improves heart function in a mouse model of sepsis^{48,49}.

Thioredoxin-interacting protein (TXNIP) links oxidative stress to NLRP3 inflammasome activation^{50–52} (Fig. 1). In healthy conditions, TXNIP is bound to thioredoxin, but increased levels of reactive oxygen species (ROS) and the unfolded protein response release TXNIP from thioredoxin, promoting NLRP3 activation and consequent inflammasome formation. TXNIP silencing, using small interfering RNAs, reduces NLRP3 activation and infarct size after AMI⁵⁰. However, in some models, such as murine bone marrow-derived cells treated with classical NLRP3 inducers, activation of NLRP3 is independent of TXNIP^{53,54}.

The role of autophagy—Autophagy is a homeostatic, proteolytic process that removes misfolded or damaged proteins and organelles and regulates inflammasome function⁵⁵. Inhibition of the autophagic pathway in human monocytes and macrophages enhances NLRP3 inflammasome formation, whereas increasing the autophagic flux reduces IL-1 β secretion^{56–58}. In a mouse model of heart failure secondary to pressure overload due to transaortic constriction (TAC), cardiomyocyte-restricted gene silencing of the renin receptor (*Atp6ap2*), a component of the vacuolar-type ATPase required for autophagy, reduced autophagic flux, increased NLRP3 activation and worsened myocardial damage and remodelling⁵⁹. NLRP3 inhibition blunted myocardial damage, implying inflammasome activation driven by an autophagy impairment⁵⁹. In mice with diabetes mellitus, MI induced by coronary artery ligation impaired autophagic flux, increased expression of NLRP3 inflammasome components and worsened cardiac remodelling⁶⁰. Induction of autophagy with rapamycin reversed these effects, to a degree similar to NLRP3 inhibition^{60,61}.

Mitochondrial injury and mitophagy—Mitochondrial dysfunction and damage are major contributors to NLRP3 activation⁶². Mitochondria generate ROS and determine cell fate under stress conditions, regulating multiple cell death pathways (i.e. necrosis, apoptosis and pyroptosis)^{62–65}. Dysfunctional mitochondria increase ROS production and K⁺ efflux, both of which are NLRP3 triggers^{62,64,65}. Three distinct pathways regulate the fate of dysfunctional and damaged mitochondria — mitophagy (which clears mitochondria through autophagy), mitochondrial fission (division) and mitochondrial fusion^{55,66,67}. Ineffective mitophagy increases activation of caspase 1 and IL-1 β due to sustained ROS production and cytoplasmic accumulation of mitochondrial DNA⁶². In activated macrophages, mitophagy is reduced by pro-IL-1 α binding to cardiolipin (a mitochondrial lipid that binds and activates NLRP3⁶⁸) exacerbating NLRP3 inflammasome activity⁶⁹. In another model, deletion of dynamin-1-like protein, 1 (DRP1) impaired mitochondrial fission and promoted NLRP3-dependent caspase 1 activation and IL-1 β secretion⁶⁶. Mitochondrial fusion on the other hand prevents NLRP3 activation⁷⁰.

The NLRP3 inflammasome in AMI

Ischaemia and reperfusion—The expression of NLRP3 inflammasome components in the healthy heart is negligible. However, human pathology studies show evidence of NLRP3 inflammasome activation during AMI⁷¹. Patients with AMI also have elevated plasma levels of NLRP3 and caspase 1⁷². The time-dependent activation of the NLRP3 inflammasome in the heart has been examined in animal studies^{40,73} (Fig. 2). In a mouse model of AMI, the expression of NLRP3 inflammasome components increased over time — activation occurred 3–24 hours after reperfusion, peaking after 1 or 3 days in mice with reperfused and non-reperfused AMI, respectively^{40,73–75}. Histology studies showed inflammasome specks in endothelial cells and cardiomyocytes from the ischaemic area and border zones early in the course of AMI, followed by activation in fibroblasts and then infiltrating leukocytes. As inflammation resolves, inflammasome specks are seen in isolated cardiomyocytes or fibroblasts^{40,76}.

Response to injury—Inflammasome formation is a finely-regulated and energy-requiring process. From an evolutionary standpoint, the formation of the inflammasome is a ‘sacrifice’

by which injured (often infected) cells activate the inflammatory response, sacrificing those ‘sentinel’ cells⁷⁷. Therefore, when the inflammasome is formed in injured cardiomyocytes it becomes a mechanism of cell death^{40,78}. Compared with wild-type mice, those that lack *Nlrp3*, *ASC* or *Casp1* genes, or have undergone *Nlrp3* siRNA silencing, have a smaller infarct size and preserved cardiac function^{50,71,79} (Fig. 3). Of note, differences between *Nlrp3* knock-out and wild-type mice were evident early during ischaemia, a finding consistent with the limited expression of inflammasome components during the first 3 hours after injury^{73,74,79} (Fig. 2).

Healing and remodelling—In mouse models, delaying treatment with an NLRP3 inhibitor by 1 hour after the beginning of reperfusion did not limit the ability to reduce infarct size. However, a delay of 3 hours led to failure to reduce infarct size^{73,80} (Fig. 2). These data suggest that inhibition of the NLRP3 inflammasome within 3 hours of reperfusion salvages the portion of myocardium that is rescued by reperfusion but remains in jeopardy and is eventually lost by pyroptosis. Accordingly, inhibition of other inflammasome components limits pyroptosis and infarct size⁷¹.

NLRP3 inhibition did not reduce infarct size in a non-reperfused AMI model⁴³. Nevertheless, inhibition of the NLRP3 inflammasome in non-reperfused AMI prevents adverse remodelling, independent of infarct size^{40,43}. Inflammasome formation is present in cardiomyocytes and fibroblasts for several days after AMI⁴⁰. IL-1 β production is increased during AMI and modulates cardiac function and remodelling (Fig. 4). IL-1 blockade with the recombinant human IL-1 receptor antagonist, anakinra (Fig. 5), IL-1trap (analogous to rilonacept) or a mouse anti-IL-1 β antibody (analogous to canakinumab) reduced cardiomyocyte cell death, preserved cardiac function and restored β -adrenergic receptor responsiveness in models of ischaemic cardiomyopathy^{81–85}.

Treatment of mice with AMI using an anti-IL-1 α antibody significantly reduced the acute inflammatory response and infarct size, whereas an IL-1 β antibody did not^{86,87}. This finding reflects the fact that IL-1 α is an IL-1 isoform that is constitutively expressed in the heart and is active in its precursor form, pro-IL-1 α ^{84,86,88}. By contrast, IL-18 activity is regulated by the inflammasome. IL-18 contributes to contractile dysfunction in isolated human myocardial strips exposed to simulated ischaemia–reperfusion injury⁸⁹. In mouse models of AMI, treatment with an anti-IL-18 antibody or recombinant IL-18 binding protein (IL-18BP) reduce infarct size, ischaemia–reperfusion injury, and myocardial inflammation^{90–92}.

Cardiac dysfunction and heart failure—Activation of NLRP3 (triggering), without induction of the scaffold and effector proteins (priming) is insufficient to induce cardiac dysfunction, due to low constitutive expression of inflammasome components³². As such, patients with mutations in the *NLRP3* gene (previously known as *CIAS1*), causing constitutively active, cryopyrin-associated periodic syndromes, show no generalized signs of cardiac dysfunction, but might be vulnerable to loss of cardiac function during the clinically active phases of the disease⁹³. Pro-inflammatory stimuli cause cardiac dysfunction through activation of the NLRP3 inflammasome and release of IL-1 β and IL-18^{32,93–95}. IL-1 β induces simultaneous changes in the expression and function of L-type calcium channels,

phospholamban and sarcoplasmic/endoplasmic reticulum calcium ATPase 2, leading to a functional desensitization of the β -adrenergic receptors, consistent with changes in patients with heart failure⁹⁶ (Fig. 4). IL-18 seems to mediate many of the effects of IL-1 β ⁹⁷. Of note, cardiac dysfunction induced by IL-1 β is, at least in part, reversible⁹⁸.

The NLRP3 inflammasome in non-ischaemic injury

As discussed above, the inflammatory response to injury is a stereotyped response in which the NLRP3 inflammasome is central. Priming and triggering can occur through the membrane and cytoplasmic sensors (i.e. Toll-like receptors and P2X purinoceptor 2 on the membrane and NOD-like receptors in the cell (Fig. 1). Several non-ischaemic processes can induce injury to the heart and cardiovascular system and stimulate an NLRP3 inflammasome-mediated response³⁸.

Atherothrombosis—The NLRP3 inflammasome has a role in the atherothrombotic process that leads to myocardial ischaemia and infarction⁹⁹. Atherosclerosis refers to changes in the vessel wall characterized by inflammation and accumulation of intracellular and extracellular cholesterol crystals. When the atherosclerotic plaque is complicated by thrombosis (atherothrombosis), blood flow in the vessel is abruptly threatened causing ischaemia and infarction (Fig. 3). Cholesterol and calcium phosphate crystals found in atherosclerotic plaque lead to lysosomal instability, cathepsin B release and NLRP3 inflammasome activation¹⁰⁰. Deletion of the *Nlrp3*, *Asc* and *Il-1 α / β* genes in the bone marrow of mice with hypercholesterolaemia prevented the development and modified the characteristics of atherosclerotic plaques^{100–103}. In addition, caspase 4 expression in human peripheral blood mononuclear cells increases with the severity of coronary artery disease, and deletion of its ortholog in mice, caspase 11, reduced the progression of atherosclerosis¹⁰⁴.

Myocarditis—Myocarditis is an inflammatory process that occurs in response to direct injury of the myocardium by a virus, or a pathological immune response (antigen mimicry or autoimmunity). Myocardial injury stimulates the stereotyped NLRP3 inflammasome-mediated response that promotes further damage and progression to heart failure^{105,106} (Fig. 4). Caspase 11 activation, part of the non-canonical inflammasome activation pathway, has been reported in the hearts of mice with coxsackievirus B3-induced myocarditis¹⁰⁷.

Sarcoidosis—The formation of the NLRP3 inflammasome has also been described in cardiac sarcoidosis. This distinct form of myocarditis is generally chronic, often part of a systemic illness and characterized by myocardial injury mediated by non-caseating granulomatous inflammation with the formation of multinucleated giant cells¹⁰⁸ (Fig. 4). Kron et al. found that granulomas in samples of myocardial tissue from three patients with cardiac sarcoidosis and end-stage heart failure contained the inflammasome components ASC, NLRP3 and caspase 1¹⁰⁸. Similarly, the presence of NLRP3 inflammasome components in granulomas was also seen in lung samples, and the induction of sarcoid granulomas in mouse lungs using trehalose 6,6'-dimycolate was attenuated by *Nlrp3* deletion, pharmacological NLRP3 inhibition or IL-1 β blockade¹⁰⁹.

Sepsis and septic cardiomyopathy—Sepsis refers to a systemic inflammatory response to infection, in which inflammation is excessive and often a mechanism for multiple organ failure. Cardiac dysfunction during sepsis is referred to as septic cardiomyopathy. Activation of the NLRP3 inflammasome in the heart during sepsis provides a source of cytokines, inducing a paracrine and systemic effect on contractility^{95,110} (Fig. 4).

Cardiotoxicity—The myocardium is sensitive to toxic injury, stimulating NLRP3 inflammasome-mediated sterile inflammation. A classic example of non-ischaemic, non-infectious injury is caused by the cancer chemotherapeutic agent, doxorubicin, which induces the NLRP3 inflammasome in leukocytes and cardiac cells¹¹¹ (Fig. 4). *Nlrp3*^{−/−} or *Casp1*^{−/−} mice are protected from doxorubicin-induced cardiomyopathy¹¹², and treatment with an NLRP3 inhibitor has been shown to prevent cardiac dysfunction and fibrosis in wild-type mice⁴³.

Chronic non-ischaemic cardiomyopathy—Non-ischaemic cardiomyopathy refers to a broad category of conditions, in which injury to the myocardium is not the result of coronary artery disease or AMI. Cardiac remodelling and heart failure are often the result of pressure overload secondary to hypertension or valvular diseases (Fig. 4). Chronic pressure overload stimulates a pro-inflammatory response in the heart with NLRP3 inflammasome activation¹¹³. Calcium/calmodulin-dependent protein kinase II δ seems to be necessary for NLRP3 inflammasome activation in response to pressure overload due to TAC or infusion of angiotensin II in cardiomyocytes^{41,42}. NLRP3 signalling in cardiomyocytes promoted maladaptive left ventricular remodelling¹¹⁴. TAC-induced β -adrenergic signalling contributed to inflammasome priming and NLRP3 activation, leading to ventricular dysfunction, fibrosis and hypertrophy in rats¹¹⁴.

Diabetic cardiomyopathy refers to structural changes in the hearts of patients with diabetes that are not explained by other factors, and that lead heart failure. The consequences of the pro-inflammatory stimuli in diabetes are, at least partly, mediated by the NLRP3 inflammasome¹¹⁵. In mice, a high fat diet (HFD) induces obesity and type 2 diabetes, cardiac remodelling and heart failure with preserved ejection fraction (HFpEF). The adverse metabolic effects, as well as cardiac remodelling and dysfunction, induced by a HFD are mitigated in *Nlrp3*^{−/−} or *Asc*^{−/−} mice¹¹⁶. The use of an NLRP3 inhibitor also improved diastolic function in mice fed a HFD¹¹⁷. In a Dahl/salt-sensitive rat model of HFpEF, colchicine treatment increased survival and reduced cardiac dysfunction and fibrosis¹¹⁸. In a HFD mouse model of the nitric oxide synthetases inhibitor, N^ω-nitro-L-arginine methyl ester (L-NAME), the development of HFpEF was associated with increased inflammasome priming and active IL-1 β and IL-18 in heart tissue¹¹⁹. Atrial tissue from patients with arrhythmias express elevated levels of NLRP3 inflammasome components and active caspase 1¹²⁰. Mutant mice expressing cardiomyocyte-specific constitutively active NLRP3 developed arrhythmias that were prevented by NLRP3 inhibition¹²⁰.

Cardiomyopathies can also be genetically determined and mediated by low-grade chronic injury, as in Duchenne muscular dystrophy. Activation of the NLRP3 inflammasome in such conditions can amplify injury and promote progression to cardiomyopathy¹²¹.

Pericarditis—Injury to the mesothelial cells constituting the pericardial sac often causes an inflammatory response. In some cases, such inflammation is perpetuated by activation of the NLRP3 inflammasome and IL-1 α and IL-1 β released by dying cells and inflammatory cells, respectively^{122,123} (Fig. 6).

NLRP3 inflammasome inhibitors

Preclinical data—Several pharmacological agents are being developed to inhibit NLRP3 inflammasome activation through various mechanisms, including NLRP3–NEK7 interaction and NLRP3 oligomerization. Table 1 and Fig. 5 show the characteristics and proposed sites of action of the NLRP3 inflammasome inhibitors tested in animal models of AMI. Although these inhibitors vary in structure, site of action, efficacy and specificity, the ATPase activity of NLRP3 is a preferred site of action for several agents. The data generated in studies of NLRP3 inflammasome inhibitors, paired with matching genetic mouse models, are important for translational and clinical research. Some of the inhibitors have not been tested in CVD and, therefore, their utility is unknown.

Sulfonamides and related molecules.: Glyburide is a sulfonylurea that increases insulin release by pancreatic β cells and is used to treat diabetes. Glyburide inhibits the NLRP3 inflammasome in vitro without suppressing its ATPase activity^{124,125}. However, at the concentration required to inhibit the NLRP3 in vivo, glyburide causes lethal hypoglycaemia in mice¹²⁵. JC121 (also known as 16673–34-0) and JC124 are sulfonamides that are derived from glyburide, but lack the cycloexylurea moiety group. JC121 and JC124 inhibit the NLRP3 inflammasome, without affecting insulin or inducing hypoglycaemia^{43,125,126}. These agents prevented NLRP3 oligomerization, significantly reduced infarct size and preserved cardiac function in mice with ischaemia–reperfusion injury and those with non-ischaemic injury^{43,125,126}.

MCC950 is a sulfonylurea that selectively inhibits NLRP3 activation and its ATPase activity^{15,127}. MCC950 significantly reduced infarct size in a pig model of ischaemia induced by balloon occlusion of the left anterior descending artery¹²⁸. The effects of MCC950 in AMI have been confirmed in a mouse model of permanent left main coronary occlusion, in which it reduced fibrosis, ameliorated remodelling and improved cardiac function¹²⁹. MCC950 was also shown to reduce left ventricular dysfunction, hypertrophy, fibrosis and inflammation in a model of TAC in obese mice¹³⁰. Furthermore, MCC950 improved survival and reduced post-resuscitation myocardial dysfunction in rats undergoing cardiac arrest and resuscitation¹³¹. The development of atherosclerosis in susceptible mice fed a HFD is promoted by the NLRP3 inflammasome and its cytokines, IL-1 β and IL-18³. MCC950 was reported to reduce atherosclerosis in apolipoprotein E-deficient mice fed a HFD¹³². In another study, MCC950 prevented aortic aneurism and dissection in mice fed a HFD and given angiotensin II, by reducing caspase 1 and IL-1-dependent activation of matrix metalloproteinase 9¹³³.

OLT1177 (dapansutrile).: A β -sulfonyl nitrile compound that selectively inhibits NLRP3 ATPase activity^{134,135}. Administration of OLT1177 reduced infarct size in mice that underwent experimental ischaemia–reperfusion injury⁸⁰. OLT1177 has also been tested in

mice with severe ischaemic cardiomyopathy. Treatment started 1 week after permanent coronary artery ligation improved diastolic function and increased cardiac reserve compared with control mice¹³⁶.

RRx-001.: A dinitroazetidine anticancer compound that covalently binds to haemoglobin, increases oxygen delivery and catalyses the production of nitric oxide and ROS in tumour cells. RRx-001 promotes epigenetic modulation and upregulates the expression of the antioxidant protein nuclear factor erythroid 2-related factor 2¹³⁷. In addition, RRx-001 covalently binds to NLRP3 and prevents its interaction with NEK7¹³⁸. In vivo, RRx-001 reduced systemic inflammation in separate mouse models of sepsis, colitis and autoimmune encephalomyelitis, all diseases driven by NLRP3 activity¹³⁸.

BAY 11–7082.: An NF- κ B inhibitor that also inhibits NLRP3 ATPase activity¹³⁹. Mice with experimental ischaemia–reperfusion AMI that were given BAY 11–7082 intraperitoneally, 10 minutes prior to reperfusion, displayed reduced myocardial inflammasome formation and decreased infarct size⁵⁰. Similarly, BAY 11–7082 administered 30 minutes prior to reperfusion in rats, significantly reduced infarct size and preserved cardiac function¹⁴⁰.

INF4E.: An acrylamide derivative that covalently binds to NLRP3 and blocks its ATPase activity¹⁴¹. INF4E administered ex vivo (50 μ M) 20 minutes prior to ischaemia on a Langendorff apparatus, reduced inflammasome activity and infarct size⁷⁵.

CY-09.: Inhibits NLRP3 and its oligomerization through covalent binding to the ATPase domain¹⁴². In mice that underwent permanent occlusion of the coronary artery, CY-09 given three times per week for 4 weeks reduced fibrosis and improved heart function¹⁴³.

Colchicine.: Used for millennia to treat inflammatory disease, colchicine is known to interfere with microtubule assembly and is recognized as an inhibitor of inflammasome aggregation (ASC and NLRP3 polymerization through the pyrin domain)¹⁴⁴. Colchicine also inhibits the activation of P2X7 by extracellular ATP¹⁴⁴. In mice with non-reperfused AMI, colchicine (0.1 mg/kg/day) significantly reduced inflammasome activation measured after 24 hours, decreased infarct size and ventricular remodelling and increased 7-day survival¹⁴⁵.

Ibrutinib.: A small molecule drug that irreversibly binds to BTK, thereby inhibiting Toll-like receptor signalling and NLRP3 inflammasome activation. In a mouse model of sepsis, ibrutinib (30 mg/kg) given intravenously 1 hour after surgery ameliorated ventricular dysfunction associated with sepsis⁴⁹.

Clinical data—Clinical experience with targeted NLRP3 inflammasome inhibitors is limited. Indirect evidence of the central role of the NLRP3 inflammasome is derived from non-selective NLRP3 inhibitors, such as colchicine, or blockers of IL-1 and IL-18.

Colchicine.: Colchicine is frequently used in patients with acute and chronic inflammatory diseases^{144,146}. In patients with ST-segment elevation AMI, colchicine significantly reduced infarct size in some trials, but not in others (Table 2)^{147–149}. Devereux et al. reported that colchicine given after percutaneous coronary intervention with a bare-metal stent in patients

with diabetes was associated with reduced in-stent restenosis and neointimal hyperplasia¹⁵⁰. In COLCOT¹⁵¹, a study of patients with recent (<30 days) AMI, colchicine 0.5 mg daily significantly reduced the risk of ischaemic cardiovascular events when compared with placebo (Table 2). A similar benefit was seen with colchicine 0.5 mg daily administered to patients with stable coronary artery disease in the LoDoCo¹⁵² and LoDoCo2¹⁵³ trials, with a significant reduction in the incidence of atherothrombotic complications compared with placebo in both studies (Table 2). However, colchicine 0.5 mg twice daily did not induce a benefit compared with placebo in patients with stable, chronic heart failure¹⁵⁴ (Table 2). Data from several trials of patients with pericarditis indicate that colchicine effectively treats the initial episode and prevents recurrences^{155–159} (Table 3). However, an unexpected small increase in the rates of non-cardiac death and pneumonia was seen in three separate colchicine trials^{151,153,160}, as well as in a meta-analysis including all available studies¹⁶¹. As of June 2023, colchicine is approved by the FDA for the prevention of atherothrombotic complications in patients with established CVD or multiple cardiovascular risk factors and, although not approved for the treatment of pericarditis, colchicine is considered first-line therapy for initial and recurrent events.

IL-1 blockers.: Several IL-1 blockers are available for clinical use and are being studied in patients with CVD³. Anakinra (Kineret, Sobi), a recombinant human IL-1 receptor antagonist, was first studied in VCU-ART¹⁶², in 10 patients with ST-segment elevated AMI, at the standard dose of 100 mg daily for up to 14 days. Two follow-up phase II studies (VCU-ART2¹⁶³ and VCU-ART3¹⁶⁴) followed, with 30 and 99 patients, respectively. Anakinra was well tolerated, blunted the acute systemic inflammatory response associated with myocardial injury (measured by C-reactive protein serum levels) and reduced the incidence of heart failure and related complications¹⁶⁵ (Table 2; Fig. 7). In the MRC-ILA Heart study¹⁶⁶ of 182 patients with non-ST-segment elevation AMI, anakinra administered for 14 days reduced the acute inflammatory response, albeit with a paradoxical increase in late atherothrombotic events (Table 2; Fig. 7). When studied in patients with acute and chronic systolic and diastolic heart failure, anakinra (once or twice daily) reduced C-reactive protein levels, improved cardiorespiratory fitness, Doppler echocardiographic parameters and quality of life measures and showed a trend toward a reduction in hospitalizations^{167–171} (Table 2; Fig. 6). A systematic review of studies reporting on IL-1-targeted therapies showed that anakinra is effective in treating myocarditis and pericarditis associated with Still disease (a rare type of inflammatory arthritis)¹⁷². A case report from 2016 suggests a role for anakinra in the treatment of viral and idiopathic myocarditis¹⁷³. The first randomized clinical trial of IL-1 blockade in patients with acute myocarditis (the ARAMIS trial¹⁷⁴) was completed in 2023. In patients with pericarditis, anakinra reduces systemic inflammation and improves pain within 24 hours¹⁷⁵, and in patients with recurrent pericarditis refractory to first-line therapy (including colchicine and prednisone), anakinra treated the acute event and prevented recurrence¹⁷⁶ (Table 3). Finally, a small pilot study of patients with rheumatoid arthritis showed that anakinra improved vascular and left ventricular function^{177,178}.

Canakinumab (Ilaris, Novartis) is an antibody that selectively blocks IL-1 β (Table 2; Fig. 5). In CANTOS¹⁷⁹, the largest cytokine trial ever completed, 10,061 patients with

prior AMI and elevated C-reactive protein (>2 mg/l) were randomly assigned to receive canakinumab (50 mg, 150 mg or 300 mg) or placebo every 3 months (median treatment duration 3.5 years). In the canakinumab 150 mg arm, the incidence of cardiovascular death, AMI, stroke, urgent revascularization and heart failure-related hospitalizations was significantly reduced, independent of the effects on classic cardiovascular risk factors^{179–182}. Canakinumab improved cardiac function and cardiorespiratory fitness in patients with heart failure and reduced ejection fraction (HFrEF), and walking distance in patients with low-extremity peripheral arterial disease^{183,184}. The benefits of canakinumab seemed to be most pronounced in the subgroup of patients with somatic mutations associated with clonal hematopoiesis¹⁸⁵.

Rilonacept (Arcalyst, Regeneron Pharmaceuticals, Inc.) is a recombinant chimeric fusion protein functioning as a ‘trap’ for IL-1 β and IL-1 α ³ (Table 3; Fig. 5). In the RHAPSODY trial¹⁸⁶ of patients with recurrent pericarditis, rilonacept (320 mg loading dose, then 160 mg weekly) effectively treated the acute episode and significantly reduced the recurrence rate (Table 3). Consequently, rilonacept is now approved by the FDA for the treatment of recurrent pericarditis¹⁸⁶.

Goflikicept (RPH104) is also a recombinant chimeric fusion trap protein, structurally different from rilonacept, binding both IL-1 β and IL-1 α ¹⁸⁷ (Table 3; Fig. 5). Goflikicept significantly reduced the risk of recurrence in patients with recurrent or refractory pericarditis in a phase II–III trial¹⁸⁷. Goflikicept is also being studied in a phase II trial of patients with ST-segment elevation AMI¹⁸⁸.

IL-18 and IL-6 blockers.: Data from CANTOS¹⁸⁹ have shown that substantial residual inflammatory risk, related to both IL-18 and IL-6, remains after IL-1 β inhibition with canakinumab. Potential future therapies to address this residual risk include GSK1070806, a monoclonal antibody directed against IL-18, and tadekinig α , a recombinant human IL-18 binding protein that sequesters IL-18. However, these drugs are not available for clinical use and data on cardiovascular outcomes are lacking^{190,191} (Fig. 5).

IL-6 is a secondary cytokine, acting downstream of IL-1 β and other cytokines, and often considered a surrogate for IL-1 activity¹⁹². Several IL-6 blockers are available, or under development, for clinical use across a wide range of inflammatory conditions. Tocilizumab (Actemra/Ro-Actemra, Chugai Pharmaceutical Co. Ltd (Roche)), an IL-6 receptor blocking antibody, was shown to increase the myocardial salvage ratio in the ASSAIL-MI trial¹⁹³ of patients with ST-segment elevation AMI, and to reduce myocardial injury in patients with out-of-hospital cardiac arrest (the IMICA trial¹⁹⁴). Tocilizumab, however, interfered with the cardiovascular and metabolic benefits of exercise training in abdominally obese, but otherwise healthy, adults¹⁹⁵. Another IL-6 blocking antibody, ziltivekimab, was shown to reduce biomarkers of inflammation and thrombosis relevant to atherosclerosis in the phase II, dose-ranging RESCUE trial¹⁹⁶. Ziltivekimab 15 mg monthly is now under investigation in two large phase III cardiovascular outcome studies (the ZEUS¹⁹⁷ and HERMES¹⁹⁸ trials).

Targeted inflammasome inhibitors.: As discussed above, several targeted inflammasome inhibitors are under development (Table 1), but the clinical data for these agents are scarce

at this time. OLT1177 (dapansutrile) has a favourable safety profile in healthy individuals as well as in patients with HFrEF¹⁹⁹. Efficacy was dose-dependent, with favourable signals for 2000 mg per day in patients with heart failure¹⁹⁹ or acute monoarticular gout flares²⁰⁰. DFV890 (IFM-2427) is a sulfonimidamide with a structure similar to MCC950, but with some key modifications, which is being studied across a wide spectrum of inflammatory disease. DFV890 had acceptable safety and biological activity in a phase IIa trial of patients with severe COVID 19-associated pneumonia²⁰¹, and is in phase II testing to treat osteoarthritis²⁰². ZYIL1, a NLRP3 inhibitor derived from MCC950, is in the early phase of clinical development. ZYIL1 showed an acceptable safety profile and biological activity in healthy volunteers and is being studied in patients with the autoinflammatory disease cryopyrin-associated periodic syndromes (CAPS)^{203,204}. No data are yet available on the effects of ZYIL1 in CVD. Selnoflast (RG6418/IZD334) is a sulfonamide carboxamide compound that showed a good safety profile in a phase Ib study of patients with ulcerative colitis²⁰⁵ (a disease driven by NLRP3 inflammasome activity). This agent is under investigation in phase I clinical trials of healthy volunteers and patients with CAPS²⁰⁶. However, a study in individuals at high cardiovascular risk was discontinued prematurely²⁰⁷. HT-6184, which targets NEK7, is being tested in healthy individuals²⁰⁸. Several small molecule inhibitors of NLRP3 are being studied in clinical trials for various indications — VTX2735 for CAPS²⁰⁹, NT-0249 and NT-0796 for neuro-inflammation²¹⁰ and RRx-001 for advanced small cell lung cancer²¹¹.

Conclusions

The NLRP3 inflammasome is a macromolecular intracellular structure that functions as a sensor for injury. This complex amplifies the inflammatory response and tissue injury by regulating the processing and release of IL-1 β and IL-18, and causing inflammatory cell death (pyroptosis). The preclinical experience with genetically-modified mouse models and the use of targeted inhibitors has stimulated a clinical research programme in CVD. During the past 5 years, selective NLRP3 inhibitors have been studied in phase I–II clinical trials. Colchicine, a non-selective NLRP3 inflammasome inhibitor, has been shown to be efficacious not only in the treatment of pericarditis, for which it is considered part of the standard of care, but also in atherothrombotic risk reduction in patients with coronary artery disease, and is now approved for this indication by the FDA. IL-1 blockers, already clinically available and routinely used for various chronic inflammatory conditions, have been shown in phase Ib–III to reduce cardiovascular risk and morbidity across a wide range of cardiovascular pathologies, including AMI, heart failure, myocarditis and, most recently, recurrent pericarditis, for which rilonacept now has a FDA-approved indication. Inhibitors of IL-6, downstream of IL-1, are also clinically available and are being studied in phase Ib–III trials of patients with CVD. Although not yet available for clinical use, IL-18 blockers represent a potential future approach to treatment.

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Key points

- The NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome is a macromolecular, intracellular structure that functions as a sensor for injury.
- NLRP3 inflammasome activation amplifies the inflammatory response and tissue injury by regulating the processing and release of IL-1 β and IL-18, and causing cell death by pyroptosis.
- Preclinical studies with genetically-modified mouse models and the use of targeted inhibitors have shown that inhibiting activation of the NLRP3 inflammasome reduces inflammatory injury and adverse remodelling.
- Colchicine, a non-selective NLRP3 inflammasome inhibitor, has been shown to be efficacious in the treatment of pericarditis and in reducing atherothrombotic risk in patients with coronary artery disease.
- IL-1 blockers have been shown in phase Ib–III to reduce cardiovascular risk and morbidity across a wide range of cardiovascular diseases, including myocardial infarction, heart failure, acute myocarditis and recurrent pericarditis.
- Targeted NLRP3 inflammasome inhibitors and blockers of IL-18 and IL-6, downstream of IL-1, are in clinical testing for a variety of cardiovascular diseases.

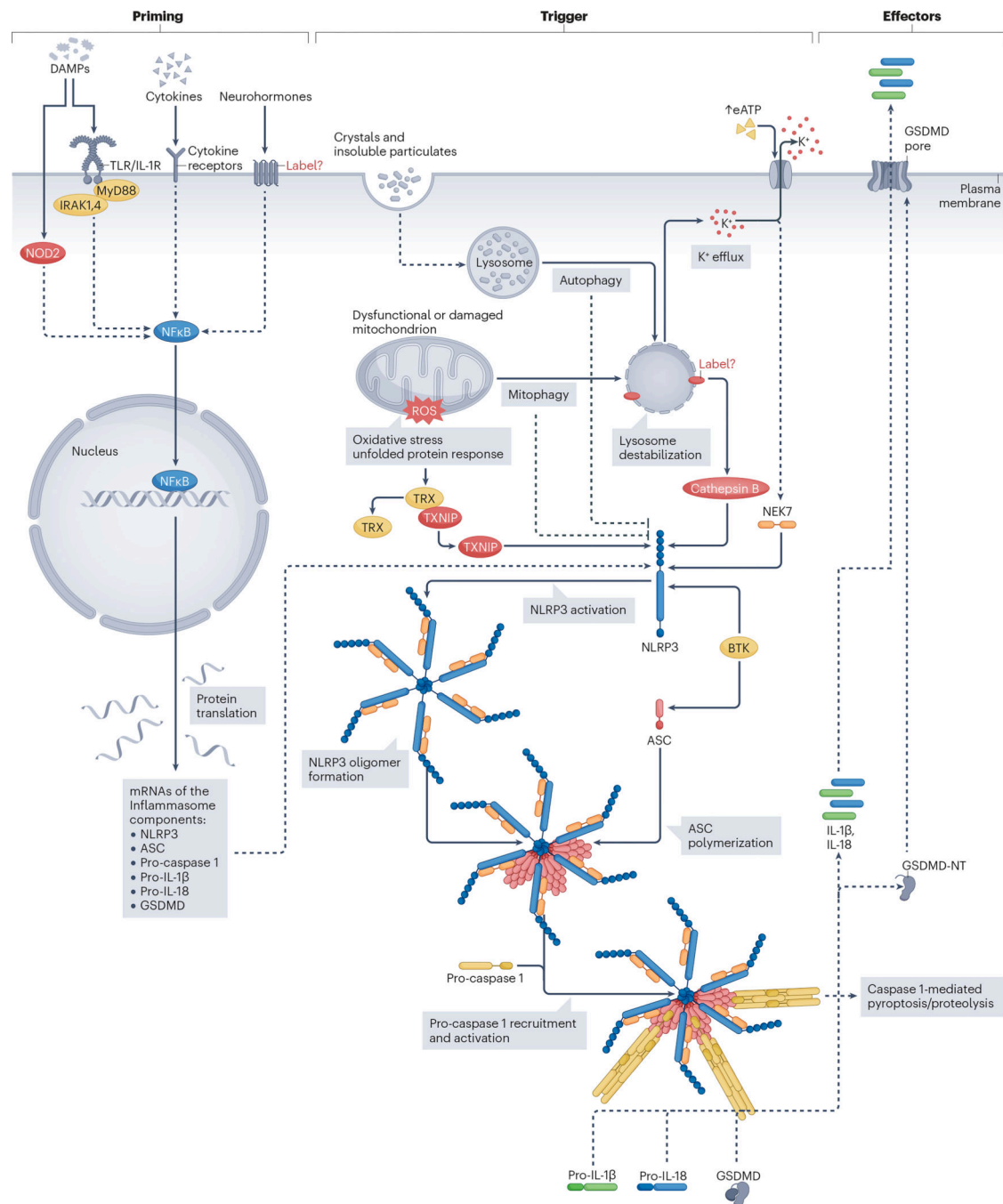


Fig. 1 | Activation of the NLRP3 inflammasome pathways in cardiomyocytes.

The NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome forms in myocardial cells following a two-step process. Firstly, ‘priming’ that leads to transcription and translation of the NLRP3 inflammasome components and substrates (IL-1β and IL-18). Nuclear factor-κB (NF-κB) activation drives the priming process, initiated by the membrane Toll-like receptors (TLRs) or IL-1 receptor (IL-1R) family. Downstream signalling is mediated by myeloid differentiation primary response protein MyD88 (MyD88) and interleukin-1 receptor-associated kinases (IRAKs). Nucleotide-

binding oligomerization domain-containing protein 2 (NOD2) promotes a similar priming signal. Secondly, activation or ‘triggering’ of NLRP3. NLRP3 activation is mediated by extracellular and intracellular pathways. Increasing the concentration of extracellular ATP (eATP) activates the P2X purinoceptor 7 and leads to K⁺ efflux, a step that triggers the activation of NLRP3. The serine/threonine-protein kinase NEK7 senses K⁺ efflux and binds to NLRP3, allowing its activation. Lysosomal destabilization by crystals and indigestible material represent another mechanism that leads to NLRP3 activation, through leakage of the lysosomal enzyme cathepsin B and by induction of K⁺. Mitochondrial damage and dysfunction produce reactive oxygen species (ROS), leading to dissociation of thioredoxin-interacting protein (TXNIP) from thioredoxin (TRX), and TXNIP binding to NLRP3. Ineffective clearance of dysfunctional mitochondria through mitophagy contributes to lysosomal destabilization. By contrast, effective mitophagy and autophagy limit the activation of NLRP3. Tyrosine-protein kinase BTK (also known as Bruton kinase) also binds to NLRP3, as well as to apoptosis speck-like protein containing a caspase recruiting domain (ASC), contributing to inflammasome activation. The active NLRP3 bound to NEK7 oligomerizes and forms a disc-like structure that is platform for the polymerization of ASC into a central filament, facilitating assembly of caspase 1 into filaments that form a star-like structure. Active caspase 1 cleaves the inactive pro-IL-1 β and pro-IL-18 into the active forms, IL-1 and IL-18. Caspase 1 also cleaves gasdermin D (GSDMD) producing N-terminal fragments (GSDMD-NT) that oligomerize and form pores in the cell membrane, leading to the extracellular release of active IL-1 β and IL-18 and pyroptosis. DAMPs, damage-associated molecular patterns.

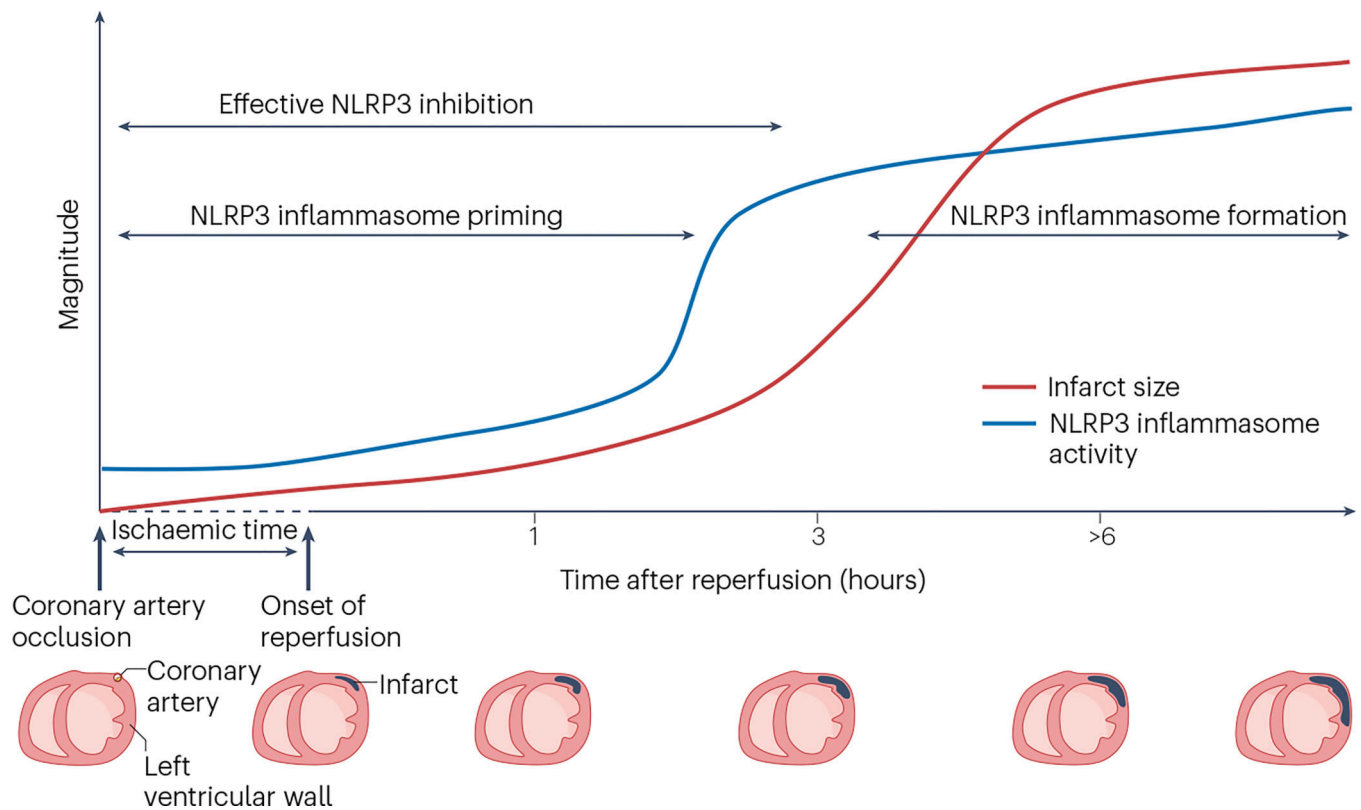


Fig. 2 |. Temporal expression of the NLRP3 inflammasome components and window of opportunity for effective inhibition in ischaemia–reperfusion injury.

Progressive expression of the NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome components (black line) and of infarct area growth (orange line) over time are shown. The infarct area (orange) in the ventricular wall is shown below the graph. For the initial hour after ischaemia–reperfusion injury, the expression of NLRP3 components and the inflammasome activity in the myocardium remains low. The expression of the inflammasome components increases between 1 and 3 hours after injury, leading to effective priming and facilitation of inflammasome formation during this time and in subsequent hours. The size of the infarct continues to grow after reperfusion. The time that precedes the activation of the inflammasome represents a therapeutic window for intervention with NLRP3 inhibitors before the inflammasome forms.

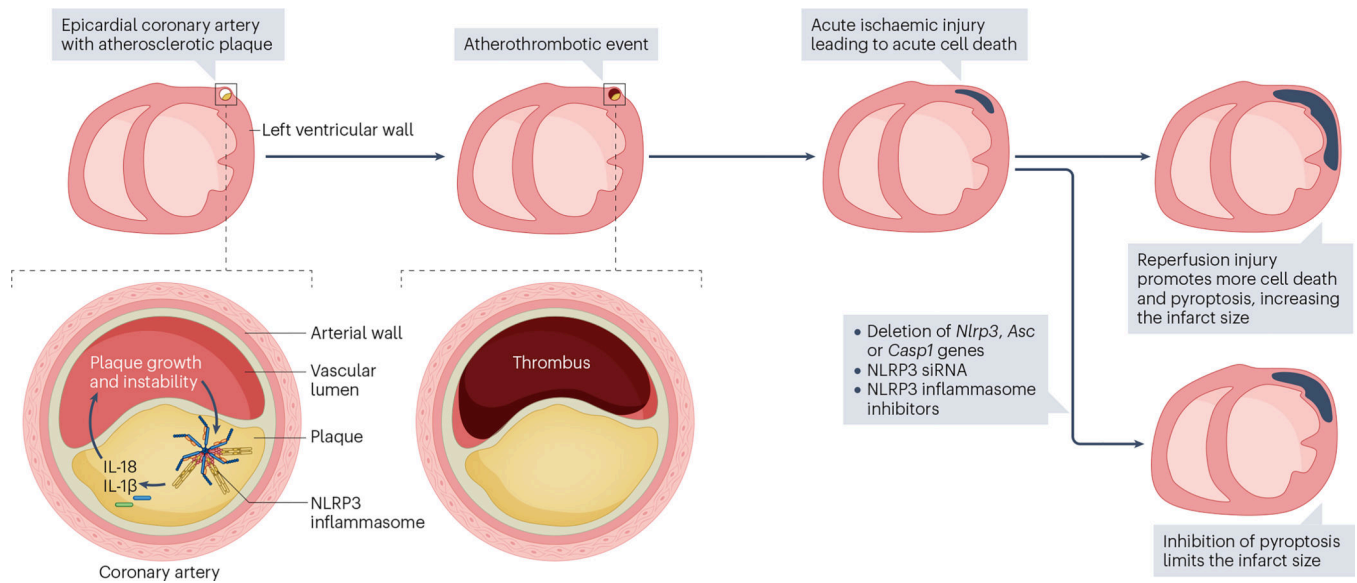


Fig. 3 |. Prevention of NLRP3 inflammasome formation reduces damage in animal models of ischaemia–reperfusion injury.

Coronary plaque growth and instability are promoted by NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome formation. An atherothrombotic event causes ischaemic ventricular damage, leading to the death of cardiac muscle. During reperfusion, inflammasome-mediated damage promotes further damage and infarct growth. Preventing the formation of the inflammasome — by deleting genes of the inflammasome components (NLRP3, apoptosis speck-like protein containing a caspase recruiting domain (ASC) or caspase 1), by reducing expression of NLRP3 using small interfering RNA (siRNA) or by administering selective NLRP3 inhibitors — reduces infarct size.

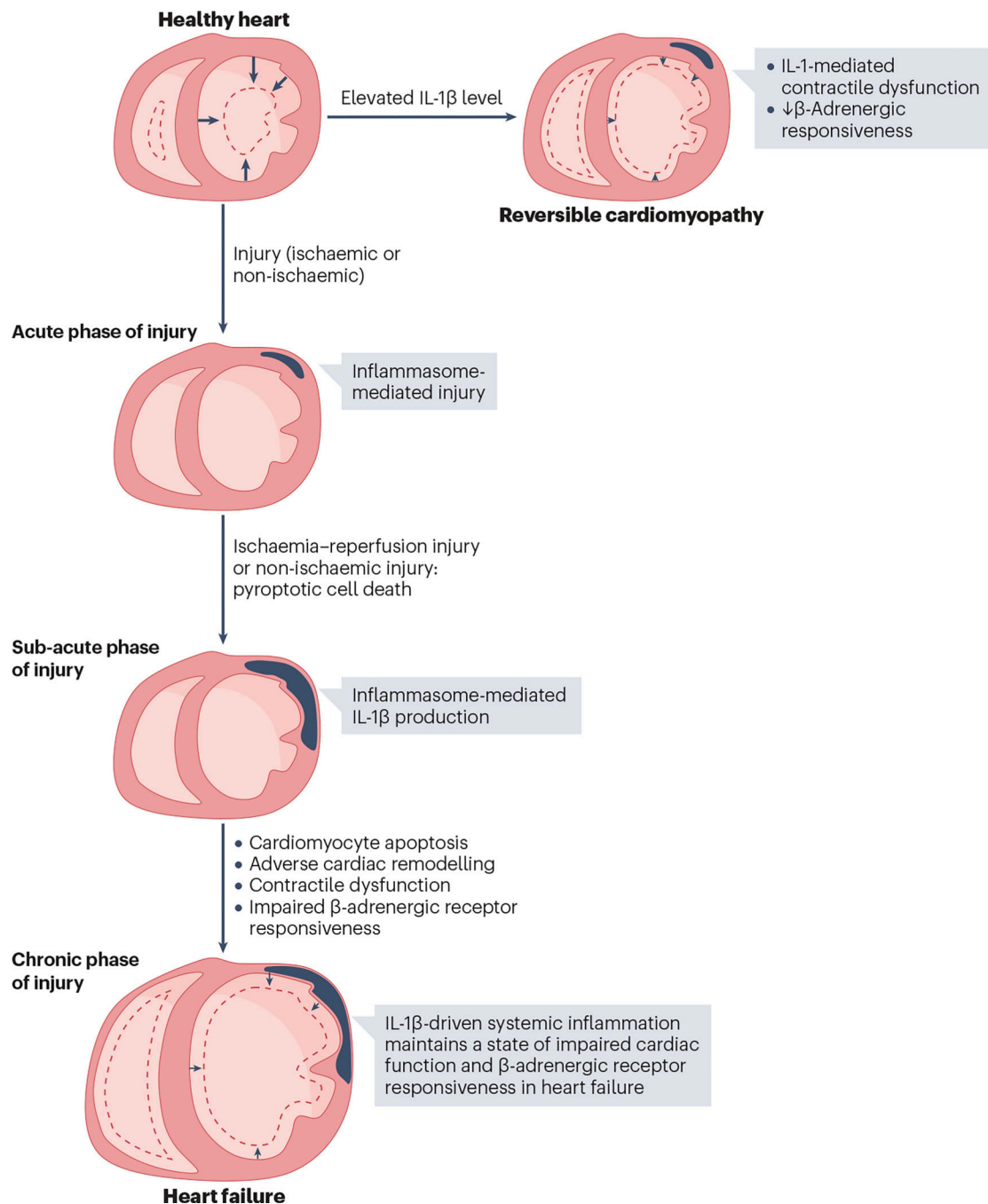


Fig. 4 |. Role of IL-1 β in acute injury and progression to heart failure.

The left and right ventricular cavities in diastole and in systole (dotted lines) are shown. IL-1 β decreases myocardial contractility and the response to β -adrenergic receptor agonists in cardiomyocytes, even in the absence of acute myocardial injury. In the acute phase that follows ischaemic or non-ischaemic myocardial injury, NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome formation promotes myocardial cell death due to pyroptosis. In the subacute phase, IL-1 β induces cardiomyocyte apoptosis, favouring adverse cardiac remodelling and heart failure. Enhanced IL-1 β activity in the subacute

and chronic phases contribute to impaired myocardial contractility and β -adrenergic responsiveness in heart failure.

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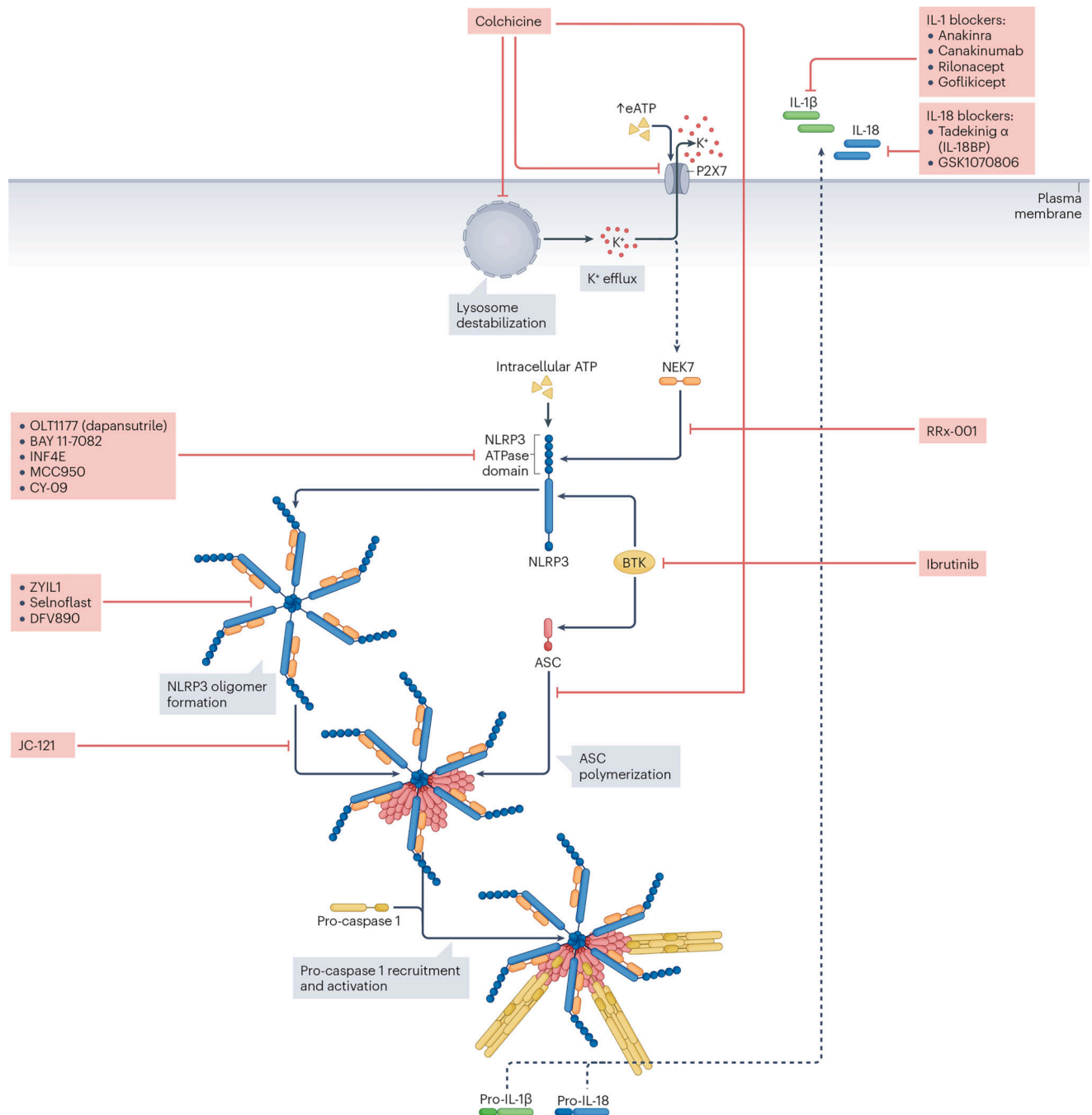


Fig. 5 |. Mechanism of action of NLRP3 inflammasome inhibitors tested in experimental models of ischaemic and non-ischaemic injury.

Several NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome inhibitors have been tested in animal models of acute myocardial infarction. Colchicine can act downstream of NLRP3 by inhibiting the polymerization of apoptosis speck-like protein containing a caspase recruiting domain (ASC). OLT1177, BAY 11-7082, INF4E, MCC950 and CY-09 inhibit the ATPase activity of NLRP3. ZYL1, selnoflast, DFV890 and JC-121 (also known as 16673-34-0) prevent oligomerization of NLRP3 and the interaction with ASC. Ibrutinib inhibits activation of NLRP3 mediated by tyrosine-protein kinase BTK (also

known as Bruton kinase). RRx-001 binds NLRP3 and inhibits its interaction with serine/threonine-protein kinase NEK7. IL-1 β activity is blocked by the IL-1 inhibitors anakinra (a recombinant IL-1 receptor antagonist), canakinumab (an anti-IL-1 β antibody), rilonacept (an IL-1 trap) and goflikicept (a different IL-1 trap). IL-18 activity is blocked by tadekinig α (a recombinant form of IL-18 binding protein; IL-18BP) and by GSK1070806 (an anti-IL-18 antibody). eATP, extracellular ATP.

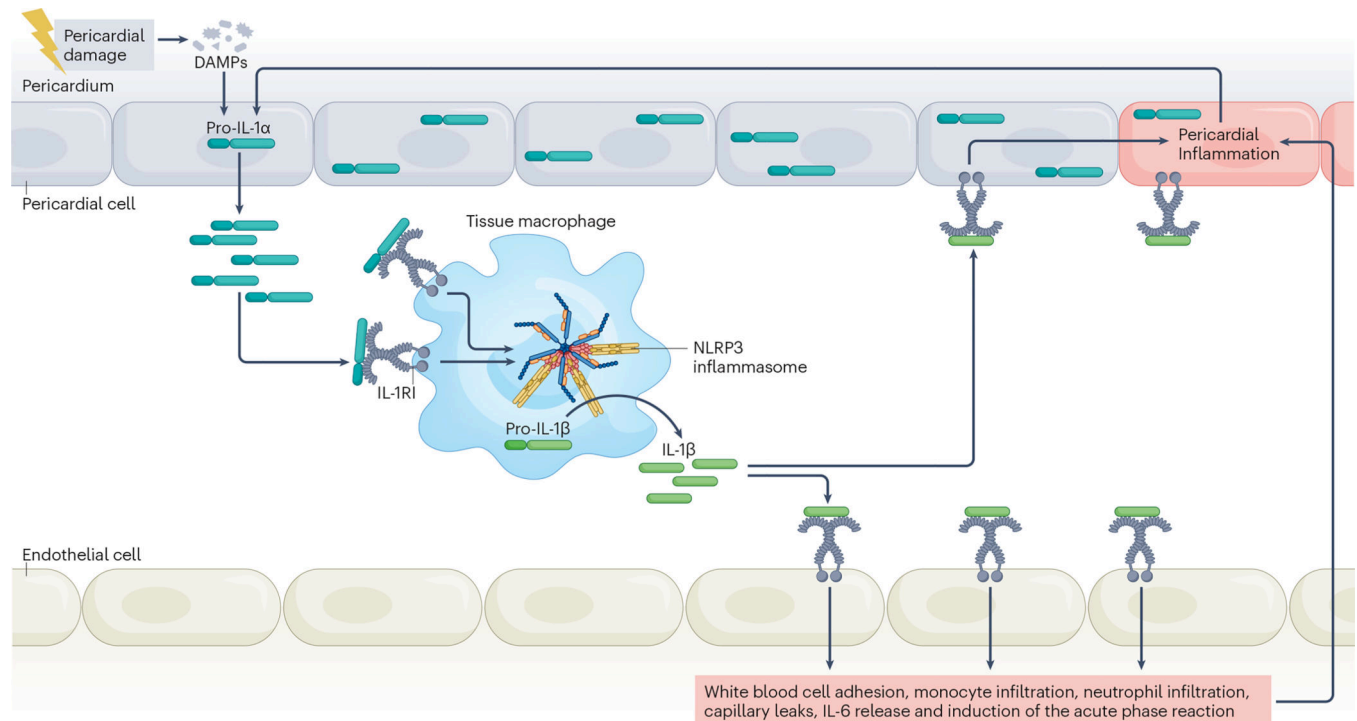


Fig. 6 |. Role of IL-1 α and IL-1 β in the pathophysiology of recurrent pericarditis.

Injury to the pericardial cells induces the release of intracellular contents. (Pro)-IL-1 α released outside the cell is already active, binds the IL-1 receptor type I (IL-1RI) on macrophages and activates the signal, functioning as an alarmin. Macrophages respond to the alarmins by forming and triggering the NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome that processes and releases active IL-1 β in its active form. This process amplifies the inflammatory response and induces further injury, which in turn leads to the release of more pro-IL-1 α . IL-1 β also induces the release of IL-6, which mediates the acute phase reaction associated with inflammation. DAMPs, damage-associated molecular patterns.

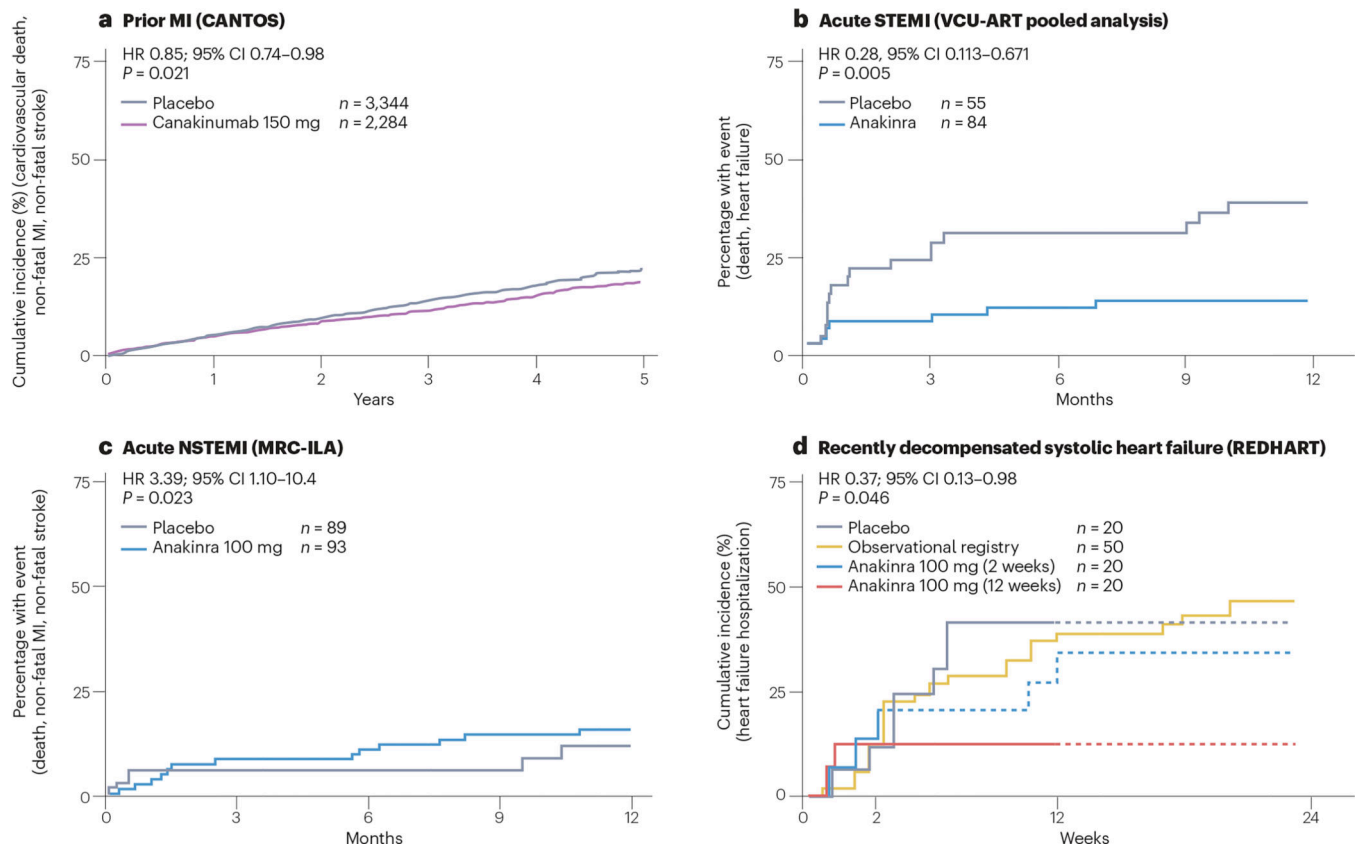
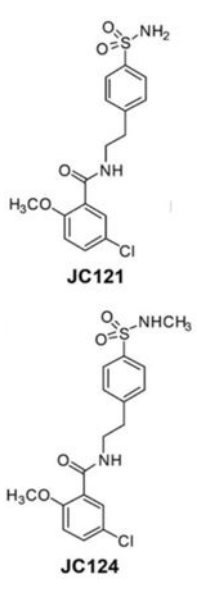
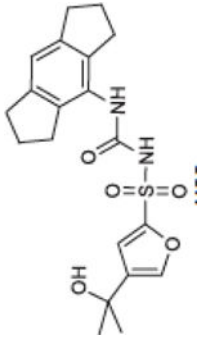
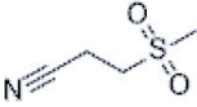


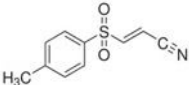
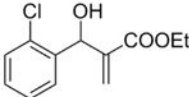
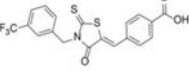
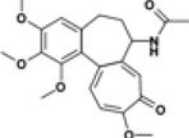
Fig. 7 |. Overview of phase II–III trials of IL-1 blockers in acute myocardial infarction and heart failure.

a, The CANTOS trial. Canakinumab 150 mg every 3 months significantly reduced the incidence of cardiovascular death, non-fatal myocardial infarction and stroke in patients with prior myocardial infarction (MI) and residual inflammatory risk. **b**, A pooled analysis of the VCU-ART trials. Anakinra 100 mg daily (or twice daily) for 14 days significantly reduced the incidence of heart failure or death in patients with ST-segment elevation MI (STEMI). **c**, The MRC-ILA Heart trial. Anakinra 100 mg daily for 14 days was associated with an unexpected increase in late cardiac and cerebral ischaemic events in patients with non-ST segment elevation MI (NSTEMI). **d**, The REDHART trial. Anakinra 100 mg daily for 12 weeks, but not for 2 weeks only, showed a favourable rate of hospital readmission for heart failure in patients with recently decompensated systolic heart failure as compared with placebo and an observational registry.

Table 1 |

NLRP3 inflammasome inhibitors in experimental models of AMI

Name, chemical name	Chemical structure	Mechanism of action	Doses, route	Animal model	Findings	Refs
JC-121 (or 16673–34-0) 5-chloro-2-methoxy-N-[2-(4-sulfamoylphenyl) ethyl]benzamide and JC-124 5-chloro-N-[2-(4-methyl-sulfamoyl-phenyl)-ethyl]-2-methoxy-benzamide	 JC121 JC124	Prevention of oligomerization in the interaction between NLRP3 and ASC	5–100 mg/kg, intraperitoneal. Given prior to or up to 1 h in reperfused/non-reperfused AMI	Mouse, coronary artery ligation AMI	Reduction in infarct size at 24 h (measured by pathology, echocardiography and troponin I plasma levels) Improvement in cardiac remodelling and left ventricular dysfunction in reperfused and non-reperfused AMI Reduction in caspase 1 activation and cardiomyocyte pyroptosis in the early phases of AMI, and reduction in apoptosis and myocardial fibrosis in the late phases of AMI	43, 125, 126
MCC950 N-((1,2,3,5,6,7-hexahydro-s-indacen-4-yl)carbamoyl)-4-(2-hydroxypropan-2-yl)furan-2-sulfonamide; N-[[[(1,2,3,5,6,7-hexahydro-s-indacen-4-yl)amino]carbonyl]-4-(1-hydroxy-1-methylethyl)-2-furansulfonamide		Inhibition of NLRP3 activation and ATPase activity	Pig model: 3 or 6 mg/kg, intravenous. Given 15 min before reperfusion, then repeated every 24 h for 6 days; (mouse model): 10 mg/kg intraperitoneal, given after ischaemia and for 14 days (mouse model)	Pig model: balloon angioplasty inflation; mouse model: surgical coronary artery ligation	Reduction in infarct size (measured by pathology, echocardiography and troponin I plasma levels) Improvement in cardiac remodelling and prevention of left ventricular dysfunction Reduction in local and systemic inflammatory response	128, 129
OLT1177 (dapansutrile) 3-(methanesulfonyl)propanenitrile		Inhibition of ATPase activity	Ligation model: 6, 60 or 600 mg/kg, intraperitoneal. Given at the same time as, or up to 30 min after, reperfusion; ischaemic cardiomyopathy model: 3.75 or 7.5 mg/kg in chow diet started 7 days after MI and given daily up to 10 weeks	Mouse, surgical coronary artery ligation or chronic ischaemic cardiomyopathy	Reduction in infarct size (measured by pathology and echocardiography) and improvement in contractile reserve Reduction in end diastolic pressure	80, 136

Name, chemical name	Chemical structure	Mechanism of action	Doses, route	Animal model	Findings	Refs
BAY 11-7082 3-[(4-methylphenyl)sulfonyl]-(2E)-propenenitrile		Inhibition of ATPase activity	Mouse model: pretreatment (dose not specified) prior to ischaemia or reperfusion; rat model: pretreatment (130 µg/kg) 30 min before ischaemia	Mouse model: ischaemia–reperfusion; rat model: ischaemia–reperfusion	Reduction in infarct size (measured by pathology) Reduction in inflammasome activity (caspase 1 activity, IL-1β levels) Preservation of cardiac function	50, 139, 140
INF4E Ethyl 2-((2-Chlorophenyl)(hydroxy)methyl)acrylate		Inhibition of ATPase activity	50 µM given 20 minutes prior to ischaemia	Ex vivo myocardial model	Reduction of infarct size at 1 h (measured by pathology, cardiac markers, and contractility) Reduction in caspase 1 activity, and levels of cleaved IL-1β and gasdermin D levels at 1 h.	75
CY-09 4-[[4-Oxo-2-thioxo-3-[[3-(trifluoromethyl)phenyl]methyl]-5-thiazolidinylidene]methyl]benzoic acid		Prevention of NLRP3 oligomerization	5 mg/kg, intraperitoneal. Given three times per week for 4 weeks	Mouse, permanent surgical coronary artery ligation	Reduction in fibrosis and improvement in ventricular function (measured by pathology and echocardiography)	143
Colchicine N-[(7S)-1,2,3,10-tetramethoxy-9-oxo-6,7-dihydro-5H-benzo[a]heptalen-7-yl]acetamide		Inhibition of NLRP3 and ASC polymerization	0.1 mg/kg, oral. Given 1 h after surgery then daily for the following 6 days	Mouse, permanent surgical coronary artery ligation	Reduced infarct size (measured by pathology) Reduction in cytokines and in infiltrating granulocytes and macrophages Reduction in inflammasome activity (expression of inflammasome components, caspase 1 activity) Prevention of adverse remodelling and heart failure Improved survival	144, 145

AMI, acute myocardial infarction; ASC, apoptosis speck-like protein containing a caspase recruiting domain; NLRP3, NACHT, LRR and PYD domains-containing protein 3.

Table 2 |

Clinical trials of colchicine and IL-1 blockers in AMI, CAD and HF

Study (year)	Indication (n)	Study design and drug regimen	Main findings for study drug	Refs
Colchicine				
LoDoCo (2013)	Stable CAD (532)	Randomization 1:1 to colchicine 0.5 mg or placebo daily (median duration 2.36 years)	Reduction in the combined cardiovascular end point, due to a large reduction in the incidence of ACS	152
Colchicine for the prevention of restenosis of bare-metal stents in diabetic patients (2013)	Stable CAD and diabetes mellitus undergoing bare-metal stent implantation (196)	Randomization 1:1 to colchicine 0.5 mg twice daily or placebo for 6 months	Reduction in angiographic in-stent restenosis; significantly less lumen area loss	150
Colchicine in stable chronic HF (2014)	Stable systolic HF (LVEF ≤40%) (267)	Randomized, double-blinded, trial of colchicine 0.5 mg daily or placebo for 6 months	No significant effect on the >1 class NYHA improvement; no significant effect on treadmill exercise time; significant reduction in CRP levels	154
Anti-inflammatory treatment with colchicine in AMI (2015)	ST-segment elevation AMI (151)	Randomization 1:1 to colchicine or placebo; loading dose 2.0 mg (1.5 mg initially followed by 0.5 mg after 1 h) and continuing with 0.5 mg twice daily or placebo for 5 days	Reduction in infarct size (measured by cardiac magnetic resonance and by area-under-the-curve for cardiac markers); reduction in peak C reactive protein levels	151
COLCOT (2019)	Stable CAD (4,745)	Randomization 1:1 to colchicine 0.5 mg or placebo daily (median duration 22.6 months)	Reduction in the composite of death from cardiovascular causes, resuscitated cardiac arrest, AMI, stroke or urgent hospitalization for angina leading to coronary revascularization, due to a large reduction in urgent revascularization	151
LoDoCo2 (2020)	Stable CAD (5,522)	Randomization 1:1 to colchicine 0.5 mg or placebo daily (median duration 28.6 months)	Reduction in the composite of cardiovascular death, AMI, ischaemic stroke and ischaemia-driven revascularization, due to a large reduction in the rate of AMI and revascularization	153
			The incidence of death from non-cardiovascular causes was higher in the colchicine group than in the placebo group (0.7 vs. 0.5 events per 100 person-years; HR 1.51, 95% CI 0.99–2.31).	
COPS (2020)	ACS (795)	Randomization 1:1 to colchicine 0.5 mg twice daily for 1 month followed by 0.5 mg daily or placebo for 11 months	No significant effect on the composite end point of death from any cause, ACS, ACS-driven urgent revascularization, and non-cardioembolic ischaemic stroke	160
			The incidence of death from any cause was higher in the colchicine group than in the placebo group (HR 8.20, 95% CI 1.03–65.6).	
Colchicine and major adverse cardiac events after ACS (2021)	ACS (361)	Randomization 1:1 to colchicine 0.5 mg or placebo daily for 6 months	Reduction in major adverse cardiac events due to a large reduction in recurrent ACS	212
COPE-PCI (2022)	Stable angina or non-ST-segment elevation AMI (50)	Randomization 1:1 to colchicine 1.0 mg followed by 0.5 mg after 1 h or matching placebo 6–24 hours before intervention	No significant difference in the number of patients with coronary microvascular dysfunction; no significant effect on CRP levels; reduction in troponin levels and blood neutrophil count	148, 213
PodCAST-PCI (2022)	ST-segment elevation AMI (321)	Randomization 1:1 to colchicine 1.0 mg followed by 0.5 mg daily or placebo until discharge	No significant effect on no-reflow, infarct size or levels of inflammatory biomarkers	149
IL-1 blockers				

Study (year)	Indication (n)	Study design and drug regimen	Main findings for study drug	Refs
VCU-ART (2010)	ST-segment elevation AMI (10)	Randomization 1:1 to anakinra 100 mg once daily or placebo for 14 days	Anakinra was safe and favourably affected left ventricular remodelling. Anakinra also blunted the inflammatory response during AMI	162
VCU-ART2 (2013)	ST-segment elevation AMI (30)	Randomization 1:1 to anakinra 100 mg once daily or placebo for 14 days	Blunting of the inflammatory response during AMI and a trend toward reduced incidence of adverse remodelling or HF at 3 months and at long-term follow up	163
VCU-ART3 (2020)	ST-segment elevation AMI (99)	Randomization 1:1:1 to anakinra 100 mg once daily, 100 mg twice daily, or placebo for 14 days	Blunting of the inflammatory response during AMI; reduced incidence of new-onset HF and hospitalization for HF; no difference between anakinra once daily versus twice daily	164, 165
MRC-ILA Heart Study (2015)	Non-ST-segment elevation AMI (182)	Randomization 1:1 to anakinra 100 mg or placebo once daily for 14 days	Blunting of the inflammatory response during AMI; no differences in major adverse cardiac events at 30 days and 3 months, but more events occurring after 6 months in the anakinra group	166
AIR-HF (2012)	Stable NYHA II–III systolic HF with CRP >2 mg/l (10)	Single arm, open-label; treatment with anakinra 100 mg once daily for 2 weeks	Reduction in CRP levels; improvement in peak aerobic exercise capacity and ventilator efficiency at 2 weeks	167
ADHF (2016)	Acute decompensated systolic HF with CRP >5 mg/l (30)	Randomized, double-blinded, trial of anakinra 100 mg or placebo twice daily for 3 days and then once daily for 2 weeks	Reduction in CRP levels at 72 h and 14 days and a trend toward more favourable effects on signs of congestion and LVEF	170
REDHART (2017)	Recently (within 2 weeks of hospital discharge) decompensated systolic HF with CRP >2 mg/l (60)	Randomized, double-blinded trial of anakinra 100 mg once daily continued for 2 weeks or for 12 weeks or placebo for 2 weeks (1:1:1)	Reduction in CRP levels; improvement in peak aerobic exercise capacity and quality of life questionnaires, and a trend toward reduced HF readmissions at 6 months, with 12-week treatment	168
D-HART (2014)	Stable NYHA II–III diastolic HF with LVEF >50% and CRP >2 mg/l (12)	Randomized, double-blinded, cross-over trial of anakinra 100 mg or placebo once daily for 2 weeks	Reduction in CRP levels; improvement in peak aerobic exercise capacity at 2 weeks	167
D-HART2 (2018)	Stable NYHA II–III diastolic HF with LVEF >50% and CRP >2 mg/l (31)	Randomized, double-blinded trial of anakinra 100 mg once daily or placebo for 12 weeks (2:1)	No improvement in peak aerobic exercise capacity or ventilatory efficiency. Reduction in CRP and NT-proBNP levels and increase in exercise time.	171
CANTOS (2017)	Prior AMI with CRP >2 mg/l (at least 30 days after AMI) (10,060)	Randomized, double-blinded trial of canakinumab 50 mg, 150 mg or 300 mg or placebo (1:1:1:1.5) (median duration 3.5 years)	For canakinumab 150 mg: significant reduction in the incidence of the composite end point of cardiac death, non-fatal AMI or non-fatal stroke (canakinumab 150 mg); significant reduction in the incidence of recurrent AMI, unstable angina and need for revascularization	179, 181
			Significant reduction in the rate of HF-related hospitalizations and death (p for trend = 0.025)	
			For canakinumab 150 mg and 300 mg: small reduction in cancer-related mortality	
			Small increase in the risk of fatal infections with canakinumab (all doses combined)	

ACS, acute coronary syndromes; AMI, acute myocardial infarction; CAD, coronary artery disease; CRP, C reactive protein; HF, heart failure; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

Table 3 |

Clinical trials of colchicine and IL-1 blockers in pericarditis

Study (year)	Indication (n)	Study design and drug regimen	Main findings for study drug	Refs
Colchicine				
COPE trial (2005)	Acute pericarditis (120)	Randomization 1:1 to colchicine loading dose of 1.0–2.0 mg followed by 0.5–1.0 mg daily plus aspirin versus aspirin alone for 3 months	Resolution of symptoms at 72 h and reduction in recurrence rate at 18 months	155
CORP trial (2011)	Recurrent pericarditis – first episode (120)	Randomization 1:1 to colchicine loading dose of 1.0–2.0 mg followed by 0.5–1.0 mg daily or placebo in addition to standard of care for 3 months	Resolution of symptoms at 72 h and reduction in recurrence rate at 18 months	157
ICAP trial (2013)	Acute pericarditis – first episode (240)	Randomization 1:1 to colchicine 0.5–1.0 mg or placebo for 3 months	Resolution of symptoms at 72 h; reduction in the rate of incessant or recurrent pericarditis and in hospitalizations; increase in number of patients in remission at 1 week	158
CORP2 trial (2014)	Recurrent pericarditis – multiple episodes (240)	Randomization 1:1 to colchicine 0.5–1.0 mg daily or placebo in addition to standard of care for 6 months	Resolution of symptoms at 72 h; reduction in the rate of incessant or recurrent pericarditis and in pericarditis-related hospitalizations	159
IL-1 blockers				
AIRTRIP trial (2016)	Recurrent, colchicine resistant, corticosteroid dependent pericarditis (21)	Open-label run-in with anakinra 100 mg daily for 2 months, followed by randomized withdrawal to anakinra continuation or placebo for 6 months	Reduction of recurrences; increase in flare-free survival; frequent injection site reactions	176
Acute effects of anakinra in patients with acute pericarditis (2020)	Symptomatic acute pericarditis despite initial treatment with colchicine and NSAIDs (5)	Open-label treatment with anakinra 100 mg	Significant reduction in pain at 6 and 24 h; reduction of IL-6 at 24 h	175
RHAPSODY trial (2021)	Recurrent pericarditis, unresponsive to standard treatment (86 run-in; 61 randomized)	Open-label run-in with rilonacept 320 mg loading dose, followed by 160 mg weekly, followed by randomized withdrawal to rilonacept continuation or placebo	Resolution of acute flares (open label run-in phase); reduction in the recurrence rate	186
Goflikicept in recurrent pericarditis (2022)	Recurrent pericarditis, unresponsive to standard treatment (22)	Open-label run-in with goflkicept 80 mg every 2 weeks (with or without loading dose), followed by randomized withdrawal to goflkicept continuation or placebo	Resolution of acute flares (open label run-in phase); reduction in the recurrence rate	187

NSAIDs, non-steroidal anti-inflammatory drugs.