

## The NLRP3 inflammasome in acute myocardial infarction

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**Abstract** | The heart is extremely sensitive to ischaemic injury. During an acute myocardial infarction (AMI) event, the injury is initially caused by reduced blood supply to the tissues, which is then further exacerbated by an intense and highly specific inflammatory response that occurs during reperfusion. Numerous studies have highlighted the central role of the NACHT, LRR, and PYD domains-containing protein 3 (NLRP3) inflammasome in this process. The inflammasome, an integral part of the innate immune system, is a macromolecular protein complex that finely regulates the activation of caspase 1 and the production and secretion of powerful pro-inflammatory cytokines such as IL-1 $\beta$  and IL-18. In this Review, we summarize evidence supporting the therapeutic value of NLRP3 inflammasome-targeted strategies in experimental models, and the data supporting the role of the NLRP3 inflammasome in AMI and its consequences on adverse cardiac remodelling, cytokine-mediated systolic dysfunction, and heart failure.

Acute myocardial infarction (AMI) remains one of the common causes of hospitalization and death worldwide<sup>1</sup>. The sudden reduction in coronary artery flow secondary to coronary artery atherothrombosis results in an abrupt reduction in cellular energy, leading to subsequent cell necrosis. Current treatment strategies for AMI are centred on restoring flow in the coronary artery (reperfusion) by dissolving the thrombus with medications and/or implantation of an intravascular stent<sup>1</sup>. Although reperfusion strategies are successful in limiting injury to the heart, reducing infarct size, and improving overall prognosis, patients with AMI remain at short-term and long-term increased risk of heart failure<sup>1,2</sup>. Therefore, improved treatment strategies for AMI are urgently needed for the prevention of adverse cardiac remodelling and eventual heart failure. In this Review, we describe the data from experimental studies in small and large animals showing that NACHT, LRR, and PYD domains-containing protein 3 (NLRP3) inflammasome-targeted therapies might be a viable strategy for the reduction of infarct size and prevention of heart failure following AMI. Additional studies with selective NLRP3 inhibitors are eagerly awaited.

### Acute inflammatory response in AMI

The paradigm of inflammation following injury involves the elimination of the offending agent (in case of an infection), removal of cell debris, repair of the injured tissue, and promotion of tissue regeneration (if applicable). As the injury to the heart during AMI is sterile, the resulting inflammation is responsible for

the resorption of the wound and the formation of the scar, whereas tissue regeneration, while present, seems not to be particularly effective<sup>3,4</sup>. Before the introduction of reperfusion strategies to treat AMI, the disease often led to transmural myocardial necrosis, which exposes the patient to increased risk of wall rupture. In such cases, inflammation was considered a necessary consequence of AMI, as it allows the infarct to heal and the scar to form; anti-inflammatory therapies were thus thought to increase the risk of wall rupture<sup>3</sup>. However, researchers have since discovered that excessive or unopposed inflammation can equally impair the formation of a solid scar and pose a similar risk of rupture<sup>3,5–7</sup>.

With the advent of reperfusion therapy, the inflammatory response to ischaemia and reperfusion has proven also to be more complex than previously thought<sup>3</sup>. This extended paradigm proposes that inflammation has a role not only in removal of cellular debris and repair of injured tissue, but that it also contributes to further injury. The inflammation-mediated worsening of the injury occurs in a paracrine manner to the myocardial tissue that was initially salvaged by reperfusion in the infarct border zone. In addition, inflammation can further induce a systemic response that favours adverse remodelling, similar to the neurohormonal activation seen after AMI<sup>3</sup>.

### NLRP3 as a sensor of injury

As an integral part of the innate immune system, the inflammasome is a macromolecular protein complex

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## Key points

- The inflammasome is a macromolecular structure in the cell responsible for sensing danger and triggering a local or systemic inflammatory response
- Upon activation, the inflammasome produces large amounts of active cytokines (primarily IL-1 $\beta$ ) for extracellular secretion, and those cytokines mediate the acute phase of an inflammatory response, such as fever
- The most widely characterized inflammasome sensor in the heart is NACHT, LRR, and PYD domains-containing protein 3 (NLRP3), which is activated in response to noninfectious stimuli such as cell debris during acute myocardial infarction
- Activation of the NLRP3 inflammasome triggers further myocardial damage indirectly through the release of IL-1 $\beta$  and directly through promotion of inflammatory cell death via pyroptosis
- Experimental studies have shown that strategies inhibiting the activation of the NLRP3 inflammasome in the early reperfusion period after acute myocardial infarction reduce the overall size of the infarct and preserve normal cardiac function
- IL-1 blockade can prevent the recurrence of acute myocardial infarction in patients who have experienced a previous event and might improve exercise capacity and cardiac function in patients with heart failure

that finely regulates the activation of caspase 1 and the production and secretion of powerful pro-inflammatory cytokines such as IL-1 $\beta$  and IL-18 (REF. 8). The sensing component of the inflammasome is a member of the nucleotide-binding oligomerization domain-containing protein (NOD)-like receptor (NLR) proteins<sup>8,9</sup>. The classic structure of an NLR is tripartite with leucine-rich repeats domain at the C-terminal, a central NOD, and an effector domain at the N-terminal, which interacts with downstream signalling molecules<sup>8,9</sup>.

NLRP3 senses 'danger' in the form of lysosomal destabilization or a decline in intracellular K<sup>+</sup> concentration<sup>8</sup>. The presence of any microbial or nonmicrobial pathogen that cannot be digested by the lysosomal machinery leads to the activation of a secondary defence mechanism that involves the leakage of cathepsin B into the cytoplasm and K<sup>+</sup> efflux from the cell, to induce conformational changes to the NLRP3 (REF. 8). Such changes lead to oligomerization and recruitment of a macromolecular assembly with a central scaffold protein known as the apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), so-called because of its characteristic formation of a large perinuclear speck under light microscopy when the inflammasome is formed in leukocytes<sup>8,10</sup>. A model for the formation of the ASC specks has been proposed based on findings from electron microscopy<sup>11</sup>. The initial oligomerization of NLRP3 that occurs in a ring-like conformation forms a platform for the polymerization of ASC into filaments. Given that these filaments are insoluble in common detergents used in cell biology, the structure is very stable. In addition to NLRP3 and ASC, another important component of the inflammasome is caspase 1, an enzymatic effector. During the formation of the inflammasome, a large number of caspase 1 filaments branch out from the central core of polymerized ASC, generating a star-like structure. This high concentration of caspase 1 might favour contact with substrates<sup>11</sup>. Activation of caspase 1 is required for the formation

of the inflammasome. Although caspase 1 has several functions unrelated to the inflammasome, its main role in the inflammasome is to cleave pro-IL-1 $\beta$  into its active form, and as such, caspase 1 is also known as the IL-1 $\beta$ -converting enzyme<sup>12,13</sup>.

IL-1 $\alpha$  and IL-18 are other members of the IL-1 family of cytokines<sup>8,12</sup>. Caspase 1 can also activate IL-18 by cleavage of the inactive pro-IL-18 (REFS 8, 12). Contrary to pro-IL-1 $\beta$  and pro-IL-18, pro-IL-1 $\alpha$  lacks a cleavage site for caspase 1, but is already active in this form<sup>14</sup>. Nevertheless, cleavage of pro-IL-1 $\alpha$  seems to increase the affinity of IL-1 $\alpha$  for the IL-1 receptor<sup>14</sup>. In rare instances, pro-IL-1 $\alpha$  is cleaved by the Ca<sup>2+</sup>-dependent enzyme calpain<sup>14</sup>. Other extracellular enzymes (such as neutrophil elastase, granzyme B, and chymase) can also cleave pro-IL-1 $\alpha$ <sup>14</sup>. Furthermore, caspase 1 activity can regulate the secretion of pro-IL-1 $\alpha$ <sup>15</sup>. Activation of caspase 1 within the inflammasome results in a form of inflammatory cell death known as pyroptosis<sup>8,12</sup>. In contrast to apoptosis (typical programmed cell death), death by pyroptosis is associated with cell swelling, increased membrane permeability, and cell rupture, leading to the extracellular release of pro-inflammatory mediators such as pro-IL-1 $\alpha$ <sup>8,10,12,14</sup>. The loss of cell membrane integrity is mediated by gasdermin D, a substrate of caspase 1 that forms N-terminal fragment oligomers within the cell membrane after its cleavage, followed by the formation of pores<sup>16</sup>. Gasdermin D pores are permeable to macromolecules and mediate the unconventional extracellular release of mature IL-1 $\beta$ , IL-18, and active caspase 1. Whether gasdermin D mediates the caspase 1-dependent release of IL-1 $\alpha$  has not been determined. In addition, caspase 1 cleaves several proteins involved in the Krebs cycle, resulting in a dramatic decrease in cell energy production that eventually leads to cell swelling and rupture<sup>17</sup>.

## NLRP3 inflammasome in the heart

The activation of the NLRP3 inflammasome in the heart involves two independent steps<sup>18</sup>. NLRP3 and other important components of the inflammasome are not constitutively expressed in cardiomyocytes, and their expression is regulated by nuclear factor (NF)- $\kappa$ B<sup>8,18</sup> (FIG. 1). Pro-inflammatory stimuli such as cellular debris or microbial products that bind to Toll-like receptors or cytokines can induce the expression of NLRP3 and the other inflammasome components in cardiomyocytes, leukocytes, and other noncardiomyocyte resident cells such as fibroblasts and endothelial cells<sup>10,19</sup>. The cellular debris and microbial products are collectively referred to as damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs)<sup>8</sup>. When priming is completed and the inflammasome components are expressed, a trigger is required for the activation of the NLRP3, which is largely, but not exclusively, dependent on intracellular K<sup>+</sup> concentration. Lysosomal destabilization activates a signalling pathway that indirectly leads to increased membrane permeability for K<sup>+</sup> and increased intracellular efflux. Extracellular ATP triggers K<sup>+</sup> efflux binding to the P2X purinoreceptor 7 (P2X7). The change

in  $K^+$  concentration leads to a conformational change of the NLRP3 that allows recruitment of ASC<sup>8</sup> (FIG. 1). Furthermore, cathepsin B leakage from lysosomal and autophagic vesicles contributes to NLRP3 activation and post-AMI myocardial injury<sup>20</sup>.

#### Activation by kinases and oxidative stress

Several intermediate steps dependent on additional cytoplasmic adaptor proteins or kinases are also involved in the activation process. The serine/threonine-protein kinase Nek7, a member of the never in mitosis A

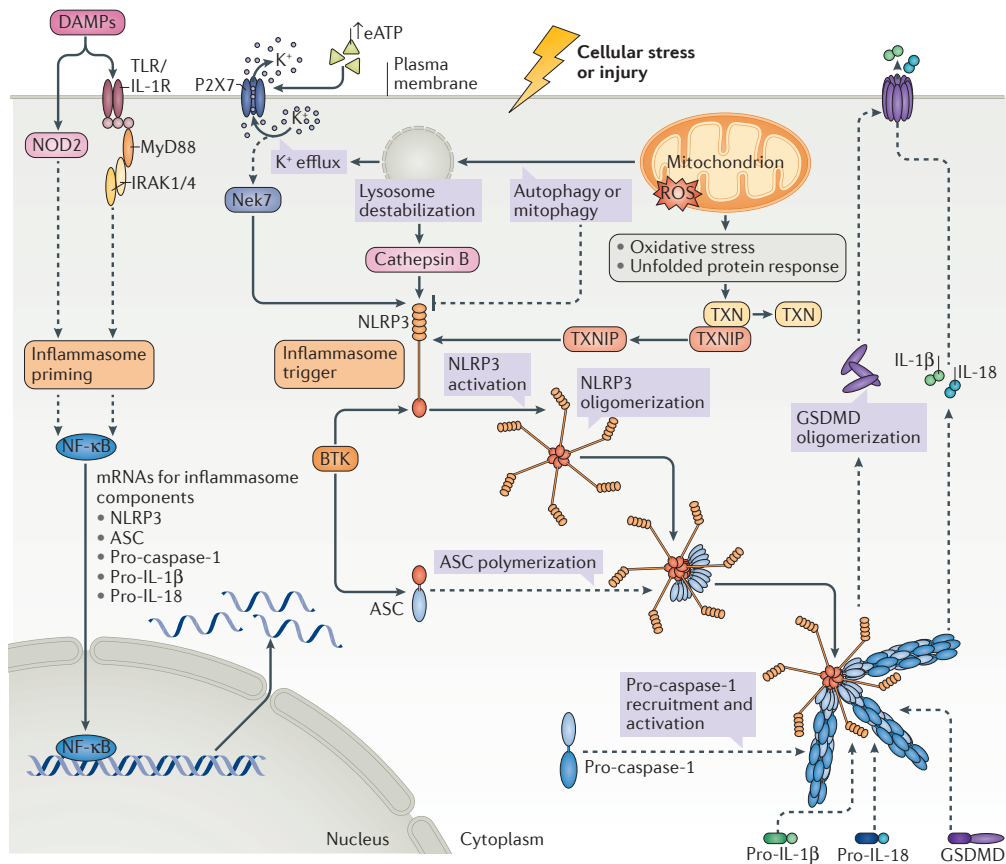


**Figure 1 | NLRP3 inflammasome formation pathways.** The formation of the inflammasome in the heart requires two independent steps: priming and triggering. The priming signal is dependent on the activity of nuclear factor (NF)-κB through stimulation of the membrane Toll-like receptors (TLRs) and the downstream signalling mediated by myeloid differentiation primary response protein MyD88 and interleukin-1 receptor-associated kinases (IRAKs). The TLRs sense extracellular threats through damage-associated molecular patterns (DAMPs) and prime the cells to respond to the potential injurious conditions by increasing the transcription and translation of inflammasome components and the associated cytokines (IL-1β and IL-18). These cytokines function in a paracrine fashion on the membrane cytokine receptor and converge on the same pathways to amplify the signal. A similar priming effect is mediated by nucleotide-binding oligomerization domain-containing protein 2 (NOD2). The primary signal is necessary but insufficient to form the inflammasome in cardiomyocytes in the absence of the trigger signal. The activation of NACHT, LRR, and PYD domains-containing protein 3 (NLRP3) is mediated by extracellular and/or intracellular pathways. The increase in extracellular ATP (eATP) activates the P2X purinoreceptor 7 (P2X7) and leads to  $K^+$  efflux, a step that triggers NLRP3 activation. Lysosomal destabilization by indigestible material is another mechanism that leads to NLRP3 activation by leakage of the lysosomal enzyme cathepsin B and by induction of  $K^+$  efflux. The serine/threonine-protein kinase Nek7 senses the  $K^+$  efflux and binds NLRP3, allowing its activation. Thioredoxin-interacting protein (TXNIP) links oxidative stress and the unfolded protein response to NLRP3 activation. TXNIP is freed from thioredoxin (TXN) and binds NLRP3. The mitochondria also have an important role in producing reactive oxygen species (ROS). Furthermore, ineffective clearance of mitochondrial debris by mitophagy contributes to lysosomal destabilization. By contrast, effective mitophagy and autophagy suppress the activation of NLRP3. The tyrosine-protein kinase BTK also binds NLRP3 and apoptosis-associated speck-like protein containing a CARD (ASC), leading to inflammasome activation after ischaemia. The active NLRP3 oligomerizes, forming a circular structure that functions as a platform for the polymerization of ASC into filaments, which in turn work as a central core from which caspase 1 filaments branch, forming a star-like structure. Active caspase 1 cleaves the inactive pro-IL-1β and pro-IL-18 into the active forms, IL-1β and IL-18. Gasdermin D (GSDMD) is an additional substrate of caspase 1 that oligomerizes upon cleavage and forms pores in the cell membrane with the N-terminal fragments, which allow the extracellular release of active IL-1β and IL-18.

(NIMA)-related kinase family, acts downstream of P2X7 and K<sup>+</sup> efflux. Nek7 binds to NLRP3 and regulates NLRP3 oligomerization and activation<sup>21</sup>. Nek7 is highly expressed in heart muscle and might be a target for intervention to inhibit inflammasome formation in the heart<sup>21</sup>. Oxidative stress can also activate the inflammasome in a process mediated by thioredoxin-interacting protein (TXNIP)<sup>22</sup>. The production of reactive oxygen species (ROS) and the induction of the unfolded protein response together cause the detachment of TXNIP from thioredoxin, which functions as an antioxidant by reducing oxidized sulfhydryl groups. Free TXNIP then participates in NLRP3 activation and subsequent inflammasome formation. The inhibition of TXNIP using small interfering RNA in the heart has been shown to reduce inflammasome activation and infarct size after ischaemia–reperfusion injury<sup>22</sup>. This process couples the response to oxidative stress to initiation of the inflammatory response (FIG. 1).

The tyrosine–protein kinase BTK interacts with both NLRP3 and ASC and is also required for the activation of NLRP3 *in vivo*<sup>23</sup>. Furthermore, the inhibition of BTK with ibrutinib has been shown to suppress inflammasome activation and reduce infarct size after cerebral ischaemia<sup>23</sup>.

#### Role of mitochondria

Mitochondria are one of the major sources of ROS and have an important role in determining cell fate under conditions of stress<sup>24</sup>. In addition, mitochondria are involved in necrotic and apoptotic cell death<sup>25</sup>. In the past 6 years, the role of the mitochondria in the regulation of the inflammasome and their association with pyroptosis have become well understood<sup>26,27</sup>. Mitochondrial dysfunction directly activates the NLRP3 inflammasome by producing ROS and by inducing K<sup>+</sup> efflux<sup>26,27</sup>. Mitophagy, the clearance of damaged and dysfunctional mitochondria through the autophagic pathway, has also been shown to regulate inflammasome activity<sup>28,29</sup>. Ineffective mitophagy perpetuates the cellular surge in ROS, but also promotes the cytoplasmic accumulation of mitochondrial DNA, which enhances caspase 1 activation and IL-1 $\beta$  production<sup>28</sup>. In addition, mitochondrial fission (division) and fusion also contribute to the regulation of mitochondrial homeostasis and inflammasome activation<sup>29,30</sup>. Impairment of mitochondrial fission, caused by the deletion of dynamin 1-like protein, leads to an increase in NLRP3-dependent caspase 1 activation and IL-1 $\beta$  secretion<sup>29</sup>. Finally, cardiolipin, a mitochondrial lipid, can bind to and activate the NLRP3 inflammasome<sup>31</sup>. Conversely, inhibition of cardiolipin production suppresses the activation of the inflammasome.

#### Role of autophagy

The autophagic pathway is another regulator of inflammasome function<sup>28,32</sup>. Autophagy is a proteolytic process that involves the removal of misfolded and damaged proteins and organelles and is important for the maintenance of the cellular homeostasis<sup>28</sup>. In primary human monocytes and macrophages, inhibition of autophagy enhances

NLRP3 inflammasome formation, whereas activation of autophagy limits the secretion of active IL-1 $\beta$ <sup>32</sup>. During myocardial ischaemia–reperfusion injury, autophagy limits myocardial damage; conversely, inhibition of autophagy exacerbates cardiac remodelling<sup>33</sup>. However, whether autophagy regulates inflammasome activity during myocardial ischaemia–reperfusion needs to be determined.

#### Differential roles of the inflammasome

Although every cell type in the heart might be capable of triggering the NLRP3 inflammasome if appropriately stimulated, the stimuli needed to activate the inflammasome and the effects of the active inflammasome might be different. Circulating monocytes constitutively express many components of the inflammasome and require minimal priming to produce large amounts of IL-1 $\beta$ <sup>34</sup>. Cardiomyocytes have low expression of NLRP3 and caspase 1 and do not express and secrete high levels of IL-1 $\beta$  even when stimulated with high doses of pro-inflammatory mediators. However, both monocytes and cardiomyocytes can express and activate IL-18, and cardiomyocytes die of pyroptosis following caspase 1 activation<sup>8,34,35</sup>. The difference between the secretion of IL-1 $\beta$  and secretion of IL-18 might involve differences in how they are transcriptionally regulated. Indeed, IL-1 $\beta$  mRNA levels in cardiomyocytes are very low, often undetectable, and unaffected by treatment such as lipopolysaccharide incubation<sup>34</sup>.

#### NLRP3 inflammasome in AMI

##### NLRP3 inflammasome formation

In patients, evidence of NLRP3 inflammasome activation in the context of AMI is limited to several autopsy cases<sup>19</sup> and to measurement of plasma levels of NLRP3 and caspase 1 (REF. 36). However, the time-dependent activation of the NLRP3 inflammasome in the heart has been investigated in preclinical studies using animal models. The expression of NLRP3 and the activity of the inflammasome in the heart were low when measured within 3 h after AMI<sup>37,38</sup> in a mouse model of ischaemia–reperfusion injury. In this model, the NLRP3 inflammasome was formed in the myocardium within 3–24 h of the AMI<sup>37</sup> and contributed to both the inflammatory response and the exacerbation of the ischaemia–reperfusion injury<sup>8,10,37–40</sup>. Increased expression and activity of the inflammasome was found to peak after 1 and 3 days in mice with reperfused and nonreperfused AMI, respectively. During the early phases of formation, the inflammasome specks can be seen in the endothelial cells, cardiomyocytes, and fibroblasts. As the leukocytes infiltrate the heart, the majority of specks can be seen within the granulocytes and macrophages. During the postinjury healing phase, as the infiltrate resolves, the aggregates are seen more commonly in isolated cardiomyocytes or fibroblasts<sup>8,10</sup>.

Experimental data suggest that the effects of NLRP3 activation in the heart are cell-type-specific<sup>41,42</sup>. The induction of inflammasome formation in fibroblasts, endothelial cells, or leukocytes results in the secretion of IL-1 $\beta$ . However, cardiomyocytes do not consistently

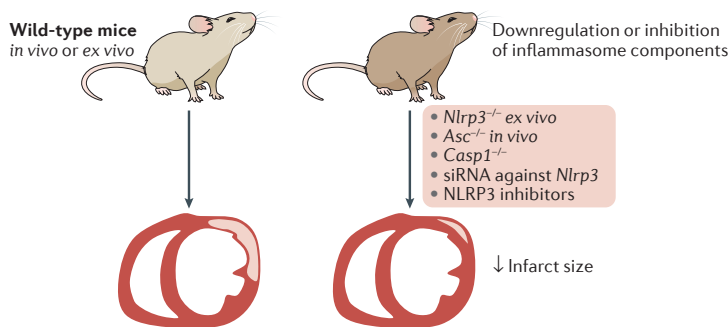


**Figure 2 | Strategies to reduce infarct size by inhibiting the NLRP3 inflammasome in experimental animal models of myocardial ischaemia–reperfusion injury.** The left and right ventricular cavities and the infarct areas of the hearts of untreated wild-type mice and genetically modified or treated mice are shown. The formation of the inflammasome is a critical step following ischaemia–reperfusion injury. Preventing the formation of the inflammasome by genetically deleting any of the inflammasome components (NACHT, LRR, and PYD domains-containing protein 3 [NLRP3], apoptosis-associated speck-like protein containing a CARD [ASC], or caspase 1), using small interfering RNA (siRNA) specific for NLRP3, or administering selective NLRP3 inhibitors has been shown to reduce infarct size.

secrete high levels of IL-1 $\beta$ . Nevertheless, NLRP3 inflammasome formation in cardiomyocytes activates caspase 1 and induces pyroptosis<sup>41,42</sup>.

NLRP3 is not the only inflammasome sensor that can trigger caspase 1 to release IL-1 $\beta$  and IL-18. Other well-characterized NLRs include NLRP1, NLR family CARD domain-containing protein 4 (NLRC4), and the interferon-inducible protein AIM2, but the role of these receptors in the context of cardiovascular diseases is less clear<sup>8</sup>. Another important member of this family of innate immune receptors, NOD2, has been shown to exacerbate myocardial injury in a mouse model of permanent coronary occlusion<sup>43</sup>. NOD2 expression was increased in the myocardium of wild-type mice, whereas its deletion reduced myocardial damage and the expression of pro-inflammatory markers, including IL-1 $\beta$ <sup>43</sup>. NOD2 induces NF- $\kappa$ B activation and thus contributes to inflammasome priming<sup>9,43</sup>.

#### The inflammasome in disease progression

The role of the NLRP3 inflammasome in AMI progression should not come as a surprise, considering that inflammasome activity is involved in the typical response to injury. Given that AMI is a prototypical example of sterile inflammation, the response to injury and the recruitment of inflammatory cells are expected to be at least partly dependent on the NLRP3 inflammasome<sup>8</sup>. Whether the formation of the inflammasome in the injured cardiomyocytes is a beneficial and protective mechanism or a mechanism of further injury remains unclear. Since 2011, numerous experimental studies have investigated this question<sup>10,19</sup>. Inflammasome formation is an energy-requiring process that occurs in injured cells to rapidly induce an inflammatory response to the area. This inflammatory cascade of events resembles the ‘defensive’ process, which, from an evolutionary standpoint, results from a microbial invasion at the cost of sacrificing those ‘sentinel’ cells.

However, in the context of sterile inflammation, this inflammatory response results in further injury and very little benefit<sup>8</sup>. When exposed to ischaemia–reperfusion injury, mice that lack the *Nlrp3* gene or have undergone siRNA silencing to suppress *Nlrp3* expression have a smaller infarct size and better cardiac function than wild-type mice<sup>19,22,44</sup> (FIG. 2). Accordingly, mice deficient in ASC or caspase 1 are protected against ischaemia–reperfusion injury and have reduced myocardial levels of active IL-1 $\beta$ , suggestive of reduced inflammasome activity<sup>19</sup> (FIG. 2). Consistent with the time course of the expression of NLRP3 and the activity of the inflammasome in the heart, which are both low at the onset of ischaemia<sup>37,38,45</sup>, the NLRP3 knockout mouse was not protected against injury within the first few hours after ischaemia was induced<sup>38,45</sup>.

#### Novel inhibitors of the inflammasome

Although the molecular structure of NLRP3 is known, the exact mechanism by which K<sup>+</sup> concentration induces the conformational change and aggregation of the inflammasome is not well understood. The sulfonylurea glyburide was shown to inhibit IL-1 $\beta$  release from leukocytes independently of its capacity to induce insulin release from the pancreatic  $\beta$  cells, and it also inhibited the NLRP3 inflammasome *in vitro* without suppressing its ATPase activity<sup>46</sup>. However, when used at the doses required to inhibit the inflammasome *in vivo*, glyburide caused lethal hypoglycaemia in mice<sup>39</sup>.

FIGURE 3 and TABLE 1 show the characteristics and the proposed sites of action of the different experimental NLRP3 inflammasome inhibitors that have been tested in models of AMI. Marchetti and colleagues designed a novel inhibitor derived from glyburide, but lacking the cyclohexylurea moiety group, which is responsible for insulin secretion<sup>39</sup>. Therefore, this molecule could not induce insulin release, and after modification of side residues, could selectively inhibit the NLRP3 (REF. 39). When given in a single dose or in repeated doses before ischaemia or after reperfusion, this NLRP3 inhibitor significantly reduced infarct size and preserved cardiac function, thus demonstrating the critical role of early activation of the NLRP3 inflammasome for downstream inflammatory signalling<sup>39,47</sup>. The inhibitor is specific for NLRP3 and prevents NLRP3 oligomerization after its activation. Marchetti and colleagues also obtained similar results using a different NLRP3 inhibitor (OLT1177)<sup>48</sup>. In a mouse model, OLT1177 administration resulted in a 60% reduction in infarct size compared with vehicle-treated controls (A. A., unpublished data). OLT1177 was initially developed as a topical treatment for degenerative arthritis, but is now being tested in a phase II clinical trial for the treatment of acute gouty arthritis after successful phase I clinical testing<sup>49</sup>.

In 2010, Juliana and colleagues reported that Bay 11–7082, a compound known to inhibit NF- $\kappa$ B, could also inhibit the ATPase activity of NLRP3, preventing the formation of the inflammasome in response to NLRP3-specific stimuli<sup>50</sup>. In a separate mouse study, Bay 11–7082 administered intraperitoneally 10 min before reperfusion in an ischaemia–reperfusion AMI

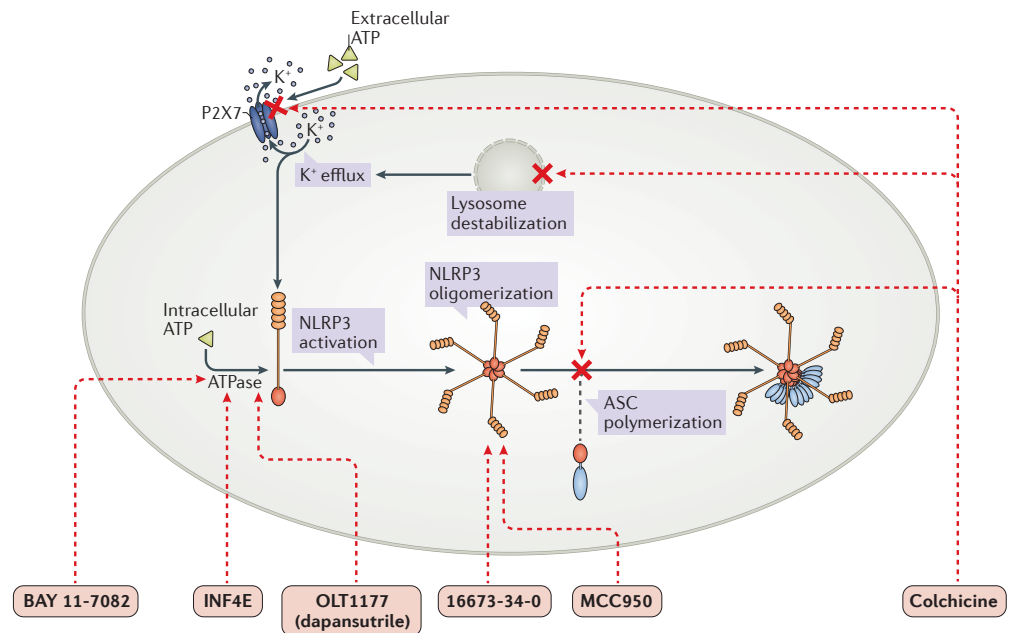


**Figure 3 | Mechanism of action of NLRP3 inhibitors tested in experimental models of acute myocardial infarction.** Several chemical compounds have been developed with inhibitory effects on inflammasome activity, some of which have been tested in animal models of acute myocardial infarction and in clinical trials. BAY-11-7072, INF4E, and OLT1177 can inhibit the ATPase activity of NACHT, LRR, and PYD domains-containing protein 3 (NLRP3). OLT1177, 16673-34-0, and MCC950 are compounds that inhibit NLRP3 and prevent its oligomerization. Colchicine has multiple mechanisms of inhibition, acting upstream of NLRP3 to prevent the opening of the P2X purinoreceptor 7 (P2X7) channel, to destabilize the lysosome, and to inhibit NLRP3 activation. Colchicine can also act downstream of NLRP3 by inhibiting the polymerization of apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC).

model significantly reduced inflammasome formation in the heart and decreased infarct size<sup>22</sup>. When given 30 min before induction of ischaemia in a rat model of ischaemia–reperfusion, the Bay 11–7082 compound also significantly reduced infarct size and preserved cardiac function<sup>51</sup>. Cocco and colleagues developed acrylamide derivatives that covalently bind to NLRP3 to inhibit its ATPase activity<sup>52</sup>. A dose of 50 µmol/l of the compound administered *ex vivo* to a mouse model of AMI 20 min before induction of ischaemia resulted in a reduction in inflammasome activity and infarct size<sup>52</sup>. Furthermore, MCC950, a small molecule known for its anti-inflammatory properties, was shown to selectively inhibit the binding of NLRP3 to ASC in mice *in vivo*, independently of NLRP3 ATPase activity<sup>53</sup>. MCC950 also reduced infarct size in a porcine model of ischaemia induced by balloon occlusion of the left anterior descending coronary artery<sup>54</sup>.

Colchicine is a nonspecific inhibitor of the NLRP3 inflammasome<sup>55</sup>. Originally thought to function only as an inhibitor of microtubule polymerization and leukocyte diapedesis, a large part of the efficacy of colchicine as an anti-inflammatory drug is related to its inhibition of the NLRP3 inflammasome<sup>56</sup>. Colchicine prevents inflammasome activity at two levels: it inhibits the activation of the P2X7 receptor, and the polymerization of ASC by interfering with the interaction between the pyrin domains<sup>56</sup> (FIG. 3). Colchicine has been shown to

suppress the transport of mitochondria and the subsequent colocalization of ASC to NLRP3, which suggests that microtubules create the sites for the inflammasome components to interact, which eventually leads to NLRP3 inflammasome activation<sup>57</sup>. In a nonreperfused AMI mouse model, a high dose of colchicine (0.1 mg/kg) administered daily for 7 days significantly reduced inflammasome activation after 24 h, decreased infarct size and ventricular remodelling, and prolonged 7-day survival<sup>58</sup> (FIG. 3).

Although the pharmacological inhibitors discussed have inherent limitations in specificity, they are important tools for the validation of genetic mouse models and are therefore essential for clinical translation. Additional validation and comparative studies between different experimental models are needed<sup>59</sup>.

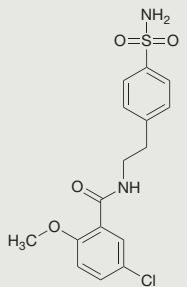
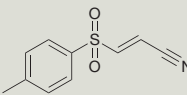
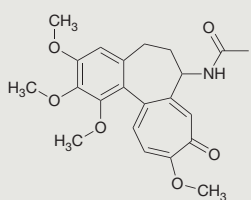
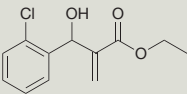
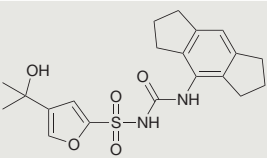
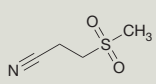
**NLRP3 inflammasome inhibition: beyond infarct size reduction.** In agreement with the timeline of the expression and the activation of the NLRP3 inflammasome, Toldo and colleagues have shown that delaying treatment with an NLRP3 inhibitor (16673-34-0) by 60 min after the beginning of reperfusion did not limit its cardioprotective benefits<sup>37</sup>. However, the benefit of NLRP3 inflammasome inhibition was lost if treatment was given >3 h after the beginning of reperfusion, suggesting that the formation of the NLRP3 inflammasome in the heart occurs between 1 and 3 h after reperfusion

in the mouse (FIG. 4). Similarly, in the model of non-reperfused AMI, in which the infarct size is dictated by the area of ischaemia and reperfusion-induced inflammatory cell death is absent, the NLRP3 inhibitor did not reduce infarct size<sup>47</sup>. The mechanism by which the NLRP3 inflammasome inhibitors reduce infarct size is likely not to be attributable to reduced release of mature IL-1 $\beta$  for several reasons. First, IL-1 $\beta$  might not remain elevated for several hours after AMI. Furthermore, IL-1 $\beta$  blockers were unable to reduce infarct size when administered during reperfusion<sup>60</sup>. In addition, NLRP3

inflammasome inhibition seems to affect only the portion of the myocardium that was temporarily rescued by reperfusion, but eventually dies by pyroptosis following inflammasome formation as part of reperfusion injury. Finally, whereas inhibition of the components of the inflammasome prevents pyroptosis, inhibition of IL-1 $\beta$  activity did not have any effect<sup>8</sup>.

Regardless of whether the ischaemic myocardium undergoes reperfusion, the NLRP3 inflammasome remains active for several days after AMI. The ASC specks can be seen in individual cardiomyocytes, fibroblasts,

Table 1 | NLRP3 inflammasome inhibitors in experimental AMI

| Name (chemical name)                                                                                                                                                                                                | Chemical structure                                                                  | Doses/route/animal                                                                                                                                                 | Experimental findings in the context of AMI                                                                                                                                                                                                                                                                                                                                                                                                         | Refs                          |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|
| 16673-34-0<br>5-Chloro-2-methoxy-N-[2-(4-sulfamoylphenyl)ethyl]benzamide                                                                                                                                            |    | 5–100 mg/kg given before or up to 60 min after reperfusion in a mouse coronary artery ligation model                                                               | <ul style="list-style-type: none"> <li>Reduction in infarct size at 24 h (measured by pathology, echocardiography, and troponin I plasma levels)</li> <li>Improvement in cardiac remodelling and left ventricular dysfunction in reperfused and nonreperfused AMI</li> <li>Reduction in caspase 1 activation and cardiomyocyte pyroptosis in the early phases of AMI and of apoptosis and myocardial fibrosis in the later phases of AMI</li> </ul> | 37,39,47                      |
| BAY 11-7082<br>3-[(4-Methylphenyl)sulfonyl]-(2E)-propenenitrile                                                                                                                                                     |  | Pretreatment with the drug (dose not specified) before ischaemia or before reperfusion in a mouse or rat model of ischaemia and reperfusion                        | <ul style="list-style-type: none"> <li>Reduction in infarct size</li> <li>Reduction in inflammasome activity (caspase 1 activity, IL-1<math>\beta</math> levels)</li> <li>Preservation of cardiac function</li> </ul>                                                                                                                                                                                                                               | 22,50,51                      |
| Colchicine<br>N-[(7S)-1,2,3,10-tetramethoxy-9-oxo-6,7-dihydro-5H-benzo[a]heptalen-7-yl]acetamide                                                                                                                    |  | 0.1 mg/kg given daily for 7 days starting before ischaemia in a reperfused AMI mouse model                                                                         | <ul style="list-style-type: none"> <li>Reduced infarct size (measured by pathology)</li> <li>Reduction in inflammasome activity (expression of inflammasome components, caspase 1 activity)</li> <li>Prevention of adverse remodelling and heart failure</li> <li>Prolonged survival</li> </ul>                                                                                                                                                     | 55,56,58                      |
| INF4E<br>Ethyl 2-[(2-chlorophenyl)(hydroxymethyl)acrylate                                                                                                                                                           |  | 50 $\mu$ mol/l given in an ex vivo myocardial ischaemia model 20 min before induction of ischaemia                                                                 | <ul style="list-style-type: none"> <li>Reduction of infarct size at 60 min (measured by pathology, cardiac markers, and contractility)</li> <li>Reduction in caspase 1 activity, cleaved IL-1<math>\beta</math> levels, and gasdermin D levels at 60 min</li> </ul>                                                                                                                                                                                 | 40,52                         |
| MCC950<br>N-[(1,2,3,5,6,7-hexahydro-s-indacen-4-yl)carbonyl]-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 |  | 3–6 mg/kg given intravenously 15 min before reperfusion, then repeated after 24 h and the following 5 days in a pig balloon angioplasty infarction ischaemia model | <ul style="list-style-type: none"> <li>Reduction in infarct size (measured by pathology, echocardiography, and troponin I plasma levels)</li> <li>Improvement in cardiac remodelling and prevention of left ventricular dysfunction</li> <li>Reduction in local and systemic inflammatory response</li> </ul>                                                                                                                                       | 53,54                         |
| OLT1177 (dapansutrile)<br>3-(methanesulfonyl)propanenitrile                                                                                                                                                         |  | 6–600 mg/kg given before or up to 30 min after reperfusion in a mouse surgical coronary artery ligation model                                                      | <ul style="list-style-type: none"> <li>Reduction in infarct size (measured by pathology and echocardiography)</li> </ul>                                                                                                                                                                                                                                                                                                                            | 48, (A. A., unpublished data) |

AMI, acute myocardial infarction; NLRP3, NACHT, LRR and PYD domains-containing protein 3.

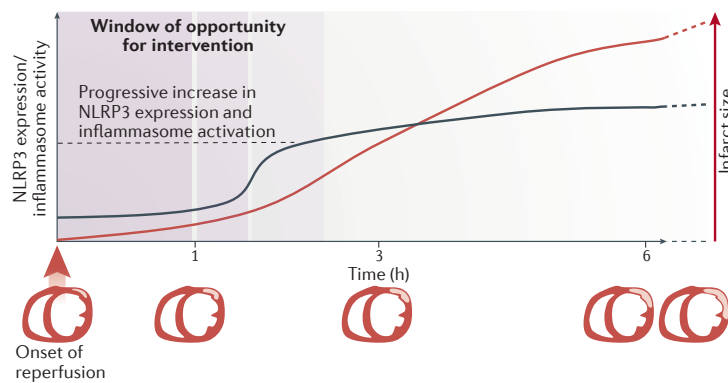


**Figure 4 | Window of opportunity for intervention using NLRP3 inhibitors in ischaemia–reperfusion injury.** The left and right ventricular cavities and the progression of the infarct areas over time are shown. At the onset of reperfusion injury and for the initial hour, NACHT, LRR, and PYD domains-containing protein 3 (NLRP3) myocardial expression and inflammasome activity are low, increasing between 1 and 3 h after reperfusion injury. The size of the infarct continues to grow after reperfusion. The time that precedes the activation of the inflammasome is a therapeutic window for intervention with NLRP3 inhibitors.

and leukocytes throughout the heart, and the inflammatory response is systemic. Inhibition of the NLRP3 inflammasome in nonreperfused AMI results in more favourable cardiac remodelling without affecting infarct size<sup>47</sup>. This pattern resembles those seen with IL-1 $\beta$  blockade strategies and is characterized by a reduction in apoptosis and preservation of cardiac contractility<sup>61–63</sup>. Treatment after the onset of ischaemia using anakinra, a recombinant IL-1 receptor antagonist, or a mouse anti-IL-1 $\beta$  antibody (analogue of canakinumab) significantly reduced cardiomyocyte apoptosis in the border zone of the infarcted area, and also decreased left ventricular dilatation and dysfunction without reducing infarct size<sup>61–63</sup>. IL-1 $\beta$  blockade has also been shown to improve left ventricular contractility and  $\beta$ -adrenergic receptor responsiveness in models of ischaemic cardiomyopathy<sup>64</sup>. FIGURE 5 summarizes the role of IL-1 $\beta$  in adverse cardiac remodelling and cardiac dysfunction.

Compared with IL-1 $\beta$ , IL-1 $\alpha$  has a less established role in AMI. In contrast to IL-1 $\beta$ , pro-IL-1 $\alpha$  is constitutively expressed in the heart and is active in its precursor form<sup>65</sup>. When released during cell death, pro-IL-1 $\alpha$  functions as a signal for tissue and cell damage. Accordingly, a strategy that inhibits IL-1 $\alpha$  might prove more useful in limiting the first wave of the inflammatory injury than one that involves IL-1 $\beta$  blockade<sup>66</sup>.

Similarly, the role of IL-18 in AMI is largely unknown<sup>67</sup>. IL-18 activity has been shown to contribute to contractile dysfunction in human ischaemic myocardial strips<sup>68</sup>. In a mouse model of AMI, pretreatment with an IL-18 blocking antibody reduced infarct size<sup>69</sup>. Furthermore, IL-18 blockade following reperfusion injury in a mouse model of heterotopic heart transplantation reduced myocardial inflammation and improved graft survival<sup>70</sup>. Additional studies are required to determine the safety and efficacy of IL-18 blockade in preclinical models of AMI.

### linflammasome and cardiac dysfunction

The expression of the components of the NLRP3 inflammasome is constitutively low in the majority of tissue-resident cells. As mentioned above, activation of the NLRP3 inflammasome requires two independent steps: priming and triggering<sup>18</sup>. The use of a transgenic mouse with constitutively active NLRP3 has highlighted how NLRP3 activation alone is insufficient to induce cardiac dysfunction<sup>18</sup>. When priming occurs together with activation of NLRP3, cardiac dysfunction, progression of nonischaemic cardiomyopathy, and death ensue<sup>18</sup>. The need for priming explains why patients with cryopyrin-associated periodic syndromes, who carry a mutation in the *Nlrp3* gene that causes NLRP3 to be constitutively active, generally do not seem to have cardiac dysfunction but might be more vulnerable to cardiac dysfunction during the clinically active phases of the disease<sup>71</sup>.

IL-1 $\beta$ , the main product of the active inflammasome, had already been identified as a soluble cardiodepressant factor 21 years ago, initially in patients with sepsis<sup>72</sup> and more recently in those with acute decompensated heart failure<sup>73</sup>. The effects of IL-1 $\beta$  administration on impaired contractility follow the timeline of a fever, with a peak inflammatory response occurring 3–4 h after administration, an effect that requires new protein synthesis<sup>61</sup>. IL-1 $\beta$ -induced impairment in contractility is reversible even after repeated treatments<sup>61,73,74</sup> and results from a fault of the electrical–mechanical association at a cellular level, whereby the expression and function of L-type calcium channels, phospholamban, and sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (SERCA2) are simultaneously altered, leading to functional desensitization of the  $\beta$ -adrenergic receptors<sup>61,75</sup>. Impaired contractility, altered expression and function of calcium-regulating proteins, and  $\beta$ -adrenergic desensitization, as seen with IL-1 $\beta$  treatment, are all hallmarks of heart failure.

### Inflammasome in nonischaemic injury

During the process of NLRP3 inflammasome priming and triggering, the membrane and cytoplasmic sensors are activated in a similar pattern after cell death, depending on whether ischaemia was the cause of cell death. Toll-like receptors and purinergic 2X receptors on the membrane recognize cell debris and extracellular ATP, respectively, both byproducts of cell death, and provide signals for the priming and triggering of the inflammasome<sup>8</sup>. The inflammatory response to injury is, therefore, a rather nonspecific type of response that occurs for both ischaemic and nonischaemic injury. Experimental studies have shown that the NLRP3 inflammasome has a central pathogenic role in nonischaemic cardiomyopathy in a mouse model with cardiac-specific heterozygous overexpression of the calcineurin transgene, which results in cardiac hypertrophy, inflammation, apoptosis, and ventricular dilatation<sup>76,77</sup>. Myocardial injury owing to coxsackievirus infection also leads to NLRP3 activation and formation of the inflammasome, which is ultimately responsible for the progression of cardiac dysfunction<sup>78</sup>.

In experimental models of sepsis, cardiac fibroblasts respond to pro-inflammatory stimuli by forming the NLRP3 inflammasome and producing IL-1 $\beta$ , which then acts in a paracrine fashion on the cardiomyocytes to induce  $\beta$ -adrenergic receptor desensitization and contractile dysfunction<sup>75</sup>. Myocardial injury due to doxorubicin, the most commonly used antineoplastic chemotherapeutic agent, is also associated with activation of the NLRP3 inflammasome; doxorubicin-induced cardiotoxicity can be reduced with an NLRP3 inflammasome inhibitor (16673-34-0)<sup>47</sup>. An increase in NLRP3 inflammasome activity in the heart has also been seen in animal models of pressure overload, such as those of transverse aortic constriction<sup>79</sup>.

### Indirect NLRP3 inhibition in AMI

Data on the role of the NLRP3 inflammasome in patients with AMI are scarce and are mostly based on biomarker studies and on indirect evidence resulting from trials that used nonselective NLRP3 inhibitors or IL-1 blockers. Indeed, with the exception of OLT117, which is currently being investigated in a phase II clinical trial for the treatment of acute gouty arthritis, and of colchicine, which is not a selective inhibitor, no other

selective NLRP3 inhibitors are currently available in the clinic. Two IL-1 blockers have been tested in phase I–III clinical trials for AMI or heart failure<sup>61</sup>.

All members of the IL-1 family (IL-1 $\beta$ , IL-1 receptor antagonist, soluble ST2 [interleukin-1 receptor-like 1], and IL-18) and surrogates (IL-6 and C-reactive protein [CRP]) serve as very good biomarkers and predictors of unfavourable outcomes in patients with AMI<sup>80</sup>. NLRP3 and caspase 1 mRNA transcription in circulating monocytes are increased in patients with acute coronary syndrome or stable angina compared with healthy controls. At the protein level, NLRP3 also increased in parallel to an increase in the active forms of caspase 1, IL-1 $\beta$ , and IL-18 (REF. 36). Although not probative, the available clinical data in patients with AMI, together with preclinical data in animals, create a compelling argument for further assessment of selective NLRP3 inhibitors.

### Colchicine

Colchicine was historically used off label to treat Mediterranean fever and was approved by the FDA in 2009 for the treatment of Mediterranean fever and acute gouty arthritis, after evidence of its capacity to inhibit the assembly and activation of NLRP3 inflammasome in response to monosodium urate crystals<sup>55</sup>. Consistent with a central role of the NLRP3 inflammasome during ischaemia–reperfusion injury, colchicine (1.5 mg initial dose, followed by 0.5 mg given 1 h later and 0.5 mg given twice daily for 5 days) significantly reduced the size of the infarct in patients with ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention<sup>81</sup> (TABLE 2). Notably, however, 26% of 77 patients assigned to colchicine discontinued treatment before completion owing to poor tolerance, which is common for doses of colchicine >1.0 mg daily. In another clinical trial, 532 patients with stable coronary artery disease were randomly assigned 1:1 to low-dose colchicine (0.5 mg daily) or placebo for a median of 2.3 years<sup>82</sup>. Colchicine significantly reduced the incidence of a combined adverse cardiovascular end point compared with placebo, owing to a large reduction in the incidence of acute coronary syndromes (4.6% versus 13.6%; OR 0.33, 95% CI 0.18–0.63,  $P < 0.001$ )<sup>82</sup>.

### Anakinra in AMI and heart failure

The first study of IL-1 blockade in patients with AMI was a 2010 pilot feasibility study involving ten patients with ST-segment elevation myocardial infarction<sup>83</sup>. A second proof-of-concept study involving 30 patients was published 3 years later<sup>84</sup>. Administration of anakinra (100 mg daily for 14 days) in patients with ST-segment elevation myocardial infarction was well tolerated and blunted the acute systemic inflammatory response. The clinical follow-up of the small number of patients enrolled revealed a favourable clinical profile with lower CRP levels and a trend towards reduced incidence of adverse remodelling and heart failure at 3 months and at long-term follow up with anakinra<sup>83–85</sup> (TABLE 2). Anakinra is now being further explored in a

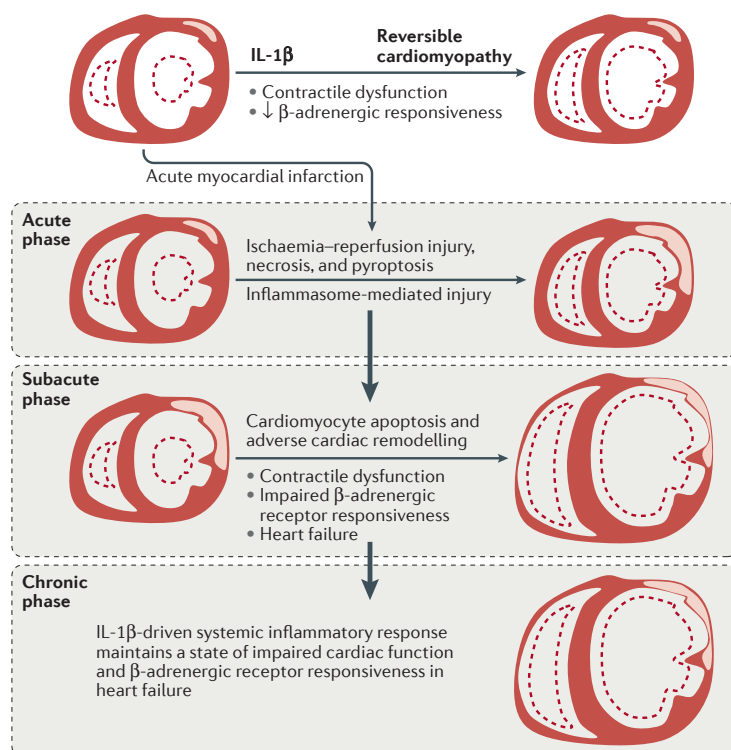


**Figure 5 | Role of IL-1 $\beta$  in acute myocardial infarction.** The left and right ventricular cavities in diastole and in systole (dotted lines) are shown. IL-1 $\beta$  reduces myocardial contractility and the myocardial response to  $\beta$ -adrenergic receptor agonists in the heart, even in the absence of myocardial infarction. In the subacute phase that follows acute myocardial infarction, IL-1 $\beta$  induces cardiomyocyte apoptosis, favouring adverse myocardial remodelling and heart failure. Enhanced IL-1 $\beta$  activity in the subacute and chronic phases that follow myocardial infarction contribute to impaired myocardial contractility and  $\beta$ -adrenergic responsiveness in heart failure.

larger phase II trial in patients with ST-segment elevation myocardial infarction<sup>86</sup>. In 2014, the results of a phase II clinical trial involving 182 patients with non-ST-segment elevation myocardial infarction showed a similar reduction in the acute inflammatory response with anakinra (100 mg daily for 14 days)<sup>87</sup> (TABLE 2). The clinical events at 30 days and 3 months were unaffected by anakinra treatment, but by 12 months, surprisingly, the anakinra-treated patients had experienced significantly more recurrent ischaemic events than untreated

control patients<sup>87,88</sup>. Based on known cardiodepressive effects of IL-1 $\beta$ , small proof-of-concept pilot studies have explored the beneficial effects of IL-1 blockade in heart failure. In a series of studies in patients with acute or chronic systolic or diastolic heart failure, anakinra (100 mg once or twice daily) reduced the systemic inflammatory response (defined by a >50% reduction in plasma CRP level) and improved exercise capacity, Doppler echocardiographic parameters, and/or quality-of-life measures<sup>73,89–92</sup> (TABLE 2).

Table 2 | Clinical trials of colchicine and IL-1 blockers in AMI and heart failure

| Study (year)                                              | Indication (n)                                                                                  | Study design and drug regimen                                                                                                                                                                | Main findings                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Refs  |
|-----------------------------------------------------------|-------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| Anti-inflammatory treatment with colchicine in AMI (2015) | ST-segment elevation AMI (151)                                                                  | Randomization 1:1 colchicine or placebo; loading dose of 2 mg (initial dose of 1.5 mg followed by 0.5 mg after 1 h) and continuing treatment with 0.5 mg twice daily, or placebo, for 5 days | <ul style="list-style-type: none"> <li>Reduction in infarct size (measured using cardiac magnetic resonance and by area under the curve for cardiac markers)</li> <li>Reduction of peak CRP level</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 81    |
| Low-dose colchicine in coronary artery disease (2013)     | Stable coronary artery disease (532)                                                            | Randomization 1:1 colchicine or placebo (0.5 mg daily for a median of 2.4 years)                                                                                                             | <ul style="list-style-type: none"> <li>Reduction in combined adverse cardiovascular end points, owing to a large reduction in the incidence of acute coronary syndrome</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 82    |
| VCU-ART (2010) and VCU-ART2 (2013)                        | ST-segment elevation AMI (n = 10 for VCU-ART and n = 30 for VCU-ART2)                           | Randomization 1:1 anakinra or placebo (100 mg once daily for 14 days)                                                                                                                        | <ul style="list-style-type: none"> <li>Blunting of the inflammatory response during AMI</li> <li>Trend towards reduced incidence of adverse remodelling/heart failure at 3 months and at long-term follow-up with anakinra</li> <li>The larger VCU-ART3 clinical trial (n = 99) is ongoing and aims to evaluate two different anakinra regimens</li> </ul>                                                                                                                                                                                                                                                                                                                                      | 83–85 |
| MRC-ILA Heart Study (2015)                                | Non-ST-segment elevation AMI (182)                                                              | Randomization 1:1 anakinra or placebo (100 mg once daily for 14 days)                                                                                                                        | <ul style="list-style-type: none"> <li>Blunting of the inflammatory response during AMI</li> <li>No differences in major adverse cardiac events at 30 days and 3 months, but more events occurring after 6 months in the anakinra-treated group</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                      | 87    |
| AIR-HF (2012)                                             | Stable NYHA class II–III systolic HF with CRP >2 mg/l (10)                                      | Single-arm, open-label treatment with anakinra (100 mg once daily for 2 weeks)                                                                                                               | <ul style="list-style-type: none"> <li>Reduction in CRP levels</li> <li>Improvement in peak aerobic exercise capacity at 2 weeks</li> <li>Improvement in ventilator efficiency at 2 weeks</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 73    |
| DHART (2014)                                              | Stable NYHA class II–III diastolic HF with LVEF >50% and CRP >2 mg/l (12)                       | Randomized, double-blind, crossover trial of anakinra versus placebo for 2 weeks (100 mg given once daily for 2 weeks)                                                                       | <ul style="list-style-type: none"> <li>Reduction in CRP levels</li> <li>Improvement in peak aerobic exercise capacity at 2 weeks</li> <li>Improvement in quality-of-life scores</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 89    |
| ADHF (2016)                                               | Acute decompensated systolic HF with CRP >5 mg/l (30)                                           | Randomized, double-blind trial assessing anakinra versus placebo for 2 weeks (100 mg given twice daily for 3 days, then once daily for 2 weeks)                                              | <ul style="list-style-type: none"> <li>Reduction in CRP levels at 72 h and 14 days with anakinra</li> <li>Trend towards favourable effects on congestive signs and LVEF with anakinra</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 90    |
| REDHART (2017)                                            | Recently decompensated systolic HF with CRP >2 mg/l (within 2 weeks of hospital discharge) (60) | Randomized, double-blind trial of anakinra continued for 2 weeks, anakinra for 12 weeks, or placebo for 2 weeks (100 mg once daily)                                                          | <ul style="list-style-type: none"> <li>Reduction in CRP levels</li> <li>Improvement in peak aerobic exercise capacity and quality-of-life questionnaires with anakinra treatment given for 12 weeks</li> <li>Trend towards reduced heart failure readmissions at 6 months with 12-week anakinra treatment</li> </ul>                                                                                                                                                                                                                                                                                                                                                                            | 91    |
| CANTOS (2017)                                             | Prior AMI with CRP >2 mg/l (at least 30 days after AMI) (10,060)                                | Randomized, double-blind trial of canakinumab 50 mg, 150 mg, or 300 mg, or placebo for a median of 3.5 years (1:1:1:1.5)                                                                     | <ul style="list-style-type: none"> <li>Significant reduction in the incidence of the composite end point of cardiac death, nonfatal AMI, or nonfatal stroke with canakinumab 150 mg versus placebo</li> <li>Significant reduction in the incidence of recurrent AMI, unstable angina, and need for revascularization with canakinumab 150 mg versus placebo</li> <li>Trend towards reduced cardiac and all-cause mortality with canakinumab 150 mg versus placebo</li> <li>Small increase in the risk of fatal infections with canakinumab (all doses combined) versus placebo</li> <li>Small reduction in cancer-related mortality with canakinumab 150 mg or 300 mg versus placebo</li> </ul> | 95    |

AMI, acute myocardial infarction; CRP, C-reactive protein; HF, heart failure; LVEF, left ventricular ejection fraction; NLRP3, NACHT, LRR, and PYD domains-containing protein 3.

### Canakinumab in AMI

Canakinumab is a human monoclonal antibody developed to block IL-1 $\beta$  activity and is currently FDA-approved for the treatment of systemic juvenile idiopathic arthritis and some periodic fever syndromes, including cryopyrin-associated periodic syndromes and familial Mediterranean fever<sup>93</sup>. The efficacy of canakinumab in improving outcomes in patients with AMI was tested in the multicentre CANTOS trial<sup>94,95</sup>. Investigators of the CANTOS trial<sup>94,95</sup> enrolled 10,061 patients with prior AMI and elevated CRP level (>2 mg/l). Patients were randomly assigned to placebo or canakinumab (50, 150, or 300 mg every 3 months for a median of 3.5 years), with the goal of reducing the number of recurrent atherothrombotic events<sup>94,95</sup> (TABLE 2). Using a hierarchical statistical approach, the analysis concluded that the 150 mg canakinumab group had significantly reduced occurrence of the primary end point of cardiovascular death, AMI, and stroke (HR 0.85, 95% CI 0.74–0.98,  $P=0.021$ ) compared with placebo<sup>95</sup>. These patients also had significantly fewer instances of the secondary end point, which included unstable angina leading to revascularization (HR 0.83,  $P=0.005$ ), as well as the composite end point of death from any cause, AMI, and stroke (HR 0.85,  $P=0.01$ ). The benefit in the composite end point was largely driven by a reduction in nonfatal AMI (HR 0.76,  $P=0.005$ ). Treatment with canakinumab was associated with an overall favourable safety profile with no significant excess in serious adverse events<sup>95</sup>.

No data on AMI-related or heart-failure-related morbidity or mortality were reported. These results from the CANTOS trial show, for the first time, that specifically targeting an inflammatory mediator can improve cardiovascular and mortality outcomes in patients with AMI. To date, these data are the strongest evidence supporting the proposed role of the NLRP3 inflammasome and IL-1 $\beta$  in the pathogenesis of heart disease.

### Conclusions

The NLRP3 inflammasome is a ubiquitous intracellular pattern recognition receptor that is critically involved in the response to injury during AMI and is responsible for the production of IL-1 $\beta$  and the ensuing systemic inflammatory response. Experimental studies in small and large animals show that NLRP3 inflammasome-targeted therapies might be a viable strategy for the reduction of infarct size and prevention of heart failure following AMI. However, no selective NLRP3 inhibitors are clinically available at present. Currently, IL-1 blockers are being explored in patients with AMI. In small phase II trials in AMI and heart failure, anakinra was well tolerated and effectively blunted the acute inflammatory response. In a large, multicentre, phase III clinical trial of patients with prior AMI and residual inflammatory risk, canakinumab reduced the risk of recurrent acute coronary syndromes and need for revascularization. Additional studies with selective NLRP3 inhibitors are eagerly awaited.

- Anderson, J. L. & Morrow, D. A. Acute myocardial infarction. *N. Engl. J. Med.* **376**, 2053–2064 (2017).
- Eapen, Z. J. *et al.* Defining heart failure end points in ST-segment elevation myocardial infarction trials: integrating past experiences to chart a path forward. *Circ. Cardiovasc. Qual. Outcomes* **5**, 594–600 (2012).
- Seropian, I. M., Toldo, S., van Tassell, B. W. & Abbate, A. Anti-inflammatory strategies for ventricular remodeling following ST-segment elevation acute myocardial infarction. *J. Am. Coll. Cardiol.* **63**, 1593–1603 (2014).
- Westman, P. C. *et al.* Inflammation as a driver of adverse left ventricular remodeling after acute myocardial infarction. *J. Am. Coll. Cardiol.* **67**, 2050–2060 (2016).
- Gao, X. M., White, D. A., Dart, A. M. & Du, X. J. Post-infarct cardiac rupture: recent insights on pathogenesis and therapeutic interventions. *Pharmacol. Ther.* **134**, 156–179 (2012).
- Abbate, A. *et al.* Alterations in the interleukin-1/interleukin-1 receptor antagonist balance modulate cardiac remodeling following myocardial infarction in the mouse. *PLoS ONE* **6**, e27923 (2011).
- Savvatis, K. *et al.* Interleukin-23 deficiency leads to impaired wound healing and adverse prognosis after myocardial infarction. *Circ. Heart Fail.* **7**, 161–171 (2014).
- Toldo, S. *et al.* The inflammasome in myocardial injury and cardiac remodeling. *Antioxid. Redox Signal.* **22**, 1146–1161 (2015).
- Sutterwala, F. S., Haasken, S. & Cassel, S. L. Mechanism of NLRP3 inflammasome activation. *Ann. NY Acad. Sci.* **1319**, 82–95 (2014).
- Mezzaroma, E. *et al.* The inflammasome promotes adverse cardiac remodeling following acute myocardial infarction in the mouse. *Proc. Natl Acad. Sci. USA* **108**, 19725–19730 (2011).
- Lu, A. *et al.* Unified polymerization mechanism for the assembly of ASC-dependent inflammasomes. *Cell* **156**, 1193–1206 (2014).
- Dinarello, C. A. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* **117**, 3720–3732 (2011).
- Westermann, D. *et al.* Cardioprotective and anti-inflammatory effects of interleukin converting enzyme inhibition in experimental diabetic cardiomyopathy. *Diabetes* **56**, 1834–1841 (2007).
- Rider, P., Carmi, Y., Voronov, E. & Apte, R. N. Interleukin-1 $\alpha$ . *Semin. Immunol.* **25**, 430–438 (2013).
- Gross, O. *et al.* Inflammasome activators induce interleukin-1 $\alpha$  secretion via distinct pathways with differential requirement for the protease function of caspase-1. *Immunity* **36**, 388–400 (2012).
- Liu, X. *et al.* Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature* **535**, 153–158 (2016).
- Shao, W., Veretssian, G., Doiron, K., Hussain, S. N. & Saleh, M. The caspase-1 digestome identifies the glycolysis pathway as a target during infection and septic shock. *J. Biol. Chem.* **282**, 36321–36329 (2007).
- Toldo, S. *et al.* Independent roles of the priming and the triggering of the NLRP3 inflammasome in the heart. *Cardiovasc. Res.* **105**, 203–212 (2015).
- Kawaguchi, M. *et al.* Inflammasome activation of cardiac fibroblasts is essential for myocardial ischemia/reperfusion injury. *Circulation* **123**, 594–604 (2011).
- Liu, A., Gao, X., Zhang, Q. & Cui, L. Cathepsin B inhibition attenuates cardiac dysfunction and remodeling following myocardial infarction by inhibiting the NLRP3 pathway. *Mol. Med. Rep.* **8**, 361–366 (2013).
- He, Y., Zeng, M. Y., Yang, D., Motro, B. & Núñez, G. NEK7 is an essential mediator of NLRP3 activation downstream of potassium efflux. *Nature* **530**, 354–357 (2016).
- Liu, Y. *et al.* TXNIP mediates NLRP3 inflammasome activation in cardiac microvascular endothelial cells as a novel mechanism in myocardial ischemia/reperfusion injury. *Basic Res. Cardiol.* **109**, 415 (2014).
- Ito, M. *et al.* Bruton's tyrosine kinase is essential for NLRP3 inflammasome activation and contributes to ischaemic brain injury. *Nat. Commun.* **6**, 7360 (2015).
- Starkov, A. A. The role of mitochondria in reactive oxygen species metabolism and signaling. *Ann. NY Acad. Sci.* **1147**, 37–52 (2008).
- Kim, J. S., He, L., Olan, T. & Lemasters, J. J. Role of the mitochondrial permeability transition in apoptotic and necrotic death after ischemia/reperfusion injury to hepatocytes. *Curr. Mol. Med.* **3**, 527–535 (2003).
- Zhou, R., Yazdi, A. S., Menu, P. & Tschopp, J. A role for mitochondria in NLRP3 inflammasome activation. *Nature* **469**, 221–225 (2011).
- Sadatomi, D. *et al.* Mitochondrial function is required for extracellular ATP-induced NLRP3 inflammasome activation. *J. Biochem.* **161**, 503–512 (2017).
- Sun, Q., Fan, J., Billiar, T. R. & Scott, M. J. Inflammasome and autophagy regulation — a two-way street. *Mol. Med.* <http://dx.doi.org/10.2119/molmed.2017.00077> (2017).
- Park, S. *et al.* Defective mitochondrial fission augments NLRP3 inflammasome activation. *Sci. Rep.* **5**, 15489 (2015).
- Mills, E. L., Kelly, B., O'Neill, L. A. J. Mitochondria are the powerhouses of immunity. *Nat. Immunol.* **18**, 488–498 (2017).
- Iyer, S. S. *et al.* Mitochondrial cardiolipin is required for Nlrp3 inflammasome activation. *Immunity* **39**, 311–323 (2013).
- Shi, C. S. *et al.* Activation of autophagy by inflammatory signals limits IL-1 $\beta$  production by targeting ubiquitinated inflammasomes for destruction. *Nat. Immunol.* **13**, 255–263 (2012).
- Wu, X. *et al.* Impaired autophagy contributes to adverse cardiac remodeling in acute myocardial infarction. *PLoS ONE* **9**, e112891 (2014).
- Netea, M. G. *et al.* Differential requirement for the activation of the inflammasome for processing and release of IL-1 $\beta$  in monocytes and macrophages. *Blood* **113**, 2324–2335 (2009).
- Mezzaroma, E., Toldo, S. & Abbate, A. Role of NLRP3 (cryopyrin) in acute myocardial infarction. *Cardiovasc. Res.* **99**, 225–226 (2013).
- Wang, L., Qu, P., Zhao, J. & Chang, Y. NLRP3 and downstream cytokine expression elevated in the monocytes of patients with coronary artery disease. *Arch. Med. Sci.* **10**, 791–800 (2014).

37. Toldo, S. *et al.* Inhibition of the NLRP3 inflammasome limits the inflammatory injury following myocardial ischemia-reperfusion in the mouse. *Int. J. Cardiol.* **209**, 215–220 (2016).
38. Sandanger, Ø. *et al.* NLRP3 inflammasome activation during myocardial ischemia reperfusion is cardioprotective. *Biochem. Biophys. Res. Commun.* **469**, 1012–1020 (2016).
39. Marchetti, C. *et al.* A novel pharmacologic inhibitor of the NLRP3 inflammasome limits myocardial injury after ischemia-reperfusion in the mouse. *J. Cardiovasc. Pharmacol.* **63**, 316–322 (2014).
40. Mastrocola, R. *et al.* Pharmacological inhibition of NLRP3 inflammasome attenuates myocardial ischemia/reperfusion injury by activation of RISK and mitochondrial pathways. *Oxid. Med. Cell. Longev.* **2016**, 5271251 (2016).
41. Takahashi, M. NLRP3 inflammasome as a novel player in myocardial infarction. *Int. Heart J.* **55**, 101–105 (2014).
42. Mezzaroma, E., Marchetti, C. & Toldo, S. Letter by Mezzaroma, E. *et al.* regarding article, “NLRP3 inflammasome as a therapeutic target in myocardial infarction”. *Int. Heart J.* **55**, 379 (2014).
43. Li, X. *et al.* NOD2 deficiency protects against cardiac remodeling after myocardial infarction in mice. *Cell. Physiol. Biochem.* **32**, 1857–1866 (2013).
44. Sandanger, Ø. *et al.* The NLRP3 inflammasome is up-regulated in cardiac fibroblasts and mediates myocardial ischaemia-reperfusion injury. *Cardiovasc. Res.* **99**, 164–174 (2013).
45. Jong, W. M. *et al.* Nlrp3 plays no role in acute cardiac infarction due to low cardiac expression. *Int. J. Cardiol.* **177**, 41–43 (2014).
46. Lamkanfi, M. *et al.* Glyburide inhibits the cryopyrin/Nalp3 inflammasome. *J. Cell Biol.* **187**, 61–70 (2009).
47. Marchetti, C. *et al.* Pharmacologic inhibition of the NLRP3 inflammasome preserves cardiac function after ischemic and nonischemic injury in the mouse. *J. Cardiovasc. Pharmacol.* **66**, 1–8 (2015).
48. Marchetti, C., Swartzwelder, B., Koenders, M., Dinarello, C. A. & Joosten, L. A. OP0090 The human safe NLRP3 inflammasome inhibitor OLT1177 suppresses joint inflammation in murine models of experimental arthritis. *Ann. Rheum. Dis.* **76**, 89 (2017).
49. OLATEC Lead compound. OLATEC <http://www.olatec.com/lead-compound.html> (2017).
50. Juliana, C. *et al.* Anti-inflammatory compounds parthenolide and Bay 11–7082 are direct inhibitors of the inflammasome. *J. Biol. Chem.* **285**, 9792–9802 (2010).
51. Kim, Y. S. *et al.* BAY 11–7082, a nuclear factor- $\kappa$ B inhibitor, reduces inflammation and apoptosis in a rat cardiac ischemia-reperfusion injury model. *Int. Heart J.* **51**, 348–353 (2010).
52. Cocco, M. *et al.* Design, synthesis, and evaluation of acrylamide derivatives as direct NLRP3 inflammasome inhibitors. *ChemMedChem.* **11**, 1790–1803 (2016).
53. Coll, R. C. *et al.* A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. *Nat. Med.* **21**, 248–255 (2015).
54. van Hout, G. P. *et al.* The selective NLRP3-inflammasome inhibitor MCC950 reduces infarct size and preserves cardiac function in a pig model of myocardial infarction. *Eur. Heart J.* **38**, 828–836 (2017).
55. Mauro, A. G., Thurber, C. & Abbate, A. Colchicine in acute myocardial infarction: “teaching new tricks to an old dog”. *Transl Med.* **5**, e133 (2015).
56. Leung, Y. Y., Yao Hui, L. L. & Kraus, V. B. Colchicine — update on mechanisms of action and therapeutic uses. *Semin. Arthritis Rheum.* **45**, 341–350 (2015).
57. Misawa, T. *et al.* Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome. *Nat. Immunol.* **14**, 454–460 (2013).
58. Fujisue, K. *et al.* Colchicine improves survival, left ventricular remodeling, and chronic cardiac function after acute myocardial infarction. *Circ. J.* **81**, 1174–1182 (2017).
59. Toldo, S., Marchetti, C. & Abbate, A. Re. “NLRP3 inflammasome activation during myocardial ischemia reperfusion is cardioprotective”. *Biochem. Biophys. Res. Commun.* **470**, 811–812 (2016).
60. Sager, H. B. *et al.* Targeting interleukin-1 $\beta$  reduces leukocyte production after acute myocardial infarction. *Circulation* **132**, 1880–1890 (2015).
61. van Tassell, B. W., Toldo, S., Mezzaroma, E. & Abbate, A. Targeting interleukin-1 in heart disease. *Circulation* **128**, 1910–1923 (2013).
62. Abbate, A. *et al.* Anakinra a recombinant human interleukin-1 receptor antagonist, inhibits apoptosis in experimental acute myocardial infarction. *Circulation* **117**, 2670–2683 (2008).
63. Toldo, S. *et al.* Interleukin-1 $\beta$  blockade improves cardiac remodeling after myocardial infarction without interrupting the inflammasome in the mouse. *Exp. Physiol.* **98**, 734–745 (2013).
64. Toldo, S. *et al.* Interleukin-1 $\beta$  blockade improves left ventricular systolic/diastolic function and restores contractility reserve in severe ischemic cardiomyopathy in the mouse. *J. Cardiovasc. Pharmacol.* **64**, 1–6 (2014).
65. Lugrin, J. *et al.* Cutting edge: IL-1 $\alpha$  is a crucial danger signal triggering acute myocardial inflammation during myocardial infarction. *J. Immunol.* **194**, 499–503 (2015).
66. Mauro, A. G. *et al.* Reduction of myocardial ischemia-reperfusion injury by inhibiting interleukin-1 alpha. *J. Cardiovasc. Pharmacol.* **69**, 156–160 (2017).
67. O’Brien, L. C. *et al.* Interleukin-18 as a therapeutic target in acute myocardial infarction and heart failure. *Mol. Med.* **20**, 221–229 (2014).
68. Pomerantz, B. J., Reznikov, L. L., Harken, A. H. & Dinarello, C. A. Inhibition of caspase 1 reduces human myocardial ischemic dysfunction via inhibition of IL-18 and IL-1 $\beta$ . *Proc. Natl Acad. Sci. USA* **98**, 2871–2876 (2001).
69. Venkatachalam, K. *et al.* Neutralization of interleukin-18 ameliorates ischemia/reperfusion-induced myocardial injury. *J. Biol. Chem.* **284**, 7853–7865 (2009).
70. Gu, H. *et al.* The protective role of interleukin-18 binding protein in a murine model of cardiac ischemia/reperfusion injury. *Transpl. Int.* **28**, 1436–1444 (2015).
71. Pörksen, G. *et al.* Periodic fever, mild arthralgias, and reversible moderate and severe organ inflammation associated with the V198M mutation in the CIAS1 gene in three German patients — expanding phenotype of CIAS1 related autoinflammatory syndrome. *Eur. J. Haematol.* **73**, 123–127 (2004).
72. Kumar, A. *et al.* Tumor necrosis factor alpha and interleukin 1beta are responsible for *in vitro* myocardial cell depression induced by human septic shock serum. *J. Exp. Med.* **183**, 949–958 (1996).
73. Van Tassell, B. W. *et al.* Enhanced interleukin-1 activity contributes to exercise intolerance in patients with systolic heart failure. *PLoS ONE* **7**, e33438 (2012).
74. Van Tassell, B. W., Seropian, I. M., Toldo, S., Mezzaroma, E. & Abbate, A. Interleukin-1 $\beta$  induces a reversible cardiomyopathy in the mouse. *Inflamm. Res.* **62**, 637–640 (2013).
75. Zhang, W. *et al.* Cardiac fibroblasts contribute to myocardial dysfunction in mice with sepsis: the role of NLRP3 inflammasome activation. *PLoS ONE* **9**, e107639 (2014).
76. Bracey, N. A. *et al.* The Nlrp3 inflammasome promotes myocardial dysfunction in structural cardiomyopathy through interleukin-1 $\beta$ . *Exp. Physiol.* **98**, 462–472 (2013).
77. Abbate, A. The heart on fire: inflammasome and cardiomyopathy. *Exp. Physiol.* **98**, 585 (2013).
78. Wang, Y., Gao, B. & Xiong, S. Involvement of NLRP3 inflammasome in CVB3-induced viral myocarditis. *Am. J. Physiol. Heart Circ. Physiol.* **307**, H1438–H1447 (2014).
79. Li, R. *et al.* Triptolide attenuates pressure overload-induced myocardial remodeling in mice via the inhibition of NLRP3 inflammasome expression. *Biochem. Biophys. Res. Commun.* **485**, 69–75 (2017).
80. Seropian, I. M., Sonnino, C., Van Tassell, B. W., Biasucci, L. M. & Abbate, A. Inflammatory markers in STElevation acute myocardial infarction. *Eur. Heart J. Acute Cardiovasc. Care* **5**, 382–395 (2016).
81. Devereux, S. *et al.* Anti-inflammatory treatment with colchicine in acute myocardial infarction: a pilot study. *Circulation* **132**, 1395–1403 (2015).
82. Nidorf, S. M., Eikelboom, J. W., Budgeon, C. A. & Thompson, P. L. Low-dose colchicine for secondary prevention of cardiovascular disease. *J. Am. Coll. Cardiol.* **61**, 404–410 (2013).
83. Abbate, A. *et al.* Interleukin-1 blockade with anakinra to prevent adverse cardiac remodeling after acute myocardial infarction (Virginia Commonwealth University Anakinra Remodeling Trial [VCU-ART] Pilot study). *Am. J. Cardiol.* **105**, 1371–1377 (2010).
84. Abbate, A. *et al.* Effects of interleukin-1 blockade with anakinra on adverse cardiac remodeling and heart failure after acute myocardial infarction [from the Virginia Commonwealth University-Anakinra Remodeling Trial (2) [VCU-ART2] pilot study]. *Am. J. Cardiol.* **111**, 1394–1400 (2013).
85. Abbate, A. *et al.* Comparative safety of Interleukin-1 blockade with anakinra in patients with ST-segment elevation acute myocardial infarction (from the VCU-ART and VCU-ART2 pilot studies). *Am. J. Cardiol.* **115**, 288–292 (2015).
86. US National Library of Medicine. *ClinicalTrials.gov* <https://www.clinicaltrials.gov/ct2/show/NCT01950299> (2017).
87. Morton, A. C. *et al.* The effect of interleukin-1 receptor antagonist therapy on markers of inflammation in non-ST elevation acute coronary syndromes: the MRC-ILA Heart Study. *Eur. Heart J.* **36**, 377–384 (2015).
88. Abbate, A. & Dinarello, C. A. Anti-inflammatory therapies in acute coronary syndromes: is IL-1 blockade a solution? *Eur. Heart J.* **36**, 337–339 (2015).
89. Van Tassell, B. W. *et al.* Effects of interleukin-1 blockade with anakinra on aerobic exercise capacity in patients with heart failure and preserved ejection fraction (from the D-HART pilot study). *Am. J. Cardiol.* **113**, 321–327 (2014).
90. Van Tassell, B. W. *et al.* Interleukin-1 blockade in acute decompensated heart failure: a randomized, double-blind, placebo-controlled pilot study. *J. Cardiovasc. Pharmacol.* **67**, 544–551 (2016).
91. Van Tassell, B. *et al.* Interleukin-1 blockade in recently decompensated systolic heart failure: results from the REcently Decompensated Heart failure Anakinra Response trial (REDHART). *Circ. Heart Fail.* (in press).
92. Van Tassell, B. *et al.* Interleukin-1 blockade in heart failure with preserved ejection fraction: rationale and design of the Diastolic Heart failure Anakinra Response Trial 2 (DHART2). *Clin. Cardiol.* **40**, 626–632 (2017).
93. ILARIS. Learn how prescription ILARIS may help. *ILARIS* <http://www.ilaris.com/index.jsp> (2017).
94. Ridker, P. M., Thuren, T., Zalewski, A. & Libby, P. Interleukin-1 $\beta$  inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). *Am. Heart J.* **162**, 597–605 (2011).
95. Ridker, P. M. *et al.* Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N. Engl. J. Med.* **377**, 1119–1131 (2017).

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## Author contributions

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