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(71) Applicant: **NOVARTIS AG** [CH/CH]; Lichtstrasse 35, 4056 Basel (CH).

(72) Inventors: **CHUTKOW, William**; Novartis Institutes for Biomedical Research, Inc., 250 Massachusetts Avenue, Cambridge, Massachusetts 02139 (US). **HEALEY, Margaret L.**; Novartis Institutes for Biomedical Research, Inc., 250 Massachusetts Avenue, Cambridge, Massachusetts 02139 (US). **SVENSSON, JR., Eric C.**; 619 S. Elmwood Avenue, Oak Park, Illinois 60304 (US). **XU, Huilei**; Novartis Institutes for Biomedical Research, Inc., 250 Massachusetts Avenue, Cambridge, Massachusetts 02139 (US).

(74) Agent: **NOVARTIS AG**; Lichtstrasse 35, 4056 Basel (CH).

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(54) Title: METHODS OF SELECTING AND TREATING PATIENTS AT ELEVATED RISK OF MAJOR ADVERSE CARDIAC EVENTS

(57) Abstract: The present description relates to canakinumab for use in reducing the risk of or preventing cardiovascular (CV) events such as major adverse cardiovascular events (MACE) in a patient with a gain-of-function mutation in *P2RX7*. Such a patient may, for example, have one or more copies of rs1718119 (e.g., may be homozygous for rs1718119). The patient may have elevated hsCRP levels; and/or may have previously suffered a myocardial infarction (MI).

METHODS OF SELECTING AND TREATING PATIENTS AT ELEVATED RISK OF MAJOR ADVERSE CARDIAC EVENTS

5 TECHNICAL FIELD

The present application relates to predictive methods, personalized therapies, kits, transmittable forms of information, uses, and methods for treating patients having or at risk of having cardiovascular events such as major adverse cardiac events (MACE) and having a gain-of-function mutation in P2X7.

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BACKGROUND

Cardiovascular disease (CVD) remains the leading cause of mortality and morbidity worldwide. Atherosclerosis is responsible for the majority of cardiovascular disorders with inflammation as one of its driving processes. Atherothrombosis is characterized by atherosclerotic lesion disruption with superimposed thrombus formation and is the major cause of acute coronary syndromes (ACS) and cardiovascular death. Arterial inflammation and endothelial dysfunction play key roles at all stages of the atherothrombotic process. Inflammatory mediators are intimately implicated with the cascade of events leading to atherosclerotic plaque initiation, progression, and rupture. Vascular endothelial cells express a variety of adhesion molecules that recruit monocytes when chronically exposed to noxious stimuli or pathological conditions. Adverse conditions such as hyperlipidemia are associated with enrichment of a pro-inflammatory subset of monocytes. These monocytes apparently enter the intima under the influence of chemotactic stimuli and engulf modified low density lipoprotein (LDL) and cholesterol crystals (Düwell P *et al*, Nature. 2010; 464(7293): 1357-61). The material internalized by phagocytes induces phagolysosomal damage and subsequent leakage of contents into cytosol to activate inflammasomes, including the nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome.

The P2X7 (also known as P2X₇) receptor, encoded by the *P2RX7* gene, is a member of a family of cationic channels that plays a critical role in mediating disparate physiological functions of extracellular ATP, including the regulation of immune responses and inflammation. See: North RA, Physiol Rev 2002, 82:1013-1067; Hattori and Gouaux, Nature 2012, 485:207-212. Activation of P2X7 receptors has been shown to trigger maturation of the NLRP3 inflammasome and release of certain inflammatory mediators including IL1 β , TNF α , and PGE₂. See: Di VF, Trends Pharmacol Sci 2007, 28:465-472; Surprenant *et al.*, Science 1996, 272:735-738; Ferrari *et al.*, J Immunol 1997, 159:1451-1458; MacKenzie A, Immunity 2001, 15:825-835; Solle M *et al.*, J Biol Chem 2001, 276:125-132. Gain-of-function (GOF) and loss-of-function (LOF) SNPs in the *P2RX7*

gene have been demonstrated to be responsible of GOF and LOF phenotypes in the P2X7 receptor. See Ursu *et al.*, Molecular Pain 2014, 10:37.

Interleukins such as interleukin 1 beta (IL-1beta) are key mediators in the chronic vascular inflammatory response in cardiovascular (CV) disease and have been demonstrated in animal models and in humans to be potent modulators of pro-inflammatory processes. The fact that these cytokines and their receptors are highly expressed and are functional in almost all cell types implicated in the pathogenesis of atherosclerosis including smooth muscle cells, certain subset of macrophages and T cells as well as endothelium supports the role of interleukins in vascular disease. The nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome appears to be activated by cholesterol crystals or hypoxia, subsequently promoting cleavage and secretion of IL-1beta and IL-18 and leading to atherosclerotic deposits in animal models. See Satish and Agrawal, Translational Research 2020, 215:75-85.

Despite the success of statin therapy in reducing hyperlipidemia and thereby lowering the risk of myocardial infarction, stroke and cardiovascular death, many patients, including post-myocardial infarction patients receiving statin therapy, continue to suffer from life threatening vascular events. This high risk for cardiovascular events, such as recurrent cardiovascular events, despite the use of aggressive secondary prevention strategies is at least partly due to inflammation (Ridker PM. Eur Heart J. 2016;37(22):1720-2). Thus, therapies that decrease inflammation, improve vascular function, decrease atherosclerotic burden, and ultimately translate to a decrease in cardiovascular events (including recurring cardiovascular events), especially for those patients in higher risk groups, present a significant unmet medical need. Additionally, there is a significant unmet medical need for methods of identifying patients for whom such therapies would be most effective, especially for those patients in higher risk groups.

SUMMARY

Inflammation contributes to all phases of the atherothrombotic process and certain patients have increased vascular risk despite use of aggressive secondary prevention strategies. The present disclosure relates, in part, to the finding that direct inhibition of inflammation by administration of an NLRP3 inflammasome pathway inhibitor (canakinumab, an anti-IL1beta antibody) reduces the risk of or prevents recurrence of cardiovascular events in patients with a gain-of-function mutation in the *P2RX7* gene (*e.g.*, patients who are homozygous for SNP rs1718119). Such patients may be post-myocardial infarction patients. Such patients may be, for example, patients who have already had one or more myocardial infarctions and/or have an hsCRP level of at least 2 mg/L.

Since downregulation of IL-1beta reduces the risk of or prevents recurrence of cardiovascular events in patients who are positive for a gain-of-function mutation in *P2RX7* (*e.g.*, patients who are homozygous for SNP rs1718119), it is expected that inhibition of the NLRP3 inflammasome pathway

(which results in the release of IL-1 β cytokines) also reduces the risk of or prevents recurrence of cardiovascular events (including major adverse cardiac events (MACE)) in such patients. In particular, inhibition of upstream NLRP3 pathway proteins (*e.g.*, inhibition of the NLRP3 protein with an NLRP3 inhibitor) is expected to be useful in the treatment of, reducing the risk of, or preventing recurrence of, cardiovascular events such as major adverse cardiac events (MACE) in patients. Such patients may be post-myocardial infarction patients. Such patients may be, for example, patients who have already had one or more myocardial infarctions and/or have an hsCRP level of at least 2 mg/L.

Accordingly, described herein are methods for reducing the risk of or preventing cardiovascular (CV) events such as major adverse cardiac events (MACE) in a subject that has one or more gain-of-function mutations in *P2RX7*, *e.g.*, a subject that is homozygous for the rs1718119 SNP. In some embodiments, the subject may have had a previous MI. In some embodiments, the subject may have an hsCRP level of at least 2 mg/L. In some embodiments, the subject may have had a previous MI and may have an hsCRP level of at least 2 mg/L. In some embodiments, the subject may be administered an effective amount of at least one inflammasome inhibitor. In some embodiments, the NLRP3 inflammasome pathway inhibitor is selected from: an NLRP3 inhibitor, an IL-1 β inhibitor, an IL-6 inhibitor, an IL-18 inhibitor, or a combination thereof (*e.g.*, a dual IL-1 β /IL-18 inhibitor). In some embodiments, the NLRP3 inflammasome pathway inhibitor is selected from: an NLRP3 inhibitor, an anti-IL-1 β antibody, an anti-IL-6 antibody, an anti-IL-18 antibody, and an anti IL-1 β /IL-18 bispecific antibody. In some embodiments, the NLRP3 inflammasome pathway inhibitor is an NLRP3 inhibitor, optionally wherein the NLRP3 inhibitor is a compound of any one of Formulae 101A, 102A, 1AA, 2AA, 3AA, 4AA, 5AA and 6AA. In some embodiments, the NLRP3 inflammasome pathway inhibitor is an anti-IL-1 β antibody, optionally wherein the anti-IL-1 β antibody is canakinumab. In some embodiments, the NLRP3 inflammasome pathway inhibitor is an anti-IL-18 antibody. In some embodiments, the NLRP3 inflammasome pathway inhibitor is an anti IL-1 β /IL-18 bispecific antibody.

In one aspect, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7* (*e.g.*, a subject that is homozygous for the rs1718119 SNP), wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject reduces the incidence of a major adverse cardiovascular event (MACE), comprising: (a) assaying a biological sample from the subject for at least one of: a gain-of-function mutation of *P2RX7*, the level of *P2RX7* expression, the level of P2X7 protein, or the level of P2X7 activity; and (b) thereafter, selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has at least one of: a gain-of-function mutation of *P2RX7*, an increased level of *P2RX7* expression, an increased level of P2X7 protein, or an increased level of P2X7 activity relative to a control.

In one aspect, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7* (e.g., a subject that is homozygous for the rs1718119 SNP), wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject reduces the incidence of a major adverse cardiovascular event (MACE), comprising: (a) assaying an isolated biological sample from the subject for at least one of: a gain-of-function mutation of *P2RX7*, the level of *P2RX7* expression, the level of P2X7 protein, or the level of P2X7 activity; and (b) thereafter, selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has at least one of: a gain-of-function mutation of *P2RX7*, an increased level of *P2RX7* expression, an increased level of P2X7 protein, or an increased level of P2X7 activity relative to a control.

In one aspect, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7* (e.g., a subject that is homozygous for the rs1718119 SNP), wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject reduces the incidence of a major adverse cardiovascular event (MACE), comprising: (a) assaying a biological sample from the subject for at least one of: a gain-of-function mutation of *P2RX7*, the level of *P2RX7* expression, the level of P2X7 protein, or the level of P2X7 activity; and (b) thereafter, selecting the subject for administration of a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has at least one of: a gain-of-function mutation of *P2RX7*, an increased level of *P2RX7* expression, an increased level of P2X7 protein, or an increased level of P2X7 activity relative to a control.

In one aspect, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7* (e.g., a subject that is homozygous for the rs1718119 SNP), wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject reduces the incidence of a major adverse cardiovascular event (MACE), comprising: (a) assaying an isolated biological sample from the subject for at least one of: a gain-of-function mutation of *P2RX7*, the level of *P2RX7* expression, the level of P2X7 protein, or the level of P2X7 activity; and (b) thereafter, selecting the subject for administration of a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has at least one of: a gain-of-function mutation of *P2RX7*, an increased level of *P2RX7* expression, an increased level of P2X7 protein, or an increased level of P2X7 activity relative to a control.

In one aspect, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7* (e.g., a subject that is homozygous for the rs1718119 SNP), wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject improves the hazard risks of major adverse cardiovascular event (MACE) incidents for the subject, comprising: (a) assaying a biological sample from the subject for at least one of: a gain-of-function mutation of *P2RX7*, the level of *P2RX7* expression, the level of P2X7 protein, or the level P2X7 activity; and (b) thereafter,

selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has at least one of: a gain-of-function mutation of *P2RX7*, an increased level of *P2RX7* expression, an increased level of P2X7 protein, or an increased level of P2X7 activity relative to a control.

5 In one aspect, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7* (e.g., a subject that is homozygous for the rs1718119 SNP), wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject improves the hazard risks of major adverse cardiovascular event (MACE) incidents for the subject, comprising: (a) assaying an isolated biological sample from the subject for at least one of: a gain-of-function mutation of *P2RX7*,
10 the level of *P2RX7* expression, the level of P2X7 protein, or the level P2X7 activity; and (b) thereafter, selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has at least one of: a gain-of-function mutation of *P2RX7*, an increased level of *P2RX7* expression, an increased level of P2X7 protein, or an increased level of P2X7 activity relative to a control.

15 In one aspect, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7* (e.g., a subject that is homozygous for the rs1718119 SNP), wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject improves the hazard risks of major adverse cardiovascular event (MACE) incidents for the subject, comprising: (a) assaying a biological sample from the subject for at least one of: a gain-of-function mutation of *P2RX7*, the level
20 of *P2RX7* expression, the level of P2X7 protein, or the level P2X7 activity; and (b) thereafter, selecting the subject for administration of a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has at least one of: a gain-of-function mutation of *P2RX7*, an increased level of *P2RX7* expression, an increased level of P2X7 protein, or an increased level of P2X7 activity relative to a control.

25 In one aspect, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7* (e.g., a subject that is homozygous for the rs1718119 SNP), wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject improves the hazard risks of major adverse cardiovascular event (MACE) incidents for the subject, comprising: (a) assaying an isolated biological sample from the subject for at least one of: a gain-of-function mutation of *P2RX7*,
30 the level of *P2RX7* expression, the level of P2X7 protein, or the level P2X7 activity; and (b) thereafter, selecting the subject for administration of a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has at least one of: a gain-of-function mutation of *P2RX7*, an increased level of *P2RX7* expression, an increased level of P2X7 protein, or an increased level of P2X7 activity relative to a control.

35 In some embodiments, the increased expression and/or levels are relative to those in a subject with zero copies of a *P2RX7* gain of function mutation (e.g., a subject that does not have the

rs1718119 SNP). In some embodiments, the increased expression and/or levels are relative to those in a subject with one copy of a *P2RX7* gain of function mutation (e.g., a subject that is heterozygous for the rs1718119 SNP and does not have any additional gain-of-function mutations in *P2RX7*).

In some embodiments, the gain-of-function mutation is an rs1718119 SNP.

5 In one aspect, provided herein is a method of selectively treating a subject having an increased risk of MACE, comprising selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject on the basis of the subject having at least one copy of a gain of function mutation of *P2RX7* (e.g., having one copy or two copies of a gain of function mutation of *P2RX7*).

10 In one aspect, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: (a) selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of a the subject having at least one copy of a gain of function mutation of *P2RX7* (e.g., an rs1718119 SNP); and (b) thereafter, administering a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor to the subject. In one aspect, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: (a) selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of a the subject having two copies of a gain of function mutation of *P2RX7* (e.g., an rs1718119 SNP); and (b) thereafter, administering a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor to the subject.

20 In one aspect, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: (a) selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of a the subject having two copies of a gain of function mutation of *P2RX7* (e.g., a subject that is homozygous for the rs1718119 SNP); and (b) thereafter, administering a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor to the subject.

25 In one aspect, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: (a) assaying a biological sample from the subject for the presence of at least one copy of a gain of function mutation of *P2RX7* (e.g., the rs1718119 SNP); and (b) subsequently selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of the biological sample from the subject having the at least one copy of a gain of function mutation of *P2RX7*. In one embodiment, the method further comprises: (c) subsequently administering a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor to the subject.

30 In one aspect, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: (a) assaying a

biological sample from the subject for the presence of two copies of a gain of function mutation of *P2RX7* (e.g., the rs1718119 SNP); and (b) subsequently selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of the biological sample from the subject having two copies of a gain of function mutation of *P2RX7*. In one embodiment, the method further comprises: (c) subsequently administering a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor to the subject.

In one aspect, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of a biological sample from the subject has at least one copy of a gain of function mutation of *P2RX7*. In one embodiment, the method further comprises: (c) subsequently administering a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor to the subject.

In one aspect, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis that a biological sample from the subject has two copies of a gain of function mutation of *P2RX7* (e.g., the subject is homozygous for the rs1718119 SNP). In one embodiment, the method further comprises: (c) subsequently administering a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor to the subject.

In one aspect, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: (a) assaying a biological sample from the subject for the presence of two copies of a gain of function mutation of *P2RX7* (e.g., homozygosity of the rs1718119 SNP); and (b) subsequently selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of the biological sample from the subject having two copies of a gain of function mutation of *P2RX7* (e.g., homozygosity of the rs1718119 SNP). In one embodiment, the method further comprises: (c) subsequently administering a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor to the subject.

In one aspect, provided herein is a method of predicting the likelihood that a subject having an increased risk of MACE will respond to treatment with an NLRP3 inflammasome pathway inhibitor, comprising assaying a biological sample from the subject for the presence of at least one copy of a gain of function mutation of *P2RX7*, wherein the presence of the at least one copy of a gain of function mutation of *P2RX7* is indicative of an increased likelihood that the subject will respond to treatment with the NLRP3 inflammasome pathway inhibitor.

In some embodiments, the subject has two copies of the gain of function mutation of *P2RX7* (e.g., the subject is homozygous for the rs1718119 SNP).

In some embodiments, the method further comprises the step of obtaining the biological sample from the subject, wherein the step of obtaining is performed prior to the step of assaying.

In some embodiments, the step of assaying comprises assaying the biological sample for a nucleic acid product of the gain of function mutation of *P2RX7*.

5 In some embodiments, the step of assaying comprises assaying the biological sample for a genomic sequence of the gain of function mutation of *P2RX7*.

In some embodiments, the gain of function mutation of *P2RX7* is an rs1718119 allele.

In any of the aspects or embodiments described herein, the subject may be homozygous for the rs1718119 SNP.

10 In some embodiments, the biological sample is selected from the group consisting of synovial fluid, blood, serum, feces, plasma, urine, tear, saliva, cerebrospinal fluid, a leukocyte sample, and a tissue sample.

In any of the aspects or embodiments described herein, the sample or biological sample assayed may be an isolated biological sample (*i.e.*, for *in vitro* analysis).

15 In some embodiments, the step of assaying comprises a technique selected from the group consisting of Northern blot analysis, polymerase chain reaction (PCR), reverse transcription-polymerase chain reaction (RT-PCR), TaqMan-based assays, direct sequencing, dynamic allele-specific hybridization, high-density oligonucleotide SNP arrays, restriction fragment length polymorphism (RFLP) assays, primer extension assays, oligonucleotide ligase assays, analysis of
20 single strand conformation polymorphism, temperature gradient gel electrophoresis (TGGE), denaturing high performance liquid chromatography, high-resolution melting analysis, DNA mismatch-binding protein assays, SNPLex®, capillary electrophoresis, Southern Blot, immunoassays, immunohistochemistry, ELISA, flow cytometry, Western blot, HPLC, and mass spectrometry.

In one aspect, provided herein is a method for producing a transmittable form of information
25 for predicting the responsiveness of a subject having an increased risk of MACE to treatment with an NLRP3 inflammasome pathway inhibitor, comprising: (a) determining an increased likelihood of the subject responding to treatment with the NLRP3 inflammasome pathway inhibitor based on the presence of at least one copy of a gain of function mutation of *P2RX7* in a biological sample from the subject; and (b) recording the result of the determining step on a tangible or intangible media form for
30 use in transmission. In one aspect, provided herein is a method for producing a transmittable form of information for predicting the responsiveness of a subject having an increased risk of MACE to treatment with an NLRP3 inflammasome pathway inhibitor, comprising: (a) determining an increased likelihood of the subject responding to treatment with the NLRP3 inflammasome pathway inhibitor based on the presence of two copies of a gain of function mutation of *P2RX7* in a biological sample
35 from the subject; and (b) recording the result of the determining step on a tangible or intangible media form for use in transmission.

In one aspect, provided herein is a method for producing a transmittable form of information for predicting the responsiveness of a subject having an increased incidence of MACE to treatment with an NLRP3 inflammasome pathway inhibitor, comprising: (a) determining an increased
 5 likelihood that the subject will respond to treatment with the NLRP3 inflammasome pathway inhibitor based on an increased level of *P2X7* expression, increased level of P2X7 protein, or an increased level of P2X7 activity in a biological sample from the subject relative to a control; and (b) recording the result of the determining step on a tangible or intangible media form for use in transmission.

10 In some embodiments, the MACE is a non-fatal myocardial infarction (MI), non-fatal stroke, or hospitalization for unstable angina requiring unplanned revascularization. In some embodiments, the MACE is cardiovascular (CV) death.

In some embodiments, the NLRP3 inflammasome pathway inhibitor is selected from: an interleukin-1beta (IL-1beta) inhibitor, an interleukin-18 (IL-18) inhibitor, an interleukin 6 (IL-6) inhibitor, a nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inhibitor, or
 15 a combination thereof (*e.g.*, a dual inhibitor such as a bispecific antibody).

In some embodiments, the IL-1beta inhibitor is an anti-IL-1beta antibody, and the anti-IL-1beta antibody is selected from the group comprising: (a) an antibody comprising the three HCDRs of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3; (b) an antibody comprising the three LCDRs of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6; (c) an antibody comprising the three HCDRs of SEQ ID
 20 NO:1, SEQ ID NO:2, SEQ ID NO:3 and the three LCDRs of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6; (d) an anti-IL1 β antibody comprising a HC domain comprising SEQ ID NO: 7; (e) an anti-IL1 β antibody comprising a LC domain comprising SEQ ID NO: 8; and (f) an anti-IL1 β antibody comprising a HC domain comprising SEQ ID NO: 7 and a LC domain comprising SEQ ID NO: 8.

In some embodiments, the anti-IL-1beta antibody comprises the three HCDRs of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3. In some embodiments, the anti-IL-1beta antibody comprises the three LCDRs of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6. In some embodiments, the anti-IL-1beta antibody comprises the three HCDRs of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and the three LCDRs of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6. In some embodiments, the anti-IL-1beta antibody comprises a HC domain comprising SEQ ID NO: 7. In some embodiments, the anti-
 30 IL-1beta antibody comprises a HC domain comprising SEQ ID NO: 7 and a LC domain comprising SEQ ID NO: 8.

In some embodiments, the IL-1beta inhibitor is a monoclonal antibody. In some embodiments, the IL-1beta inhibitor is a human antibody. In some embodiments, the IL-1beta inhibitor is a monoclonal human antibody. In some embodiments, the IL-1beta inhibitor is canakinumab. In some embodiments, the subject has previously suffered a myocardial infarction
 35 (MI). In some embodiments, the subject has previously suffered at least one myocardial infarction

(MI). In some embodiments, the subject is concomitantly receiving standard of care treatment for reducing the risk of or preventing cardiovascular events such as MACE. In some embodiments, the the subject has a high sensitivity C-reactive protein (hsCRP) level of ≥ 2 mg/L.

5 In one aspect, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, comprising testing for the presence of one or more copies of a gain of function mutation of *P2RX7* in a biological sample from the subject and administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the sample tests positive for one or more copies of the gain of function mutation of *P2RX7*.
10 In one aspect, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, comprising administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if a biological sample from the subject tests positive for one or more copies of a gain of function mutation of *P2RX7*.

In one aspect, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, comprising testing for the presence of one or more
15 copies of a gain of function mutation of *P2RX7* in a biological sample from the subject and administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the sample tests positive for two copies of the gain of function mutation of *P2RX7*. In one aspect, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, comprising administering a therapeutically effective amount of an
20 NLRP3 inflammasome pathway inhibitor to the subject if a biological sample from the subject tests positive for two copies of a gain of function mutation of *P2RX7*.

In one aspect, provided herein is a kit for use in predicting the likelihood that a subject having an increased risk of MACE will respond to treatment with an NLRP3 inflammasome pathway inhibitor comprising, (a) at least one probe capable of detecting the presence of at least one gain of
25 function mutation of *P2RX7* (e.g., the rs1718119 SNP); and (b) instructions for using the probe to assay a biological sample from the subject for the presence of the at least one mutation, wherein the presence of the at least one mutation is indicative of an increased likelihood that the subject will respond to treatment with the NLRP3 inflammasome pathway inhibitor.

In one aspect, provided herein is a kit for use in treating a subject having an increased risk of
30 MACE comprising, (a) a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor; (b) at least one probe capable of detecting the presence of at least one gain of function mutation of *P2RX7*; (c) instructions for using the probe to assay a biological sample from the subject for the presence of the at least one mutation, (d) instructions for administering the NLRP3 inflammasome pathway inhibitor to the subject if the biological sample from the subject has the
35 presence of the at least one mutation; and (e) optionally, means for administering the NLRP3 inflammasome pathway inhibitor to the subject.

In some embodiments, the probe is an oligonucleotide that specifically hybridizes to a region of a nucleic acid coding for the at least one mutation, an antibody that detects a polypeptide product of the at least one mutation, or an oligonucleotide that specifically hybridizes to a region of a nucleic acid coding for an equivalent genetic marker of the at least one mutation.

5 In one aspect, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, comprising (a) assaying a biological sample from the subject; (b) determining if the subject has a gain of function mutation of *P2RX7* (e.g., the rs1718119 SNP), and (c) if the mutation is present, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is to be administered to the subject.

10 In one aspect, provided herein is an *in vitro* method for determining efficacy of treatment of a subject for MACE by administration of an NLRP3 inflammasome pathway inhibitor, comprising: (a) determining *in vitro* whether the sample indicates that the subject has a gain of function mutation of *P2RX7*; and (b) administering an effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain of function mutation of *P2RX7*.

15 In one aspect, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, comprising: (a) assaying a sample from the subject; (b) determining if the subject has at least one copy of a gain of function allele of *P2RX7* (e.g., the rs1718119 SNP); and (c) if the subject has at least one copy of a gain of function allele of *P2RX7* (e.g., the rs1718119 SNP), a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is to be administered to the subject.

20 In one aspect, provided herein is an NLRP3 inflammasome pathway inhibitor for use in treating a subject at risk of MACE, characterized in that: (a) the subject is selected for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of the subject having at least one copy of a gain of function allele of *P2RX7* (e.g., the rs1718119 SNP); and (b) thereafter, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is administered to the subject.

25 In one aspect, provided herein is an NLRP3 inflammasome pathway inhibitor for use in treating a subject at risk of MACE, characterized in that: (a) a biological sample from the subject is assayed for at least one copy of a gain of function allele of *P2RX7* (e.g., the rs1718119 SNP); and (b) a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is selectively administered to the subject on the basis of the biological sample from the subject having at least one copy of a gain of function allele of *P2RX7*.

30 In one aspect, provided herein is an NLRP3 inflammasome pathway inhibitor for use in treating a subject at risk of MACE, characterized in that: (a) a biological sample from the subject is assayed for at least one copy of a gain of function allele of *P2RX7* (e.g., the rs1718119 SNP); and (b) a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is selectively

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administered to the subject on the basis of the biological sample from the subject having at least one copy of a gain of function allele of *P2RX7*.

In one aspect, provided herein is an NLRP3 inflammasome pathway inhibitor for use in treating a subject at risk of MACE, characterized in that: (a) the subject is selected for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of the subject having two copies of a gain of function allele of *P2RX7* (e.g., the rs1718119 SNP); and (b) thereafter, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is administered to the subject.

In one aspect, provided herein is an NLRP3 inflammasome pathway inhibitor for use in treating a subject at risk of MACE, characterized in that: (a) a biological sample from the subject is assayed for two copies of a gain of function allele of *P2RX7* (e.g., the rs1718119 SNP); and (b) a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is selectively administered to the subject on the basis of the biological sample from the subject having at least one copy of a gain of function allele of *P2RX7*.

In one aspect, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, wherein said subject has at least one copy of a gain of function allele of *P2RX7* (e.g., the rs1718119 SNP). In one aspect, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, wherein said subject has two copies of a gain of function allele of *P2RX7* (e.g., the rs1718119 SNP).

In some embodiments, the NLRP3 inflammasome pathway inhibitor is selected from: an NLRP3 inhibitor, an anti-IL-1beta antibody, an anti-IL-18 antibody, and an anti IL-1beta/IL-18 bispecific antibody. In some embodiments, the NLRP3 inflammasome pathway inhibitor is an NLRP3 inhibitor, optionally wherein the NLRP3 inhibitor is a compound of any one of Formulae 101A, 102A, 1AA, 2AA, 3AA, 4AA, 5AA and 6AA. In some embodiments, the NLRP3 inflammasome pathway inhibitor is an anti-IL-1beta antibody, optionally wherein the anti-IL-1beta antibody is canakinumab. In some embodiments, the NLRP3 inflammasome pathway inhibitor is an anti-IL-18 antibody. In some embodiments, the NLRP3 inflammasome pathway inhibitor is an anti IL-1beta/IL-18 bispecific antibody.

In some embodiments, canakinumab is administered to the subject about every three months. In some embodiments, 50 mg of canakinumab is administered to the subject. In some embodiments, 150 mg of canakinumab is administered to the subject. In some embodiments, 300 mg of canakinumab is administered to the subject. In some embodiments, the canakinumab is administered subcutaneously.

In some embodiments, the subject has two copies of the gain of function allele of *P2RX7*. In some embodiments, the subject has two copies of the gain of function allele of *P2RX7*. In some embodiments, the gain of function mutation of *P2RX7* is an rs1718119 allele. In certain embodiments, the subject is homozygous for the rs1718119 SNP.

In some embodiments, the subject has had at least one previous myocardial infarction (MI).

In some embodiments, the subject has an hsCRP level of ≥ 2 mg/L.

Further features and advantages of the described uses, methods, and kits will become apparent from the following detailed description.

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BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic representing the CANTOS Trial Diagram.

Fig. 2 is a graph demonstrating the cumulative incidence of MACE over time in subjects with wild-type *P2RX7* treated with placebo or canakinumab (Hazard Ratio (HR) of 0.91, p value of 0.36).

Fig. 3 is a graph demonstrating the cumulative incidence of MI and CV death (MI/CVD) over time in subjects with a 0, 1, or 2 copies of a *P2RX7* gain of function allele (SNP rs1718119; Hazard Ratio (HR) of 1.54, p value of 0.039).

Fig. 4 is a graph demonstrating the cumulative incidence of MACE over time in subjects with a homozygous *P2RX7* gain of function allele treated with placebo or canakinumab (SNP rs1718119; Hazard Ratio (HR) of 0.68, p value of 0.046).

Fig. 5 is a graph demonstrating the cumulative incidence of MI and CV death (MI/CVD) over time in subjects with a 0, 1, or 2 copies of a *P2RX7* hypomorphic allele (SNP rs7958311; Hazard Ratio (HR) of 0.41, p value of 0.054).

DETAILED DESCRIPTION

The present application relates to canakinumab for use in reducing the risk of or preventing cardiovascular (CV) events such as major adverse cardiovascular events (MACE) in a patient with a gain-of-function mutation in *P2RX7* (*e.g.*, is homozygous for the rs1718119 SNP). Such a patient may, for example, have one or more copies of the specific gain-of-function mutation in *P2RX7*. In some embodiments, the patients may be heterozygous for the rs1718119 SNP. In some embodiments, the patient may be homozygous for rs1718119. In some embodiments, the patient may have elevated hsCRP levels (*e.g.*, an hsCRP level of at least 2 mg/L). In some embodiments, the patient may have previously suffered at least one myocardial infarction (MI). In some embodiments, the patient may have elevated hsCRP levels (*e.g.*, an hsCRP level of at least 2 mg/L) and previously suffered at least one myocardial infarction (MI). In some embodiments, the patient has elevated hsCRP levels (*e.g.*, an hsCRP level of at least 2 mg/L) and previously suffered at least one myocardial infarction (MI) and be homozygous for rs1718119.

The methods, uses, and kits described herein arose from further analysis of the data generated from the CANTOS trial (Ridker PM *et al*, Am Heart J. 2011;162(4):597-605 and as described in WO2013/049278; WO2019/38737; and WO2019/38740, the contents of each of which is hereby incorporated by reference in its entirety), a randomized, double-blind, placebo-controlled, event-

driven trial, designed to evaluate whether the administration of quarterly subcutaneous canakinumab can prevent recurrent cardiovascular events among stable post-myocardial infarction patients with elevated hsCRP. The enrolled 10,061 patients with myocardial infarction and inflammatory atherosclerosis had high sensitivity C-reactive protein (hsCRP) of ≥ 2 mg/L. Three escalating
5 canakinumab doses (50 mg, 150 mg, and 300 mg given subcutaneously every 3 months) were compared to placebo.

Provided herein are, *inter alia*, methods for reducing the risk of or preventing cardiovascular (CV) events such as major adverse cardiovascular events (MACE) in a subject, wherein the subject has a gain-of-function mutation in the *P2RX7* gene (*e.g.*, has at least one copy of the rs1718119 SNP).

10 The subject may have a previous myocardial infarction (MI). The subject may have an hsCRP of at least 2 mg/L.

In any of the below described embodiments and any of the aspects, embodiments, uses, kits, and methods described herein, the NLRP3 inflammasome pathway inhibitor may be any known NLRP3 inflammasome pathway inhibitor and, *e.g.*, may be selected from: an NLRP3 inhibitor (*e.g.*,
15 any one of Formulae 101A, 102A, 1AA, 2AA, 3AA, 4AA, 5AA and 6AA), an IL-1beta inhibitor (*e.g.*, an anti-IL-1beta antibody such as canakinumab), an IL-18 inhibitor (*e.g.*, an anti-IL-18 antibody), an IL-6 inhibitor (*e.g.*, an anti-IL-6 antibody) or a combination thereof (*e.g.*, an anti IL-1beta/IL-18 bispecific antibody).

In some embodiments, provided herein is a method of treating a subject with a gain of
20 function mutation in *P2RX7*, wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject reduces the incidence of a major adverse cardiovascular event (MACE), comprising: (a) assaying a biological sample from the subject for a gain-of-function mutation of *P2RX7*; and (b) thereafter, selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain-of-function mutation of
25 *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119.

In some embodiments, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7*, wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject reduces the incidence of a major adverse cardiovascular event (MACE), comprising: (a) assaying a biological sample from the subject for a gain-of-function mutation of
30 *P2RX7*; and (b) thereafter, selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain-of-function mutation of *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has had a previous MI.

In some embodiments, provided herein is a method of treating a subject with a gain of
35 function mutation in *P2RX7*, wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject reduces the incidence of a major adverse cardiovascular event (MACE),

comprising: (a) assaying a biological sample from the subject for a gain-of-function mutation of *P2RX7*; and (b) thereafter, selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain-of-function mutation of *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has an
5 hsCRP level of at least 2.

In some embodiments, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7*, wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject reduces the incidence of a major adverse cardiovascular event (MACE), comprising: (a) assaying a biological sample from the subject for a gain-of-function mutation of
10 *P2RX7*; and (b) thereafter, selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain-of-function mutation of *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has had a previous MI and an hsCRP level of at least 2.

In some embodiments, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7*, wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject reduces the incidence of a major adverse cardiovascular event (MACE), comprising: (a) assaying an isolated biological sample from the subject for a gain-of-function mutation of *P2RX7*; and (b) thereafter, selectively administering a therapeutically effective amount of
15 an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain-of-function mutation of *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119.

In some embodiments, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7*, wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject reduces the incidence of a major adverse cardiovascular event (MACE), comprising: (a) assaying an isolated biological sample from the subject for a gain-of-function
25 mutation of *P2RX7*; and (b) thereafter, selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain-of-function mutation of *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has had a previous MI.

In some embodiments, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7*, wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject reduces the incidence of a major adverse cardiovascular event (MACE), comprising: (a) assaying an isolated biological sample from the subject for a gain-of-function
30 mutation of *P2RX7*; and (b) thereafter, selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain-of-function mutation of *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the
35 subject has an hsCRP level of at least 2.

In some embodiments, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7*, wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject reduces the incidence of a major adverse cardiovascular event (MACE), comprising: (a) assaying an isolated biological sample from the subject for a gain-of-function mutation of *P2RX7*; and (b) thereafter, selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain-of-function mutation of *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has had a previous MI and an hsCRP level of at least 2.

In some embodiments, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7*, wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject improves the hazard risks of major adverse cardiovascular event (MACE) incidents for the subject, comprising: (a) assaying a biological sample from the subject for a gain-of-function mutation of *P2RX7*; and (b) thereafter, selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain-of-function mutation of *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119.

In some embodiments, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7*, wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject improves the hazard risks of major adverse cardiovascular event (MACE) incidents for the subject, comprising: (a) assaying a biological sample from the subject for a gain-of-function mutation of *P2RX7*; and (b) thereafter, selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain-of-function mutation of *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has had a previous MI.

In some embodiments, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7*, wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject improves the hazard risks of major adverse cardiovascular event (MACE) incidents for the subject, comprising: (a) assaying a biological sample from the subject for a gain-of-function mutation of *P2RX7*; and (b) thereafter, selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain-of-function mutation of *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject an hsCRP level of at least 2.

In some embodiments, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7*, wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject improves the hazard risks of major adverse cardiovascular event (MACE) incidents for the subject, comprising: (a) assaying a biological sample from the subject for a gain-of-function mutation of *P2RX7*; and (b) thereafter, selectively administering a therapeutically effective

amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain-of-function mutation of *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has had a previous MI and an hsCRP level of at least 2.

5 In some embodiments, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7*, wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject improves the hazard risks of major adverse cardiovascular event (MACE) incidents for the subject, comprising: (a) assaying an isolated biological sample from the subject for a gain-of-function mutation of *P2RX7*; and (b) thereafter, selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a
10 gain-of-function mutation of *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119.

In some embodiments, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7*, wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject improves the hazard risks of major adverse cardiovascular event (MACE) incidents for the subject, comprising: (a) assaying an isolated biological sample from the subject for a
15 gain-of-function mutation of *P2RX7*; and (b) thereafter, selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain-of-function mutation of *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has had a previous MI.

In some embodiments, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7*, wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject improves the hazard risks of major adverse cardiovascular event (MACE) incidents for the subject, comprising: (a) assaying an isolated biological sample from the subject for a gain-of-function mutation of *P2RX7*; and (b) thereafter, selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a
25 gain-of-function mutation of *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has an hsCRP level of at least 2.

In some embodiments, provided herein is a method of treating a subject with a gain of function mutation in *P2RX7*, wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject improves the hazard risks of major adverse cardiovascular event (MACE) incidents for the subject, comprising: (a) assaying an isolated biological sample from the subject for a
30 gain-of-function mutation of *P2RX7*; and (b) thereafter, selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain-of-function mutation of *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has had a previous MI and an hsCRP level of at least 2.

35 In some embodiments, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: (a) selecting

the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of a the subject having two copies of a gain of function mutation of *P2RX7*; and (b) thereafter, administering a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor to the subject, wherein the gain-of-function mutation of *P2RX7* is rs1718119.

5 In some embodiments, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: (a) selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of a the subject having two copies of a gain of function mutation of *P2RX7*; and (b) thereafter, administering a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor to the subject,
10 wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has had a previous MI.

 In some embodiments, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: (a) selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of a the
15 subject having two copies of a gain of function mutation of *P2RX7*; and (b) thereafter, administering a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor to the subject, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has an hsCRP level of at least 2.

 In some embodiments, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: (a) selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of a the subject having two copies of a gain of function mutation of *P2RX7*; and (b) thereafter, administering a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor to the subject,
20 wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has had a previous MI and an hsCRP level of at least 2.

 In some embodiments, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: (a) assaying a biological sample from the subject for the presence of at least one copy of a gain of function mutation of *P2RX7*; and (b) subsequently selecting the subject for treatment with the NLRP3 inflammasome
30 pathway inhibitor on the basis of the biological sample from the subject having the at least one copy of a gain of function mutation of *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119.

 In some embodiments, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: (a) assaying a biological sample from the subject for the presence of at least one copy of a gain of function mutation
35 of *P2RX7*; and (b) subsequently selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of the biological sample from the subject having the at least one copy of

a gain of function mutation of *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has had a previous MI.

5 In some embodiments, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: (a) assaying a biological sample from the subject for the presence of at least one copy of a gain of function mutation of *P2RX7*; and (b) subsequently selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of the biological sample from the subject having the at least one copy of a gain of function mutation of *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has an hsCRP level of at least 2.

10 In some embodiments, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: (a) assaying a biological sample from the subject for the presence of at least one copy of a gain of function mutation of *P2RX7*; and (b) subsequently selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of the biological sample from the subject having the at least one copy of a gain of function mutation of *P2RX7*, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has had a previous MI and an hsCRP level of at least 2.

15 In some embodiments, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: (a) assaying a biological sample from the subject for the presence of at least one copy of a gain of function mutation of *P2RX7*; (b) subsequently selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of the biological sample from the subject having the at least one copy of a gain of function mutation of *P2RX7*; and (c) subsequently administering a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor to the subject, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has had a previous MI and an
25 hsCRP level of at least 2.

In some embodiments, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: (a) assaying a biological sample from the subject for the presence of at least one copy of a gain of function mutation of *P2RX7*; (b) subsequently selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of the biological sample from the subject having the at least one copy of a gain of function mutation of *P2RX7*; and (c) subsequently administering a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor to the subject, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has had a previous MI.

30 In some embodiments, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: (a) assaying a biological sample from the subject for the presence of at least one copy of a gain of function mutation

of *P2RX7*; (b) subsequently selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of the biological sample from the subject having the at least one copy of a gain of function mutation of *P2RX7*; and (c) subsequently administering a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor to the subject, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has an hsCRP level of at least 2.

In some embodiments, provided herein is a method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising: (a) assaying a biological sample from the subject for the presence of at least one copy of a gain of function mutation of *P2RX7*; (b) subsequently selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of the biological sample from the subject having the at least one copy of a gain of function mutation of *P2RX7*; and (c) subsequently administering a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor to the subject, wherein the gain-of-function mutation of *P2RX7* is rs1718119, and wherein the subject has had a previous MI and an hsCRP level of at least 2.

In some embodiments, provided herein is a method for producing a transmittable form of information for predicting the responsiveness of a subject having an increased risk of MACE to treatment with an NLRP3 inflammasome pathway inhibitor, comprising: (a) determining an increased likelihood of the subject responding to treatment with the NLRP3 inflammasome pathway inhibitor based on the presence of at least one copy of a gain of function mutation of *P2RX7* in a biological sample from the subject; and (b) recording the result of the determining step on a tangible or intangible media form for use in transmission.

In some embodiments, provided herein is a method for producing a transmittable form of information for predicting the responsiveness of a subject having an increased risk of MACE to treatment with an NLRP3 inflammasome pathway inhibitor, comprising: (a) determining an increased likelihood of the subject responding to treatment with the NLRP3 inflammasome pathway inhibitor based on the presence of at least one copy of a gain of function mutation of *P2RX7* in a biological sample from the subject; and (b) recording the result of the determining step on a tangible or intangible media form for use in transmission, and wherein the subject has had a previous MI.

In some embodiments, provided herein is a method for producing a transmittable form of information for predicting the responsiveness of a subject having an increased risk of MACE to treatment with an NLRP3 inflammasome pathway inhibitor, comprising: (a) determining an increased likelihood of the subject responding to treatment with the NLRP3 inflammasome pathway inhibitor based on the presence of at least one copy of a gain of function mutation of *P2RX7* in a biological sample from the subject; and (b) recording the result of the determining step on a tangible or intangible media form for use in transmission, and wherein the subject has an hsCRP level of at least 2.

In some embodiments, provided herein is a method for producing a transmittable form of information for predicting the responsiveness of a subject having an increased risk of MACE to treatment with an NLRP3 inflammasome pathway inhibitor, comprising: (a) determining an increased likelihood of the subject responding to treatment with the NLRP3 inflammasome pathway inhibitor based on the presence of at least one copy of a gain of function mutation of *P2RX7* in a biological sample from the subject; and (b) recording the result of the determining step on a tangible or intangible media form for use in transmission, and wherein the subject has had a previous MI and an hsCRP level of at least 2.

In some embodiments, provided herein is a method for producing a transmittable form of information for predicting the responsiveness of a subject having an increased incidence of MACE to treatment with an NLRP3 inflammasome pathway inhibitor, comprising: (a) determining an increased likelihood that the subject will respond to treatment with the NLRP3 inflammasome pathway inhibitor based on an increased level of *P2RX7* expression, increased level of P2X7 protein, or an increased level of P2X7 activity in a biological sample from the subject relative to a control; and (b) recording the result of the determining step on a tangible or intangible media form for use in transmission.

In some embodiments, provided herein is a method for producing a transmittable form of information for predicting the responsiveness of a subject having an increased incidence of MACE to treatment with an NLRP3 inflammasome pathway inhibitor, comprising: (a) determining an increased likelihood that the subject will respond to treatment with the NLRP3 inflammasome pathway inhibitor based on an increased level of *P2RX7* expression, increased level of P2X7 protein, or an increased level of P2X7 activity in a biological sample from the subject relative to a control; and (b) recording the result of the determining step on a tangible or intangible media form for use in transmission, and wherein the subject has had a previous MI.

In some embodiments, provided herein is a method for producing a transmittable form of information for predicting the responsiveness of a subject having an increased incidence of MACE to treatment with an NLRP3 inflammasome pathway inhibitor, comprising: (a) determining an increased likelihood that the subject will respond to treatment with the NLRP3 inflammasome pathway inhibitor based on an increased level of *P2RX7* expression, increased level of P2X7 protein, or an increased level of P2X7 activity in a biological sample from the subject relative to a control; and (b) recording the result of the determining step on a tangible or intangible media form for use in transmission, and wherein the subject has an hsCRP level of at least 2.

In some embodiments, provided herein is a method for producing a transmittable form of information for predicting the responsiveness of a subject having an increased incidence of MACE to treatment with an NLRP3 inflammasome pathway inhibitor, comprising: (a) determining an increased likelihood that the subject will respond to treatment with the NLRP3 inflammasome pathway inhibitor based on an increased level of *P2RX7* expression, increased level of P2X7 protein, or an increased

level of *P2RX7* activity in a biological sample from the subject relative to a control; and (b) recording the result of the determining step on a tangible or intangible media form for use in transmission, and wherein the subject has had a previous MI and an hsCRP level of at least 2.

5 In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, comprising testing for the presence of one or more copies of a gain of function mutation of *P2RX7* in a biological sample from the subject and administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the sample tests positive for one or more copies of the gain of function mutation of *P2RX7*.

10 In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, comprising testing for the presence of one or more copies of a gain of function mutation of *P2RX7* in a biological sample from the subject and administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the sample tests positive for one or more copies of the gain of function mutation of *P2RX7*, and wherein the subject has had a previous MI.

15 In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, comprising testing for the presence of one or more copies of a gain of function mutation of *P2RX7* in a biological sample from the subject and administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the sample tests positive for one or more copies of the gain of function mutation of *P2RX7*,
20 and wherein the subject has an hsCRP level of at least 2.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, comprising testing for the presence of one or more copies of a gain of function mutation of *P2RX7* in a biological sample from the subject and administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the
25 subject if the sample tests positive for one or more copies of the gain of function mutation of *P2RX7*, and wherein the subject has had a previous MI and an hsCRP level of at least 2.

In some embodiments, provided herein is a kit for use in predicting the likelihood that a subject having an increased risk of MACE will respond to treatment with an NLRP3 inflammasome pathway inhibitor comprising, (a) at least one probe capable of detecting the presence of at least one
30 gain of function mutation of *P2RX7*; and (b) instructions for using the probe to assay a biological sample from the subject for the presence of the at least one mutation, wherein the presence of the at least one mutation is indicative of an increased likelihood that the subject will respond to treatment with the NLRP3 inflammasome pathway inhibitor.

In some embodiments, provided herein is a kit for use in predicting the likelihood that a
35 subject having an increased risk of MACE will respond to treatment with an NLRP3 inflammasome pathway inhibitor comprising, (a) at least one probe capable of detecting the presence of at least one

gain of function mutation of *P2RX7*; and (b) instructions for using the probe to assay a biological sample from the subject for the presence of the at least one mutation, wherein the presence of the at least one mutation is indicative of an increased likelihood that the subject will respond to treatment with the NLRP3 inflammasome pathway inhibitor, and wherein the subject has had a previous MI.

5 In some embodiments, provided herein is a kit for use in predicting the likelihood that a subject having an increased risk of MACE will respond to treatment with an NLRP3 inflammasome pathway inhibitor comprising, (a) at least one probe capable of detecting the presence of at least one gain of function mutation of *P2RX7*; and (b) instructions for using the probe to assay a biological sample from the subject for the presence of the at least one mutation, wherein the presence of the at least one mutation is indicative of an increased likelihood that the subject will respond to treatment with the NLRP3 inflammasome pathway inhibitor, and wherein the subject has an hsCRP level of at least 2.

10 In some embodiments, provided herein is a kit for use in predicting the likelihood that a subject having an increased risk of MACE will respond to treatment with an NLRP3 inflammasome pathway inhibitor comprising, (a) at least one probe capable of detecting the presence of at least one gain of function mutation of *P2RX7*; and (b) instructions for using the probe to assay a biological sample from the subject for the presence of the at least one mutation, wherein the presence of the at least one mutation is indicative of an increased likelihood that the subject will respond to treatment with the NLRP3 inflammasome pathway inhibitor, and wherein the subject has had a previous MI and an hsCRP level of at least 2.

15 In some embodiments, provided herein is a kit for use in treating a subject having an increased risk of MACE comprising, (a) a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor; (b) at least one probe capable of detecting the presence of at least one gain of function mutation of *P2RX7*; (c) instructions for using the probe to assay a biological sample from the subject for the presence of the at least one mutation, (d) instructions for administering the NLRP3 inflammasome pathway inhibitor to the subject if the biological sample from the subject has the presence of the at least one mutation; and (e) optionally, means for administering the NLRP3 inflammasome pathway inhibitor to the subject.

20 In some embodiments, provided herein is a kit for use in treating a subject having an increased risk of MACE comprising, (a) a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor; (b) at least one probe capable of detecting the presence of at least one gain of function mutation of *P2RX7*; (c) instructions for using the probe to assay a biological sample from the subject for the presence of the at least one mutation, (d) instructions for administering the NLRP3 inflammasome pathway inhibitor to the subject if the biological sample from the subject has the presence of the at least one mutation; and (e) optionally, means for

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administering the NLRP3 inflammasome pathway inhibitor to the subject, wherein the subject has had a previous MI.

In some embodiments, provided herein is a kit for use in treating a subject having an increased risk of MACE comprising, (a) a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor; (b) at least one probe capable of detecting the presence of at least one gain of function mutation of *P2RX7*; (c) instructions for using the probe to assay a biological sample from the subject for the presence of the at least one mutation; (d) instructions for administering the NLRP3 inflammasome pathway inhibitor to the subject if the biological sample from the subject has the presence of the at least one mutation; and (e) optionally, means for administering the NLRP3 inflammasome pathway inhibitor to the subject, wherein the subject has an hsCRP level of at least 2.

In some embodiments, provided herein is a kit for use in treating a subject having an increased risk of MACE comprising, (a) a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor; (b) at least one probe capable of detecting the presence of at least one gain of function mutation of *P2RX7*; (c) instructions for using the probe to assay a biological sample from the subject for the presence of the at least one mutation; (d) instructions for administering the NLRP3 inflammasome pathway inhibitor to the subject if the biological sample from the subject has the presence of the at least one mutation; and (e) optionally, means for administering the NLRP3 inflammasome pathway inhibitor to the subject, wherein the subject has had a previous MI and an hsCRP level of at least 2.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for the use in the treatment or prevention of MACE in a subject, comprising (a) assaying a biological sample from the subject; (b) determining if the subject has a gain of function mutation of *P2RX7*, and (c) if the mutation is present, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is to be administered to the subject.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for the use in the treatment or prevention of MACE in a subject, comprising (a) assaying a biological sample from the subject; (b) determining if the subject has a gain of function mutation of *P2RX7*, and (c) if the mutation is present, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is to be administered to the subject, wherein the subject has had a previous MI.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for the use in the treatment or prevention of MACE in a subject, comprising (a) assaying a biological sample from the subject; (b) determining if the subject has a gain of function mutation of *P2RX7*, and (c) if the mutation is present, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is to be administered to the subject, wherein the subject has an hsCRP level of at least 2.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for the use in the treatment or prevention of MACE in a subject, comprising (a) assaying a biological sample from the subject; (b) determining if the subject has a gain of function mutation of *P2RX7*, and (c) if the mutation is present, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is to be administered to the subject, wherein the subject has had a previous MI and an hsCRP level of at least 2.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for the use in the treatment or prevention of MACE in a subject, comprising (a) assaying an isolated biological sample from the subject; (b) determining if the subject has a gain of function mutation of *P2RX7*, and (c) if the mutation is present, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is to be administered to the subject.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for the use in the treatment or prevention of MACE in a subject, comprising (a) assaying an isolated biological sample from the subject; (b) determining if the subject has a gain of function mutation of *P2RX7*, and (c) if the mutation is present, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is to be administered to the subject, and wherein the subject has had a previous MI.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for the use in the treatment or prevention of MACE in a subject, comprising (a) assaying an isolated biological sample from the subject; (b) determining if the subject has a gain of function mutation of *P2RX7*, and (c) if the mutation is present, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is to be administered to the subject, and wherein the subject has an hsCRP level of at least 2.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for the use in the treatment or prevention of MACE in a subject, comprising (a) assaying an isolated biological sample from the subject; (b) determining if the subject has a gain of function mutation of *P2RX7*, and (c) if the mutation is present, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is to be administered to the subject, and wherein the subject has had a previous MI and an hsCRP level of at least 2.

In one aspect, provided herein is an *in vitro* method for determining efficacy of treatment of a subject for MACE by administration of an NLRP3 inflammasome pathway inhibitor, comprising determining *in vitro* whether the sample indicates that the subject has a gain of function mutation of *P2RX7*. In some embodiments, the gain of function mutation of *P2RX7* is an rs1718119 allele. In some embodiments, the subject is heterozygous for the rs1718119 allele. In some embodiments, the subject is homozygous for the rs1718119 allele. In some embodiments, the subject has had a previous MI and/or an hsCRP level of at least 2.

In one aspect, provided herein is an *in vitro* method for determining efficacy of treatment of a subject for MACE by administration of an NLRP3 inflammasome pathway inhibitor, comprising determining *in vitro* whether the sample indicates that the subject has a gain of function mutation of *P2RX7*; wherein the gain of function mutation of *P2RX7* is an rs1718119 allele; and wherein the subject is heterozygous for the rs1718119 allele. In some embodiments, the subject has had a previous MI and/or an hsCRP level of at least 2.

In one aspect, provided herein is an *in vitro* method for determining efficacy of treatment of a subject for MACE by administration of an NLRP3 inflammasome pathway inhibitor, comprising determining *in vitro* whether the sample indicates that the subject has a gain of function mutation of *P2RX7*; wherein the gain of function mutation of *P2RX7* is an rs1718119 allele; wherein the subject is heterozygous for the rs1718119 allele; and wherein the subject has had a previous MI and/or an hsCRP level of at least 2.

In some embodiments, provided herein is an *in vitro* method for determining efficacy of treatment of a subject for MACE by administration of an NLRP3 inflammasome pathway inhibitor, comprising: (a) determining *in vitro* whether the sample indicates that the subject has a gain of function mutation of *P2RX7*, and (b) administering an effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain of function mutation of *P2RX7*.

In some embodiments, provided herein is an *in vitro* method for determining efficacy of treatment of a subject for MACE by administration of an NLRP3 inflammasome pathway inhibitor, comprising: (a) determining *in vitro* whether the sample indicates that the subject has a gain of function mutation of *P2RX7*, and (b) administering an effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain of function mutation of *P2RX7*, wherein the subject has had a previous MI.

In some embodiments, provided herein is an *in vitro* method for determining efficacy of treatment of a subject for MACE by administration of an NLRP3 inflammasome pathway inhibitor, comprising: (a) determining *in vitro* whether the sample indicates that the subject has a gain of function mutation of *P2RX7*, and (b) administering an effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain of function mutation of *P2RX7*, wherein the subject has an hsCRP level of at least 2.

In some embodiments, provided herein is an *in vitro* method for determining efficacy of treatment of a subject for MACE by administration of an NLRP3 inflammasome pathway inhibitor, comprising: (a) determining *in vitro* whether the sample indicates that the subject has a gain of function mutation of *P2RX7*, and (b) administering an effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain of function mutation of *P2RX7*, wherein the subject has had a previous MI and an hsCRP level of at least 2.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, comprising: (a) assaying a sample from the subject; (b) determining if the subject has at least one copy of a gain of function allele of *P2RX7*; and (c) if the subject has at least one copy of a gain of function allele of *P2RX7*, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is to be administered to the subject.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, comprising: administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject after determining that the subject has at least one copy of a gain of function allele of *P2RX7*. In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, comprising: administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject after determining that the subject has at least one copy of a gain of function allele of *P2RX7*; wherein the gain of function mutation of *P2RX7* is an rs1718119 allele. In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, comprising: administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject after determining that the subject has at least one copy of a gain of function allele of *P2RX7*; wherein the gain of function mutation of *P2RX7* is an rs1718119 allele; and wherein the subject is heterozygous for the rs1718119 allele.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, comprising: administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject after determining that the subject has at least one copy of a gain of function allele of *P2RX7*; wherein the gain of function mutation of *P2RX7* is an rs1718119 allele; and wherein the subject is heterozygous for the rs1718119 allele; and wherein the subject has had a previous MI and/or an hsCRP level of at least 2..

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, comprising: (a) assaying a sample from the subject; (b) determining if the subject has at least one copy of a gain of function allele of *P2RX7*; and (c) if the subject has at least one copy of a gain of function allele of *P2RX7*, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is to be administered to the subject, wherein the subject has had a previous MI.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, comprising: (a) assaying a sample from the subject; (b) determining if the subject has at least one copy of a gain of function allele of *P2RX7*; and (c) if the subject has at least one copy of a gain of function allele of *P2RX7*, a therapeutically effective

amount of the NLRP3 inflammasome pathway inhibitor is to be administered to the subject, wherein the subject has an hsCRP level of at least 2.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, comprising: (a) assaying a sample from the subject; (b) determining if the subject has at least one copy of a gain of function allele of *P2RX7*; and (c) if the subject has at least one copy of a gain of function allele of *P2RX7*, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is to be administered to the subject, wherein the subject has had a previous MI and an hsCRP level of at least 2.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in treating a subject at risk of MACE, characterized in that: (a) the subject is selected for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of the subject having at least one copy of a gain of function allele of *P2RX7*; and (b) thereafter, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is administered to the subject.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in treating a subject at risk of MACE, characterized in that: (a) the subject is selected for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of the subject having at least one copy of a gain of function allele of *P2RX7*; and (b) thereafter, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is administered to the subject, wherein the subject has had a previous MI.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in treating a subject at risk of MACE, characterized in that: (a) the subject is selected for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of the subject having at least one copy of a gain of function allele of *P2RX7*; and (b) thereafter, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is administered to the subject, wherein the subject has an hsCRP level of at least 2.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in treating a subject at risk of MACE, characterized in that: (a) the subject is selected for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of the subject having at least one copy of a gain of function allele of *P2RX7*; and (b) thereafter, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is administered to the subject, wherein the subject has had a previous MI and an hsCRP level of at least 2.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in treating a subject at risk of MACE, characterized in that: (a) a biological sample from the subject is assayed for at least one copy of a gain of function allele of *P2RX7*; and (b) a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is selectively administered to the subject on

the basis of the biological sample from the subject having at least one copy of a gain of function allele of *P2RX7*.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in treating a subject at risk of MACE, characterized in that: (a) a biological sample from the subject is assayed for at least one copy of a gain of function allele of *P2RX7*; and (b) a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is selectively administered to the subject on the basis of the biological sample from the subject having at least one copy of a gain of function allele of *P2RX7*, wherein the subject has had a previous MI.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in treating a subject at risk of MACE, characterized in that: (a) a biological sample from the subject is assayed for at least one copy of a gain of function allele of *P2RX7*; and (b) a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is selectively administered to the subject on the basis of the biological sample from the subject having at least one copy of a gain of function allele of *P2RX7*, wherein the subject has an hsCRP level of at least 2.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in treating a subject at risk of MACE, characterized in that: (a) a biological sample from the subject is assayed for at least one copy of a gain of function allele of *P2RX7*; and (b) a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is selectively administered to the subject on the basis of the biological sample from the subject having at least one copy of a gain of function allele of *P2RX7*, wherein the subject has had a previous MI and an hsCRP level of at least 2.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, wherein said subject has at least one copy of a gain of function allele of *P2RX7*.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, wherein said subject has at least one copy of a gain of function allele of *P2RX7*, and wherein the subject has had a previous MI.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, wherein said subject has at least one copy of a gain of function allele of *P2RX7*, and wherein the subject has an hsCRP level of at least 2.

In some embodiments, provided herein is an NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, wherein said subject has at least one copy of a gain of function allele of *P2RX7*, and wherein the subject has had a previous MI and an hsCRP level of at least 2.

In some embodiments, an NLRP3 inflammasome pathway inhibitor for use in the described methods, uses, and kits may be, for example, an IL-1 β inhibitor, an IL-18 inhibitor, an NLRP3 inhibitor, an IL-6 inhibitor, or a combination thereof.

Canakinumab (international nonproprietary name (INN) number 8836) is described in WO02/16436, US7993878, US8273350, the contents of each of which is hereby incorporated by reference in its entirety for this purpose. Canakinumab is also known as ACZ885 and Ilaris®. Canakinumab is also published as CAS number 914613-48-2 and DrugBank Accession Number DB06168. Canakinumab is a fully human monoclonal anti-human IL-1beta (also written as “IL-1β” or “IL-1b”) antibody of the IgG1/k isotype, and has been developed for the treatment of IL-1β driven inflammatory diseases. It is designed to specifically bind to human IL-1beta and block the interaction of this cytokine with its receptors (*i.e.*, acting as an IL-1beta inhibitor). The antagonism of IL-1beta-mediated inflammation using canakinumab in lowering high sensitivity C-reactive protein (hsCRP) and other inflammatory marker levels has shown an acute phase response in patients with, for example, Cryopyrin-Associated Periodic Syndrome (CAPS) and rheumatoid arthritis. This evidence has been replicated in patients with type 2 diabetes mellitus (T2DM) using canakinumab and with other IL-1β antibody therapies in development, although in T2DM reduction in hsCRP levels did not translate to increased efficaciousness over standard of care treatment. The CANTOS study demonstrated that treatment with canakinumab significantly reduces the risk of experiencing recurrent cardiovascular events in stable post-myocardial patients with elevated hsCRP by lowering residual inflammatory risk through administration of canakinumab without effecting the levels of HDL cholesterol, LDL cholesterol and triglycerides. The present analysis demonstrates that specific gain-of-function mutations of the *P2RX7* gene create a population of patients with increased risk of MACE that can be particularly helped through the administration of canakinumab (*e.g.*, by significantly lowering the risk of a major adverse cardiovascular event).

For ease of reference the amino acid sequences of the canakinumab monoclonal antibody heavy chain CDRs, light chain CDRs, heavy chain variable region, and light chain variable region are provided in Table 1.

Table 1. Sequence information for canakinumab

SEQ ID NO:	Description of sequence	Detailed amino acid or nucleotide sequences
1	Heavy chain CDR1	VYGMN
2	Heavy chain CDR2	IIWYDGDNQYYADSVK
3	Heavy chain CDR3	DLRTGP
4	Light chain CDR1'	RASQSIGSSLH
5	Light chain CDR2'	ASQSFS
6	Light chain CDR3'	HQSSSLP

7	Heavy chain variable region	QVQLVESGGGVVQPGRSLRLSCAASGFTFSVYGMNWVRQAPGKGLEWVAII WYDGDNQYYADSVKGRFTISRDN SKNTLYLQMNGLRAEDTAVVYCARDLR TGPFDYWGQGTLLTVSS
8	Light chain variable region	EIVLTQSPDFQSVPKEKVTITCRASQSIGSSLHWYQQKPDQSPKLLIKYASQS FSGVPSRFSGSGSGTDFTLTINSLEAEDAAAYYCHQSSSLPFTFGPGTKVDIK

In one embodiment, the NLRP3 inflammasome pathway inhibitor is an IL-1beta inhibitor such as an anti-IL-1beta antibody or antigen-binding fragment thereof. In some embodiments, the anti-IL1beta antibody or antigen-binding fragment thereof comprises at least one immunoglobulin heavy chain variable domain (VH) comprising hypervariable regions CDR1, CDR2 and CDR3, the CDR1 having the amino acid sequence SEQ ID NO:1, the CDR2 having the amino acid sequence SEQ ID NO:2, and the CDR3 having the amino acid sequence SEQ ID NO:3. In one embodiment, the anti-IL1beta antibody or antigen-binding fragment thereof comprises at least one immunoglobulin light chain variable domain (VL') comprising hypervariable regions CDR1', CDR2' and CDR3', the CDR1' having the amino acid sequence SEQ ID NO:4, the CDR2' having the amino acid sequence SEQ ID NO:5 and the CDR3' having the amino acid sequence SEQ ID NO:6. In one embodiment, the anti-IL1beta antibody or antigen-binding fragment thereof comprises at least one immunoglobulin V_H domain and at least one immunoglobulin V_L domain, wherein: a) the immunoglobulin V_H domain comprises (*e.g.*, in sequence): i) hypervariable regions CDR1, CDR2 and CDR3, the CDR1 having the amino acid sequence SEQ ID NO:1, the CDR2 having the amino acid sequence SEQ ID NO:2, and the CDR3 having the amino acid sequence SEQ ID NO:3; and b) the immunoglobulin V_L domain comprises (*e.g.*, in sequence) hypervariable regions CDR1', CDR2' and CDR3', the CDR1' having the amino acid sequence SEQ ID NO:4, the CDR2' having the amino acid sequence SEQ ID NO:5, and the CDR3' having the amino acid sequence SEQ ID NO:6. In one embodiment, the anti-IL1beta antibody or antigen-binding fragment thereof comprises: a) an immunoglobulin heavy chain variable domain (VH) comprising the amino acid sequence set forth as SEQ ID NO:7; b) an immunoglobulin light chain variable domain (VL) comprising the amino acid sequence set forth as SEQ ID NO:8; c) an immunoglobulin VH domain comprising the amino acid sequence set forth as SEQ ID NO:7 and an immunoglobulin VL domain comprising the amino acid sequence set forth as SEQ ID NO:8; d) an immunoglobulin VH domain comprising the hypervariable regions set forth as SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3; e) an immunoglobulin VL domain comprising the hypervariable regions set forth as SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6; or f) an immunoglobulin VH domain comprising the hypervariable regions set forth as SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3 and an immunoglobulin VL domain comprising the hypervariable regions set forth as SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

In some embodiments, the patient has a high sensitivity C-reactive protein (hsCRP) level of ≥ 2 mg/L assessed before first administration of an NLRP3 inflammasome pathway inhibitor (*e.g.*, an anti-IL-1beta antibody; *e.g.*, canakinumab). In some embodiments, the patient has a high sensitivity

- C-reactive protein (hsCRP) level of ≥ 3 mg/L assessed before first administration of an NLRP3 inflammasome pathway inhibitor (*e.g.*, an anti-IL-1beta antibody; *e.g.*, canakinumab). In some embodiments, the patient has a high sensitivity C-reactive protein (hsCRP) level of ≥ 4 mg/L assessed before first administration of an NLRP3 inflammasome pathway inhibitor (*e.g.*, an anti-IL-1beta antibody; *e.g.*, canakinumab). In some embodiments, the patient has a high sensitivity C-reactive protein (hsCRP) level of ≥ 5 mg/L assessed before first administration of an NLRP3 inflammasome pathway inhibitor (*e.g.*, an anti-IL-1beta antibody; *e.g.*, canakinumab). In some embodiments, the patient has a high sensitivity C-reactive protein (hsCRP) level of ≥ 6 mg/L assessed before first administration of an NLRP3 inflammasome pathway inhibitor (*e.g.*, an anti-IL-1beta antibody; *e.g.*, canakinumab). In some embodiments, the patient has a high sensitivity C-reactive protein (hsCRP) level of ≥ 7 mg/L assessed before first administration of an NLRP3 inflammasome pathway inhibitor (*e.g.*, an anti-IL-1beta antibody; *e.g.*, canakinumab). In some embodiments, the patient has a high sensitivity C-reactive protein (hsCRP) level of ≥ 8 mg/L assessed before first administration of an NLRP3 inflammasome pathway inhibitor (*e.g.*, an anti-IL-1beta antibody; *e.g.*, canakinumab). In some embodiments, the patient has a high sensitivity C-reactive protein (hsCRP) level of ≥ 9 mg/L assessed before first administration of an NLRP3 inflammasome pathway inhibitor (*e.g.*, an anti-IL-1beta antibody; *e.g.*, canakinumab). In some embodiments, the patient has a high sensitivity C-reactive protein (hsCRP) level of ≥ 10 mg/L assessed before first administration of an NLRP3 inflammasome pathway inhibitor (*e.g.*, an anti-IL-1beta antibody; *e.g.*, canakinumab).
- In some embodiments, the NLRP3 inflammasome pathway inhibitor is administered to the subject in an effective amount, *e.g.*, an amount that may lower or reduce the level of hsCRP in the subject. In some embodiments, the hsCRP level may be measured approximately one, two, three, four, five, six, seven, or eight months after first administration of the NLRP3 inflammasome pathway inhibitor (*e.g.*, an anti-IL-1beta antibody; *e.g.*, canakinumab).
- In one embodiment, the reduced level of hsCRP assessed after first administration (*e.g.*, six months after first administration) of the NLRP3 inflammasome pathway inhibitor (*e.g.*, an anti-IL-1beta antibody; *e.g.*, canakinumab) is < 1.9 , < 1.8 , < 1.7 , < 1.6 , < 1.5 , < 1.4 , < 1.3 , < 1.2 , < 1.1 , < 1.0 , < 0.9 , < 0.8 , < 0.7 , < 0.6 , or < 0.5 mg/L. In one embodiment, the reduced level of hsCRP assessed approximately 6 months after first administration of the NLRP3 inflammasome pathway inhibitor (*e.g.*, an IL-1beta inhibitor such as an anti-IL-1beta antibody; *e.g.*, canakinumab) is < 1.8 mg/L. In another embodiment, the reduced level of hsCRP assessed approximately 6 months after first administration of the NLRP3 inflammasome pathway inhibitor (*e.g.*, an IL-1beta inhibitor such as an anti-IL-1beta antibody; *e.g.*, canakinumab) is < 1.5 mg/L.
- In one embodiment, the reduced level of hsCRP assessed approximately 9 months after first administration of the NLRP3 inflammasome pathway inhibitor (*e.g.*, an IL-1beta inhibitor such as an anti-IL-1beta antibody; *e.g.*, canakinumab) is < 1.9 , < 1.8 , < 1.7 , < 1.6 , < 1.5 , < 1.4 , < 1.3 , < 1.2 , < 1.1 ,

<1.0, <0.9, <0.8, <0.7, <0.6, or <0.5 mg/L. In one embodiment, the reduced level of hsCRP assessed approximately 9 months after first administration of v is <1.8 mg/L. In another embodiment, the reduced level of hsCRP assessed approximately 9 months after first administration of the NLRP3 inflammasome pathway inhibitor (*e.g.*, an IL-1beta inhibitor such as an anti-IL-1beta antibody; *e.g.*, canakinumab) is <1.5 mg/L.

In one embodiment, any use, method, or kit described herein comprises administering about 150, 175, 200, 225, 250, 275, 300 mg or any combination thereof of canakinumab.

In one embodiment, 150 mg or 300 mg canakinumab is administered to a subject in need thereof (*e.g.*, a subject who has at least one copy of a gain-of-function mutation in *P2RX7*, *e.g.*, a subject who is homozygous for the rs1718119 SNP). In a specific embodiment, 150 mg of canakinumab is administered. In a specific embodiment, 300 mg of canakinumab is administered. In a specific embodiment, about 150 mg of canakinumab is administered. In a specific embodiment, about 300 mg of canakinumab is administered. In some embodiments, the subject has had one or more previous myocardial infarctions (MIs). In a specific embodiment of any use described herein, canakinumab is administered at the earliest 30 days after a previous MI. In some embodiments, the subject has an hsCRP level of at least 2 mg/L prior to the administration of canakinumab.

Included herein are various aspects and embodiments of the uses, methods, and kits described herein. The skilled person realizes that the embodiments in the following pages are all combinable with each other and combining features from various embodiments of these pages will be considered to be adequately disclosed to the skilled person.

In some embodiments, said patient is concomitantly receiving standard of care treatment reducing the risk of or preventing cardiovascular events (*e.g.*, MACE). Such standard of care treatment includes but is not limited to lipid lowering agents such as a HMG-CoA reductase inhibitor, *e.g.*, a statin such as lovastatin, pravastatin, simvastatin, fluvastatin, atorvastatin, cerivastatin, mevastatin, pitavastatin, rosuvastatin or mixtures thereof or mixtures with ezetimibe, niacin, amlodipine besylate, inhibitors of proprotein convertase subtilisin/kexin type 9 (PCSK9i) such as alirocumab (Praluent®), evolocumab (Repatha®), bococizumab, inhibitors of cholesteryl ester transfer protein (CETP) such as anacetrapib, torcetrapib, dalcetrapib, anti-hypertensives such as a calcium channel blocker (*e.g.*, amlodipine, diltiazem, nifedipine, nicardipine, verapamil) or beta-adrenergic blocking drugs such as esmolol, metoprolol, nadolol, penbutolol or anti-hypertensives such as labetalol, metoprolol, hydralazine, nitroglycerin, nicardipine, sodium nitroprusside, clevidipine or a diuretic such as a thiazide diuretic, chlorthalidone, furosemide, hydrochlorothiazide, indapamide, metolazone, amiloride hydrochloride, spironolactone, triamterene, or an angiotensin-converting enzyme (ACE) inhibitor such as ramipril, ramiprilat, captopril, lisinopril or an angiotensin II receptor blocker such as losartan, valsartan, olmesartan, irbesartan, candesartan, telmisartan, eprosartan or an angiotensin receptor-neprilysin inhibitor (ARNI) such as sacubitril/valsartan (Entresto®), or an

anticoagulant such as acenocoumarol, coumatetralyl, dicoumarol, ethyl biscoumacetate, phenprocoumon, warfarin heparin, low molecular weight heparin such as bemiparin, certoparin, dalteparin, enoxaparin, nadroparin, parnaparin, reviparin, tinzaparin or an inhibitor of platelet aggregation such clopidogrel, elinogrel, prasugrel, cangrelor, ticagrelor, ticlopidine, cilostazol, dipyridamole, picotamide, abciximab, eptifibatide, tirofiban or terutroban or a Prostaglandin analogue (PGI₂) such as beraprost, prostacyclin, iloprost or treprostinil, or COX inhibitors such as aspirin, aloxiprin or carbasalate calcium, indobufen or triflusal or cloricromen or ditazole or 1,3-indandiones such as clorindione, diphenadione or phenindion, or tiocloamarol, or direct thrombin (II) inhibitors such as hirudin, bivalirudin, lepirudin, desirudin (bivalent) or argatroban or dabigatran (monovalent) or oligosaccharides such as fondaparinux, idraparin, or heparinoids such as danaparoid, sulodexide, dermatan sulfate or direct Xa inhibitors xabans such as apixaban, betrixaban, edoxaban, otamixaban, rivaroxaban or REG1 or defibrotide or ramatroban or antithrombin III or protein C (drotrecogin alfa) or fibrinolytics plasminogen activators: r-tPA such as alteplase, reteplase, tenecteplase or UPA such as urokinase or saruplase or streptokinase or anistreplase or monteplase or other serine endopeptidases or ancrod or fibrinolysin; or brinase or citrate or EDTA or oxalate or digitalis, or digoxin, or nesiritide, or oxygen, or a nitrate such as glyceryl trinitrate (GTN)/nitroglycerin, isosorbide dinitrate, isosorbide mononitrate or an analgesic such as morphine sulfate or a renin inhibitor such as aliskiren or an endothelin A receptor inhibitor or an aldosterone inhibitor.

20 *General:*

All patents, published patent applications, publications, references and other material referred to herein are incorporated by reference herein in their entirety.

As used herein, the term “comprising” encompasses “including” as well as “consisting,” *e.g.* a composition “comprising” X may consist exclusively of X or may include something additional, *e.g.*, X + Y.

As used herein, the term “administering” in relation to a compound, *e.g.*, an NLRP3 inflammasome pathway inhibitor (*e.g.*, an IL-1 β inhibitor such as an anti-IL-1 β antibody; *e.g.*, canakinumab) or standard of care agent, is used to refer to delivery of that compound by any route of delivery. In some embodiments, the compound that is administered is canakinumab, and in specific embodiments the canakinumab is administered subcutaneously.

As used herein, the term “about” in relation to a numerical value x means, for example, +/- 10%.

As used herein, the word “substantially” does not exclude “completely,” *e.g.*, a composition which is “substantially free” from Y may be completely free from Y. Where necessary, the word “substantially” may be omitted from the definition of the disclosure.

As used herein, the term “3 months” includes a time period that extends one week before and one week after the 3 months (3 months +/- 1 week). The term “approximately 3 months” or “about three months” includes a time period of 90 days +/- 15 days or 90 days +/- 10 days.

As used herein, the term “6 months” includes a time period that extends one week before and one week after the 6 months (6 months +/- 1 week). The term “approximately 6 months” or “about six months” includes a time period of 120 days +/- 15 days or 120 days +/- 10 days.

As used herein, the term “NLRP3 inflammasome pathway” is a critical component of the innate immune system that mediates caspase-1 activation and the secretion of proinflammatory cytokines IL-1beta and IL-18 in response to microbial infection and cellular damage. See, *e.g.*, Kelley *et al.*, Int J Mol Sci 2019; 20(13) 3328, the contents of which are incorporated herein for this purpose.

As used herein, the term “NLRP3 inflammasome pathway inhibitor” refers to an entity that binds to and decreases the activity of at least one protein in the NLRP3 inflammasome pathway. Such a protein may be upstream (*e.g.*, the NLRP3 protein) or downstream (*e.g.*, IL-1beta, IL-18, IL-6 cytokines) of the NLRP3 inflammasome complex. Said entity may be, but is not limited to, a chemical compound, a mono specific antibody, or a bispecific antibody. The term “NLRP3 inflammasome pathway inhibitor” may be used interchangeably herein with “inflammasome inhibitor”.

As used herein, the term “IL-1beta” or “IL-1 β ” or “IL-1b” (with or without hyphenation) refers to interleukin 1 beta, a cytokine protein that in humans is encoded by the *IL1B* gene and is meant to include, without limitation, nucleic acids, polynucleotides, oligonucleotides, sense and antisense polynucleotide strands, complementary sequences, peptides, polypeptides, proteins, homologous and/or orthologous molecules, isoforms, precursors, mutants, variants, derivatives, splice variants, alleles, different species, and active fragments thereof. IL-1beta precursor protein is cleaved by cytosolic caspase 1 (interleukin 1 beta convertase) to form mature IL-1beta. NCBI reference sequences NM_000576.3; NP_000567.1.

As used herein, the term “IL-1beta inhibitor” or “inhibitor of IL-1beta” refers to an agent that is able to bind to and to partially or completely block the activity of IL-1beta. For example, such an inhibitor may be an “IL-1beta binding antibody” (which may also be referred to as an “anti-IL-1beta antibody”). Such an anti-IL-1beta antibody is capable of binding to the IL-1beta antigen either alone or when it is associated with other molecules and inhibiting its activity. The binding reaction may be shown by standard methods (qualitative assays) including, for example, a bioassay for determining the inhibition of IL-1beta binding to its receptor or any kind of binding assays, with reference to a negative control test in which an antibody of unrelated specificity but of the same isotype is used. Advantageously, the binding of the IL-1beta binding antibodies may be shown in a competitive binding assay. An exemplary IL-1beta inhibitor described herein is canakinumab.

As used herein, the term “IL-18” refers to interleukin 18, which is a pro-inflammatory cytokine, and is meant to include, without limitation, nucleic acids, polynucleotides, oligonucleotides, sense and antisense polynucleotide strands, complementary sequences, peptides, polypeptides, proteins, homologous and/or orthologous molecules, isoforms, precursors, mutants, variants, derivatives, splice variants, alleles, different species, and active fragments thereof. As used herein, the term “IL-18 inhibitor” or “inhibitor of IL-18” refers to an agent that is able to bind to and to partially or completely block the activity of IL-18. IL-18 inhibitors for the uses, methods, and kits described herein may be any known inhibitors, such as anti-IL-18 antibodies and IL-18bp (*e.g.*, a recombinant human IL-18 binding protein such as tadekinig alfa).

As used herein, the term “IL-6” refers to interleukin 6, which is a pro-inflammatory cytokine, and is meant to include, without limitation, nucleic acids, polynucleotides, oligonucleotides, sense and antisense polynucleotide strands, complementary sequences, peptides, polypeptides, proteins, homologous and/or orthologous molecules, isoforms, precursors, mutants, variants, derivatives, splice variants, alleles, different species, and active fragments thereof. As used herein, the term “IL-6 inhibitor” or “inhibitor of IL-6” refers to an agent that is able to bind to and to partially or completely block the activity of IL-6. IL-6 inhibitors for the uses, methods, and kits described herein may be any known inhibitors, such as anti-IL-6R antibodies (*e.g.*, tocilizumab) and anti-IL-6 antibodies (*e.g.*, siltuximab). IL-6 inhibitors for use in the methods, uses, and kits described herein may also be selected from, *e.g.*, olokizumab, elsilimomab, BMS-945429, sirukumab, and levilimab.

As used herein, the term “NLRP3” refers to the nucleotide-binding oligomerization domain-like receptor protein 3 (also known as the NLR Family Pyrin Domain Containing 3), which is a pyrin-like protein that plays a crucial role in innate immunity and inflammation as a component of the NLRP3 inflammasome pathway. This term is meant to include, without limitation, nucleic acids, polynucleotides, oligonucleotides, sense and antisense polynucleotide strands, complementary sequences, peptides, polypeptides, proteins, homologous and/or orthologous NLRP molecules, isoforms, precursors, mutants, variants, derivatives, splice variants, alleles, different species, and active fragments thereof.

As used herein, the term “NLRP3 inhibitor”, “NLRP3 antagonist” or “inhibitor of NLRP3” is an agent, a genetic mutation, or altered signaling pathways in a mammalian cell that results in a decrease in one or both of (i) the activity of an NLRP3 inflammasome (*e.g.*, any of the exemplary activities of an NLRP3 inflammasome described herein) (*e.g.*, as compared to the level of NLRP3 inflammasome activity in the absence of the agent) and (ii) the expression level of NLRP3 inflammasomes in a mammalian cell (*e.g.*, using any of the exemplary methods of detection described herein) (*e.g.*, as compared to the expression level of NLRP3 inflammasomes in a mammalian cell not contacted with the agent). Typically, an NLRP3 antagonist has a <1 micromolar activity in cellular

assay systems, assessed for example using a nigericin-stimulated IL-1 β secretion assay in THP-1 cells, defined herein. Exemplary NLRP3 antagonists are described elsewhere herein.

The term “biomarker”, as used herein, refers generally to a molecule, *i.e.*, a gene (or nucleic acid encoding said gene), protein, the expression of which in a biological sample from a patient can be detected by standard methods in the art, and is predictive or denotes a condition of the patient from which it was obtained. Exemplary biomarkers include, but are not limited to, P2RX7, hsCRP, and IL-6.

As used herein, the term “assaying” is used to refer to the act of detecting, identifying, screening, or determining, which act may be performed by any conventional means. For example, a sample may be assayed for the presence of a particular marker by using an ELISA assay, a Northern blot, imaging, serotyping, cellular typing, gene sequencing, phenotyping, haplotyping, immunohistochemistry, western blot, mass spectrometry, *etc.* The term “detecting” (and the like) means the act of extracting particular information from a given source, which may be direct or indirect. In some embodiments of the predictive methods disclosed herein, the presence of a given thing (*e.g.*, allele, level of protein, *etc.*) is detected in a biological sample indirectly, *e.g.*, by querying a database. The terms “assaying” and “determining” contemplate a transformation of matter, *e.g.*, a transformation of a biological sample, *e.g.*, a blood sample or other tissue sample, from one state to another by means of subjecting that sample to physical testing. The term “assaying” is used to mean that a sample may be tested (either directly or indirectly) for either the presence or level of a given marker (*e.g.*, P2RX7 or P2X7, hsCRP, or IL-6). It will be understood that, in a situation where the level of a substance denotes a probability, then the level of such substance may be used to guide a therapeutic decision. For example, one may determine the level of hsCRP in a patient by assaying for its presence by quantitative or relatively-quantitative means (*e.g.*, levels relative to the levels in other samples). For example, one may determine the level of P2X7 in a patient by assaying for its presence by quantitative or relatively-quantitative means (*e.g.*, levels relative to the levels in other samples). Certain methods described herein involve, *inter alia*, determining the level of a particular marker, *e.g.*, P2X7, in a patient. Certain methods described herein involve, *inter alia*, determining the level of a particular marker, *e.g.*, hsCRP, in a patient. Certain methods described herein involve, *inter alia*, determining the presence of a particular SNP, *e.g.*, rs1718119, in a patient. In some embodiments, any of the samples assayed for the uses and methods described herein are analyzed in an *in vitro* setting (*i.e.*, are assayed after isolation).

As used herein, the terms “P2X7”, “P2X₇”, “P2X7 receptor”, and “P2X₇ receptor” refer to the P2X purinoceptor 7 protein (also known as the purinergic receptor P2X7) encoded by the P2RX7 gene (NCBI Reference Sequence NG_011471.2). The terms P2RX7 and P2X7 are interchangeable (*i.e.*, this term may appear in both italicized and non-italicized font and refers to the P2RX7 gene). This protein is a member of a family of cationic channels that plays a critical role in mediating disparate

physiological functions of extracellular ATP, including the regulation of immune responses and inflammation. See: North RA, *Physiol Rev* 2002, 82:1013-1067; Hattori and Gouaux, *Nature* 2012, 485:207-212. Activation of P2X7 receptors has been shown to trigger maturation of the inflammasome and release of certain inflammatory mediators including IL1beta, TNFalpha, and PGE2. See: Di VF, *Trends Pharmacol Sci* 2007, 28:465-472; Surprenant *et al.*, *Science* 1996, 272:735-738; Ferrari *et al.*, *J Immunol* 1997, 159:1451-1458; MacKenzie A, *Immunity* 2001, 15:825-835; Solle M *et al.*, *J Biol Chem* 2001, 276:125-132. In certain embodiments of the uses and methods described herein, P2X7 levels are assessed in a biological sample, *e.g.*, blood, obtained from the patient. A biological sample from the patient may be assayed for the level of P2X7 (*e.g.*, an *in vitro* sample). In certain embodiments of the uses and methods described herein, a patient or a biological sample from a patient may be assayed or analyzed in order to determine whether the patient has one or more polymorphisms of the *P2RX7* gene. For example, one or more biological samples from a patient may be assayed or analyzed in order to determine if the patient has one or more gain-of-function mutations in the *P2RX7* gene. In certain embodiments, one or more biological samples (*e.g.*, a blood sample) may be assayed or analyzed in order to determine whether the patient has one or more copies of the rs1718119 SNP. In certain embodiments, one or more biological samples (*e.g.*, a blood sample) may be assayed or analyzed in order to determine whether the patient is homozygous for the rs1718119 SNP. Such analyses may be used to determine whether the patient is at elevated risk for MACE and/or whether the patient may benefit from treatment with an effective amount of an NLRP3 inflammasome pathway inhibitor (*e.g.*, an IL-1beta inhibitor such as an IL-1beta inhibitor such as canakinumab).

As used herein, the terms “C-reactive protein” and “CRP” refers to serum C-reactive protein, which is used as an indicator of the acute phase response to inflammation. In certain embodiments of the uses and methods described herein, hsCRP levels are assessed in a biological sample, *e.g.*, blood, obtained from the patient. A biological sample from the patient is assayed for the level of hsCRP (*e.g.*, an *in vitro* sample). As used herein, the term “hsCRP” or “hsCRP level” refers to the level of CRP in the blood as measured by high sensitivity CRP testing. The level of CRP or hsCRP in plasma may be given in any concentration, *e.g.*, mg/dl, mg/L, nmol/L. Levels of CRP or hsCRP may be measured by a variety of well-known methods, *e.g.*, radial immunodiffusion, electroimmunoassay, immunoturbidimetry, ELISA, turbidimetric methods, fluorescence polarization immunoassay, and laser nephelometry. Testing for CRP may employ a standard CRP test or a high sensitivity CRP (hsCRP) test (*i.e.*, a high sensitivity test that is capable of measuring low levels of CRP in a sample, *e.g.*, using laser nephelometry). Kits for detecting levels of CRP or hsCRP may be purchased from various companies, *e.g.*, Calbiotech, Inc, Cayman Chemical, Roche Diagnostics Corporation, Abzyme, DADE Behring, Abnova Corporation, Aniera Corporation, Bio-Quant Inc., Siemens Healthcare Diagnostics, etc.

As used herein, the term “gain-of-function mutation” refers to a mutation that confers new or enhanced activity on a protein (*e.g.*, P2X7). As described herein, such a gain-of-function mutation may result in increases in the level of *P2RX7* expression, the level of P2X7 protein, and/or the level of P2X7 activity. As a non-limiting example, such a mutation may result in increased activity of the

5 NLRP3 inflammasome pathway.

As used herein, the term “polymorphism” refers to one of two or more variants of a particular DNA sequence. A common type of polymorphism involves variation at a single base pair known as a “single nucleotide polymorphism” or “SNP.” As an example, rs1718119 is a SNP of *P2RX7* referred to herein that represents variation at a single base pair.

10 As used herein, the term “allele” refers to an alternate form of a gene (in diploids, one member of a pair) that is located at a defined position on a specific chromosome. Alleles may be, for example, a SNP (as described elsewhere herein) or may be longer. An organism which has two different alleles of the gene is called heterozygous or a heterozygote, while an organism (*e.g.*, a patient or subject) which has two copies of the same allele of the gene is called homozygous or a

15 homozygote.

As used herein, the term “patient” is used interchangeably with the term “subject” and includes any human or nonhuman animal. The term “nonhuman animal” includes all vertebrates, *e.g.*, mammals and non-mammals, such as nonhuman primates, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, etc. In a specific embodiment, the compositions, methods, and uses described

20 herein are in reference to a human patient or human subject.

As used herein, a subject is “in need of” a treatment if such subject who is afflicted with the condition, disease, disorder, or syndrome of interest (*e.g.*, any of the cardiovascular conditions described herein) or who is at risk of the same (*e.g.*, faces an increased risk of suffering from the condition, disease, disorder, or syndrome of interest (*e.g.*, any of the cardiovascular conditions

25 described herein) and who would benefit biologically, medically, or in quality of life from such treatment. In some embodiments, the subject is “in need of” one or more of the treatments described herein (*e.g.*, an effective amount of an NLRP3 inflammasome pathway inhibitor, *e.g.*, an IL-1beta inhibitor, *e.g.*, canakinumab) if the subject has one or more (*e.g.*, two) copies of the rs1718119 allele.

As used herein, the term “prevent”, “preventing”, or “prevention” in connection to a disease,

30 condition, disorder, or syndrome refers to the prophylactic treatment of a subject who is at risk of developing a condition (*e.g.*, a specific disease, condition, disorder, or syndrome or clinical symptom thereof) resulting in a decrease in the probability that the subject will develop the condition. For example, the patient may be at risk of a an adverse cardiovascular event (*e.g.*, a MACE) such as a myocardial infarction (MI). Such a treatment would be used to prevent a future MACE (*e.g.*, a

35 myocardial infarction or cardiovascular death) in such a patient.

The terms “treat”, “treating”, and “treatment” refer to both therapeutic treatment and prophylactic or preventive measures, wherein the object is to ameliorate the disease, condition, disorder, or syndrome (*i.e.*, slowing or arresting or reducing the development of the disease or at least one of the clinical symptoms thereof) by alleviating or ameliorating at least one physical parameter including those which may not be discernible by the patient. The terms “treat”, “treating”, or “treatment” also refer to modulating the disease or disorder, either physically, (*e.g.*, stabilization of a discernible symptom), physiologically, (*e.g.*, stabilization of a physical parameter), or both and/or to preventing or delaying the onset or development or progression of the disease or disorder.

As used herein, the term “cardiovascular death” includes sudden cardiac death, death due to acute myocardial infarction (AMI), death due to heart failure, death due to stroke, and death due to other cardiovascular causes.

As used herein, “sudden cardiac death” is a sudden death that occurs in a previously stable patient who does not have a prior terminal condition, such as malignancy not in remission or end-stage chronic lung disease.

Death due to acute myocardial infarction (AMI): refers to a death within 30 days after a myocardial infarction (MI) related to consequences seen immediately after the myocardial infarction, such as progressive congestive heart failure (CHF), inadequate cardiac output, or recalcitrant arrhythmia.

Death due to heart failure or cardiogenic shock refers to death occurring in the context of clinically worsening symptoms and/or signs of heart without evidence of another cause of death and includes sudden death occurring during an admission for worsening heart failure as well as death from progressive heart failure or cardiogenic shock following implantation of a mechanical assist device.

Death due to stroke (intracranial hemorrhage or non-hemorrhagic stroke) refers to death occurring up to 30 days after a suspected stroke based on clinical signs and symptoms as well as neuroimaging and/or autopsy, and where there is no conclusive evidence of another cause of death.

As used herein, “death due to other cardiovascular causes” refers to death due to a cardiovascular cause not included in the above categories (*e.g.*, dysrhythmia, pulmonary embolism, cardiovascular intervention, aortic aneurysm rupture, or peripheral arterial disease). Mortal complications of cardiac surgery or non-surgical revascularization, even if “non-cardiovascular” in nature, should be classified as cardiovascular deaths.

As used herein the term “death of undetermined cause” (presumed cardiovascular) refers to all deaths not attributed to the categories of cardiovascular death or to a non-cardiovascular cause are considered presumed cardiovascular deaths. As used herein, “non-cardiovascular death” is defined as any death not covered by cardiac death or vascular death and is categorized as follows: pulmonary causes, renal causes, gastrointestinal causes, infection (including sepsis), non-infectious causes,

malignancy, accident/trauma, suicide, non-cardiovascular system organ failure (*e.g.* hepatic), hemorrhage, not intracranial or other.

As used herein, the term “recurrent CV events” is a repeated CV event taking place after the myocardial infarction qualifying a patient for treatment and is selected from non-fatal MI, non-fatal stroke, cardiovascular (CV) death and hospitalization for unstable angina requiring unplanned revascularization.

As used herein, the term “myocardial infarction (MI)” refers to “acute myocardial infarction”: the term myocardial infarction (MI) is used when there is evidence of myocardial necrosis in a clinical setting consistent with myocardial ischemia. The term MI includes an ST-elevated MI (STEMI) or a non-ST-elevated MI (NSTEMI). Under these conditions any one of the following criteria meets the diagnosis for MI:

The term “spontaneous MI” refers to the detection of rise and/or fall of cardiac biomarkers with at least one value above the 99th percentile of the upper reference limit (URL) together with evidence of myocardial ischemia with at least one of the following: symptoms of ischemia, ECG changes indicative of new ischemia (ST Elevation - New ST elevation at the J-point in two contiguous leads with the cut-off points: ≥ 0.2 mV in men or ≥ 0.15 mV in women in leads V2-V3 and/or ≥ 0.1 mV in other leads, ST depression and T-wave changes – New horizontal or down-sloping ST depression ≥ 0.05 mV in two contiguous leads; and/or T inversion ≥ 0.1 mV in two contiguous leads with prominent R waves or R/S ratio >1 ., development of pathological Q waves in the ECG (Any Q-wave in leads V2-V3 ≥ 0.02 seconds or QS complex in leads V2 and V3, Q-wave ≥ 0.03 seconds and ≥ 0.1 mV deep or QS complex in leads I, II, aVL, aVF, or V4-V6 in any two leads of a contiguous lead grouping (I, aVL, V6, V4-V6, II, III, aVF), imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.

The term “percutaneous coronary intervention (PCI) related myocardial infarct” refers to PCI in patients with normal baseline troponin values elevations of cardiac biomarkers above the 99th percentile URL within 24 hours of the procedure are indicative of peri-procedural myocardial necrosis. By convention increases of biomarkers greater than $3 \times 99^{\text{th}}$ percentile URL are consistent with PCI related myocardial infarction. If the cardiac biomarker is elevated prior to PCI $\geq 20\%$ increase of the value in that second cardiac biomarker within 24 hours of the PCI and documentation that cardiac biomarkers were decreasing (two samples at least 6 hours apart) prior to the suspected recurrent MI is also consistent with PCI related MI. Symptoms of cardiac ischemia are not required

The term “CABG related myocardial infarct” refers to CABG in patients with normal baseline troponin, elevations of cardiac biomarkers above 5 times the 99th percentile of the normal reference range during the first 72 hours after CABG, when associated with either new pathological Q waves in at least 2 contiguous leads on the ECG that persist through 30 days or new left bundle branch block

(LBBB) or angiographically documented new graft or native coronary artery occlusion or imaging evidence of new loss of viable myocardium

If the cardiac biomarker is elevated prior to CABG a $\geq 20\%$ increase of the value in the second cardiac biomarker within 72 hours of CABG AND documentation that the cardiac biomarkers were decreasing (2 samples at least 6 hours apart) prior to the suspected recurrent MI plus either new pathological Q waves in at least 2 contiguous leads on the ECG or new LBBB, angiographically documented new graft or native artery occlusion or imaging evidence or new loss of viable myocardium is consistent with a peri-procedural myocardial infarct after CABG. Symptoms of cardiac ischemia are not required.

Criteria for Prior Myocardial Infarction (also referred to as a previous myocardial infarction): Any of the following criteria meets the diagnosis for prior myocardial infarction: development of new pathological Q waves with or without symptoms, imaging evidence of a region of loss of viable myocardium that is thinned and fails to contract in the absence of a non-ischemic cause, pathological findings of a healed or healing myocardial infarction

ECG changes associated with prior Myocardial Infarction:

- Any Q wave in leads V2-V3 ≥ 0.02 seconds or QS complex in leads V2 and V3
- Q-wave ≥ 0.03 seconds and ≥ 0.1 mV deep or QS complex in leads I, II, aVL, aVF, or V4-V6 in any two leads of a contiguous lead grouping (I, aVL, V6, V4-V6, II, III, and aVF)
- R-wave ≥ 0.04 seconds in V1-V2 and R/S ≥ 1 with a concordant positive T-wave in the absence of a conduction defect

Criterion for Reinfarction: In patients where recurrent MI is suspected from clinical signs or symptoms following the initial infarction, an immediate measurement of the employed cardiac biomarker is recommended. A second sample should be obtained 3-6 hours later. Recurrent infarction is diagnosed if there is a $\geq 20\%$ increase of the value in the second sample. This value should exceed the 99th percentile URL. However if cardiac biomarkers are elevated prior to the suspected new MI, there must also be documentation of decreasing values (two samples at least 6 hours apart) prior to the suspected new MI. If the values are falling criteria for reinfarction by further measurement of biomarkers together with features of the ECG or imaging can be applied.

ECG diagnosis of reinfarction following the initial infarction: may be confounded by the initial evolutionary ECG changes. Reinfarction should be considered when the ST elevation ≥ 0.1 mV reoccurs in an inpatient having a lesser degree of ST elevation or new pathognomonic Q-waves, in at least two contiguous leads, particularly when associated with ischemic symptoms for 10 minutes or longer. The re-evaluation of the ST segment can, however also be seen in threatening myocardial rupture and should lead to additional diagnostic work-up. ST depression or LBBB on their own should not be considered valid criteria for Myocardial Infarction.

If biomarkers are increasing or peak is not reached then there is insufficient data to diagnose recurrent MI.

Clinical Classification of different types of Myocardial Infarction :

- Type 1 – Spontaneous MI related to ischemia due to a primary coronary event such as plaque erosion and/or rupture, fissuring or dissection.
- Type 2 – MI secondary to ischemia due to either increased oxygen demand or decreased supply, *e.g.* coronary artery spasm, anemia, hypotension, coronary embolism, arrhythmias, hypertension or hypotension.
- Type 3 – Sudden unexpected cardiac death including cardiac arrest, often with symptoms suggestive of myocardial ischemia accompanied by presumably new ST elevation, or new LBBB, or evidence of fresh thrombus in a coronary artery by angiography and/or at autopsy, but death occurring before blood samples could be obtained or at a time before the appearance of cardiac biomarkers in the blood.
- Type 4a – MI associated with PCI (Percutaneous Coronary Intervention).
- Type 4b – MI associated with stent thrombosis as documented by autopsy or angiography.
- Type 5 – MI associated with CABG (Coronary artery bypass grafting)

The term “silent MI”: the following criteria will be used by the central ECG reading vendor to define interval “silent” (no clinical symptoms or signs) MI between baseline and yearly ECGs (Surawicz B *et al*, Chou's electrocardiography in clinical practice : adult and pediatric. Philadelphia: Saunders; 2001):

Myocardial infarctions are reported only on the basis of pathologic Q waves. Pathologic Q waves are defined as Q wave duration > 40ms and Q/R ratio = 1/3.

Any Q wave in V1 or V2 that is followed by an R wave should be considered abnormal.

When pathologic Q waves (*e.g.*, as a result of myocardial infarction) are present, ST elevation or T wave inversion may be used to classify the infarction as New or Acute. However, ST elevation or T wave inversion in the absence of pathologic Q waves are not sufficient criteria for diagnosis of myocardial infarction.

- Anterolateral MI - Pathologic Q waves in leads V3-V6.
- Anterior MI - Pathologic Q waves in V3 and V4.
- Anteroseptal MI - Pathologic Q waves or QS in leads V1-V4.
- Extensive Anterior MI - Pathologic Q waves in leads I, aVL, and V1-V6.
- High lateral MI - Pathologic Q waves in leads I and aVL.
- Inferior MI - Pathologic Q waves or QS in at least two of the inferior leads: aVF, III, II.
- Lateral MI - Pathologic Q waves in leads I, aVL, and V5-V6.
- Septal MI - Pathologic Q waves or QS in leads V1-V2, (V3). In the presence of LAHB or LVH a Q or QS in V3 is required.

- Posterior MI - Initial R wave duration 40 ms in V1 or V2, and R > S and upright T wave; Inferior or Lateral MI are usually also present.

As used herein, the term “stroke” is defined as the rapid onset of a new persistent neurological deficit attributed to an obstruction in cerebral blood flow and/or cerebral hemorrhage with no apparent non-vascular cause (*e.g.* tumor, trauma, infection). Available neuroimaging studies will be considered to support the clinical impression and to determine if there is a demonstrable lesion compatible with an acute stroke. Non-fatal strokes will be classified as ischemic, hemorrhagic or unknown.

As used herein the term “unstable angina requiring unplanned revascularization” is defined as no elevation in cardiac biomarkers and clinical presentation (one of the following) with cardiac symptoms lasting ≥ 10 minutes and considered to be myocardial ischemia on final diagnosis (rest angina or new onset (< 2 months) severe angina (CCS classification severity $\geq III$; Grading of Angina Pectoris According to Canadian Cardiovascular Society Classification) or increasing angina (in intensity, duration and/or frequency) and severe recurrent ischemia requiring urgent revascularization: as defined by an episode of angina prompting the performance of coronary revascularization on the index hospitalization or an episode of recurrent angina after discharge that resulted in re-hospitalization during which coronary revascularization was performed; and at least one of the following: new or worsening ST or T segment changes on ECG, ST Elevation (new ST elevation at the J point in two anatomically contiguous leads with the cut-off points: ≥ 0.2 mV in men (> 0.25 mV in men < 40 years) or ≥ 0.15 mV in women in leads V2-V3 and/or ≥ 0.1 mV in other leads), ST depression and T-wave Evidence of ischemia on stress testing with cardiac imaging, evidence of ischemia on stress testing without cardiac imaging but with angiographic evidence of $\geq 70\%$ lesion, and/or thrombus in the epicardial coronary artery or initiation/increased dosing of anti-anginal therapy, angiographic evidence of $\geq 70\%$ lesion and/or thrombus in an epicardial coronary artery.

As used herein “coronary revascularization” is defined as an invasive procedure, which usually follows coronary angiography, wherein either percutaneous transluminal intervention, followed by Stent Placement, Balloon Angioplasty, or CABG is performed to relieve obstructed coronary arteries. A team of medical professionals lead by either an invasive cardiologist (percutaneous transluminal intervention, followed by stent placement, balloon angioplasty) or a thoracic surgeon (CABG), who performs the described procedures.

As used herein the term “non-coronary revascularization” is defined as vascular surgery or percutaneous intervention. Vascular surgery is defined as the placement of a conduit with or without proximal and/or distal anastomoses. Percutaneous intervention is defined as balloon inflation with or without stenting.

As used herein, the term “atherosclerosis” occurs when fatty material and a substance called plaque builds up on the walls of the arteries, causing the lumen to narrow.

As used herein, the term “MACE”, which can refer interchangeably to “major adverse cardiac events” and “major adverse cardiovascular events” comprises non-fatal heart attack, non-fatal stroke, and cardiovascular (CV) death.

The phrase “receiving data” is used to mean obtaining possession of information by any available means, *e.g.*, orally, electronically (*e.g.*, by electronic mail, encoded on diskette or other media), written, etc.

As used herein, “selecting” and “selected” in reference to a patient is used to mean that a particular patient is specifically chosen from a larger group of patients on the basis of (due to) the particular patient having a predetermined criteria, *e.g.*, the patient has a gain-of-function mutation in *P2RX7* (*e.g.*, the patient has 1 or 2 copies of the rs1718119 SNP in the *P2RX7* gene). Similarly, “selectively treating” refers to providing treatment to a patient having or at risk of having a particular condition, disease, or disorder (*e.g.*, MACE), where that patient is specifically chosen from a larger group of patients on the basis of the particular patient having a predetermined criteria, *e.g.*, the patient has a gain-of-function mutation in *P2RX7* (*e.g.*, the patient has 1 or 2 copies of the rs1718119 SNP in the *P2RX7* gene). Similarly, “selectively administering” refers to administering a drug to a patient that is specifically chosen from a larger group of patients on the basis of (due to) the particular patient having a predetermined criteria, *e.g.*, a particular genetic or other biological marker; *e.g.*, the patient has a gain-of-function mutation in *P2RX7* (*e.g.*, the patient has 1 or 2 copies of the rs1718119 SNP in the *P2RX7* gene). By selecting, selectively treating and selectively administering, it is meant that a patient is delivered a personalized therapy based on the patient’s particular biology, rather than being delivered a standard treatment regimen based solely on the patient having a particular disease. Selecting, in reference to a method of treatment as used herein, refers to the deliberate choice to administer an NLRP3 inflammasome pathway inhibitor (*e.g.*, an IL-1beta inhibitor such as an anti-IL-1beta antibody, *e.g.*, canakinumab) to a patient based on the patient having a gain-of-function mutation in *P2RX7* (*e.g.*, the patient has 1 or 2 copies of the rs1718119 SNP in the *P2RX7* gene). Thus, selective treatment differs from standard treatment, which delivers a particular drug to all patients, regardless of their allelic status.

As used herein, “predicting” indicates that the methods described herein provide information to enable a health care provider to determine the likelihood that an individual having or at risk of having MACE will respond to or will respond more favorably to treatment with NLRP3 inflammasome pathway inhibitor (*e.g.*, an IL-1beta inhibitor such as an anti-IL-1beta antibody, *e.g.*, canakinumab). It does not refer to the ability to predict response with 100% accuracy. Instead, the skilled artisan will understand that it refers to an increased probability.

As used herein, “likelihood” and “likely” is a measurement of how probable an event is to occur. It may be used interchangeably with “probability”. Likelihood refers to a probability that is more than speculation, but less than certainty. Thus, an event is likely if a reasonable person using

common sense, training or experience concludes that, given the circumstances, an event is probable. In some embodiments, once likelihood has been ascertained, the patient may be treated (or treatment continued, or treatment proceed with a dosage increase) with an NLRP3 inflammasome pathway inhibitor (*e.g.*, an IL-1beta inhibitor such as an anti-IL-1beta antibody, *e.g.*, canakinumab) or the patient may not be treated (or treatment discontinued, or treatment proceed with a lowered dose) with an NLRP3 inflammasome pathway inhibitor (*e.g.*, an IL-1beta inhibitor such as an anti-IL-1beta antibody, *e.g.*, canakinumab).

The phrase “increased likelihood” refers to an increase in the probability that an event will occur. For example, some methods herein allow prediction of whether a patient will display an increased likelihood of responding to treatment with an NLRP3 inflammasome pathway inhibitor (*e.g.*, an IL-1beta inhibitor such as an anti-IL-1beta antibody, *e.g.*, canakinumab) or an increased likelihood of responding better to treatment with an NLRP3 inflammasome pathway inhibitor (*e.g.*, an IL-1beta inhibitor such as an anti-IL-1beta antibody, *e.g.*, canakinumab) in comparison to a patient who does not have a gain-of-function mutation in *P2RX7* (*e.g.*, the patient has no copies of the rs1718119 SNP in the *P2RX7* gene).

As used herein “SNP” refers to “single nucleotide polymorphism”. A single nucleotide polymorphism is a DNA sequence variation occurring when a single nucleotide in the genome (or other shared sequence) differs between members of a biological species or paired chromosomes in an individual. Most SNPs have only two alleles, and one is usually more common in the population. A SNP may be present in an exon or an intron of a gene, an upstream or downstream untranslated region of a gene, or in a purely genomic location (*i.e.*, non-transcribed). When a SNP occurs in the coding region of a gene, the SNP may be silent (*i.e.*, a synonymous polymorphism) due to the redundancy of the genetic code, or the SNP may result in a change in the sequence of the encoded polypeptide (*i.e.*, a non-synonymous polymorphism). Herein, SNPs are identified by their Single Nucleotide Polymorphism Database (dbSNP) rs number, *e.g.*, “rs1718119” or “rs7958311”. The dbSNP is a free public archive for genetic variation within and across different species developed and hosted by the National Center for Biotechnology Information (NCBI) in collaboration with the National Human Genome Research Institute (NHGRI).

A polymorphic site, such as a SNP, is usually preceded by and followed by conserved sequences in the genome of the population of interest and thus the location of a polymorphic site can often be made in reference to a consensus nucleic acid sequence (*e.g.*, of thirty to sixty nucleotides) that bracket the polymorphic site, which in the case of a SNP is commonly referred to as the “SNP context sequence”. Context sequences for the SNPs disclosed herein may be found in the NCBI SNP database available at: [www \[dot\] ncbi \[dot\] nlm \[dot\] nih \[dot\] gov/snp](http://www.ncbi.nlm.nih.gov/snp). Alternatively, the location of the polymorphic site may be identified by its location in a reference sequence (*e.g.*, GeneBank deposit) relative to the start of the gene, mRNA transcript, BAC clone or even relative to the initiation

codon (ATG) for protein translation. The skilled artisan understands that the location of a particular polymorphic site may not occur at precisely the same position in a reference or context sequence in each individual in a population of interest due to the presence of one or more insertions or deletions in that individual's genome as compared to the consensus or reference sequence. It is routine for the skilled artisan to design robust, specific and accurate assays for detecting the alternative alleles at a polymorphic site in any given individual, when the skilled artisan is provided with the identity of the alternative alleles at the polymorphic site to be detected and one or both of a reference sequence or context sequence in which the polymorphic site occurs. Thus, the skilled artisan will understand that specifying the location of any polymorphic site described herein by reference to a particular position in a reference or context sequence (or with respect to an initiation codon in such a sequence) is merely for convenience and that any specifically enumerated nucleotide position literally includes whatever nucleotide position the same polymorphic site is actually located at in the same locus in any individual being tested for the genetic marker using any of the genotyping methods described herein or other genotyping methods known in the art.

In addition to SNPs, genetic polymorphisms include translocations, insertions, substitutions, deletions, etc., that occur in gene enhancers, exons, introns, promoters, 5' UTR, 3' UTR, etc.

As used herein "rs1718119" refers to an SNP located within an exon of the human *P2RX7* gene (NCBI Reference Sequence NG_011471.2). The rs1718119 polymorphic site is located at chromosomal position chr12:121177300 (GRCh38.p12), in exon 11. In some embodiments of the disclosed methods, uses, and kits, the patient has at least one rs1718119 polymorphism. In some embodiments of the disclosed methods, uses, and kits, the patient has two rs1718119 polymorphisms. Patients with at least one copy (*e.g.*, two copies) of the rs1718119 SNP (a gain-of-function mutation in *P2RX7*) have an increased risk of MACE and MI; such patients may benefit from treatment with an NLRP3 inflammasome pathway inhibitor (*e.g.*, an IL-1beta inhibitor, *e.g.*, canakinumab). In some embodiments, the polymorphism is Ala348Thr in *P2RX7*.

As recognized by the skilled artisan, nucleic acid samples containing a particular SNP may be complementary double stranded molecules and thus reference to a particular site on the sense strand refers as well to the corresponding site on the complementary antisense strand. Similarly, reference to a particular genotype obtained for a SNP on both copies of one strand of a chromosome is equivalent to the complementary genotype obtained for the same SNP on both copies of the other strand.

As used herein, "genomic sequence" refers to a DNA sequence present in a genome, and includes a region within an allele, an allele itself, or a larger DNA sequence of a chromosome containing an allele of interest.

An "equivalent genetic marker" refers to a genetic marker that is correlated to an allele of interest, *e.g.*, it displays linkage disequilibrium (LD) or is in genetic linkage with the allele of interest. Equivalent genetic markers may be used to determine if a patient has such a marker, rather than

directly interrogating a biological sample from the patient for the allele per se. Various programs exist to help determine LD for particular SNPs, e.g., HaploBlock (available at [bioinfo \[dot\] cs \[dot\] technion \[dot\] ac \[dot\] il/haploblock/](https://bioinfo.cs.cmu.edu/technion/ac/haploblock/)), HapMap, WGA Viewer.

The term “probe” refers to any composition of matter that is useful for specifically detecting another substance. A probe can be an oligonucleotide (including a conjugated oligonucleotide) that specifically hybridizes to the genomic sequence of *P2RX7*, or a nucleic acid product of *P2RX7*. A conjugated oligonucleotide refers to an oligonucleotide covalently bound to chromophore or molecules containing a ligand (*e.g.*, an antigen), which is highly specific to a receptor molecule (*e.g.*, an antibody specific to the antigen). The probe can also be a PCR primer, *e.g.*, together with another primer, for amplifying a particular region within *P2RX7*. Further, the probe can be an antibody that specifically binds to polypeptide products of these alleles. Further, the probe can be any composition of matter capable of detecting (*e.g.*, binding or hybridizing) an equivalent genetic marker of *P2RX7*. In some embodiments, the probe specifically hybridizes to a nucleic acid sequence (*e.g.*, genomic DNA) or specifically binds to a polypeptide sequence of an allele of interest (*e.g.*, a gain-of-function allele of *P2RX7*). In some embodiments, the probe is at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, or 35 nucleic acids in length.

The phrase “specifically hybridizes” is used to refer to hybridization under stringent hybridization conditions. Stringent conditions are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6; the contents of which are incorporated herein by reference for this purpose. Aqueous and nonaqueous methods are described in that reference and either can be used. One example of stringent hybridization conditions is hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by at least one wash in 0.2X SSC, 0.1% SDS at 50°C. A second example of stringent hybridization conditions is hybridization in 6X SSC at about 45°C, followed by at least one wash in 0.2X SSC, 0.1% SDS at 55°C. Another example of stringent hybridization conditions is hybridization in 6X SSC at about 45°C, followed by at least one wash in 0.2X SSC, 0.1% SDS at 60°C. A further example of stringent hybridization conditions is hybridization in 6X SSC at about 45°C, followed by at least one wash in 0.2X SSC, 0.1% SDS at 65°C. High stringent conditions include hybridization in 0.5 M sodium phosphate, 7% SDS at 65°C, followed by at least one wash at 0.2X SSC, 1% SDS at 65°C.

The phrase “a region of a nucleic acid” is used to indicate a smaller sequence within a larger sequence of nucleic acids. For example, a gene is a region of a chromosome, an exon is a region of a gene, etc.

The term “specifically binds” in the context of polypeptides is used to mean that a probe binds a given polypeptide target (*e.g.*, P2X7) rather than binding non-P2X7 polypeptides. However, “specifically binds” does not exclude some cross reactivity with non-P2X7 polypeptides, as long as

that cross reactivity does not interfere with the capability of the probe to provide a useful measure of the presence of the given polypeptide target.

The term “capable” is used to mean that ability to achieve a given result, *e.g.*, a probe that is capable of detecting the presence of a particular substance means that the probe may be used to detect the particular substance.

An “oligonucleotide” refers to a short sequence of nucleotides, *e.g.*, 2-100 bases.

The term “biological sample” as used herein refers to a sample from a patient, which may be used for the purpose of identification, diagnosis, prediction, or monitoring. Examples of biological samples which may be used in connection with the methods and uses described herein may include, but are not limited to: synovial fluid, blood, blood-derived product (such as buffy coat, serum, and plasma), lymph, urine, tear, saliva, hair bulb cells, cerebrospinal fluid, buccal swabs, feces, synovial fluid, synovial cells, sputum, or tissue samples (*e.g.*, cartilage samples). In addition, one of skill in the art would realize that some samples would be more readily analyzed following a fractionation or purification procedure, for example, isolation of DNA from whole blood. In specific embodiments, it will be understood that the biological sample will be an *in vitro* sample for testing purposes (*i.e.*, for analysis).

The phrase “identifying a patient”, “selecting a patient”, “identifying a subject”, or “selecting a subject” as used herein refers to using the information or data generated relating to the level of P2X7 and/or the level of hsCRP as referred to herein in a sample of a patient to identify or selecting the patient as more likely to benefit or less likely to benefit from a therapy comprising an NLRP3 inflammasome pathway inhibitor (*e.g.*, an IL-1b inhibitor such as canakinumab). In one embodiment, a patient is considered to respond to a therapy comprising an NLRP3 inflammasome inhibitor (*e.g.*, an IL-1beta inhibitor such as canakinumab; and, thus, to be more likely to benefit from said therapy), if said therapy reduces the risk of said patient of experiencing a cardiovascular (CV) event such as a MACE (major adverse cardiac event). In one embodiment, said risk is reduced by at least 20%, by at least 21%, by at least 22%, by at least 23%, by at least 24%, by at least 25%, by at least 26%, by at least 27%, by at least 28%, by at least 29%, by at least 30%, by at least 31%, by at least 32%, by at least 33%, by at least 34%, or by at least 35%. In some embodiments, said risk is reduced by at least 25%. In some embodiments, said risk is reduced by at least 30%. Also, a patient is considered not to respond to a therapy comprising an NLRP3 inflammasome pathway inhibitor (*e.g.*, an IL-1b inhibitor such as canakinumab; and, thus, to be more likely not to benefit from said therapy), if said therapy does not reduce the risk of experiencing a recurrent cardiovascular (CV) event after first administration of an NLRP3 inflammasome pathway inhibitor (*e.g.*, an IL-1b inhibitor such as canakinumab). In this case, unnecessary health care costs or patient exposure can be avoided, if the medicament is not administered to unresponsive patients.

Any chemical formula given herein is also intended to represent unlabeled forms as well as isotopically labeled forms of the compounds. Isotopically labeled compounds have structures depicted by the formulae given herein except that one or more atoms are replaced by an atom having a selected atomic mass or mass number. Isotopes that can be incorporated into compounds of the disclosure include, for example, isotopes of hydrogen, carbon, nitrogen, and oxygen, such as ^3H , ^{11}C , ^{13}C , ^{14}C , and ^{15}N . Accordingly, it should be understood that methods, uses, and kits described herein can or may involve compounds that incorporate one or more of any of the aforementioned isotopes, including for example, radioactive isotopes, such as ^3H and ^{14}C , or those into which non-radioactive isotopes, such as ^2H and ^{13}C are present. Such isotopically labelled compounds are useful in metabolic studies (with ^{14}C), reaction kinetic studies (with, for example ^2H or ^3H), detection or imaging techniques, such as positron emission tomography (PET) or single-photon emission computed tomography (SPECT) including drug or substrate tissue distribution assays, or in radioactive treatment of patients. Isotopically-labeled compounds can generally be prepared by conventional techniques known to those skilled in the art, *e.g.*, using an appropriate isotopically-labeled reagents in place of the non-labeled reagent previously employed.

Contemplated and encompassed herein are also embodiments that include all pharmaceutically acceptable salts of the compounds useful according to the described methods, uses, and kits provided herein. As used herein, "pharmaceutically acceptable salt" refers to derivatives of the disclosed compounds wherein the parent compound is modified by converting an existing acid or base moiety to its salt form. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. The pharmaceutically acceptable salts can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, non-aqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are especially useful. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418 and *Journal of Pharmaceutical Science*, 66, 2 (1977), each of which is incorporated herein by reference for this purpose. For example, such pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines. For example, the salt can be a hydrochloride salt.

The phrase "pharmaceutically acceptable" as used herein refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity,

irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

It is to be understood that each embodiment may be combined with one or more other embodiments, to the extent that such a combination is consistent with the description of the
 5 embodiments. It is further to be understood that the embodiments provided above are understood to include all embodiments, including such embodiments as result from combinations of embodiments.

Techniques for Assaying, Diagnostic Methods, and Methods of Producing a Transmittable Form of Information

10 The disclosed methods are useful for the treatment, prevention, or amelioration of MACE, as well as predicting the likelihood of a patient's response to treatment with an inflammatory inhibitor; *e.g.*, an IL-1beta inhibitor, *e.g.*, canakinumab. These methods employ, *inter alia*, determining whether a patient has an gain-of-function mutation in one or more alleles of *P2RX7*, *e.g.*, through analysis of a biological sample from the patient.

15 A biological sample from the patient may be assayed for the presence of a gain-of-function allele of *P2RX7* by any applicable conventional means.

Numerous biological samples may be used to identify the presence of alleles or proteins, the level of expression of genes or proteins, and the activity of a protein, *e.g.*, blood, synovial fluid, buffy coat, serum, plasma, lymph, feces, urine, tear, saliva, cerebrospinal fluid, buccal swabs, sputum, or
 20 tissue. Various sources within a biological sample may be used in the disclosed methods, *e.g.*, one may assay genomic DNA obtained from a biological sample to detect *e.g.*, the presence of a gain-of-function mutation in *P2RX7*, or one may assay products of the gene, *e.g.*, nucleic acid products (*e.g.*, DNA, pre-mRNA, mRNA, micro RNAs, etc.) and polypeptide products (*e.g.*, expressed proteins) obtained from a biological sample.

25 Described herein are certain data useful for predicting selected subjects may respond to treatment by NLRP3 inflammasome pathway inhibition (*e.g.*, use of an IL-1beta inhibitor such as canakinumab). For example, the presence of the rs1718119 SNP may be determined by assaying genomic DNA, RNA and/or protein sequence, given that this SNP falls within an exon (exon 11) of the *P2RX7* gene and produces a missense amino acid change. Accordingly, a skilled artisan will
 30 understand that one may identify whether a subject has such a SNP by assaying a nucleic acid product of *P2RX7*, a polypeptide product of *P2RX7* (P2X7), or an equivalent genetic marker of *P2RX7*. In certain embodiments, a genomic sequence of *P2RX7* is analyzed to determine whether a subject the described gain-of-function mutation.

As described in the Examples, the findings herein lead to the conclusion that increased levels
 35 of *P2RX7* expression, levels of P2X7 protein and/or levels of P2X7 activity may be useful to predict improved response to NLRP3 inflammasome pathway inhibitors (*e.g.*, IL-1beta inhibitors such as

canakinumab) for MACE. Levels of *P2RX7* expression, P2X7 protein and/or P2X7 activity may be directly measured by various techniques described herein or known to skilled practitioners. The presence of a *P2RX7* polymorphism that results in an increased level of *P2RX7* expression, level of P2X7 protein or level of P2X7 activity may be detected by a variety of genotyping techniques.

- 5 Typically, such genotyping techniques employ one or more oligonucleotides that are complementary to a region containing, or adjacent to, the polymorphic site (*e.g.*, SNP) of interest. The sequence of an oligonucleotide used for genotyping a particular polymorphic site of interest is typically designed based on a context sequence or a reference sequence.

- 10 Numerous methods and devices are available to identify the presence of a gain-of-function mutation in *P2RX7*. For example, DNA (genomic and cDNA) for SNP detection can be prepared from a biological sample by methods well known in the art, *e.g.*, phenol/chloroform extraction, PUREGENE DNA[®] purification system from GentAS Systems (Qiagen, CA). Detection of a DNA sequence may include examining the nucleotide(s) located at either the sense or the anti-sense strand within that region. The presence of polymorphisms in a patient may be detected from DNA (genomic
- 15 or cDNA) obtained from PCR using sequence-specific probes, *e.g.*, hydrolysis probes from Taqman, Beacons, Scorpions; or hybridization probes that detect the marker or polymorphism. For the detection of the polymorphism, sequence specific probes may be designed such that they specifically hybridize to the genomic DNA for the alleles of interest or, in some cases, an RNA of interest. Primers and probes for polymorphic sites (*e.g.*, SNP) may be designed based on context sequences
- 20 found in the NCBI SNP database available at: [www \[dot\] ncbi \[dot\] nlm \[dot\] nih \[dot\] gov/snp](http://www.ncbi.nlm.nih.gov/snp). These probes may be labeled for direct detection or contacted by a second, detectable molecule that specifically binds to the probe. The PCR products also can be detected by DNA-binding agents. Said PCR products can then be subsequently sequenced by any known DNA sequencing method available. Alternatively the presence of allele can be detected by sequencing using any sequencing methods such
- 25 as, but not limited to, Sanger-based sequencing, pyrosequencing or next generation sequencing (Shendure J. and Ji, H., Nature Biotechnology (1998), Vol. 26, Nr 10, pages 1135-1145). Optimized allelic discrimination assays for SNPs may be purchased from, *e.g.*, Applied Biosystems (Foster City, California, USA).

- Various techniques can be applied to interrogate a particular polymorphism (*e.g.*, SNP),
- 30 including any described herein and, *e.g.*, hybridization-based methods, such as dynamic allele-specific hybridization (DASH) genotyping, polymorphic site (*e.g.*, SNP) detection through molecular beacons (Abravaya K., et al. (2003) Clin Chem Lab Med. 41:468-474), Luminex xMAP technology[®], Illumina Golden Gate[®] technology and commercially available high-density oligonucleotide SNP arrays (*e.g.*, the Affymetrix Human SNP 5.0 GeneChip[®] performs a genome-wide assay that can
- 35 genotype over 500,000 human SNPs), BeadChip[®] kits from Illumina, *e.g.*, Human660W-Quad and Human 1.2M-Duo); enzyme-based methods, such as restriction fragment length polymorphism

(RFLP), PCR-based methods (*e.g.*, Tetra-primer ARMS-PCR), Invader assays (Olivier M. (2005) *Mutat Res.* 573(1-2):103-10), various primer extension assays (incorporated into detection formats, *e.g.*, MALDI-TOF Mass spectrometry, electrophoresis, blotting, and ELISA-like methods), TaqMan® assays, and oligonucleotide ligase assays; and other post-amplification methods, *e.g.*, analysis of single strand conformation polymorphism (Costabile et al. (2006) *Hum. Mutat.* 27(12):1163-73), temperature gradient gel electrophoresis (TGGE), denaturing high performance liquid chromatography, high-resolution melting analysis, DNA mismatch-binding protein assays (*e.g.*, MutS protein from *Thermus aquaticus* binds different single nucleotide mismatches with different affinities and can be used in capillary electrophoresis to differentiate all six sets of mismatches), SNPLex® (proprietary SNP detecting system available from Applied Biosystems), capillary electrophoresis, mass spectrometry, and various sequencing methods, *e.g.*, pyrosequencing and next generation sequencing, etc. Commercial kits for SNP genotyping include, *e.g.*, Fluidigm Dynamic Array® IFCs (Fluidigm), TaqMan® SNP Genotyping Assay (Applied Biosystems), MassARRAY® iPLEX Gold (Sequenom), Type-it Fast® SNP Probe PCR Kit (Qiagen), *etc.*

In some embodiments, the presence of a polymorphic site (*e.g.*, SNP) in a patient is detected using a hybridization assay. In a hybridization assay, the presence of the genetic marker is determined based on the ability of the nucleic acid from the sample to hybridize to a complementary nucleic acid molecule, *e.g.*, an oligonucleotide probe. A variety of hybridization assays are available. In some, hybridization of a probe to the sequence of interest is detected directly by visualizing a bound probe, *e.g.*, a Northern or Southern assay. In these assays, DNA (Southern) or RNA (Northern) is isolated. The DNA or RNA is then cleaved with a series of restriction enzymes that cleave infrequently in the genome and not near any of the markers being assayed. The DNA or RNA is then separated, *e.g.*, on an agarose gel, and transferred to a membrane. A labeled probe or probes, *e.g.*, by incorporating a radionucleotide or binding agent (*e.g.*, SYBR® Green), is allowed to contact the membrane under low-, medium- or high-stringency conditions. Unbound probe is removed and the presence of binding is detected by visualizing the labeled probe. In some embodiments, arrays, *e.g.*, the MassARRAY® system (Sequenom, San Diego, California, USA) may be used to genotype a subject.

Traditional genotyping methods may also be modified for use in genotyping. Such traditional methods include, *e.g.*, DNA amplification techniques such as PCR and variants thereof, direct sequencing, SSO hybridization coupled with the Luminex xMAP® technology, SSP typing, and SBT.

Sequence-Specific Oligonucleotide (SSO) typing uses PCR target amplification, hybridization of PCR products to a panel of immobilized sequence-specific oligonucleotides on the beads, detection of probe-bound amplified product by color formation followed by data analysis. Those skilled in the art would understand that the described Sequence-Specific Oligonucleotide (SSO) hybridization may be performed using various commercially available kits, such as those provided by One Lambda, Inc.

(Canoga Park, CA) or Lifecodes HLA Typing Kits (Tepnel Life Sciences Corp.) coupled with Luminex® technology (Luminex, Corporation, TX). LABType® SSO is a reverse SSO (rSSO) DNA typing solution that uses sequence-specific oligonucleotide (SSO) probes and color-coded microspheres to identify HLA alleles. The target DNA is amplified by polymerase chain reactions (PCR) and then hybridized with the bead probe array. The assay takes place in a single well of a 96-well PCR plate; thus, 96 samples can be processed at one time.

Sequence Specific Primers (SSP) typing is a PCR based technique which uses sequence specific primers for DNA based typing. The SSP method is based on the principle that only primers with completely matched sequences to the target sequences result in amplified products under controlled PCR conditions. Allele sequence-specific primer pairs are designed to selectively amplify target sequences which are specific to a single allele or group of alleles. PCR products can be visualized on agarose gel. Control primer pairs that matches non-allelic sequences present in all samples act as an internal PCR control to verify the efficiency of the PCR amplification. Those skilled in the art would understand that low, medium and high resolution genotyping with the described sequence-specific primer typing may be performed using various commercially available kits, such as the Olerup SSP™ kits (Olerup, PA) or (Invitrogen) or Allset and™Gold DQA1 Low resolution SSP (Invitrogen).

Sequence Based Typing (SBT) is based on PCR target amplification, followed by sequencing of the PCR products and data analysis.

In some cases, RNA, *e.g.*, mature mRNA, pre-mRNA, can also be used to determine the presence of particular polymorphisms. Analysis of the sequence of mRNA transcribed from a given gene can be performed using any known method in the art including, but not limited, to Northern blot analysis, nuclease protection assays (NPA), *in situ* hybridization, reverse transcription-polymerase chain reaction (RT-PCR), RT-PCR ELISA, TaqMan-based quantitative RT-PCR (probe-based quantitative RT-PCR) and SYBR green-based quantitative RT-PCR. In one example, detection of mRNA levels involves contacting the isolated mRNA with an oligonucleotide that can hybridize to mRNA encoded by a gene of interest (*e.g.*, *P2RX7*). The nucleic acid probe can typically be, for example, a full-length cDNA, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, or 100 nucleotides in length and sufficient to specifically hybridize under stringent conditions to the mRNA. Hybridization of an mRNA with the probe indicates that the marker in question is being expressed. In one format, the RNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated RNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. Amplification primers are defined as being a pair of nucleic acid molecules that can anneal to 5' or 3' regions of a gene (plus and minus strands, respectively, or vice-versa) and contain a short region in between. In general, amplification primers are from about 10 to 30 nucleotides in length and flank a region from about 50 to 200 nucleotides in length. Under appropriate

conditions and with appropriate reagents, such primers permit the amplification of a nucleic acid molecule comprising the nucleotide sequence flanked by the primers. PCR products can be detected by any suitable method including, but not limited to, gel electrophoresis and staining with a DNA-specific stain or hybridization to a labeled probe.

- 5 The level of expression of a gene (*e.g.*, *P2RX7*) may be determined by measuring RNA (or reverse transcribed cDNA) levels using various techniques, *e.g.*, a PCR-based assay, reverse-transcriptase PCR (RT-PCR) assay, Northern blot, etc. Quantitative RT-PCR with standardized mixtures of competitive templates can also be utilized.

- 10 In some cases, the presence of a polymorphism in a patient can be determined by analyzing polypeptide products (in this case, *P2X7*). Detection of polypeptide products can be performed using any known method in the art including, but not limited, to immunocytochemical staining, ELISA, flow cytometry, Western blot, spectrophotometry, HPLC, and mass spectrometry.

- 15 It is contemplated that testing subjects at risk of MACE (*e.g.*, subjects having one or more MI; and/or subjects with an hsCRP level of at least 2) for the levels of *P2X7* protein will be useful in a variety of pharmaceutical products and methods that involve identifying individuals (*e.g.*, patients or subjects) more likely to respond to NLRP3 inflammasome pathway inhibitor (*e.g.*, IL-1 β inhibitor, *e.g.*, canakinumab) therapy and in helping physicians decide whether to prescribe such therapies to a patient at risk of MACE.

- 20 One method for detecting polypeptide products in a sample is by means of a probe that is a binding protein capable of interacting specifically with a marker protein (*e.g.*, an antibody capable of binding *P2X7* protein). As a non-limiting set of examples, labeled antibodies, binding portions thereof, or other binding partners can be used. The antibodies can be monoclonal or polyclonal in origin, or may be biosynthetically produced. The binding partners may also be naturally occurring molecules or synthetically produced. The amount of complexed proteins is determined using standard
25 protein detection methodologies described in the art. A detailed review of immunological assay design, theory and protocols can be found in numerous texts in the art, including Practical Immunology, Butt, W. R., ed., Marcel Dekker, New York, 1984. A variety of assays are available for detecting proteins with labeled antibodies. Direct labels include fluorescent or luminescent tags, metals, dyes, radionuclides, and the like, attached to the antibody. Indirect labels include various
30 enzymes well known in the art, such as alkaline phosphatase, hydrogen peroxidase and the like. In a one-step assay, polypeptide products, if present, are immobilized and incubated with a labeled antibody. The labeled antibody binds to the immobilized target molecule. After washing to remove unbound molecules, the sample is assayed for the label.

- 35 The use of immobilized antibodies specific for the proteins or polypeptides is also contemplated by herein. The antibodies can be immobilized onto a variety of solid supports, such as magnetic or chromatographic matrix particles, the surface of an assay plate (such as microtiter wells),

pieces of a solid substrate material (such as plastic, nylon, paper), and the like. An assay strip can be prepared by coating the antibody or a plurality of antibodies in an array on solid support. This strip can then be dipped into the test sample and then processed quickly through washes and detection steps to generate a measurable signal, such as a colored spot.

5 In a two-step assay, immobilized polypeptide products of a gene of interest such as a P2X7 protein may be incubated with an unlabeled antibody. The unlabeled antibody complex, if present, is then bound to a second, labeled antibody that is specific for the unlabeled antibody. The sample is washed and assayed for the presence of the label. The choice of marker used to label the antibodies will vary depending upon the application. However, the choice of the marker is readily determinable
10 to one skilled in the art. The antibodies may be labeled, *e.g.*, with a radioactive atom, an enzyme, a chromophoric or fluorescent moiety, or a colorimetric tag. The choice of tagging label also will depend on the detection limitations desired. Enzyme assays (ELISAs) typically allow detection of a colored product formed by interaction of the enzyme-tagged complex with an enzyme substrate. Some examples of radioactive atoms include ^{32}P , ^{125}I , ^3H , and ^{14}P . Some examples of enzymes include
15 horseradish peroxidase, alkaline phosphatase, beta-galactosidase, and glucose-6-phosphate dehydrogenase. Some examples of chromophoric moieties include fluorescein and rhodamine. The antibodies may be conjugated to these labels by any known methods. For example, enzymes and chromophoric molecules may be conjugated to the antibodies by means of coupling agents, such as dialdehydes, carbodiimides, dimaleimides, and the like. Alternatively, conjugation may occur
20 through a ligand-receptor pair. Some suitable ligand-receptor pairs include, for example, biotin-avidin or -streptavidin, and antibody-antigen.

 In one aspect, contemplated herein is the use of a sandwich technique for detecting polypeptide products in biological samples. The technique requires two antibodies capable of binding the protein of interest: *e.g.*, one immobilized onto a solid support and one free in solution, but labeled
25 with some easily detectable chemical compound. Examples of chemical labels that may be used for the second antibody include but are not limited to radioisotopes, fluorescent compounds, and enzymes or other molecules which generate colored or electrochemically active products when exposed to a reactant or enzyme substrate. When samples containing polypeptide products are placed in this system, the polypeptide products binds to both the immobilized antibody and the labeled antibody.
30 The result is a “sandwich” immune complex on the surface of the support. The complexed protein is detected by washing away nonbound sample components and excess labeled antibody, and measuring the amount of labeled antibody complexed to protein on the surface of the support. The sandwich immunoassay is highly specific and very sensitive, provided that labels with good limits of detection are used.

35 The presence of polypeptide products in a sample can be detected, for example, by radioimmunoassays or enzyme-linked immunoassays, competitive binding enzyme-linked

immunoassays, dot blot, Western blot, chromatography, high performance liquid chromatography (HPLC), or other assays known in the art. In a specific example, the presence of polypeptide products can be detected by high performance liquid chromatography (HPLC). Specific immunological binding of the antibody to the protein or polypeptide can be detected directly or indirectly.

5 Dot blotting is routinely practiced by the skilled artisan to detect a desired protein using an antibody as a probe (Promega Protocols and Applications Guide, Second Edition, 1991, Page 263, Promega Corporation). Samples are applied to a membrane using a dot blot apparatus. A labeled probe is incubated with the membrane, and the presence of the protein is detected.

10 Western blot analysis is well known to the skilled artisan (Sambrook et al., Molecular Cloning, A Laboratory Manual, 1989, Vol. 3, Chapter 18, Cold Spring Harbor Laboratory). In Western blot, the sample is separated by SDS-PAGE. The gel is transferred to a membrane. The membrane is incubated with labeled antibody for detection of the desired protein.

The assays described above involve steps such as but not limited to, immunoblotting, immunodiffusion, immunoelectrophoresis, or immunoprecipitation. In some embodiments, an
15 automatic analyzer is used to determine the presence of a gain-of-function mutation in *P2RX7*.

The level of *P2X7* activity may be assayed by various methods disclosed in the art, e.g., via the methods set forth in Kochan et al. (2011) Proc Natl Acad Sci U S A. 108(19):7745-50, the contents of which are incorporated by reference for this purpose.

For comparative purposes, the level of *P2RX7* expression, level of *P2X7* protein, or level of
20 *P2X7* activity from a patient may be compared to the level of *P2RX7* expression, level of *P2X7* protein, and level of *P2X7* activity from a control. The control may be a reference level of *P2RX7* expression, level of *P2X7* protein, or level of *P2X7* activity derived from subjects, e.g., from biological samples from reference subjects who do not have a gain-of-function mutation in *P2RX7* (e.g., has no gain-of-function mutation such as rs1718119). The control may be a reference
25 level of *P2RX7* expression, level of *P2X7* protein, or level of *P2X7* activity derived from subjects, e.g., from biological samples from reference subjects who are not homozygous for a gain-of-function mutation in *P2RX7* (e.g., is not homozygous for a gain-of-function mutation such as rs1718119). In some embodiments, the control may simply be a numerical standard (e.g., mean, median, range, [+/- standard deviation]) previously derived from reference subjects.

30 Analysis of the level of *P2RX7* expression, the level of *P2X7* protein, the level of *P2X7* activity, or presence of a *P2RX7* polymorphism may be carried out separately or simultaneously while analyzing other genetic sequences (e.g., additional response markers). Thus, in one aspect of the present disclosure, an array is provided to which probes that correspond in sequence to gene products, e.g., genomic DNA, cDNAs, mRNAs, cRNAs, polypeptides and fragments thereof, can be
35 specifically hybridized or bound at a known position. As such, one may use an array to concurrently analyze a biological sample from a patient for various genomic or biochemical markers of a patient.

In performing any of the methods described herein that require determining the presence of a *P2RX7* polymorphism, the level of *P2RX7* expression, the level of P2X7 protein, or the level of P2X7 activity, such determination may be made by consulting a data repository that contains sufficient information on the patient's genetic composition to determine whether the patient has the marker of interest. The data repository can list the genotype present (or absent) in the individual. The data repository could include the individual's patient records, a medical data card, a file (*e.g.*, a flat ASCII file) accessible by a computer or other electronic or non-electronic media on which appropriate information or genetic data can be stored. As used herein, a medical data card is a portable storage device such as a magnetic data card, a smart card (which has an on-board processing unit and which is sold by vendors such as Siemens of Munich Germany), or a flash-memory card. If the data repository is a file accessible by a computer; such files may be located on various media, including: a server, a client, a hard disk, a CD, a DVD, a personal digital assistant such as a smart phone, Palm Pilot, a tape recorder, a zip disk, the computer's internal ROM (read-only-memory), or the internet or worldwide web. Other media for the storage of files accessible by a computer are available to skilled artisans.

Typically, once levels of *P2RX7* expression, levels of P2X7 protein/activity, or the presence of a *P2RX7* polymorphism is determined, physicians or genetic counselors or patients or other researchers may be informed of the result. Specifically the result can be cast in a transmittable form of information that can be communicated or transmitted to other researchers or physicians or genetic counselors or patients. Such a form can vary and can be tangible or intangible. The result in the individual tested can be embodied in descriptive statements, diagrams, photographs, charts, images or any other visual forms. For example, images of gel electrophoresis of PCR products can be used in explaining the results. Diagrams showing where a variant occurs in an individual's allele are also useful in indicating the testing results. Statements regarding levels of *P2RX7* expression, levels of P2X7 protein/activity, or the presence of a *P2RX7* polymorphism are also useful in indicating the testing results. These statements and visual forms can be recorded on a tangible media such as papers, computer readable media such as floppy disks, compact disks, etc., or on an intangible media, *e.g.*, an electronic media in the form of email or website on internet or intranet. In addition, the result can also be recorded in a sound form and transmitted through any suitable media, *e.g.*, analog or digital cable lines, fiber optic cables, etc., via telephone, facsimile, wireless mobile phone, internet phone and the like. All such forms (tangible and intangible) would constitute a "transmittable form of information". Thus, the information and data on a test result can be produced anywhere in the world and transmitted to a different location. For example, when a genotyping assay is conducted offshore, the information and data on a test result may be generated and cast in a transmittable form as described above. The test result in a transmittable form thus can be imported into the U.S. Accordingly, the present disclosure also encompasses a method for producing a transmittable form of information containing levels of *P2RX7* expression, levels of P2X7 protein/activity, or the presence of a *P2RX7*

polymorphism in an individual. This form of information is useful for predicting the responsiveness of a patient at risk of MACE to administration of an NLRP3 inflammasome pathway inhibitor (*e.g.*, an IL-1beta inhibitor such as canakinumab), for selecting a course of treatment based upon that information, and for selectively treating a patient based upon that information.

Described herein are methods of predicting the likelihood that a patient having or at risk of MACE will respond to treatment with an NLRP3 inflammasome pathway inhibitor (*e.g.*, an IL-1beta inhibitor such as canakinumab), comprising detecting the presence of a gain-of-function mutation in *P2RX7* in a biological sample from the patient. In some embodiments, the patient is homozygous for the rs1718119 SNP in the *P2RX7* gene.

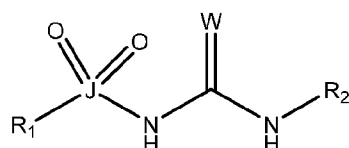
NLRP3 inflammasome pathway inhibitors

An NLRP3 inflammasome pathway inhibitor is an entity that binds to and decreases the activity of at least one protein in the NLRP3 inflammasome pathway. In some embodiments, the NLRP3 inflammasome pathway inhibitor is an NLRP3 inhibitor, an anti-IL-1beta antibody, an anti-IL-18 antibody, or an IL-1beta/IL-18 bispecific antibody.

In any of the embodiments described herein, the NLRP3 inhibitor may be any of the NLRP3 inhibitors disclosed in WO2019023147, PCT/US2019/060770, WO2016131098, WO2017140778, WO2018215818, WO2019166621, WO2019068772, WO2019211463, WO2018225018; WO2019/043610; WO2019206871 and their worldwide equivalents including those published in the United States (the contents of each of which is incorporated herein by reference for this purpose).

In some embodiments, the NLRP3 inhibitor is any of the compounds described in WO 2017/184624, WO 2017/184623, WO 2017/184604, WO 2019/023147, WO 2019/023145, WO 2019/079119, WO 2020/102096, US16/874862, or PCT/IB2020/054613 and is suitable for this purpose (*i.e.*, is an NLRP3 inhibitor).

In certain embodiments, the NLRP3 inhibitor is a compound described in WO 2016/131098, which is incorporated by reference herein for this purpose along with its U.S. published equivalent. For example, in some embodiments, the NLRP3 antagonist is a compound of formula (101A), having substituents as defined in claim 1 of WO2016/131098, or a pharmaceutically acceptable salt, solvate or prodrug thereof, *i.e.*, a compound of:

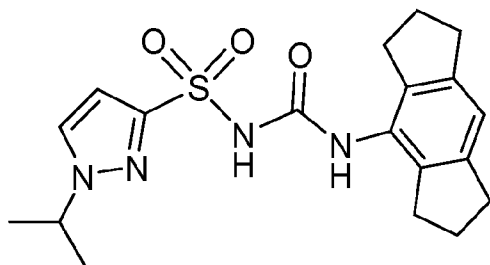


Formula (101A)

In some embodiments, NLRP3 antagonists for use in the described methods, uses, and kits include exemplary compounds in Table 3 on pages 285-315 of WO 2016/131098. In some

embodiments, NLRP3 antagonists for use in the described methods, uses, and kits include the compounds of claim 36 of WO 2016/131098.

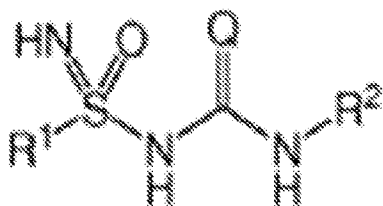
In a specific embodiment, the NLRP3 inhibitor is the molecule shown below:



5

In some embodiments, the NLRP3 antagonist is a compound described in WO 2019/068772, the contents of which are incorporated by reference herein for this purpose along with its U.S. published equivalent. For example, the NLRP3 antagonist may be a compound of formula (102A), having substituents as defined in claim 1 of WO 2019/068772, or a pharmaceutically acceptable salt, solvate or prodrug thereof, *i.e.*, a compound of:

10



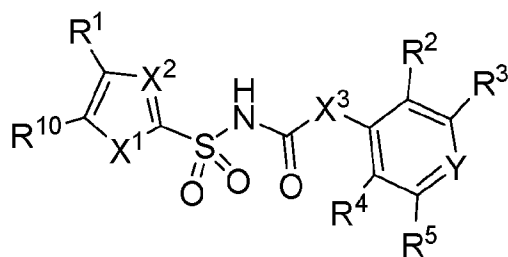
Formula (102A)

In some embodiments, NLRP3 antagonists for use in the described methods, uses, and kits include exemplary compounds described on page 44, lines 11 to page 52, line 10 and in Examples 1-28 on pages 117-158 of WO 2019/068772. In some embodiments, NLRP3 antagonists for use in the described methods, uses, and kits include exemplary compounds of claim 13 of WO 2019/068772.

15

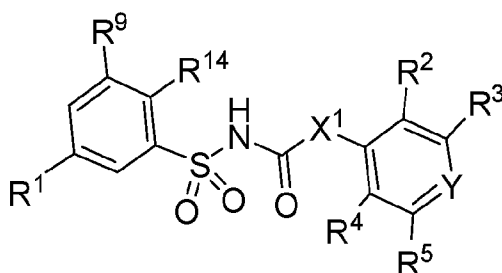
In some embodiments, the NLRP3 antagonist is a compound described in WO 2017/184624, the contents of which are incorporated by reference herein for this purpose. For example, the NLRP3 antagonist may be a compound of formula (1AA), having substituents as defined in claim 1 of WO 2017/184624, or a pharmaceutically acceptable salt, solvate or prodrug thereof, *i.e.*, a compound of:

20



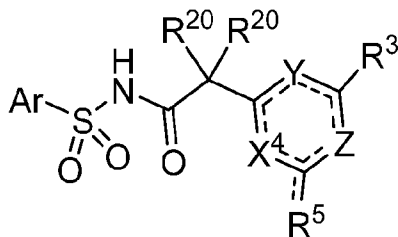
Formula 1AA

In some embodiments, the NLRP3 antagonist is a compound described in WO 2017/184623,
 5 the contents of which are incorporated by reference herein for this purpose along with its U.S.
 published equivalent. For example, the NLRP3 antagonist may be a compound of formula (2AA),
 having substituents as defined in claim 1 of WO 2017/184623, or a pharmaceutically acceptable salt,
 solvate or prodrug thereof, *i.e.*, a compound of:



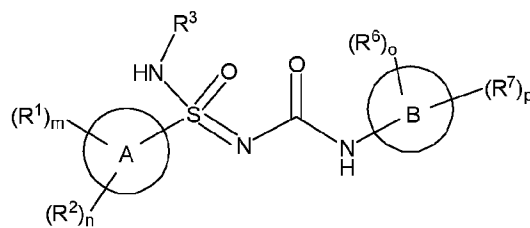
10 Formula 2AA.

In some embodiments, the NLRP3 antagonist is a compound described in WO 2017/184604,
 the contents of which are incorporated by reference herein for this purpose along with its U.S.
 published equivalent. For example, the NLRP3 antagonist may be a compound of formula (3AA),
 15 having substituents as defined in claim 1 of WO 2017/184604, or a pharmaceutically acceptable salt,
 solvate or prodrug thereof, *i.e.*, a compound of:



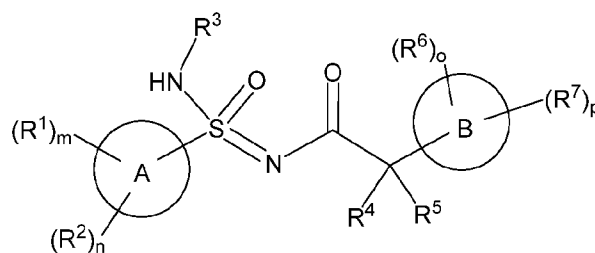
Formula 3AA

In some embodiments, the NLRP3 antagonist is a compound described in WO 2019/023147, the contents of which are incorporated by reference herein for this purpose along with its U.S. published equivalent. For example, the NLRP3 antagonist may be a compound of formula (4AA), having substituents as defined in claim 1 of WO 2019/023147, or a pharmaceutically acceptable salt, solvate or prodrug thereof, *i.e.*, a compound of:



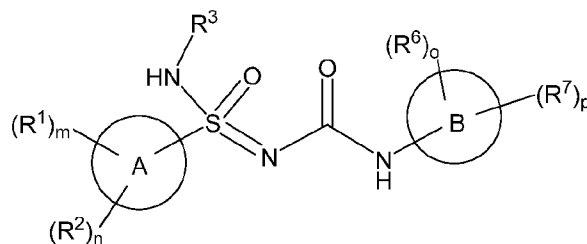
Formula 4AA.

In some embodiments, the NLRP3 antagonist is a compound described in WO 2019/023145, the contents of which are incorporated by reference herein for this purpose along with its U.S. published equivalent. For example, the NLRP3 antagonist may be a compound of formula (5AA), having substituents as defined in claim 1 of WO 2019/023145, or a pharmaceutically acceptable salt, solvate or prodrug thereof, *i.e.*, a compound of:



Formula 5AA.

In some embodiments, the NLRP3 antagonist is a compound described in WO 2020/102096, the contents of which are incorporated by reference herein for this purpose along with its U.S. published equivalent. For example, the NLRP3 antagonist may be a compound of formula (6AA), having substituents as defined in claim 1 of WO 2020/102096, or a pharmaceutically acceptable salt, solvate or prodrug thereof, *i.e.*, a compound of:



Formula 6AA.

5 Methods of Treatment and Uses of NLRP3 Inflammasome Pathway Inhibitors

The disclosed methods and uses allow clinicians to provide a personalized therapy for cardiovascular disease (*e.g.*, MACE) patients, *i.e.*, they allow determination of whether to selectively treat the patient with an NLRP3 inflammasome pathway inhibitor (*e.g.*, an IL-1beta inhibitor such as canakinumab) or whether to selectively treat the patient with another agent (*e.g.*, a standard of care agent) without the NLRP3 inflammasome pathway inhibitor.

The NLRP3 inflammasome pathway inhibitors, *e.g.*, IL-1beta inhibitors (*e.g.*, an IL-1beta antibody or antigen-binding portion thereof, *e.g.*, canakinumab) may be used *in vitro*, *ex vivo*, or incorporated into pharmaceutical compositions and administered to individuals (*e.g.*, human patients) *in vivo* to treat, ameliorate, or prevent MACE, *e.g.*, in patients who have increased levels of P2RX7 expression and/or levels of P2X7 protein/activity. A pharmaceutical composition will be formulated to be compatible with its intended route of administration (*e.g.*, oral compositions generally include an inert diluent or an edible carrier). Other nonlimiting examples of routes of administration include parenteral (*e.g.*, intravenous), intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. The pharmaceutical compositions compatible with each intended route are well known.

The NLRP3 inflammasome pathway inhibitors, *e.g.*, IL-1beta inhibitors (*e.g.*, an IL-1beta antibody or antigen-binding portion thereof, *e.g.*, canakinumab) may be used as a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may contain, in addition to the inflammsome inhibitor, carriers, various diluents, fillers, salts, buffers, stabilizers, solubilizers, and other known materials. The characteristics of the carrier will depend on the route of administration. The pharmaceutical compositions for use in the disclosed methods may also contain additional therapeutic agents for treatment of the particular targeted disorder. For example, a pharmaceutical composition may also a further standard of care agent.

Pharmaceutical compositions for use in the disclosed methods may be manufactured in conventional manner. In one embodiment, the pharmaceutical composition is provided in lyophilized form. For immediate administration it is dissolved in a suitable aqueous carrier, for example sterile

water for injection or sterile buffered physiological saline. If it is considered desirable to make up a solution of larger volume for administration by infusion rather than a bolus injection, may be advantageous to incorporate human serum albumin or the patient's own heparinised blood into the saline at the time of formulation. The presence of an excess of such physiologically inert protein prevents loss of antibody by adsorption onto the walls of the container and tubing used with the infusion solution. Other formulations comprise a liquid or lyophilized formulation.

The appropriate dosage will, of course, vary depending upon, for example, the particular NLRP3 inflammasome pathway inhibitors, *e.g.*, IL-1beta inhibitors (*e.g.*, an IL-1beta antibody or antigen-binding portion thereof, *e.g.*, canakinumab) to be employed, the host, the mode of administration and the nature and severity of the condition being treated, and on the nature of prior treatments that the patient has undergone. Ultimately, the attending health care provider will decide the amount of the NLRP3 inflammasome pathway inhibitor with which to treat each individual patient. In some embodiments, the attending health care provider may administer low doses of the NLRP3 inflammasome pathway inhibitor and observe the patient's response. In other embodiments, the initial dose(s) of NLRP3 inflammasome pathway inhibitor administered to a patient may be high, and then are titrated downward until signs of relapse occur. Larger doses of the NLRP3 inflammasome pathway inhibitor may be administered until the optimal therapeutic effect is obtained for the patient, and the dosage is not generally increased further.

20 Kits

Also contemplated herein are kits for detecting a *P2RX7* polymorphism, or the level of *P2RX7* expression, level of P2X7 protein or level of P2X7 activity in a biological sample (a test sample) from a patient. Such kits can be used to predict if a patient having or at risk of having cardiovascular disease or MACE is likely to respond (or have a higher response) to treatment with an NLRP3 inflammasome pathway inhibitors, *e.g.*, IL-1beta inhibitors (*e.g.*, an IL-1beta antibody or antigen-binding portion thereof, *e.g.*, canakinumab). For example, the kit can comprise a probe (*e.g.*, an oligonucleotide, antibody, labeled compound or other agent) capable of detecting a *P2RX7* polymorphism, or the level of *P2RX7* expression, level of P2X7 protein or level of P2X7 activity, products of those alleles and/or an equivalent genetic marker of those alleles in a biological sample. The kit may also comprise instructions for providing a prediction of the likelihood that the patient will respond to treatment with the NLRP3 inflammasome pathway inhibitor.

Probes may specifically hybridize to genomic sequences, nucleic acid products, or polypeptide products. Exemplary probes are oligonucleotides or conjugated oligonucleotides that specifically hybridize to gain-of-function alleles of *P2RX7* (*e.g.*, SNP rs1718119); an antibody that is capable of differentiating between polypeptide products encoded by the disclosed alleles; primer-extension oligonucleotides, allele-specific primers, a combination of allele-specific primers, allele-

specific probes, and primer extension primers, etc. Optionally, the kit can contain a probe that targets an internal control allele, which can be any allele presented in the general population. Detection of an internal control allele is designed to assure the performance of the kit. The disclosed kits can also comprise, *e.g.*, a buffering agent, a preservative, or a protein stabilizing agent. The kit can also
5 comprise components necessary for detecting the detectable agent (*e.g.*, an enzyme or a substrate). The kit can also contain a control sample or a series of control samples that can be assayed and compared to the test sample contained. Each component of the kit is usually enclosed within an individual container, and all of the various containers are within a single package along with instructions for use.

10 Such kits may also comprise an NLRP3 inflammasome pathway inhibitor, *e.g.*, IL-1beta inhibitors (*e.g.*, an IL-1beta antibody or antigen-binding portion thereof, *e.g.*, canakinumab) (*e.g.*, in liquid or lyophilized form) or a pharmaceutical composition comprising the NLRP3 inflammasome pathway inhibitor (described herein). In this way, such kits are useful in the selective treatment of patients having or at risk of having MACE. Additionally, such kits may comprise means for
15 administering the NLRP3 inflammasome pathway inhibitor (*e.g.*, a syringe and vial, a prefilled syringe, a prefilled pen) and instructions for use. These kits may contain additional therapeutic agents for treating MACE, *e.g.*, for delivery in combination with the enclosed NLRP3 inflammasome pathway inhibitor.

20 The phrase “means for administering” is used to indicate any available implement for systemically administering a drug to a patient, including, but not limited to, a pre-filled syringe, a vial and syringe, an injection pen, an autoinjector, an i.v. drip and bag, a pump, etc. With such items, a patient may self-administer the drug (*i.e.*, administer the drug on their own behalf) or a physician may administer the drug.

25 Other features, objects, and advantages of the methods, uses, and kits described herein will be apparent from the description and drawings, and from the claims. Each and every publication described herein (including patent publications) are incorporated by reference for the relevant subject matter.

The following Examples are merely illustrative and are not intended as limiting any way.

EXAMPLES

30 The Examples below are set forth to aid in the understanding of the description but are not intended, and should not be construed, to limit its scope in any way.

Example 1: A randomized, double-blind, placebo-controlled, event-driven trial of quarterly subcutaneous canakinumab in the prevention of recurrent cardiovascular events among stable post-myocardial infarction patients with elevated hsCRP.

This study was designed as a multi-center, randomized, parallel group, placebo-controlled, double-blind, event-driven trial to provide definitive evidence on the effects of canakinumab on cardiovascular adverse events in patients with recent MI and elevated inflammatory burden as evidenced by elevated hsCRP. This study design was the most robust clinical trial design to test the hypothesis that anti-inflammatory treatment with canakinumab reduce major adverse cardiovascular events.

Rationale of study design

Trial Population. Patients were eligible for enrollment if they had a prior history of myocardial infarction and had blood levels of hsCRP of 2 mg/L or greater despite use of aggressive secondary prevention strategies. The trial excluded from enrollment those with a history of chronic or recurrent infection, prior malignancy other than basal cell skin carcinoma, suspected or known immunocompromised state, a history of or high risk for tuberculosis or HIV-related disease, or ongoing use of other systemic anti-inflammatory treatments.

Inclusion criteria

Patients eligible for inclusion in the study had to fulfill all of the following criteria:

1. Written informed consent obtained before any assessment performed.
2. Male, or Female of non-child-bearing potential
3. Age $\geq$ 18 years at Visit 1.
4. Documented spontaneous MI (diagnosed according to the universal MI criteria with or without evidence of ST segment elevation) at least 30 days before randomization (Dewell P *et al*, Nature. 2010;464(7293):1357-61).
 - Diagnosis of the qualifying MI should be based on medical history of clinical symptoms consistent with myocardial ischemia associated with elevation of cardiac biomarkers above the 99th percentile of the upper reference limit (preferably troponin) OR development of new pathological Q waves regardless of symptoms. For details, refer to the Universal Definition of MI (Dewell P *et al*, Nature. 2010;464(7293):1357-61).
 - a. Acute MI (hospitalization records): requires documentation of a rise and/or fall of cardiac biomarkers (preferably troponin) with at least one value above the 99th percentile of the upper reference limit (URL) or above criteria diagnostic for MI and evidence of myocardial ischemia as demonstrated by at least one of the following :
 - i. Symptoms of ischemia
 - ii. ECG changes indicative of new ischemia (new ST-T changes or new LBBB)

- iii. Development of pathologic Q waves
- iv. Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality
- b. Prior MI (no hospital records for acute event available): requires documentation of any one of the following:
 - i. Development of pathological Q waves, with or without symptoms
 - ii. Imaging evidence of a region of loss of viable myocardium that is thinned and fails to contract, in the absence of a non-ischemic cause
 - iii. Pathologic findings of a healed or healing MI
 - Patients with MI resulting from PCI or CABG were not eligible
- 5. Have an hsCRP ≥ 2 mg/L (collected less than 60 days prior to Visit 2 and performed at the central laboratory, which is a minimum of 28 days after qualifying MI or after any PCI performed separately from qualifying MI) on stable (at least 4 weeks) long term (cardiovascular) medications (standard of care).

Randomization.

Patients were initially randomized to canakinumab 150 mg, canakinumab 300 mg, or placebo in a 1:1:1 ratio. After the enrollment of 741 participants, a 50 mg dose was added at regulatory request, with the randomization ratio adjusted accordingly; we sought to achieve a final randomization ratio of 1.5:1:1:1. All study-drug doses and placebo were administered subcutaneously once every three months; for the 300 mg dose, the regimen was 300 mg every two weeks for the first two doses, then once every three months. Randomization was performed with the use of a centralized computer system, with stratification by time since index myocardial infarction and by trial part (before versus after inclusion of the 50 mg dose).

End Points.

The primary efficacy end point was time to first occurrence of nonfatal myocardial infarction, any nonfatal stroke, or cardiovascular death. The trial had two key secondary efficacy end points. The first key secondary end point included the components of the primary end point as well as hospitalization for unstable angina requiring urgent revascularization. The two other pre-specified secondary end points were all-cause mortality and the composite of nonfatal myocardial infarction, any nonfatal stroke, or all-cause mortality. All components of these end points were adjudicated by an end point adjudication committee, with members masked to study-drug assignment.

Statistical Analysis.

Distributions of percent change from baseline in hsCRP and lipid levels were compared between placebo and each canakinumab group at intervals up to 48 months. Similar comparisons were made for IL-6 up to 12 months. Log-rank tests and Cox proportional-hazards models, stratified by time since index myocardial infarction and trial part, were used to analyze the pre-specified primary and key secondary cardiovascular outcomes that occurred during trial follow-up according to the intention-to-treat principle. Formal evaluation of significance for individual doses, adjusted for multiplicity, followed a closed testing procedure. Based on the closed testing procedure, and using the pre-specified allocation of alpha error, the two-sided P value thresholds for statistical significance for the primary end point were 0.01058 for the test of the 300 mg dose of canakinumab versus placebo and 0.02115 for the tests of the other two doses versus placebo. The closed testing procedure also specified that formal significance testing for the key secondary end points would be performed for any given dose only if the significance threshold for the primary end point for that dose had been met. While the primary analysis strategy was based on pair-wise comparisons of individual dose groups to the placebo group, comparisons were also made between incidence rates on placebo and incidence rates across ascending canakinumab doses (using scores of 0, 1, 3, and 6 proportional to doses in a trend analysis) and for the combined active canakinumab treatment groups versus placebo. In addition, on-treatment analyses were performed with follow-up for each patient censored 119 days after the last study injection received. The significance thresholds for these tests were not adjusted for multiplicity. Similar analyses were used for adverse events. All P values are two-sided and all confidence intervals computed at the 95% level.

Patients.

Trial enrollment began in April 2011 and was completed in March 2014; the last trial visit was in June 2017. Of 17,482 post-infarction patients who underwent screening in the central laboratory, 10,061 (57.6%) were correctly randomized and received at least one dose of trial medication (Figure 1). The most common reasons for exclusion were hsCRP less than 2 mg/L (46% of excluded subjects), active tuberculosis or tuberculosis risk factors (25.4%), and exclusionary concomitant disorders (9.9%).

The mean age of randomized participants was 61 years, 26% were women, and 40% had diabetes (Table 2). Most participants had undergone prior revascularization procedures (67% percutaneous coronary interventions, 14% coronary bypass surgery). At baseline, anti-thrombotic therapy was taken by 95%, lipid-lowering therapy by 93%, anti-ischemia agents by 91%, and inhibitors of the renin-angiotensin system by 79%. The median hsCRP at entry was 4.2 mg/L and the median LDL cholesterol was 82 mg/dL.

Table 2: Characteristics of trial participants

Characteristic	Canakinumab Dose (SC q 3 months)				
	Placebo (N=3344)	50 mg (N=2170)	150 mg (N=2284)	300 mg (N=2263)	All Doses (N=6717)
Age (yr), Mean (SD)	61.1 (10.0)	61.1 (10.1)	61.2 (10.0)	61.1 (10.1)	61.1 (10.1)
Female sex, N (%)	865 (25.9)	541 (24.9)	575 (25.2)	606 (26.8)	1722 (25.6)
Current smoking, N (%)	765 (22.9)	531 (24.5)	534 (23.4)	536 (23.7)	1601 (23.8)
Body mass index (kg/m ²)†	29.7 (26.6, 33.8)	29.9 (26.6, 33.9)	29.8 (26.5, 33.7)	29.8 (26.5, 33.8)	29.9 (26.6, 33.8)
Hypertension, N (%)	2644 (79.1)	1751 (80.7)	1814 (79.4)	1799 (79.5)	5364 (79.9)
Diabetes, N (%)	1333 (39.9)	854 (39.4)	954 (41.8)	888 (39.2)	2696 (40.1)
Qualifying myocardial infarction, N (%)					
STEMI	1807 (54.0)	1231 (56.7)	1231 (53.9)	1213 (53.6)	3675 (54.7)
Non-STEMI	1132 (33.9)	710 (32.7)	781 (34.2)	761 (33.6)	2252 (33.5)
Unknown/missing	405 (12.1)	229 (10.6)	272 (11.9)	289 (12.8)	790 (11.8)
History of PCI, N (%)	2192 (65.6)	1454 (67.0)	1555 (68.1)*	1509 (66.7)	4518 (67.3)
History of CABG, N (%)	469 (14.0)	302 (13.9)	324 (14.2)	316 (14.0)	942 (14.0)
History of congestive heart failure, N (%)	721 (21.6)	451 (20.8)	478 (20.9)	523 (23.1)	1452 (21.6)
Lipid lowering therapy, N (%)	3132 (93.7)	2038 (94.0)	2114 (92.7)	2113 (93.5)	6265 (93.3)
Statin, N (%)	3045 (91.1)	1990 (91.7)	2065 (90.6)	2057 (91.1)	6112 (91.0)
Renin-angiotensin inhibitors, N (%)	2665 (79.8)	1718 (79.3)	1817 (79.8)	1792 (79.6)	5327 (79.3)
Anti-ischemia agents,** N (%)	3080 (92.1)	1974 (91.0)	2079 (91.2)	2058 (91.1)	6111 (91.0)
Antithrombotics/Anticoagulants, N (%)	3188 (95.3)	2059 (94.9)	2157 (94.6)	2149 (95.1)	6365 (94.8)
hsCRP (mg/L)†	4.1 (2.75, 6.85)	4.25 (2.80, 7.15)	4.25 (2.85, 7.05)	4.15 (2.85, 7.15)	4.2 (2.80, 7.10)
IL-6 (ng/L)†	2.61 (1.80, 4.06)	2.53 (1.80, 4.17)	2.56 (1.74, 4.11)	2.59 (1.79, 4.08)	2.56 (1.77, 4.13)
Total cholesterol (mg/dL)†	161 (137, 190)	159 (136, 189)	159 (136, 188)	161 (137, 189)	160 (136, 189)
LDL cholesterol (mg/dL)†	82.8 (64.2, 107.5)	81.2 (62.3, 106.0)	82.4 (63.4, 106.0)	83.5 (64.0, 108.0)	82.0 (63.0, 106.7)
HDL cholesterol (mg/dL)†	44.5 (37.1, 52.6)	43.7 (37.0, 52.2)	43.7 (36.3, 52.0)*	44.0 (36.7, 53.0)	43.7 (36.7, 52.2)*
Triglycerides (mg/dL)†	139 (100, 194)	140 (102, 198)	139 (101, 196)	138 (103, 194)	139 (102, 196)
eGFR (mL/min/1.73 m ²)†	79.0 (65.0, 93.0)	79.0 (64.0, 92.0)	79.0 (64.5, 93.0)	78.0 (64.0, 93.0)	78.5 (64.0, 93.0)
Loss to follow-up N, (%)	9 (0.27)	9 (0.41)	5 (0.22)	4 (0.18)	18 (0.27)

SC=subcutaneously; SD=standard deviation; STEMI=ST elevation myocardial infarction; PCI=percutaneous coronary intervention; CABG=coronary bypass graft surgery; hsCRP=high sensitivity C-reactive protein; IL-6=interleukin 6; HDL=high density lipoprotein cholesterol; LDL=low density lipoprotein cholesterol; eGFR=estimated glomerular filtration rate* P-value <0.05 in comparison of canakinumab to placebo.

** Beta-blocking agents, nitrates, or calcium channel blocking agents

† Median (IQR) values are presented for all measured plasma variables and body mass index

Effects on inflammatory biomarkers and lipid levels.

Compared to placebo, at 48 months, hsCRP was reduced by 26%, 37%, and 41% in the canakinumab 50 mg, 150 mg, and 300 mg groups, respectively (all P-values <0.001 in comparisons of the median percent change on canakinumab to the median percent change on placebo) (Tables 3-7).

- 5 Similar effects were observed for IL-6 (measured up to 12 months). By contrast, canakinumab use resulted in no reduction in LDL cholesterol or HDL cholesterol, and a 4 to 5% median increase in triglycerides.

10 **Table 3. Effects of 3-month treatment with canakinumab on hsCRP, IL-6, and lipid levels. P-values reflect change from baseline.**

	Canakinumab Dose (SC q 3 months)				
Biomarker	Placebo	50mg	150mg	300mg	All Doses
hsCRP (mg/L) Baseline median	4.05	4.05	4.15	4.05	4.10
3-month median	3.50	2.20	1.8	1.30	1.80
Change median, %)	-13.8	-45.1	-57.6	-66.7	-56.8
P-value		<0.001	<0.001	<0.001	<0.001
IL-6 (ng/L) Baseline median	2.58	2.53	2.54	2.56	2.55
3-month median	2.60	1.98	1.64	1.43	1.64
Change median, %	0.00	-24.7	-36.4	-43.3	-35.3
P-value		<0.001	<0.001	<0.001	<0.001
LDLC (mg/dL) Baseline median	82.8	81.2	82.0	83.1	82.0
3-month median	82.0	82.4	84.0	83.1	83.1
Change median, %	0.00	1.4	1.1	1.9	1.5
P-value		0.002	0.015	0.001	<0.001
HDLC (mg/dL) Baseline median	44.5	43.7	43.7	44.0	44.0
3-month median	44.9	44.9	45.2	46.0	45.2
Change median, %	0.00	2.9	3.7	3.8	3.5
P-value		<0.001	<0.001	<0.001	<0.001
TG (mg/dL) Baseline median	138.9	140.7	138.1	137.5	138.9
3-month median	138.9	148.7	143.9	145.1	146.0
Change median, %	-0.7	4.5	4.7	5.4	4.8
P-value		<0.001	<0.001	<0.001	<0.001

LDLC = low density lipoprotein (LDL) cholesterol, HDLC = high density lipoprotein (HDL) cholesterol, TG = triglycerides, IL-6 = interleukin-6, SC=subcutaneous, q=quarterly

15 **Table 4. Effects of 12-month treatment with canakinumab on hsCRP, IL-6, and lipid levels. P-values reflect change from baseline.**

	Canakinumab Dose (SC q 3 months)				
Biomarker	Placebo	50mg	150mg	300mg	All Doses
hsCRP (mg/L) Baseline median	4.00	4.10	4.05	4.05	4.05
12-month median	3.40	2.30	1.80	1.50	1.80
Change median, %	-14.6	-45.3	-55.6	-61.0	-54.5
P-value		<0.001	<0.001	<0.001	<0.001
IL-6 (ng/L) Baseline median	2.57	2.52	2.50	2.52	2.52
12-month median	2.63	2.03	1.71	1.60	1.75

Change median, %	3.52	-19.5	-33.8	-37.7	-31.1
P-value		<0.001	<0.001	<0.001	<0.001
LDLC (mg/dL) Baseline median	83.0	80.4	82.0	83.1	82.0
12-month median	82.4	84.0	82.4	84.0	83.5
Change median, %	0.00	1.53	0.81	0.00	0.88
P-value		0.04	0.22	0.64	0.11
HDLC (mg/dL) Baseline median	44.1	44.0	43.8	44.0	44.0
12-month median	44.5	44.5	44.9	45.0	44.9
Change median, %	0.00	1.06	2.67	2.75	2.22
P-value		<0.001	<0.001	<0.001	<0.001
TG (mg/dL) Baseline median	138.9	139.8	138.9	138.1	138.9
12-month median	137.2	146.9	144.6	147.8	146.9
Change median, %	-1.0	4.0	5.7	4.7	4.7
P-value		<0.001	<0.001	<0.001	<0.001

LDLC = low density lipoprotein (LDL) cholesterol, HDLC = high density lipoprotein (HDL) cholesterol, TG = triglycerides, IL-6 = interleukin-6, SC=subcutaneous, q=quarterly

Table 5. Effects of 24-month treatment with canakinumab on hsCRP and lipid levels. P-values reflect change from baseline.

Biomarker	Canakinumab Dose (SC q 3 months)				
	Placebo	50mg	150mg	300mg	All Doses
hsCRP (mg/L) Baseline median	3.95	4.00	4.00	4.00	4.00
24-month median	3.40	2.30	1.80	1.60	1.90
Change median, %	-17.9	-43.2	-53.6	-58.1	-52.5
P-value		<0.001	<0.001	<0.001	<0.001
LDLC (mg/dL) Baseline median	83.0	80.8	82.0	82.4	82.0
24-month median	83.0	84.0	84.3	83.0	85.9
Change median, %	0.0	2.2	2.1	1.5	1.9
P-value		0.014	0.14	0.30	0.01
HDLC (mg/dL) Baseline median	44.1	44.0	44.0	44.0	44.0
24-month median	44.0	44.1	44.9	45.2	44.9
Change median, %	0.00	0.76	1.92	2.45	1.90
P-value		0.07	<0.001	<0.001	<0.001
TG (mg/dL) Baseline median	138.9	139.8	138.9	138.1	138.9
24-month median	135.9	142.5	146.0	145.9	145.1
Change median, %	-2.0	2.9	5.3	4.9	4.6
P-value		<0.001	<0.001	<0.001	<0.001

5 LDLC = low density lipoprotein (LDL) cholesterol, HDLC = high density lipoprotein (HDL) cholesterol, TG = triglycerides, SC=subcutaneous, q=quarterly

Table 6. Effects of 36-month treatment with canakinumab on hsCRP and lipid levels. P-values reflect change from baseline.

Biomarker	Canakinumab Dose (SC q 3 months)				
	Placebo	50mg	150mg	300mg	All Doses
hsCRP (mg/L) Baseline median	4.05	4.10	4.10	4.10	4.10

10

36-month median	3.50	2.40	2.00	1.70	2.00
Change median, %	-17.1	-41.4	-52.6	-58.3	-51.5
P-value		<.001	<0.001	<0.001	<0.001
LDLC (mg/dL) Baseline median	83.1	80.8	82.0	82.4	81.6
36-month median	82.8	83.1	84.0	83.5	83.9
Change median, %	-0.5	3.1	3.3	1.2	2.5
P-value		0.002	<0.001	0.04	<0.001
HDLC (mg/dL) Baseline median	44.1	44.0	43.7	44.0	44.0
36-month median	44.0	44.0	44.5	45.0	44.5
Change median, %	-1.4	0.00	1.6	2.1	1.3
P-value		0.01	<0.001	<0.001	<0.001
TG (mg/dL) Baseline median	138.9	140.7	139.8	138.1	139.4
36-month median	134.5	142.2	146.9	144.2	144.9
Change median, %	-0.6	2.4	4.5	6.2	3.9
P-value		0.01	<0.001	<0.001	<0.001

LDLC = low density lipoprotein (LDL) cholesterol, HDLC = high density lipoprotein (HDL) cholesterol, TG = triglycerides, SC=subcutaneous, q=quarterly

Table 7. Effects of 48-month treatment with canakinumab on hsCRP and lipid levels. P-values reflect change from baseline.

Biomarker	Canakinumab Dose (SC q 3 months)				
	Placebo	50mg	150mg	300mg	All Doses
hsCRP (mg/L) Baseline median	4.15	4.20	4.35	4.30	4.30
48-month median	3.60	2.50	1.90	1.90	2.10
Change median, %	-17.0	-44.4	-56.3	-57.1	-52.9
P-value		<.001	<0.001	<0.001	<0.001
LDLC (mg/dL) Baseline median	85.1	84.7	82.4	86.0	84.7
48-month median	82.4	84.0	84.7	85.0	84.3
Change median, %	-1.4	0.0	1.69	-2.8	0.0
P-value		0.19	0.004	0.67	0.045
HDLC (mg/dL) Baseline median	44.1	44.5	43.7	44.1	44.1
48-month median	43.7	44.5	44.0	44.9	44.1
Change median, %	-2.25	0.00	0.00	0.00	0.00
P-value		0.007	<0.001	<0.001	<0.001
TG (mg/dL) Baseline median	141.6	138.0	140.7	138.1	138.9
48-month median	138.9	139.8	152.2	138.9	144.2
Change median, %	-3.2	1.7	7.5	2.3	3.8
P-value		0.026	<0.001	0.020	<0.001

LDLC = low density lipoprotein (LDL) cholesterol, HDLC = high density lipoprotein (HDL) cholesterol, TG = triglycerides, SC=subcutaneous, q=quarterly

Follow-up and Effects on Clinical End Points. By the end of follow-up, 18.1% of patients in the placebo group had discontinued study drug, as compared to 18.7% of patients in the combined canakinumab groups. At a median follow-up of 3.7 years, the incidence rates for the primary end point (which included nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death) in the placebo, 50 mg, 150 mg, and 300 mg groups were 4.50, 4.11, 3.86, and 3.90 per 100 person-years, respectively (Table 8).

Table 8. Incidence rates (per 100 person years) and hazard ratios for major clinical outcomes and all-cause mortality

Clinical Outcome	Placebo (N=3344)	Canakinumab Dose (SC q 3 months)				P-value for trend across doses
		50 mg (N=2170)	150 mg (N=2284)	300 mg (N=2263)	All Doses (N=6717)	
Primary end point*						
Incidence rate, (N)	4.50 (535)	4.11 (313)	3.86 (320)	3.90 (322)	3.95 (955)	0.020
Hazard ratio	1.00	0.93	0.85	0.86	0.88	
95 % CI	(referent)	0.80-1.07	0.74-0.98	0.75-0.99	0.79-0.97	
P	(referent)	0.30†	0.021 [#]	0.031†	0.015	
Key secondary cardiovascular end point**						
Incidence rate, (N)	5.13 (601)	4.56 (344)	4.29 (352)	4.25 (348)	4.36 (1044)	0.003
Hazard ratio	1.00	0.90	0.83	0.83	0.85	
95% CI	(referent)	0.78-1.03	0.73-0.95	0.72-0.94	0.77-0.94	
P	(referent)	0.12 ^{\$}	0.005 [#]	0.004 ^{\$}	0.001	
Myocardial infarction, stroke, or death from any cause						
Incidence rate, (N)	5.56 (661)	5.17 (394)	4.77 (395)	4.88 (403)	4.93 (1192)	0.015
Hazard ratio	1.00	0.94	0.85	0.87	0.89	
95%CI	(referent)	0.83-1.07	0.75-0.96	0.77-0.99	0.81-0.97	
P	(referent)	0.35	0.010	0.030	0.013	
Myocardial infarction						
Incidence rate, (N)	2.43 (292)	2.20 (169)	1.90 (159)	2.09 (174)	2.06 (502)	0.028
Hazard ratio	1.00	0.94	0.76	0.84	0.84	
95% CI	(referent)	0.78-1.15	0.62-0.92	0.70-1.02	0.73-0.97	
P	(referent)	0.56	0.005	0.074	0.020	
Hospitalization for unstable angina requiring urgent revascularization						
Incidence rate, (N)	0.69 (85)	0.48 (38)	0.44 (38)	0.40 (34)	0.44 (110)	0.005
Hazard ratio	1.00	0.70	0.64	0.58	0.64	
95% CI	(referent)	0.47-1.03	0.44-0.94	0.39-0.86	0.48-0.85	
P	(referent)	0.071	0.021	0.006	0.002	
Any coronary revascularization						
Incidence rate, (N)	3.61 (421)	2.53 (191)	2.49 (205)	2.56 (209)	2.53 (605)	<0.001
Hazard ratio	1.00	0.72	0.68	0.70	0.70	
95% CI	(referent)	0.60-0.86	0.58-0.81	0.59-0.83	0.62-0.79	
P	(referent)	<0.001	<0.001	<0.001	<0.001	
Any Stroke						
Incidence rate, (N)	0.74 (92)	0.73 (58)	0.74 (63)	0.60 (51)	0.69 (172)	0.17
Hazard ratio	1.00	1.01	0.98	0.80	0.93	
95% CI	(referent)	0.72-1.41	0.71-1.35	0.57-1.13	0.72-1.20	
P	(referent)	0.95	0.91	0.20	0.58	
Cardiovascular Death (confirmed)						
Incidence rate, (N)	1.44 (182)	1.18 (94)	1.26 (110)	1.33 (115)	1.26 (319)	0.76
Hazard ratio	1.00	0.80	0.88	0.93	0.87	

95%CI	(referent)	0.62-1.03	0.70-1.12	0.74-1.18	0.73-1.05	
P	(referent)	0.083	0.30	0.55	0.15	
Cardiovascular death (or death of unknown cause)						
Incidence rate, (N)	1.86 (235)	1.71 (137)	1.65 (144)	1.74 (151)	1.70 (432)	0.62
Hazard ratio	1.00	0.89	0.90	0.94	0.92	
95% CI	(referent)	0.72-1.11	0.73-1.10	0.77-1.16	0.78-1.07	
P	(referent)	0.30	0.30	0.59	0.28	
Non-Cardiovascular death (confirmed)						
Incidence rate, (N)	1.11 (140)	1.14 (91)	1.08 (94)	1.02 (88)	1.08 (273)	0.45
Hazard ratio	1.00	1.02	0.97	0.92	0.97	
95%CI	(referent)	0.78-1.34	0.74-1.26	0.70-1.20	0.79-1.19	
P	(referent)	0.87	0.81	0.54	0.79	
Total Mortality						
Incidence rate, (N)	2.97 (375)	2.85 (228)	2.73 (238)	2.76 (239)	2.78 (705)	0.39
Hazard ratio	1.00	0.94	0.92	0.94	0.94	
95% CI	(referent)	0.80-1.11	0.78-1.09	0.80-1.10	0.83-1.06	
P	(referent)	0.48	0.33	0.42	0.31	

P values for trend, *P* values for the combination of all doses compared to placebo, and *P* values for all secondary end points other than the key secondary cardiovascular end point have not been adjusted for multiplicity.

* Primary end point = nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death.

5 ** Key secondary cardiovascular end point = nonfatal myocardial infarction, nonfatal stroke, hospitalization for unstable angina requiring unplanned revascularization, or cardiovascular death

Statistically significant compared to placebo, adjusted for multiplicity and accounting for two efficacy interim analyses, in accordance with the pre-specified closed-testing. The threshold *P* value for the primary end point for the 150 mg dose was 0.02115. The threshold *P* value for the key secondary cardiovascular end point for the 150 mg dose was 0.00529.

10 † Not statistically significant compared to placebo based on the prespecified closed-testing procedure. The threshold *P* value for the primary end point for the 50 mg dose was 0.02115. The threshold *P* value for the primary end point for the 300 mg dose was 0.01058.

15 ^s Exploratory analyses.

No significant effect was observed for the primary end point in the canakinumab 50 mg dose group compared to placebo (hazard ratio [HR] 0.93, *P*=0.30). By contrast, a statistically significant effect for the primary end point was observed in the canakinumab 150 mg dose group (HR 0.85, *P*=0.02075, threshold *P* value 0.02115). In the canakinumab 300 mg dose group, the hazard ratio was similar but the *P* value did not meet the prespecified significance threshold (HR 0.86, *P*=0.0314, threshold *P* value 0.01058). The *P* value for trend across the active-dose groups compared to placebo was 0.020, and the *P* value for comparison of all doses combined versus placebo was 0.015 (both results not adjusted for multiple testing).

25 Additionally, a subgroup of patients showing greater reductions in their hsCRP levels after treatment with canakinumab after 6 months show a statistically significant greater risk reduction in MACE compared to the overall treatment population. A separate causal inference analysis was conducted: the method estimates average treatment effect in the subgroup of patients who achieve hsCRP levels below the specified target at 6-months following treatment with canakinumab: The approach was used to derive the hazard rates for patients who had hsCRP level of between ≥ 2 mg/L

and <5 mg/L at three months from first administration of canakinumab and who received a further dose of canakinumab at three months and had hsCRP level of <2 mg/L at six months from first administration of canakinumab and patients who had hsCRP level of between ≥ 2 mg/L and <5 mg/L at three months from first administration of canakinumab and who received a further dose of
5 canakinumab at three months, and had hsCRP level of ≥ 2 mg/L at six months from first administration of canakinumab. The estimation of these potential outcomes differs from the multivariable adjustment described above in that it allows to ascertain the average treatment comparison in the population of patients who would achieve on treatment hsCRP values below the target levels of interest. In the application of the causal inference analysis the number of patients included in the analyses was
10 expanded to encompass all patients who were alive at the time of the 6-month assessment and could have provided a sample by relying on multiple imputation of the missing hsCRP values in order to avoid introducing bias by excluding patients who might have contributed events to the analysis but were initially excluded due to the unavailability of an assayed sample.

For canakinumab treated patients the treatment effect as the hazard rate of occurrence of the
15 endpoint of interest (MACE) was observed, but for placebo treated patients their hsCRP levels under treatment with canakinumab are unknown. Hence the placebo survival of canakinumab “responder” patients is derived, *i.e.*, canakinumab responder patients counterfactually treated with placebo, by deriving the average survival of placebo patients predicted from the covariate values of canakinumab responder patients. The baseline covariates are those that are useful for predicting hsCRP response
20 below a certain target level when treated with canakinumab: baseline hsCRP, Body-Mass Index (BMI), the SMART risk score established by the European Society for Cardiology (Dorresteijn, J. A. N. *et al*, Heart. 2013;99(12):866-72), LDL-C, baseline statin dose and indicator of medical history of recurrent MI. The hazard rates for these two groups are derived: canakinumab treated patient using observed risk, and the average over the covariate weighted survival of the placebo patients who would
25 have been responders when treated with canakinumab. Hazard rates were then obtained using non-parametric or semiparametric models (Cox regression) stratified by time since qualifying MI for survival and then estimating the hazards. The causal inference approach does not provide p-values, only bounds calculated as the quantiles corresponding to the usual two-sided 95% intervals from a bootstrap resampling procedure applied. These hazard rates were used to derive hazard ratios, with
30 confidence bounds being derived from 3,000 bootstrap iterations, which included accounting for the uncertainty of multiply imputed hsCRP values for patients not having a laboratory value at 6 months and who did not have a death date prior to or on Day 183.

Patients responding with reductions in their hsCRP levels to between ≥ 2 and <5 mg/L at three months from first administration of 150 mg canakinumab and who received a further dose of 150 mg
35 canakinumab at three months and who had reduced hsCRP levels of <2 mg/L at six months showed a 30% relative risk reduction in MACE, based on causal inference analysis assuming exponential

survival distribution, estimates based on 3,000 bootstrap samples. The covariates used for the placebo model were baseline CRP, SMART risk score, BMI, Statin dose, LDL-C and indicator of medical history of recurrent MI as covariates (Table 9).

Table 9. hsCRP at six months responder analysis for risk of MACE

	HR (95% CI)
3-month hsCRP responders (≥ 5 mg/L)*	
Canakinumab 150 mg	0.97 (0.72, 1.25)
3-month hsCRP responders (≥ 2 mg/L and < 5 mg/L)*	
Canakinumab 150 mg	0.82 (0.66, 1.01)
3-month hsCRP responders (≥ 2 mg/L and < 5 mg/L)* and 6 month hsCRP < 2 mg/L	
Canakinumab 150 mg	0.70 (0.39, 1.05)
3-month hsCRP responders (≥ 2 mg/L and < 5 mg/L)* and 6 month hsCRP ≥ 2 mg/L	
Canakinumab 150 mg	1.02 (0.75, 1.36)
3-month hsCRP responders (≥ 2 mg/L and < 4 mg/L)* and 6 month hsCRP < 2 mg/L	
Canakinumab 150 mg	0.73 (0.39, 1.15)
3-month hsCRP responders (≥ 2 mg/L and < 4 mg/L)* and 6 month hsCRP ≥ 2 mg/L	
Canakinumab 150 mg	1.16 (0.81, 1.62)
3-month hsCRP responders (≥ 2 mg/L and < 3 mg/L)* and 6 month hsCRP < 2 mg/L	
Canakinumab 150 mg	0.79 (0.35, 1.35)
3-month hsCRP responders (≥ 2 mg/L and < 3 mg/L)* and 6 month hsCRP ≥ 2 mg/L	
Canakinumab 150 mg	1.11 (0.69, 1.74)

5 * Based on causal inference analysis assuming exponential survival distribution, estimates based on 3,000 bootstrap samples

For the key secondary cardiovascular end point (which included the components of the primary end point plus hospitalization for unstable angina requiring urgent revascularization), incidence rates in the placebo, 50 mg, 150 mg, and 300 mg groups were 5.13, 4.56, 4.29, and 4.25 per 100 person-years, respectively (Table 8). For the canakinumab 150 mg dose (for which the P value met the significance threshold for the primary end point), the hazard ratio for the secondary cardiovascular endpoint was 0.83 (P=0.00525, threshold P value 0.00529). According to the closed testing procedure, formal significance testing for the prespecified secondary end point was not performed for the 50 mg and 300 mg doses. The hazard ratios for these doses were 0.90 and 0.83, respectively. The P value for trend across the active-dose groups compared to placebo was 0.003, and the P value for comparison of all doses combined versus placebo was 0.001 (both results not adjusted for multiple testing).

Analyses of the additional secondary end points, and of the components of the primary and secondary end points, were not adjusted for multiple testing (Table 8). Nominally significant reductions were seen in myocardial infarction for the 150 mg dose of canakinumab; in hospitalization for unstable angina requiring urgent revascularization for the 150 mg and 300 mg doses; and in any coronary revascularization for all three doses. All-cause mortality was neutral in comparisons of all canakinumab doses to placebo (HR 0.94, 95%CI 0.83-1.06, P=0.31). In on-treatment analyses for the primary end point, the observed hazard ratios in the placebo, 50 mg, 150 mg, and 300 mg groups were 1.0, 0.90, 0.83, and 0.79 (P-trend across groups=0.003). In comparable analyses for the key secondary

cardiovascular end point, the corresponding hazard ratios were 1.0, 0.88, 0.80, and 0.77 (P-trend across groups <0.001).

Adverse Events and Other Clinical Outcomes.

Neutropenia was more common among those allocated to canakinumab and there was a statistically significant increase in fatal events attributed to infection or sepsis when the three canakinumab groups were pooled and compared to placebo (incidence rates 0.31 versus 0.18 per 100 person years, $P=0.023$) (Table 4). Participants succumbing to infection tended to be older and more likely to have diabetes. Six confirmed cases of tuberculosis occurred in the trial with similar rates in the canakinumab and placebo groups (0.06%); five cases occurred in India and one in Taiwan.

Thrombocytopenia was more common among those allocated to canakinumab, but no difference in hemorrhage was observed. No increase in injection site reactions was observed. Consistent with known effects of IL-1 β inhibition, canakinumab resulted in significant reductions in reports of arthritis, gout, and osteoarthritis (Table). There was also a significant reduction in cancer mortality with canakinumab.

Table 10. Incidence rates (per 100-person years), number (N) of serious adverse events, and selected on-treatment safety laboratory data (% , N), stratified by study group.

	Canakinumab Dose (SC q 3 months)						
Adverse Event or Laboratory Parameter	Placebo n=3344	50mg N=2170	150mg N=2284	300mg N=2263	All doses N=6717	P-value for trend across doses vs. placebo	P-value for combined dose groups vs. placebo
Any SAE	12.0 (1202)	11.4 (741)	11.7 (812)	12.3 (836)	11.8 (2389)	0.43	0.79
Any SAE infection	2.86 (342)	3.03 (230)	3.13 (258)	3.25 (265)	3.14 (753)	0.12	0.14
Cellulitis	0.24 (30)	0.24 (19)	0.37 (32)	0.41 (35)	0.34 (86)	0.0213	0.09
Pneumonia	0.90 (112)	0.94 (74)	0.94 (80)	0.99 (84)	0.95 (238)	0.56	0.62
Urinary tract	0.22 (27)	0.18 (14)	0.24 (21)	0.20 (17)	0.21 (52)	0.84	0.87
Opportunistic infections+	0.18 (23)	0.16 (13)	0.15 (13)	0.20 (17)	0.17 (43)	0.97	0.78
Pseudomembranous Colitis	0.03 (4)	0.13 (10)	0.05 (4)	0.12 (10)	0.10 (24)	0.13	0.0302
Fatal infection/sepsis	0.18 (23)	0.31 (25)	0.28 (24)	0.34 (29)	0.31 (78)	0.09	0.0228
Any malignancy++	1.88 (231)	1.85 (144)	1.69 (143)	1.72 (144)	1.75 (431)	0.31	0.38
Fatal malignancy++	0.64 (81)	0.55 (44)	0.50 (44)	0.31 (27)	0.45 (115)	0.0007	0.016
Other adverse events							
Injection site reaction+	0.23 (29)	0.27 (21)	0.28 (24)	0.30 (26)	0.28 (71)	0.49	0.36
Arthritis	3.32 (385)	2.15 (164)	2.17 (180)	2.47 (201)	2.26 (545)	0.0020	<0.0001

Osteoarthritis	1.67 (202)	1.21 (94)	1.12 (95)	1.30 (109)	1.21 (298)	0.0393	0.0005
Gout	0.80 (99)	0.43 (34)	0.35 (30)	0.37 (32)	0.38 (96)	<0.0001	<0.0001
Drug induced liver injury (SAE)+	0.18 (23)	0.15 (12)	0.13 (11)	0.05 (4)	0.11 (27)	0.0039	0.0541
Leukopenia	0.24 (30)	0.30 (24)	0.37 (32)	0.52 (44)	0.40 (100)	0.002	0.013
Neutropenia	0.06 (7)	0.05 (4)	0.07 (6)	0.18 (15)	0.10 (25)	0.014	0.17
Any Hemorrhage	4.01 (462)	3.33 (249)	4.15 (327)	3.82 (301)	3.78 (877)	0.94	0.31
Thrombocytopenia	0.43 (53)	0.56 (44)	0.54 (46)	0.71 (60)	0.60 (150)	0.022	0.031
Hepatic variable**							
ALT > 3x normal %, (N)	1.4 (46)	1.9 (42)	1.9 (44)	2.0 (45)	2.0 (131)	0.19	0.058
AST > 3x normal %, (N)	1.1 (36)	1.5 (32)	1.5 (35)	1.5 (34)	1.5 (101)	0.30	0.11
ALP > 3x normal %, (N)	0.4 (15)	0.5 (11)	0.4 (10)	0.5 (12)	0.5 (33)	0.67	0.82
Bilirubin >2x normal %, (N)	0.8 (26)	1.0 (21)	0.7 (15)	0.7 (15)	0.8 (51)	0.34	0.83

Data are shown as incidence rates per 100 person-years (with number of patients with event) for adverse events and as percentages of patients with the condition (with number of patients) for hepatic variables to facilitate the comparison of rates between groups.

All adverse event categories are based on standardized Medical Dictionary for Regulatory Activities (MedDRA), version 20.0, queries or classification levels, except those otherwise indicated.

SAE= serious adverse event; ALT=alanine aminotransferase; AST=aspartate transaminase; ALP=alkaline phosphatase

+ Sponsor categorization of adverse events of special interest

++ Included are malignancies adjudicated by the Cancer Endpoint Adjudication Committee

10 ** Hepatic variable – percent of patients with condition (No.)

CANTOS was designed to test directly the inflammatory hypothesis of atherothrombosis. In this trial, among patients with a prior history of myocardial infarction, hsCRP levels and IL-6 levels were significantly reduced by canakinumab, with no reduction in lipid levels. While the 50 mg dose of canakinumab did not have a statistically significant effect on the primary cardiovascular end point compared to placebo, participants in the 150 mg dose group experienced relative hazard reductions of 15% for the primary end point (from 4.50 to 3.86 events per 100 person-years) and 17% for the key secondary cardiovascular end point (from 5.13 to 4.29 events per 100 person-years). The P values for both of these end points met pre-specified multiplicity-adjusted thresholds for statistical significance. Although the hazard reductions for the 300 mg dose group were similar to those for the 150 mg dose group, the prespecified thresholds for statistical significance were not met for this group. Both a pooled analysis of all canakinumab doses and a trend analysis, however, suggested a beneficial effect of canakinumab on cardiovascular outcomes. Specific targeting of IL-1 β as a cytokine-based therapy for the secondary prevention of atherosclerotic events rests on several observations. The pro-inflammatory cytokine IL-1 β plays multiple roles in atherothrombotic plaque development including induction of procoagulant activity, promotion of monocyte and leucocyte adhesion to vascular endothelial cells, and the growth of vascular smooth muscle cells (Dinarello CA *et al*, Nat

- Rev Drug Discov. 2012;11(8):633-52; Dinarello CA. Blood. 2011;117(14):3720-32; Libby P *et al*, Am J Pathol. 1986;124(2):179-85). In mice, deficiency of IL-1beta reduces lesion formation, while in cholesterol-fed pigs, exposure to exogenous IL-1beta increases intimal medial thickening (Kirii H *et al*, Arterioscler Thromb Vasc Biol. 2003;23(4):656-60; Shimokawa H *et al*, J Clin Invest. 1996;97(3):769-76). The nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome activates IL-1beta, a process promoted by cholesterol crystals, neutrophil extracellular traps, local hypoxia, and atheroprone flow (Düwell P *et al*, Nature. 2010;464(7293):1357-61; Rajamaki K *et al*, PLoS One. 2010;5(7):e11765; Xiao H *et al*, Circulation. 2013;128(6):632-42; Folco EJ *et al*, Circ Res. 2014;115(10):875-83). This activation of IL-1beta stimulates the downstream IL-6 receptor signaling pathway, implicated by Mendelian randomization studies as a potential causal pathway for atherothrombosis (Hingorani AD *et al*, Lancet. 2012;379(9822):1214-24; Sarwar N *et al*, Lancet. 2012;379(9822):1205-13). Additionally, parabiotic mouse studies (Sager HB *et al*, Circulation. 2015;132(20):1880-90) and studies of clonal hematopoiesis (Fuster JJ *et al*, Science. 2017;355(6327):842-7; Jaiswal S *et al*, N Engl J Med. 2017;377(2):111-21) have implicated IL-1beta in processes by which bone marrow activation accelerates atherosclerosis. Further, expression of specific inflammasome gene modules impacting IL-1beta associates with all-cause mortality and increased atherosclerosis in the elderly (Furman D *et al*, Nat Med. 2017;23(2):174-84).

Although the patients in CANTOS had generally well-controlled levels of LDL cholesterol, placebo event rates were high, with a cumulative incidence of over 20% at five years. Our data thus affirm that statin-treated patients with residual inflammatory risk as assessed by baseline hsCRP greater than 2 mg/L have future event rates at least as high as, if not higher than, statin-treated patients with residual risk due to LDL cholesterol. These two patient groups may differ and may require personalized approaches to treatment (Ridker PM. Eur Heart J. 2016;37(22):1720-2). Despite the fact that no reduction in cholesterol levels occurred, the magnitude of effect on cardiovascular events with canakinumab (given every 3 months) was comparable to that associated with monoclonal antibodies targeting PCSK9 (given every 2 to 4 weeks) (Sabatine MS *et al*, N Engl J Med. 2017;376(18):1713-22; Ridker PM *et al*, N Engl J Med. 2017;376(16):1527-39). Yet inhibition of IL-1 β is a narrowly focused intervention that represents only one of many potential anti-inflammatory pathways that might serve as targets for atheroprotection (Morton AC *et al*, Eur Heart J. 2015;36(6):377-84; Van Tassell BW *et al*, Circulation. 2013;128(17):1910-23; Ridker PM *et al*, Eur Heart J. 2014;35(27):1782-91). We observed a statistically significant increase in fatal infection and sepsis with canakinumab, as well as a reduction in platelet counts with no increase in bleeding. By contrast, there was a significant reduction in cancer mortality among those allocated to canakinumab, a finding consistent with experimental data relating IL-1 to the progression and invasiveness of certain tumors, in particular lung cancer (Ridker PM *et al*, Lancet. 2017;390(10105):1833-42 ; Apte RN *et al*, Cancer Metastasis Rev. 2006;25(3):387-408 ; Grivnenkov SI *et al*, Lancet 2000;355:735-740). There was no

significant difference between treatment groups in all-cause mortality. No significant hepatic toxicity was noted. The beneficial effects of canakinumab observed for arthritis, gout, and osteoarthritis are consistent with well-described effects of the IL-1 and IL-6 pathways in these disorders. In conclusion, in CANTOS, patients with a prior history of myocardial infarction and hsCRP levels of 2 mg/L or greater were randomized to one of three doses of canakinumab or placebo. Canakinumab significantly reduced hsCRP levels without reducing LDL cholesterol, HDL cholesterol and triglycerides and the 150 mg dose significantly reduced the incidence of recurrent cardiovascular events whilst having an acceptable levels of side effects.

Example 2: Analysis of Patient Population in CANTOS.

3,854 subjects from the trial described in Example 1 donated blood samples for genetic analysis (both whole exome sequencing (WES) and Infinium Global Screening Array (GSA)). DNA for subsequent analyses was extracted from blood using standard protocols. WES was performed using the Illumina HiSeq System using standard protocols (Illumina HiSeq platform, PE 2X150bp). GSA was performed using the Illumina HiSeq System using standard protocols (Infinium Global Screening Array, version: GSAMD-24v1-0).

A cox proportional hazards regression model adjusted for age, sex, time since last MI, BMI, diabetes status, smoking status, hypertension status and first 10 principle components was performed using the data with a focus on high impact coding variants.

It was determined that placebo-treated patients who were homozygous for the rs1718119 SNP in the *P2RX7* gene had a 1.54 -fold increased risk of cardiovascular death and myocardial infarctions that was statistically significant ($p=0.04$). This SNP had been previously reported to encode a gain of function allele of *P2RX7* and thus would be expected to increase the activation of the NLRP3 inflammasome pathway. A second SNP (rs7958311) in the *P2RX7* gene encoding a loss of function allele was also identified. In placebo treated patients homozygous for this SNP, the rate of cardiovascular death and myocardial infarction was reduced by 59% ($HR=0.41$, $p=0.054$).

For comparative purposes, a graph showing the cumulative incidence of MACE over time in subjects with wild-type *P2RX7* treated with placebo or canakinumab (Hazard Ratio (HR) of 0.91, p value of 0.36) is shown in Figure 2.

A gain-of-function SNP (rs1718119) in *P2RX7* was identified as correlating with MI/CVD/MACE. Cumulative incidence of MI and CV death (MI/CVD) over time in subjects with a 0, 1, or 2 copies of a *P2RX7* gain of function allele (SNP rs1718119; Hazard Ratio (HR) of 1.54, p value of 0.039) is shown in Figure 3.

The cumulative incidence of MACE over time in subjects with a homozygous *P2RX7* gain of function allele treated with placebo or canakinumab (SNP rs1718119; Hazard Ratio (HR) of 0.68, p value of 0.046) is shown in Figure 4. These results represent a significant risk reduction for the identified population.

A trending association between a hypomorphic snp (rs7958311) in *P2RX7* was identified as correlating with MI/CVD/MACE. Cumulative incidence of MI and CV death (MI/CVD) over time in subjects with a 0, 1, or 2 copies of *P2RX7* hypomorphic allele (SNP rs7958311; Hazard Ratio (HR) of 0.41, p value of 0.054) is shown in Figure 5.

5

Example 3: Procedure for identifying an NLRP3 inhibitor from IL-1 β production in PMA-differentiated THP-1 cells stimulated with Gramicidin.

THP-1 cells were purchased from the American Type Culture Collection and sub-cultured according to instructions from the supplier. Prior to experiments, cells were cultured in complete
10 RPMI 1640 (containing 10% heat inactivated FBS, penicillin (100 units/ml) and streptomycin (100 μ g/ml)), and maintained in log phase prior to experimental setup. Prior to the experiment THP-1 were treated with PMA (Phorbol 12-myristate 13-acetate) (20 ng/ml) for 16-18 hours. Compounds were dissolved in dimethyl sulfoxide (DMSO) to generate a 30mM stock. On the day of the experiment the media was removed and adherent cells were detached with trypsin for 5 minutes. Cells were then
15 harvested, washed with complete RPMI 1640, spun down, resuspended in RPMI 1640 (containing 2% heat inactivated FBS, penicillin (100 units/ml) and streptomycin (100 μ g/ml). The cells were plated in a 384-well plate at a density of 50,000 cells/well (final assay volume 50 μ l). Compounds were first dissolved in assay medium to obtain a 5x top concentration of 500 μ M. 10 step dilutions (1:3) were then undertaken in assay medium containing 1.67% DMSO. 5x compound solutions were added to
20 the culture medium to achieve desired final concentration (e.g., 100, 33, 11, 3.7, 1.2, 0.41, 0.14, 0.046, 0.015, 0.0051, 0.0017 μ M). Final DMSO concentration was at 0.37%. Cells were incubated with compounds for 1 hour and then stimulated with gramicidin (5 μ M) (Enzo) for 2 hours. Plates were then centrifuged at 340g for 5 min. Cell free supernatant (40 μ L) was collected using a 96-channel PlateMaster (Gilson) and the production of IL-1 β was evaluated by HTRF (cisbio). A vehicle
25 only control and a dose titration of CRID3 (100 - 0.0017 μ M) were run concurrently with each experiment. Data was normalized to vehicle-treated samples (equivalent to 0% inhibition) and CRID3 at 100 μ M (equivalent to 100% inhibition). NLRP3 inhibitor compounds exhibited a concentration-dependent inhibition of IL-1 β production in PMA-differentiated THP-1 cells.

30

CLAIMS

1. A method of treating a subject with a gain of function mutation in *P2RX7*, wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject reduces the incidence of a major adverse cardiovascular event (MACE), comprising:
 - 5 a) assaying a biological sample from the subject for at least one of: a gain-of-function mutation of *P2RX7*, the level of *P2RX7* expression, the level of P2X7 protein, or the level of P2X7 activity; and
 - b) thereafter, selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has at least one of: a gain-of-
10 function mutation of *P2RX7*, an increased level of *P2RX7* expression, an increased level of P2X7 protein, or an increased level of P2X7 activity relative to a control.
2. A method of treating a subject with a gain of function mutation in *P2RX7*, wherein the administration of an NLRP3 inflammasome pathway inhibitor to the subject improves the hazard risks
15 of major adverse cardiovascular event (MACE) incidents for the subject, comprising:
 - a) assaying a biological sample from the subject for at least one of: a gain-of-function mutation of *P2RX7*, the level of *P2RX7* expression, the level of P2X7 protein, or the level P2X7 activity; and
 - b) thereafter, selectively administering a therapeutically effective amount of an NLRP3
20 inflammasome pathway inhibitor to the subject if the subject has at least one of: a gain-of-function mutation of *P2RX7*, an increased level of *P2RX7* expression, an increased level of P2X7 protein, or an increased level of P2X7 activity relative to a control.
3. The method of claim 1 or claim 2, wherein the increased expression and/or levels are relative
25 to those in a subject with zero copies of a *P2X7* gain of function mutation.
4. The method of claim 1 or claim 2, wherein the increased expression and/or levels are relative to those in a subject with one copy of a *P2X7* gain of function mutation.
- 30 5. A method of selectively treating a subject having an increased risk of MACE, comprising selectively administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject on the basis of the subject having at least one copy of a gain of function mutation of *P2RX7*.
- 35 6. A method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising:

- a) selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of a the subject having at least one copy of a gain of function mutation of *P2RX7*; and
- b) thereafter, administering a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor to the subject.

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7. A method of selectively treating a subject having an increased risk of MACE with an NLRP3 inflammasome pathway inhibitor, comprising:

a) assaying a biological sample from the subject for the presence of at least one copy of a gain of function mutation of *P2RX7*; and

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b) subsequently selecting the subject for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of the biological sample from the subject having the at least one copy of a gain of function mutation of *P2RX7*.

8. The method of claim 7, wherein the method further comprises:

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c) subsequently administering a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor to the subject.

9. A method of predicting the likelihood that a subject having an increased risk of MACE will respond to treatment with an NLRP3 inflammasome pathway inhibitor, comprising assaying a

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biological sample from the subject for the presence of at least one copy of a gain of function mutation of *P2RX7*, wherein the presence of the at least one copy of a gain of function mutation of *P2RX7* is indicative of an increased likelihood that the subject will respond to treatment with the NLRP3 inflammasome pathway inhibitor.

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10. The method of any one of claims 5-9, wherein the subject has two copies of the gain of function mutation of *P2RX7*.

11. The method of any one of claims 7-10, further comprising the step of obtaining the biological sample from the subject, wherein the step of obtaining is performed prior to the step of assaying.

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12. The method of any one of claims 7-11, wherein the step of assaying comprises assaying the biological sample for a nucleic acid product of the gain of function mutation of *P2RX7*.

13. The method of claim 12, wherein the step of assaying comprises assaying the biological

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sample for a genomic sequence of the gain of function mutation of *P2RX7*.

14. The method of any one of claims 1-13, wherein the gain of function mutation of *P2RX7* is an Rs1718119 allele.
15. The method of any one of claims 7-14, wherein the biological sample is selected from the group consisting of synovial fluid, blood, serum, feces, plasma, urine, tear, saliva, cerebrospinal fluid, a leukocyte sample and a tissue sample.
16. The method of any one of claims 7-15, wherein the step of assaying comprises a technique selected from the group consisting of Northern blot analysis, polymerase chain reaction (PCR), reverse transcription-polymerase chain reaction (RT-PCR), TaqMan-based assays, direct sequencing, dynamic allele-specific hybridization, high-density oligonucleotide SNP arrays, restriction fragment length polymorphism (RFLP) assays, primer extension assays, oligonucleotide ligase assays, analysis of single strand conformation polymorphism, temperature gradient gel electrophoresis (TGGE), denaturing high performance liquid chromatography, high-resolution melting analysis, DNA mismatch-binding protein assays, SNPLex®, capillary electrophoresis, Southern Blot, immunoassays, immunohistochemistry, ELISA, flow cytometry, Western blot, HPLC, and mass spectrometry.
17. A method for producing a transmittable form of information for predicting the responsiveness of a subject having an increased risk of MACE to treatment with an NLRP3 inflammasome pathway inhibitor, comprising:
- a) determining an increased likelihood of the subject responding to treatment with the NLRP3 inflammasome pathway inhibitor based on the presence of at least one copy of a gain of function mutation of *P2RX7* in a biological sample from the subject; and
 - b) recording the result of the determining step on a tangible or intangible media form for use in transmission.
18. A method for producing a transmittable form of information for predicting the responsiveness of a subject having an increased incidence of MACE to treatment with an NLRP3 inflammasome pathway inhibitor, comprising:
- a) determining an increased likelihood that the subject will respond to treatment with the NLRP3 inflammasome pathway inhibitor based on an increased level of *P2X7* expression, increased level of P2X7 protein, or an increased level of P2X7 activity in a biological sample from the subject relative to a control; and
 - b) recording the result of the determining step on a tangible or intangible media form for use in transmission.

19. The method of any one of the preceding claims, wherein the MACE is a non-fatal myocardial infarction (MI), non-fatal stroke, or hospitalization for unstable angina requiring unplanned revascularization.
- 5 20. The method of any one of the preceding claims, wherein the MACE is cardiovascular (CV) death.
21. The method of any one of the preceding claims, wherein the NLRP3 inflammasome pathway inhibitor is selected from: an interleukin 1 beta inhibitor, an interleukin 18 inhibitor, an interleukin 6
10 inhibitor, an NLRP3 inhibitor, or a combination thereof.
22. The method of claim 21, wherein the interleukin 1 beta inhibitor is an anti-IL1beta antibody, and the anti-IL1beta antibody is selected from the group comprising:
- 15 a) an antibody comprising the three HCDRs of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3;
- b) an antibody comprising the three LCDRs of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6;
- c) an antibody comprising the three HCDRs of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and the three LCDRs of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6;
- 20 d) an anti-IL1 β antibody comprising a HC domain comprising SEQ ID NO: 7;
- e) an anti-IL1 β antibody comprising a LC domain comprising SEQ ID NO: 8; and
- f) an anti-IL1 β antibody comprising a HC domain comprising SEQ ID NO: 7 and a LC domain comprising SEQ ID NO: 8.
- 25 23. The method of claim 21 or claim 22, wherein the interleukin 1 beta inhibitor is a human antibody, such as a monoclonal human antibody.
24. The method of any one of claims 21-23, wherein the interleukin 1 beta inhibitor is canakinumab.
- 30 25. The method of any one of the preceding claims, wherein the subject has previously suffered a myocardial infarction (MI).
26. The method of any one of the preceding claims, wherein the subject is concomitantly
35 receiving standard of care treatment for reducing the risk of or preventing MACE.

27. The method of any one of the preceding claims, wherein the subject has a high sensitivity C-reactive protein (hsCRP) level of ≥ 2 mg/L.
28. An NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE
5 in a subject, comprising testing for the presence of one or more copies of a gain of function mutation of *P2RX7* in a biological sample from the subject and administering a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the sample tests positive for one or more copies of the gain of function mutation of *P2RX7*.
- 10 29. A kit for use in predicting the likelihood that a subject having an increased risk of MACE will respond to treatment with an NLRP3 inflammasome pathway inhibitor comprising,
a) at least one probe capable of detecting the presence of at least one gain of function mutation of *P2RX7*; and
b) instructions for using the probe to assay a biological sample from the subject for the
15 presence of the at least one mutation, wherein the presence of the at least one mutation is indicative of an increased likelihood that the subject will respond to treatment with the NLRP3 inflammasome pathway inhibitor.
30. A kit for use in treating a subject having an increased risk of MACE comprising,
20 a) a therapeutically effective amount of an NLRP3 inflammasome pathway inhibitor;
b) at least one probe capable of detecting the presence of at least one gain of function mutation of *P2RX7*;
c) instructions for using the probe to assay a biological sample from the subject for the presence of the at least one mutation;
25 d) instructions for administering the NLRP3 inflammasome pathway inhibitor to the subject if the biological sample from the subject has the presence of the at least one mutation; and
e) optionally, means for administering the NLRP3 inflammasome pathway inhibitor to the subject.
- 30 31. The kit of claim 29 or claim 30, wherein the probe is an oligonucleotide that specifically hybridizes to a region of a nucleic acid coding for the at least one mutation, an antibody that detects a polypeptide product of the at least one mutation, or an oligonucleotide that specifically hybridizes to a region of a nucleic acid coding for an equivalent genetic marker of the at least one mutation.
- 35 32. An NLRP3 inflammasome pathway inhibitor for the use in the treatment or prevention of MACE in a subject, comprising:

- a) assaying a biological sample from the subject;
- b) determining if the subject has a gain of function mutation of *P2RX7*; and
- c) if the mutation is present, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is to be administered to the subject.

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33. An in vitro method for determining efficacy of treatment of a subject for MACE by administration of an NLRP3 inflammasome pathway inhibitor, comprising:

- a) determining in vitro whether the sample indicates that the subject has a gain of function mutation of *P2RX7*; and

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- b) administering an effective amount of an NLRP3 inflammasome pathway inhibitor to the subject if the subject has a gain of function mutation of *P2RX7*.

34. An NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, comprising

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- a) assaying a sample from the subject;
- b) determining if the subject has at least one copy of a gain of function allele of *P2RX7*; and
- c) if the subject has at least one copy of a gain of function allele of *P2RX7*, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is to be administered to the subject.

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35. An NLRP3 inflammasome pathway inhibitor for use in treating a subject at risk of MACE, characterized in that:

- a) the subject is selected for treatment with the NLRP3 inflammasome pathway inhibitor on the basis of the subject having at least one copy of a gain of function allele of *P2RX7*; and
- b) thereafter, a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is administered to the subject.

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36. An NLRP3 inflammasome pathway inhibitor for use in treating a subject at risk of MACE, characterized in that:

- a) a biological sample from the subject is assayed for at least one copy of a gain of function allele of *P2RX7*; and
- b) a therapeutically effective amount of the NLRP3 inflammasome pathway inhibitor is selectively administered to the subject on the basis of the biological sample from the subject having at least one copy of a gain of function allele of *P2RX7*.

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37. An NLRP3 inflammasome pathway inhibitor for use in the treatment or prevention of MACE in a subject, wherein said subject has at least one copy of a gain of function allele of *P2RX7*.
38. The NLRP3 inflammasome pathway inhibitor for use in accordance with claim 37, wherein the NLRP3 inflammasome pathway inhibitor is selected from: an NLRP3 inhibitor, an anti-IL-1beta antibody, an anti-IL-18 antibody, and an anti IL-1beta/IL-18 bispecific antibody.
38. The NLRP3 inflammasome pathway inhibitor for use in accordance with claim 37, wherein the NLRP3 inflammasome pathway inhibitor is an NLRP3 inhibitor, optionally wherein the NLRP3 inhibitor is a compound of any one of Formulae 101A, 102A, 1AA, 2AA, 3AA, 4AA, 5AA and 6AA.
39. The NLRP3 inflammasome pathway inhibitor for use in accordance with claim 37, wherein the NLRP3 inflammasome pathway inhibitor is an anti-IL-1beta antibody, optionally wherein the anti-IL-1beta antibody is canakinumab.
40. The NLRP3 inflammasome pathway inhibitor for use in accordance with claim 37, wherein the NLRP3 inflammasome pathway inhibitor is an anti-IL-18 antibody.
41. The NLRP3 inflammasome pathway inhibitor for use in accordance with claim 37, wherein the NLRP3 inflammasome pathway inhibitor is an anti IL-1beta/IL-18 bispecific antibody.
41. The NLRP3 inflammasome pathway inhibitor for use in accordance with any one of claims 37-40, wherein the subject has two copies of the gain of function allele of *P2RX7*.
42. The NLRP3 inflammasome pathway inhibitor for use in accordance with any one of claims 37-40, wherein the gain of function mutation of *P2RX7* is an Rs1718119 allele.
43. The NLRP3 inflammasome pathway inhibitor for use in accordance with any one of claims 37-42, wherein the subject has had at least one previous myocardial infarction (MI).
44. The NLRP3 inflammasome pathway inhibitor for use in accordance with any one of claims 37-42, wherein the subject has an hsCRP level of ≥ 2 mg/L.

FIG. 1

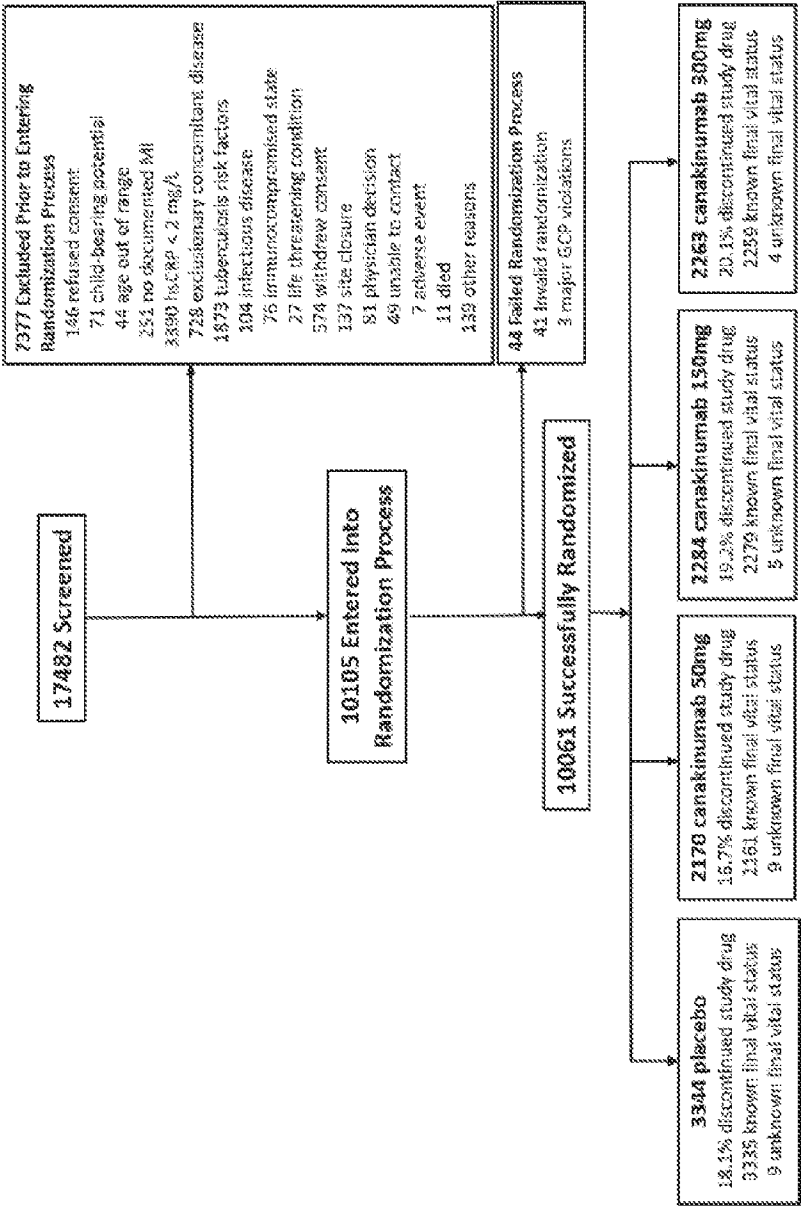


FIG. 2

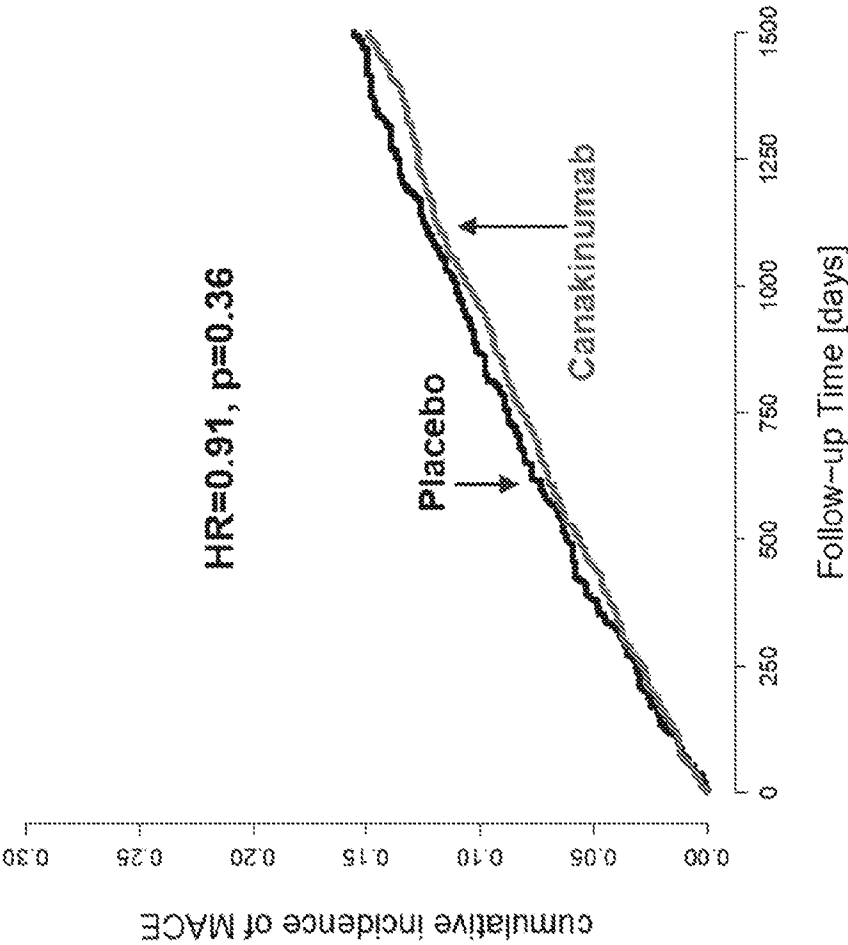


FIG. 3

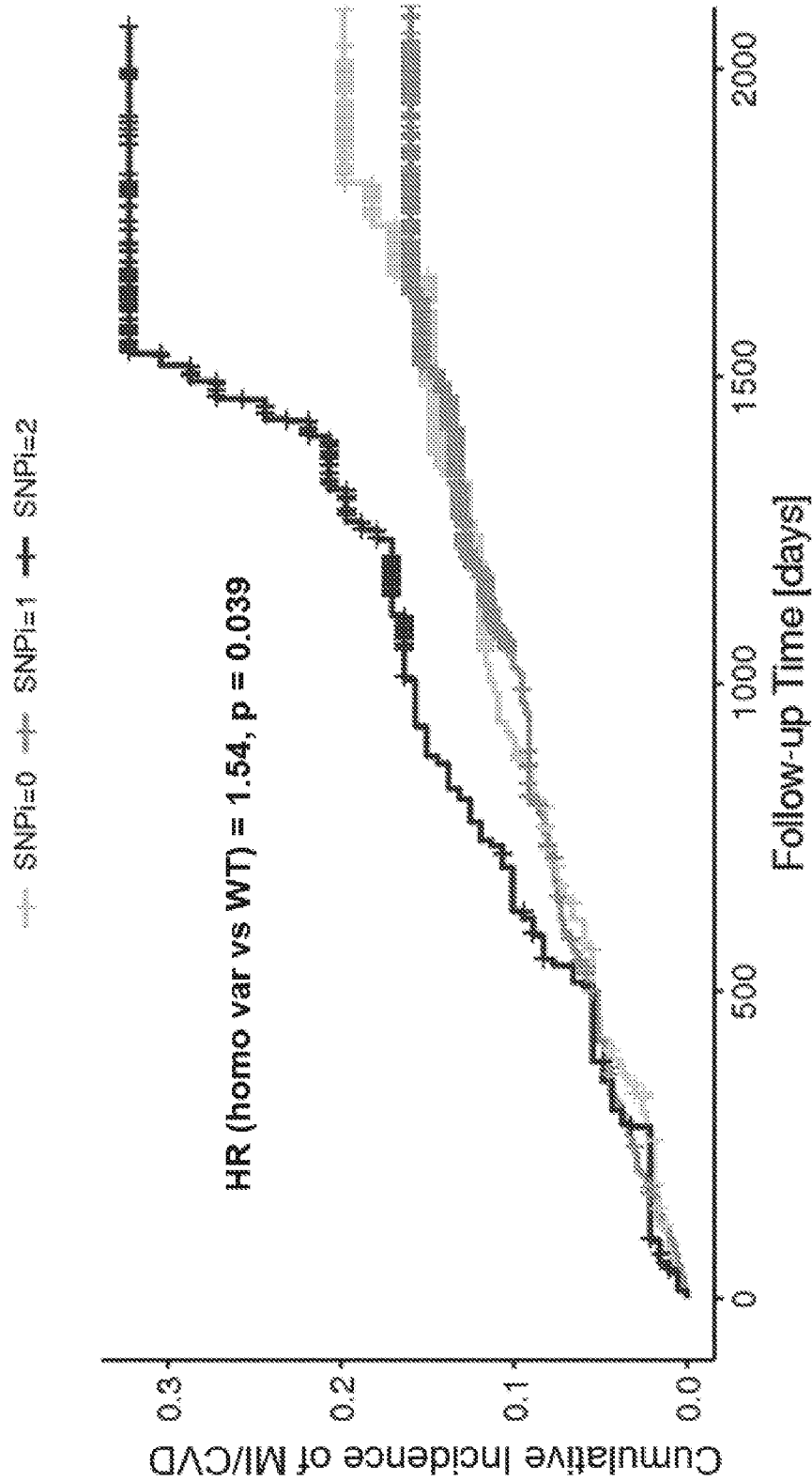


FIG. 4

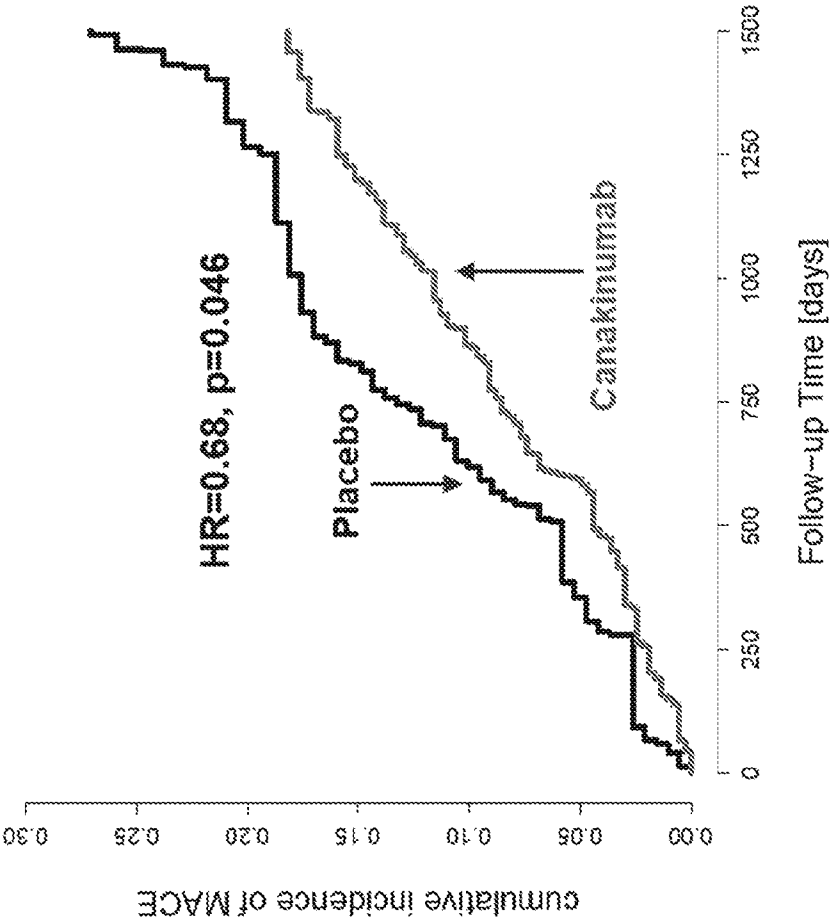
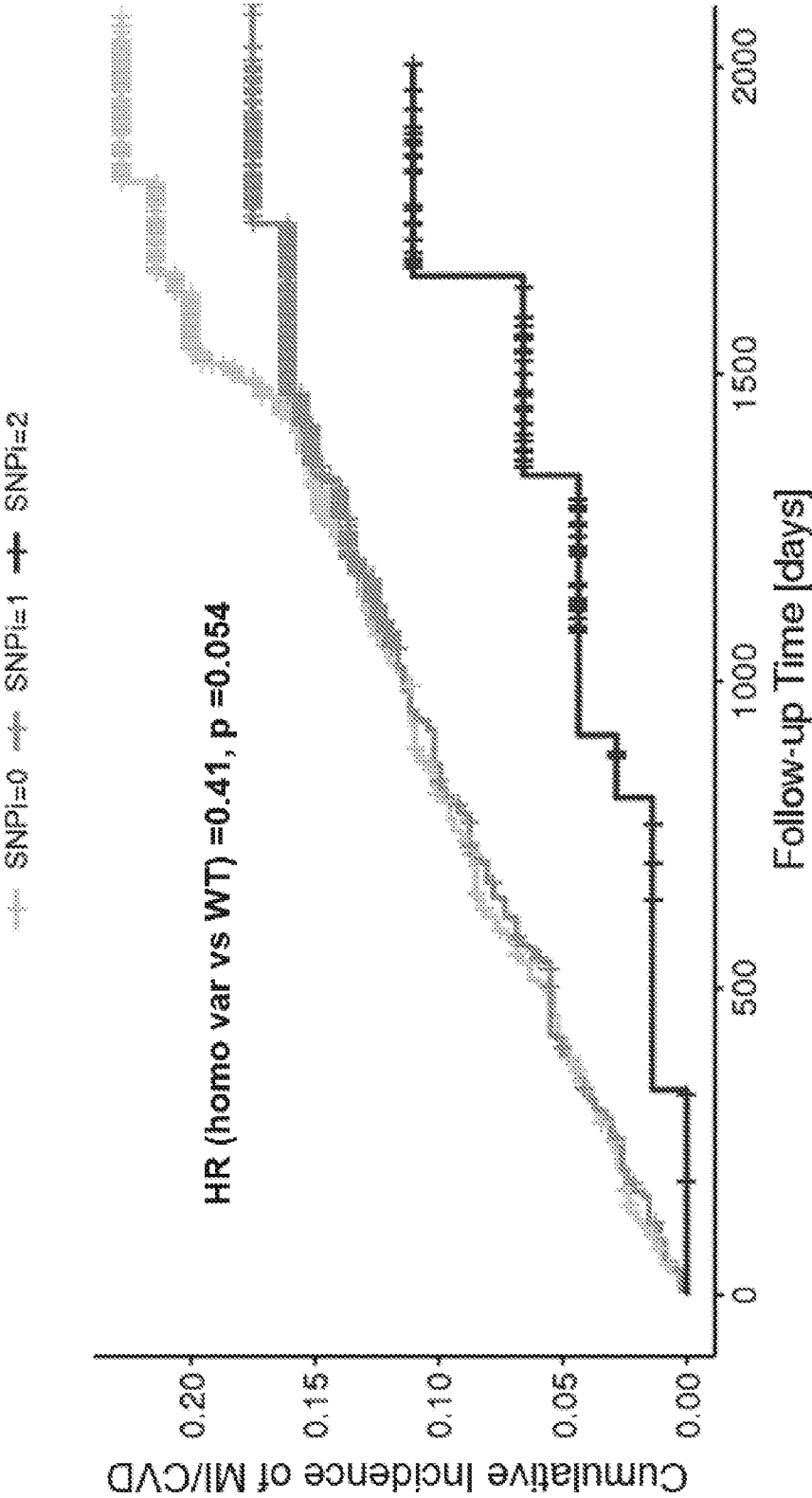


FIG. 5



INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2021/056668

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/6883
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, CHEM ABS Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Hu S ET AL: "Clinical and Experimental Rheumatology 2020 The presence of P2RX7 single nuclear polymorphism is associated with a gain of function in P2X7 receptor and", Clinical and Experimental Rheumatology, 3 August 2019 (2019-08-03), pages 442-449, XP055848969, Retrieved from the Internet: URL:https://www.clinexprheumatol.org/article.asp?a=13579 [retrieved on 2021-10-07] pages 443-444 ----- -/-	29,31



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

7 October 2021

Date of mailing of the international search report

18/10/2021

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Hennard, Christophe

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2021/056668

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YIN JIE ET AL: "P2X 7 receptor inhibition attenuated sympathetic nerve sprouting after myocardial infarction via the NLRP3/IL-1[beta] pathway", JOURNAL OF CELLULAR AND MOLECULAR MEDICINE, vol. 21, no. 11, 4 May 2017 (2017-05-04), pages 2695-2710, XP055849028, RO ISSN: 1582-1838, DOI: 10.1111/jcmm.13185 Retrieved from the Internet: URL:https://api.wiley.com/onlinelibrary/tdm/v1/articles/10.1111%2Fjcmm.13185>	29-31
Y	page 2696, "Materials and methods", column2; page 2697, "Western blotting column 2	1-44
X	----- ZHOU JUNTENG ET AL: "Inhibition of P2X7 Purinergic Receptor Ameliorates Cardiac Fibrosis by Suppressing NLRP3/IL-1 [beta] Pathway", OXIDATIVE MEDICINE AND CELLULAR LONGEVITY, vol. 2020, 22 May 2020 (2020-05-22), pages 1-13, XP055849038, US ISSN: 1942-0900, DOI: 10.1155/2020/7956274 Retrieved from the Internet: URL:http://downloads.hindawi.com/journals/omcl/2020/7956274.xml>	29-31
Y	page 3, table 1	1-44
Y	----- LIU YANG ET AL: "NLRP3 Inflammasome: A Potential Alternative Therapy Target for Atherosclerosis", EVIDENCE-BASED COMPLEMENTARY AND ALTERNATIVE MEDICINE, vol. 2020, 31 March 2020 (2020-03-31), pages 1-15, XP055849044, US ISSN: 1741-427X, DOI: 10.1155/2020/1561342 Retrieved from the Internet: URL:http://downloads.hindawi.com/journals/ecam/2020/1561342.xml> abstract; page 8, column 1 ----- -/-	1-44

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2021/056668

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	VAN HOUT GERARDUS P.J. ET AL: "The selective NLRP3-inflammasome inhibitor MCC950 reduces infarct size and preserves cardiac function in a pig model of myocardial infarction", EUROPEAN HEART JOURNAL, 17 July 2016 (2016-07-17), page ehw247, XP055849049, GB ISSN: 0195-668X, DOI: 10.1093/eurheartj/ehw247 page 829, "Methods" -----	1-44

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2021/056668

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☐ forming part of the international application as filed:
- ☐ in the form of an Annex C/ST.25 text file.
- ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☒ furnished subsequent to the international filing date for the purposes of international search only:
- ☒ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
- ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments: