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January 24, 2023

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APPLICATION NUMBER: *17/097,285*
FILING DATE: *November 13, 2020*
PATENT NUMBER: *11400063*
ISSUE DATE: *August 02, 2022*

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Doc Code: TRACK1.REQ
Document Description: TrackOne Request

PTO/AIA/424 (04-14)

CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

First Named Inventor:	Jean-Claude TARDIF	Nonprovisional Application Number (if known):	Not Yet Assigned
Title of Invention:	EARLY ADMINISTRATION OF LOW-DOSE COLCHICINE AFTER MYOCARDIAL INFARCTION		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1) and the prioritized examination fee set forth in 37 CFR 1.17(c) have been filed with the request. The publication fee requirement is met because that fee, set forth in 37 CFR 1.18(d), is currently $0. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.
2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims, and that any request for an extension of time will cause an outstanding Track I request to be dismissed.
3. The applicable box is checked below:

I. [x] **Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)**

i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.

---OR---

(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, **or** the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. [ ] **Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)**

i. A request for continued examination has been filed with, or prior to, this form.
ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature	/Jan N. Tittel/	Date	November 13, 2020
Name (Print/Typed)	Jan N. Tittel, Ph.D.	Practitioner Registration Number	52,290

Note: *This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.**

[ ] *Total of 1 forms are submitted.

PTO/AIA/15 (10-17)
Approved for use through 11/30/2020. OMB 0651-0032
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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	50761-029001
First Named Inventor	Jean-Claude TARDIF
Title	EARLY ADMINISTRATION OF LOW-DOSE COLCHICINE AFTER MYOCARDIAL INFARCTION
Priority Mail Express® Label No.	

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. [ ] **Fee Transmittal Form** (PTO/SB/17 or equivalent)
2. [ ] **Applicant asserts small entity status.** See 37 CFR 1.27
3. [ ] **Applicant certifies micro entity status.** See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
4. [X] **Specification** [*Total Pages* 50] Both the claims and abstract must start on a new page. *(See MPEP § 608.01(a) for information on the preferred arrangement)*
5. [X] **Drawing(s)** (35 U.S.C. 113) [*Total Sheets* 3]
6. **Inventor's Oath or Declaration** [*Total Pages*] *(including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))*
   a. [ ] Newly executed (original or copy)
   b. [ ] A copy from a prior application (37 CFR 1.63(d))
7. [X] **Application Data Sheet** ** See note below.* See 37 CFR 1.76 (PTO/AIA/14 or equivalent)
8. **CD-ROM or CD-R** in duplicate, large table, or Computer Program (*Appendix*)
   [ ] Landscape Table on CD
9. **Nucleotide and/or Amino Acid Sequence Submission** (*if applicable, items a. – c. are required*)
   a. [ ] Computer Readable Form (CRF)
   b. [ ] Specification Sequence Listing on:
      i. [ ] CD-ROM or CD-R (2 copies); or
      ii. [ ] Paper
   c. [ ] Statements verifying identity of above copies

ADDRESS TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450**

ACCOMPANYING APPLICATION PAPERS

10. [ ] **Assignment Papers** (cover sheet & document(s))
    Name of Assignee
11. [ ] **37 CFR 3.73(c) Statement** (*when there is an assignee*) [ ] **Power of Attorney**
12. [ ] **English Translation Document** (*if applicable*)
13. [ ] **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    [ ] Copies of citations attached
14. [ ] **Preliminary Amendment**
15. [ ] **Return Receipt Postcard** (*MPEP § 503*) (*Should be specifically itemized*)
16. [ ] **Certified Copy of Priority Document(s)** (*if foreign priority is claimed*)
17. [ ] **Nonpublication Request** Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
18. [X] **Other:** Certification and Request for Prioritized Examination (TrackOne Request) 1 page

***Note:** (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 **must** be included in an Application Data Sheet (ADS).
(2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

19. CORRESPONDENCE ADDRESS

[X] The address associated with Customer Number: 21559 **OR** [ ] Correspondence address below

Name					
Address					
City		State		Zip Code	
Country		Telephone		Email	

Signature	/Jan N. Tittel/	Date	November 13, 2020
Name (Print/Type)	Jan N. Tittel, Ph.D.	Registration No. (Attorney/Agent)	52,290

3019303

Abstract of the Invention

The invention relates to colchicine for use in a method of treating a patient after having a myocardial infarction (MI), the method including initiating the administration of colchicine at a daily low dose to the patient within about 3 days of the MI.

CLAIMS

1. A method of treating a patient after having a myocardial infarction (MI), the method comprising initiating the administration of colchicine at a daily low dose to the patient within about 3 days of the MI.

2. The method according to claim 1, wherein percutaneous coronary intervention was performed for treating the patient's MI.

3. The method according to claim 1, wherein the patient was prescribed a medication.

4. The method according to claim 3, wherein the medication is an antiplatelet agent.

5. The method according to claim 3, wherein the medication is aspirin.

6. The method according to claim 3, wherein the medication is a statin.

7. The method according to claim 1, wherein the patient is at a lower risk of a cardiovascular event, relative to a patient not being administered colchicine.

8. The method according to claim 7, wherein the cardiovascular event is an ischemic cardiovascular event.

9. The method according to claim 7, wherein the cardiovascular event is resuscitated cardiac arrest.

10. The method according to claim 7, wherein the cardiovascular event is myocardial infarction.

11. The method according to claim 7, wherein the cardiovascular event is stroke.

12. The method according to claim 7, wherein the cardiovascular event is urgent hospitalization for angina requiring coronary revascularization.

13. The method according to claim 1, wherein the patient has atherosclerotic coronary artery disease.

14. The method according to claim 1, wherein the administration of colchicine is initiated upon assessment in (a) an emergency department (ED), (b) the hospital, or (c) a medical office setting.

15. The method according to claim 1, wherein the colchicine is in the form of a tablet.

16. The method of claim 15, wherein the tablet is coated.

17. The method of claim 16, wherein the tablet is film-coated.

18. The method according to claim 1, wherein the colchicine is administered at 0.3 to 0.7 mg.

19. The method according to claim 18, wherein the colchicine is administered at 0.4 to 0.6 mg

20. The method according to claim 16, wherein the colchicine is administered at 0.5 mg.

21. The method according to claim 9, wherein the colchicine is administered at 0.5 mg.

22. The method according to claim 10, wherein the colchicine is administered at 0.5 mg.

23. The method according to claim 11, wherein the colchicine is administered at 0.5 mg.

24. The method according to claim 12, wherein the colchicine is administered at 0.5 mg.

25. The method according to claim 1, wherein the colchicine is administered once, twice or three times a day.

26. The method according to claim 25, wherein the colchicine is administered once per day.

27. The method according to claim 1, wherein the colchicine is administered without pre-loading the patient with colchicine.

28. The method according to claim 1, wherein the patient is an adult human.

29. A method of reducing the risk of a stroke in a patient after having an MI, the method comprising initiating the administration of colchicine at a daily low dose to the patient within about 30 days of an MI.

30. The method according to claim 29, wherein the method comprises administering colchicine within 4 to 7 days of the MI.

EARLY ADMINISTRATION OF LOW-DOSE COLCHICINE AFTER MYOCARDIAL INFARCTION

Background of the Invention

The invention relates to a treatment regimen for patients after suffering a myocardial infarction.

Inflammation appears to play an important role in atherosclerosis (Hansson GK, Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005;352:1685-95). Inhibition of interleukin-1β by the injectable monoclonal antibody canakinumab led to a 15% lower risk of cardiovascular events than was observed with placebo in the Canakinumab Antiinflammatory Thrombosis Outcomes Study (CANTOS) but also led to a slightly higher incidence of fatal infections (Ridker PM et al., Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med* 2017;377:1119-31). In contrast, methotrexate did not affect cardiovascular outcomes or plasma markers of inflammation in the Cardiovascular Inflammation Reduction Trial (CIRT) (Ridker PM et al., Low-dose methotrexate for the prevention of atherosclerotic events. *N Engl J Med* 2019;380:752-62). Considering these differing results and given that canakinumab is not clinically available for cardiovascular prevention, the search for a widely used alternative antiinflammatory treatment that may reduce the risk of atherosclerotic events among patients with coronary artery disease continues.

Colchicine is an inexpensive, orally administered, potent anti-inflammatory medication that was initially extracted from the autumn crocus and has been used for centuries. Its mechanism of action is through the inhibition of tubulin polymerization and microtubule generation and, possibly, effects on cellular adhesion molecules, inflammatory chemokines, and the inflammasome (Ravelli RB et al., Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. *Nature* 2004;428:198-202; Perico N et al., Colchicine interferes with L-selectin and leukocyte function-associated antigen-1 expression on human T lymphocytes and inhibits T cell activation. *J Am Soc Nephrol* 1996; 7:594-601; Pope RM and Tschopp J, The role of interleukin-1 and the inflammasome in gout: implications for therapy. *Arthritis Rheum* 2007; 56:3183-8). Colchicine is currently indicated for the treatment of gout, familial Mediterranean fever, and pericarditis (Cerquaglia C et al., Pharmacological and clinical basis of treatment of Familial Mediterranean Fever (FMF) with colchicine or analogues: an update. *Curr Drug Targets Inflamm Allergy* 2005;4:117-24; and Imazio M et al., Colchicine in addition to conventional therapy for acute pericarditis: results of the COlchicine for acute PEricarditis (COPE) trial. *Circulation* 2005;112:2012-6).

In the Low-Dose Colchicine (LoDoCo) trial, patients with stable coronary disease treated with colchicine at a dose of 0.5 mg once daily had fewer cardiovascular events than those not receiving colchicine (Nidorf SM et al., Low-dose colchicine for secondary prevention of cardiovascular disease. *J Am Coll*

Cardiol 2013;61:404-10). However, that trial enrolled only 532 patients and was not placebo-controlled.

Because acute coronary syndromes are associated with higher risks of recurrent events and exacerbated inflammation a need exists in the art for new treatment regimens.

Summary of the Invention

A clinical trial referred to as the Colchicine Cardiovascular Outcomes Trial (COLCOT) was conducted to evaluate the effects of colchicine on cardiovascular outcomes as well as its long-term safety profile in patients who had recently had a myocardial infarction.

The results of the COLCOT trial support that the use of colchicine in patients who have recently had a myocardial infarction significantly improved their quality of life in several ways. Colchicine at a daily low dose of 0.5 mg led to a statistically significant lower risk of ischemic cardiovascular events than placebo. Death from cardiovascular causes, resuscitated cardiac arrest, myocardial infarction, stroke, and urgent hospitalization for angina leading to coronary revascularization was also significantly lower among the patients who received 0.5 mg of colchicine than those who received placebo. Patients receiving low dose colchicine had reduced morbidity relative to placebo, as is demonstrated by the rates of the respective primary composite end points for the two patient populations (P = 0.02). This reduction in morbidity was particularly prominent among colchicine-receiving patients in the reduction of severe conditions, such as stroke and urgent hospitalization for angina leading to revascularization: hazard ratios are 0.26 (95% confidence interval is 0.10-0.70) and 0.50 (95% confidence interval is 0.31-0.81), respectively. Still further, unlike the use of the anti-inflammatory canakinumab for atherosclerotic events, colchicine did not increase the incidence of septic shock. Moreover, no serious adverse event of myopathy linked to colchicine occurred despite the use of statins in 99% of trial participants.

The results of the COLCOT trial have been further analyzed to determine if time to treatment initiation (TTI) had any effect on the outcome of the treatment. Three different cutoffs for TTI were used in order to determine the association between early initiation of therapy and clinical outcomes. These cutoffs were determined based on the usual journey of patients with MI. The first 30-day post-MI timeline was divided into three independent periods of time, and analyzed as such: from day 0 to 3, referring to in-hospital management; from day 4 to 7, referring to early post-discharge period and from day 8 to 30, referring to late post-discharge period.

The main COLCOT results revealed that colchicine reduced the risk of ischemic CV events by 23% in the post-MI setting. Results from the present TTI analysis support that early suppression of inflammation after MI provides even greater benefits, with a reduction of 48% in the risk of the composite primary endpoint

when colchicine was initiated between days 0 and 3. The demonstrated cost-effectiveness of low-dose colchicine also supports its large-scale use after MI. Results of the LoDoCo2 study of patients with stable coronary artery disease complement those of COLCOT in the post-MI setting.

In view of the aforementioned, the invention, in one aspect, relates to colchicine for use in a method of treating a patient after having a myocardial infarction (MI), the method comprising initiating the administration of colchicine at a daily low dose to the patient within about 3 days of the MI.

In another aspect, the invention relates to a colchicine for use in a method of treating a patient after having a MI, the method comprising initiating the administration of colchicine at a daily low dose to the patient within about 4 days of the MI.

In another aspect, the invention relates to a colchicine for use in a method of treating a patient after having a MI, the method comprising initiating the administration of colchicine at a daily low dose to the patient within about 5 days of the MI.

In another aspect, the invention relates to a colchicine for use in a method of treating a patient after having a MI, the method comprising initiating the administration of colchicine at a daily low dose to the patient within about 30 days of the MI.

In another aspect, the invention relates to a method of treating a patient after having a MI, the method comprising initiating the administration of colchicine at a daily low dose to the patient within about 3 days of the MI.

In another aspect, the invention relates to a method of treating a patient after having a MI, the method comprising initiating the administration of colchicine at a daily low dose to the patient within about 4 days of the MI.

In another aspect, the invention relates to a method of treating a patient after having a MI, the method comprising initiating the administration of colchicine at a daily low dose to the patient within about 5 days of the MI.

In another aspect, the invention relates to a method of treating a patient after having a MI, the method comprising initiating the administration of colchicine at a daily low dose to the patient within about 30 days of the MI.

In another aspect, the invention relates to the use of a daily low dose of colchicine for treating a patient

after having a MI, wherein the use is initiated within about 3 days of the MI.

In another aspect, the invention relates to the use of a daily low dose of colchicine for treating a patient after having a MI, wherein the use is initiated within about 4 days of the MI.

In another aspect, the invention relates to the use of a daily low dose of colchicine for treating a patient after having a MI, wherein the use is initiated within about 5 days of the MI.

In another aspect, the invention relates to the use of a daily low dose of colchicine for treating a patient after having a MI, wherein the use is initiated within about 30 days of the MI.

In a further aspect, the invention relates to the use of colchicine for the manufacture of a medicament for treating a patient after having a MI, wherein the colchicine is a low dose colchicine for daily administration initiated within about 3 days of the MI.

In a further aspect, the invention relates to the use of colchicine for the manufacture of a medicament for treating a patient after having a MI, wherein the colchicine is a low dose colchicine for daily administration initiated within about 4 days of the MI.

In a further aspect, the invention relates to the use of colchicine for the manufacture of a medicament for treating a patient after having a MI, wherein the colchicine is a low dose colchicine for daily administration initiated within about 5 days of the MI.

In a further aspect, the invention relates to the use of colchicine for the manufacture of a medicament for treating a patient after having a MI, wherein the colchicine is a low dose colchicine for daily administration initiated within about 30 days of the MI.

In another aspect, the present invention relates to low-dose colchicine for use in treating a patient having a MI, wherein the colchicine is for daily administration initiated within about 3 days of the MI.

In another aspect, the present invention relates to low-dose colchicine for use in treating a patient having a MI, wherein the colchicine is for daily administration initiated within about 4 days of the MI.

In another aspect, the present invention relates to low-dose colchicine for use in treating a patient having a MI, wherein the colchicine is for daily administration initiated within about 5 days of the MI.

In another aspect, the present invention relates to low-dose colchicine for use in treating a patient having

a MI, wherein the colchicine is for daily administration initiated within about 30 days of the MI.

In some embodiments, the method involves administering colchicine within 5 days of the MI (for example, at 1 day, 2 days, 3 days, or 4 days). In some embodiments, the method includes administering colchicine within 4 days of the myocardial infarction (for example, at 1 day, 2 days, or 3 days). In still other embodiments, the method includes administering colchicine within 3 days of the myocardial infarction (for example, at 1 day or 2 days).

In some embodiments, the patient administered colchicine received percutaneous coronary intervention for treating the patient's myocardial infarction.

In some embodiments, the myocardial infarction is acute MI (AMI). In some embodiments, the MI is not type 2 MI.

In some embodiments, the patient administered was previously prescribed a medication (for example, an antiplatelet agent, a statin, aspirin or a combination thereof).

In some embodiments, the patient administered is concurrently being treated with a medication (for example, an antiplatelet agent, a statin, aspirin, or a combination thereof).

In some embodiments, the patient is at a lower risk of a cardiovascular event, relative to a patient not being administered colchicine. For example, the cardiovascular event is an ischemic cardiovascular event. In some embodiments, the cardiovascular event is cardiovascular death, resuscitated cardiac arrest, myocardial infarction, stroke, or urgent hospitalization for angina requiring coronary revascularization.

In some embodiments, the patient has atherosclerotic coronary artery disease.

In some embodiments the patient does not have severe heart failure, reduced left ventricular fraction, recent stroke, type 2 MI, planned coronary artery bypass by graft (CABG), inflammatory bowel disease or chronic diarrhea.

In another aspect, the invention relates to a method of reducing the risk of or preventing a stroke in a patient after having an MI, the method comprising initiating the administration of colchicine at a daily low dose to the patient within about 30 days of an MI.

In some embodiments, the method involves administering colchicine within 4-7 days (for example, 5 days,

6 days, or 7 days) of the MI.

In some embodiments, the method involves administering colchicine within 3 days of the MI.

In another aspect, the invention relates to the use of a daily low dose of colchicine for reducing the risk of or preventing a stroke in a patient after having a MI, wherein the use is initiated within 30 days of the MI.

In another aspect, the invention relates to the use of a daily low dose of colchicine for reducing the risk of or preventing a stroke in a patient after having a MI, wherein the use is initiated between 4-7 days after the MI.

In some embodiments, the invention relates to the use of a daily low does of colchicine for reducing the risk of or preventing a stroke in a patient after having a MI, wherein the use is initiated within 3 days of the MI.

In a further aspect, the invention relates to the use of colchicine for the manufacture of a medicament for reducing the risk of or preventing a stroke in a patient after having a MI, wherein the colchicine is a low dose colchicine for daily administration initiated within 30 days of the MI.

In a further aspect, the invention relates to the use of colchicine for the manufacture of a medicament for reducing the risk of or preventing a stroke in a patient after having a MI, wherein the colchicine is a low dose colchicine for daily administration initiated between 4 and 7 days of the MI.

In another aspect, the present invention relates to the use of colchicine for the manufacture of a medicament for reducing the risk of or preventing stroke in a subject after having a MI, wherein the colchicine is for daily administration initiated within about 3 days of the MI.

In some embodiments, the administration of colchicine is initiated upon assessment in (a) an emergency department (ED), (b) the hospital, or (c) a medical office setting.

In some embodiments, colchicine is administered in the form of a tablet or a capsule (e.g., a coated tablet or capsule). In some embodiment, colchicine is administered in the form of a film-coated tablet.

In some embodiments, colchicine is administered at 0.3 to 0.7 mg. For example, colchicine is administered at 0.4 to 0.6 mg, and is preferably administered at about 0.5 mg. In some embodiments, colchicine is administered in a 0.5 mg tablet or a 0.6 mg tablet. In other embodiments, colchicine is administered in a 0.25 mg tablet.

In some embodiments, colchicine is administrated once, twice, or three times daily.

In some embodiments, colchicine is administered once per day.

In some embodiments, the colchicine is administered without pre-loading the patient with colchicine (e.g., administering a higher dose, such as 1.0 mg per day or higher (1.5 mg or 2.0 mg) in a single or multiple doses, at the onset of the treatment for one, two, or three days before switching to a lower dose of, e.g., 0.5 mg per day, for the remainder of the duration of treatment).

In some embodiments, the patient is an adult human (i.e., the patient is 18 years old or older).

In another aspect, the invention relates to colchicine for use in a method of treating a patient after having a myocardial infarction (MI), the method including the administration of colchicine at a daily low dose to the patient within about 3 days (e.g., 1 day, 2 days, and 3 days) of the MI.

Use of colchicine for treating a patient after having a myocardial infarction typically continues, as needed, throughout the life of a patient. For example, in some embodiments, the duration of treatment is for 6 months, 12 months, 18 months, 24 months, 30 months, 36 months, or even longer as is needed. In some embodiments, the duration of the treatment is for at least about 23 months.

Various colchicine formulations, including film-coated tablets, are readily available and well known in the art.

Other features and advantages of the invention will be apparent from the following Detailed Description, the Drawings, and the Claims.

Definitions

As used herein, the term “cardiovascular death” refers to death resulting from a cardiovascular disease, including coronary heart disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis, or a pulmonary embolism, or sudden cardiac event, including cardiac arrest and myocardial infarction.

As used herein, the term “myocardial infarction” refers to a cardiovascular disorder characterized by localized necrosis resulting from obstruction of the blood supply. MI may be classified as an ST elevation myocardial infarction (STEMI) or Non-ST elevation myocardial infarction (NSTEMI) based on the results of an ECG. A more explicit classification system, based on international consensus in 2012, also exists. This

classifies myocardial infarctions into five types including types 1 and 2. Type-1 AMI is caused by an acute atherothrombotic coronary event; type-2 AMI is a more heterogeneous entity, where a condition other than coronary artery disease (CAD) contributes to an acute imbalance between oxygen supply (e.g., hypoxemia, anemia, hypotension) and demand (e.g., tachycardia, hypertension).

As used herein, the term "resuscitated cardiac arrest" refers an abrupt loss of heart function resulting in a loss of blood flow to the body which requires the subject be resuscitated using any method known to one of skill in the art, including cardiopulmonary resuscitation or a defibrillator.

As used herein, the term "urgent hospitalization for angina requiring coronary revascularization" refers to an immediate need for a patient to be hospitalized due to severe chest discomfort felt due to ischemic heart disease which requires surgical intervention grafting and/or stenting approaches to improving blood flow to the heart and relieve angina.

As used herein, the term "stroke" refers to a condition which occurs when the blood supply to a part of the brain is suddenly interrupted (i.e., ischemic stroke) or when a blood vessel in the brain bursts and releases blood into the spaces surrounding the brain cells (i.e., hemorrhagic stroke). The symptoms of a stroke include numbness or weakness, especially on one side of the body corresponding to the contralateral side of the stroke, confusion, trouble understanding or producing speech, impaired vision in both eyes, impaired mobility, dizziness, or loss of balance or coordination. Stroke may be diagnosed using several techniques, such as, e.g., neurological examination, blood testing, computed tomography (CT) scan, magnetic resonance imaging (MRI) scan, Doppler ultrasound, and arteriography. Although stroke is a disease of the brain, it can affect the entire body by causing, e.g., paralysis, cognitive impairment, speech impairment, emotional dysregulation, and pain.

Brief Description of the Drawings

FIG. 1 is a schematic of a flow chart of the randomization and subject disposition of the patients in the Time-to-Treatment study.

FIG. 2 is a graph of the association between the time-to-treatment initiation and the adjusted hazard ratio (solid line) with the 95% confidence intervals of the adjusted hazard ratio (dashed line) calculated using a quadratic multivariable Cox regression model.

FIG. 3A is a graph of the cumulative incidence of cardiovascular events (time-to-treatment population). Shown are the Kaplan–Meier event curves for the primary efficacy composite end point (a composite of cardiovascular death, resuscitated cardiac arrest, MI, stroke, or urgent hospitalization for angina requiring

coronary revascularization) in the colchicine group and the placebo group for patients with a time-to-treatment initiation of ≤ 3 days. The inset shows the same data on an enlarged y-axis.

FIG. 3B is a graph of the cumulative incidence of cardiovascular events (time-to-treatment population). Shown are the Kaplan–Meier event curves for the secondary efficacy composite end point (a composite of cardiovascular death, resuscitated cardiac arrest, myocardial infarction, or stroke) in the colchicine group and the placebo group for patients with a time-to-treatment initiation of ≤3 days. The inset shows the same data on an enlarged y-axis.

Detailed Description of the Invention

We performed a randomized, double-blind trial involving patients recruited within 30 days after a myocardial infarction. The patients were randomly assigned to receive either low-dose colchicine (0.5 mg once daily) or placebo. A total of 4745 patients were enrolled; 2366 patients were assigned to the colchicine group, and 2379 to the placebo group. Patients were followed for a median of 22.6 months.

The primary efficacy end point was a composite of death from cardiovascular causes, resuscitated cardiac arrest, myocardial infarction (e.g., acute myocardial infraction or type II myocardial infarction), stroke, or urgent hospitalization for angina leading to coronary revascularization. The components of the primary end point and safety were also assessed.

Study design and patient population

COLCOT was an international multicenter, randomized, double-blinded trial that randomly assigned patients to receive either low-dose colchicine (0.5 mg once daily) or placebo. The study protocol and main results have been published (see, Tardif et al., *N Engl J Med* 2019). Patients were considered eligible if they had a recent MI (< 30 days). Main exclusion criteria were severe heart failure, reduced left ventricular ejection fraction (< 35%), recent stroke (< 3 months), type 2 MI, recent (< 3 years) or planned coronary artery bypass graft (CABG), history of cancer (< 3 years), and inflammatory bowel disease or chronic diarrhea. All patients enrolled in the trial benefited from percutaneous coronary intervention whenever indicated and guidelines-directed management of CV disease prior to randomization (see. Tardif et al., *N Engl J Med* 2019).

Clinical follow-up consisted of evaluations at 1 and 3 months after randomization and every 3 months thereafter. An independent clinical endpoint committee, blinded to trial-group assignment, adjudicated clinical endpoints. The trial was locally approved by the various institutional review boards, and all patients signed a written informed consent before enrollment.

Efficacy Endpoints

The primary efficacy endpoint was a composite of CV death, resuscitated cardiac arrest, MI, stroke, or urgent hospitalization for angina requiring coronary revascularization. The secondary endpoints consisted of the components of the primary efficacy endpoint, all-cause death, and a composite of CV death, resuscitated cardiac arrest, MI, or stroke. Exploratory endpoints included all coronary revascularizations, including both elective and urgent coronary revascularizations.

Cutoffs for Time-to-Treatment Initiation (TTI) of Colchicine

Three different cutoffs for TTI were used in order to determine the association between early initiation of therapy and clinical outcomes. These cutoffs were determined based on the usual journey of patients with MI. The first 30-day post-MI timeline was divided into three independent periods of time, and analyzed as such: from day 0 to 3, referring to in-hospital management; from day 4 to 7, referring to early post-discharge period and from day 8 to 30, referring to late post-discharge period.

Results

Baseline characteristics for patients in TTI analysis

Of the 4745 patients randomized in COLCOT, 4661 were included in the TTI analysis (colchicine, N=2322; placebo, N=2339) (Figure 1). Overall, patients were randomized at 13.5±10.1 days following the index MI, 25.6% between days 0 and 3, 15.4% between days 4 and 7 and 59.0% at day 8 or after. Baseline characteristics were similar between the colchicine and placebo groups (Table 1). Patients were mostly men (81.0%) with a mean age of 60.5 years, 20.2% had diabetes, 51.0% had a history of hypertension, 29.7% were active smokers, and 16.8% had had a prior PCI. Background therapy included aspirin, a second anti-platelet agent and a statin in 98.8%, 98.0% and 99.0% of patients, respectively. The vast majority of patients (93.0%) underwent percutaneous coronary intervention (PCI) during the index hospitalization, with no difference in terms of time to PCI between the two groups.

Table 1: Baseline Characteristics according to treatment allocation for Time-to-Treatment Initiation (TTI)

Characteristics	All patients N=4661	Colchicine group N=2322	Placebo group N=2339
Age (years) – mean ± SD	60.5 ± 10.6	60.6 ± 10.6	60.5 ± 10.6
Male sex - no. (%)	3774 (81.0%)	1861 (80.1%)	1913 (81.8%)
BMI (kg/m^2) – mean ± SD	28.3 ± 4.7	28.2 ± 4.8	28.4 ± 4.7
Current Smoking - no./total no. (%)	1382/4659 (29.7)	694/2322 (29.9%)	688/2337 (29.4%)
History of Hypertension - no. (%)	2377 (51.0%)	1160 (50.0%)	1217 (52.0%)
History of Diabetes - no. (%)	942 (20.2%)	451 (19.4%)	491 (21.0%)
Prior MI - no. (%)	751 (16.1%)	360 (15.5%)	391 (16.7%)
Prior PCI - no. (%)	783 (16.8%)	382 (16.5%)	401 (17.1%)
Prior CABG - no. (%)	146 (3.1%)	66 (2.8%)	80 (3.4%)
Prior HF - no. (%)	90 (1.9%)	48 (2.1%)	42 (1.8%)
Prior Stroke or TIA - no. (%)	119 (2.6%)	53 (2.3%)	66 (2.8%)
PCI associated with the index event - no. (%)	4336 (93.0%)	2154 (92.8%)	2182 (93.3%)
Medication use - no. (%):			
Aspirin	4605 (98.8%)	2291 (98.7%)	2314 (98.9%)
Other antiplatelet agent	4567 (98.0%)	2267 (97.6%)	2300 (98.3%)
Statin	4615 (99.0%)	2297 (98.9%)	2318 (99.1%)
Beta-blocker	4143 (88.9%)	2077 (89.4%)	2066 (88.3%)

TTI 0-3 days - no. (%)	1193 (25.6%)	604 (26.0%)	589 (25.2%)
TTI 4-7 days - no. (%)	720 (15.4%)	364 (15.7%)	356 (15.2%)
TTT ≥ 8 days - no. (%)	2748 (59.0%)	1354 (58.3%)	1394 (59.6%)
Time from index MI to randomization (days) – mean ± SD	13.5 ± 10.1	13.5 ± 10.1	13.5 ± 10.0
Time from Index MI to PCI (days) – mean ± SD	1.4 ± 2.9	1.4 ± 2.9	1.4 ± 2.9
Time from PCI to randomization (days) – mean ± SD	11.9 ± 9.9	11.9 ± 9.9	11.9 ± 9.9

Abbreviations: CABG, coronary-artery bypass graft surgery; HF, heart failure; PCI, percutaneous coronary intervention; TIA, transient ischemic attack.

Data were missing on the following characteristics: age (assessed according to date of birth; see below) for 431 patients (213 in the colchicine group and 218 in the placebo group) and body-mass index (the weight in kilograms divided by the square of the height in meters) for 5 (1 and 4 patients, respectively). Date of birth was not required field because it was considered in some countries to be sensitive data that could allow for the identification of patients.

For statistical reporting, missing information regarding the day of birth was replaced by 15, and missing information regarding the month and day of birth was replaced by July 1.

Baseline characteristics according to TTI strata are shown in Table 2. Patients in whom therapy was initiated between days 0 and 3, when compared to those at days 8 to 30, were younger (59.1±10.8 vs. 61.3±10.4 years) and more often active smokers (43.8 vs. 20.2%), had less commonly hypertension (41.1 vs. 56.2%) and diabetes (17.4 vs. 22.0%) but underwent more often PCI associated with the index MI (95.8 vs. 91.3%), all $p < 0.05$.

Table 2: Baseline characteristics according to TTI

Characteristics	TTI 0-3 days N=1193	TTI 4-7 days N=720	TTT ≥ 8 days N=2748	p*	p**
Age (years) – mean ± SD	59.1 ± 10.8	60.1 ± 11.0	61.3 ± 10.4	<0.0001	<0.0001
Male sex - no. (%)	980 (82.2%)	605 (84.0%)	2189 (80.0%)	0.014	0.071
BMI (kg/m^2) - mean ± SD	28.1 ± 4.6	27.7 ± 4.6	28.6 ± 4.8	<0.0001	0.004
Current Smoking - no. (%)	522 (43.8%)	306 (42.6%)	554 (20.2%)	<0.0001	<0.0001
History of Hypertension - no. (%)	490 (41.1%)	343 (47.6%)	1544 (56.2%)	<0.0001	<0.0001
History of Diabetes - no. (%)	208 (17.4%)	130 (18.1%)	604 (22.0%)	0.001	0.001
Prior MI - no. (%)	170 (14.3%)	111 (15.4%)	470 (17.1%)	0.070	---
Prior PCI - no. (%)	182 (15.3%)	107 (14.9%)	494 (18.0%)	0.035	0.037
Prior CABG - no. (%)	34 (2.9%)	30 (4.2%)	82 (3.0%)	0.218	---
Prior HF - no. (%)	14 (1.2%)	12 (1.7%)	64 (2.3%)	0.046	0.017
Prior Stroke or TIA - no. (%)	20 (1.7%)	21 (2.9%)	78 (2.8%)	0.084	---
PCI associated with the index event - no. (%)	1143 (95.8%)	685 (95.1%)	2508 (91.3%)	<0.0001	<0.0001
Medication use - no. (%):					
Aspirin	1181 (99.0%)	715 (99.3%)	2709 (98.6%)	0.219	---
Other antiplatelet agent	1177 (98.7%)	708 (98.3%)	2682 (97.6%)	0.072	---
Statin	1188 (99.6%)	708 (98.3%)	2719 (98.9%)	0.024	0.047
Beta-blocker	1093 (91.6%)	642 (89.2%)	2408 (87.6%)	0.001	0.0003
Time from index MI to randomization (days) – mean ± SD	2.1 ± 0.8	5.1 ± 1.1	20.8 ± 6.6	<0.0001	<0.0001

Time from Index MI to PCI (days) – mean ± SD	0.4 ± 0.7	1.4 ± 1.8	1.8 ± 3.6	<0.0001	<0.0001
Time from PCI to randomization (days) – mean ± SD	1.6 ± 0.9	3.7 ± 1.9	18.8 ± 7.3	<0.0001	<0.001

Abbreviations: CABG, coronary-artery bypass graft surgery; HF, heart failure; PCI, percutaneous coronary intervention; TIA, transient ischemic attack.

Data were missing on the following characteristics: age (assessed according to date of birth; see below) for 431 patients (213 in the colchicine group and 218 in the placebo group) and body-mass index (the weight in kilograms divided by the square of the height in meters) for 5 (1 and 4 patients, respectively).

Date of birth was not a required field because it was considered in some countries to be sensitive data that could allow for the identification of patients. For statistical reporting, missing information regarding the day of birth was replaced by 15, and missing information regarding the month and day of birth was replaced by July 1.

*: Group comparison TTI 0-3 vs. TTI 4-7 vs. TTI $\geq$ 8days

**: Group comparison TTI <3 vs. TTI $\geq$ 8 days

Effects of time-to-treatment initiation on the primary efficacy endpoint

The effects of colchicine on the primary endpoint according to TTI are shown in Table 3 and Figure 2. A primary endpoint event occurred in 4.3% of patients in the colchicine group, as compared to 8.3% of those in the placebo group when TTI was between days 0 and 3 (N=1193, HR=0.52, 95% CI 0.32-0.84, p=0.007, Figure 3A). Corresponding rates were 6.0 and 5.9% when TTI was between days 4 and 7 (N=720) and 5.7 and 7.1% when TTI was on day 8 or after (N=2748), but these differences between groups did not reach statistical significance. Table 3 also shows the percentages of patients with events and the hazard ratios for the components of the primary endpoint, including CV death (HR=1.04, 95% CI 0.15-7.37), resuscitated cardiac arrest (HR=0.33, 95% CI 0.03-3.20), MI (HR=0.58, 95% CI 0.32-1.05), stroke (HR=0.21, 95% CI 0.02-1.81) and urgent hospitalization for angina requiring coronary revascularization (HR=0.35, 95% CI 0.14-0.88).

Table 3: Efficacy endpoints according to TTI (N=4661, colchicine vs. placebo)

Endpoints	TTI 0-3 days, N=1193 Colchicine vs. placebo no. (%)	TTI 4-7 days, N=720 Colchicine vs. placebo no. (%)	TTI ≥ 8 days, N=2748 Colchicine vs. placebo no. (%)
	HR (95% CI); p	HR (95% CI); p	HR (95% CI); p
Primary composite endpoint	26 (4.3%) vs. 49 (8.3%)	22 (6.0%) vs. 21 (5.9%)	77 (5.7%) vs. 99 (7.1%)
	0.52 (0.32-0.84); p=0.007	0.96 (0.53-1.75); p=0.896	0.82 (0.61-1.11); p=0.200
CV death	2 (0.3%) vs. 2 (0.3%)	2 (0.5%) vs. 4 (1.1%)	15 (1.1%) vs. 18 (1.3%)
	1.04 (0.15-7.37); p=0.970	0.45 (0.08-2.46); p=0.356	0.89 (0.45-1.76); p=0.734
Resuscitated cardiac arrest	1 (0.2%) vs. 3 (0.5%)	2 (0.5%) vs. 1 (0.3%)	2 (0.1%) vs. 2 (0.1%)
	0.33 (0.03-3.20); p=0.340	1.90 (0.17-20.95); p=0.600	1.02 (0.14-7.22); p=0.986
MI	17 (2.8%) vs. 29 (4.9%)	16 (4.4%) vs. 9 (2.5%)	52 (3.8%) vs. 59 (4.2%)
	0.58 (0.32-1.05); p=0.071	1.67 (0.74-3.78); p=0.218	0.93 (0.64-1.35); p=0.710
Stroke	1 (0.2%) vs. 5 (0.8%)	1 (0.3%) vs. 3 (0.8%)	2 (0.1%) vs. 11 (0.8%)
	0.21 (0.02-1.81); p=0.156	0.28 (0.03-2.71); p=0.272	0.19 (0.04-0.84); p=0.029
Urgent hospitalization for angina requiring coronary revascularization	6 (1%) vs. 17 (2.9%)	4 (1.1%) vs. 6 (1.7%)	15 (1.1%) vs. 26 (1.9%)
	0.35 (0.14-0.88); p=0.026	0.63 (0.18-2.24); p= 0.476	0.61 (0.32-1.16); p=0.131
Secondary Composite Endpoint	20 (3.3%) vs. 36 (6.1%)	18 (4.9%) vs. 16 (4.5%)	67 (4.9%) vs. 77 (5.5%)
	0.55 (0.32-0.95); p=0.031	1.04 (0.53-2.03); p=0.919	0.92 (0.66-1.28); p=0.629
All-cause death	6 (1.0%) vs. 6 (1.0%)	8 (2.2%) vs. 7 (2.0%)	26 (1.9%) vs. 31 (2.2%)
	1.03 (0.33-3.19); p=0.962	1.03 (0.37-2.84); p=0.957	0.90 (0.53-1.51); p=0.684
All Coronary Revascularizations	33 (5.5%) vs. 51 (8.7%)	25 (6.9%) vs. 18 (5.1%)	72 (5.3%) vs. 94 (6.7%)
	0.63 (0.40-0.97); p=0.037	1.41 (0.76-2.61); p=0.275	0.81 (0.59-1.10); p=0.172

The effects of colchicine on the primary endpoint for a TTI of less than 4 days is shown in Table 4 and Table 5. The effects of colchicine on the primary endpoint for a TTI of less than 5 days is shown in Table 6 and Table 7. Both the TTI of less than 4 days and the TTI of less than 5 days was were calculated using a basic model (Table 4 and Table 6) as well as an adjusted model (Table 5 and Table 7) which is described below in the statistical analysis section. In all cases, the interaction p-value was not significant ($p>0.05$), meaning that the conclusion over Placebo vs. Colchicine group can be obtained directly from the respective p-values for HR. In other words, relationships within the placebo and colchicine groups with TTI are both linear/parallel and the interpretation is straightforward. For a TTI of 4 days or less, colchicine was shown to offer additional protection over the placebo in both the basic (Table 4 and Table

6) and the adjusted models (Table 5 and Table 7). The adjusted models resulted in a hazard ratio (HR) of 0.58 and a p value of 0.0126.

Table 4. Effect of Colchicine with TTI of <4 days calculated using basic model

Delay between randomization and index MI	Positively adjudicated primary endpoint	Placebo N=4310	Colchicine N=4760	All N=9070
[0-4] days	n	917	968	1885
	Yes	84 (9.16%)	38 (3.93%)	88 (5.09%)
	No	833 (90.84%)	724 (96.07%)	1557 (94.91%)
[5-30] days	n	3457	3884	7341
	Yes	175 (5.06%)	93 (2.39%)	268 (3.65%)
	No	3282 (94.94%)	3791 (97.61%)	7073 (96.35%)

Cox Regression Model for Time to First Positively Adjudicated Primary Endpoint Including Treatment Group, Delay between randomization and index MI and Delay between randomization and index MI x Randomized treatment group interaction

Interaction Effect	P-value
Delay between randomization and index MI x Randomized treatment group	0.1191

Delay between randomization and index MI	Effect	HR (95% CI)	P-value
[0-4] days	Colchicine vs. Placebo	0.57 (0.37; 0.87)	0.0098
[5-30] days	Colchicine vs. Placebo	0.83 (0.65; 1.06)	0.1379

NOTE: Model based on 0 / 3541 observations.
Counts of undetermined cause were classified as MI deaths.

Table 5. Effect of Colchicine with TTI of <4 days calculated using adjusted model

Delay between randomization and index MI	Positively adjudicated primary endpoint	Placebo N=3158	Colchicine N=3473	All N=6631
[0-4] days	n	917	968	1885
	Yes	84 (9.16%)	38 (3.93%)	88 (5.09%)
	No	833 (90.84%)	724 (96.07%)	1557 (94.91%)
[5-30] days	n	3457	3884	7341
	Yes	175 (5.06%)	93 (2.39%)	268 (3.65%)
	No	3282 (94.94%)	3791 (97.61%)	7073 (96.35%)

Cox Regression Model for Time to First Positively Adjudicated Primary Endpoint Including Treatment Group, Delay between randomization and Index MI, Delay between randomization and Index MI x Randomized treatment group Interaction, Age at randomization (years), History of diabetes, Prior coronary revascularization (prior PCI or prior CABG) and Killip Heart Failure

Interaction Effect	P-value
Delay between randomization and Index MI x Randomized treatment group	0.1513

Delay between randomization and index MI	Effect	HR (95% CI)	P-value
[0-4] days	Colchicine vs. Placebo	0.58 (0.38; 0.89)	0.0126
[5-30] days	Colchicine vs. Placebo	0.88 (0.69; 1.11)	0.2720

Notes: Model based on 0 / 3541 observations.
Counts of undetermined cause were classified as MI deaths.

Table 6. Effect of Colchicine with TTI of <5 days calculated using basic model

Delay between randomization and index MI	Participants experiencing primary endpoint	Placebo N=1736	Colchicine N=1720	All N=3456
[0-2] days	n	821	858	1680
	Yes	66 (8.0%)	46 (5.4%)	112 (6.7%)
	No	755 (92.0%)	812 (94.6%)	1568 (93.3%)
[3-30] days	n	1818	1462	3280
	Yes	133 (7.3%)	80 (5.5%)	213 (6.5%)
	No	1685 (92.7%)	1378 (94.2%)	3063 (93.4%)

Cox Regression Model for Time to First Positively Adjudicated Primary Endpoint Including Treatment Group, Delay between randomization and Index MI and Delay between randomization and Index MI x Randomized treatment group Interaction

Interaction Effect	P-value
Delay between randomization and Index MI x Randomized treatment group	0.0807

Delay between randomization and Index MI	Effect	HR (95% CI)	P-value
[0-2] days	Colchicine vs. Placebo	0.67 (0.48; 0.93)	0.0165
[3-30] days	Colchicine vs. Placebo	0.79 (0.59; 1.06)	0.1164

Notes: Model based on N = 3456 observations.
Counts of colchicine group with discontinued as in Counts.

Table 7. Effect of Colchicine with TTI of <5 days calculated using adjusted model

Delay between randomization and Index MI	Participants Adjudicated Primary Endpoint	Placebo N=1736	Colchicine N=1720	All N=3456
[0-2] days	n	821	858	1680
	Yes	66 (8.0%)	46 (5.4%)	112 (6.7%)
	No	755 (92.0%)	812 (94.6%)	1568 (93.3%)
[3-30] days	n	1818	1462	3280
	Yes	133 (7.3%)	80 (5.5%)	213 (6.5%)
	No	1685 (92.7%)	1378 (94.2%)	3063 (93.4%)

Cox Regression Model for Time to First Positively Adjudicated Primary Endpoint Including Treatment Group, Delay between randomization and Index MI, Delay between randomization and Index MI x Randomized treatment group interaction, Age at randomization (years), History of diabetes, Prior coronary revascularization (prior PCI or prior CABG) and Prior Heart Failure

Interaction Effect	P-value
Delay between randomization and Index MI x Randomized treatment group	0.1575

Delay between randomization and Index MI	Effect	HR (95% CI)	P-value
[0-2] days	Colchicine vs. Placebo	0.68 (0.45; 1.02)	0.0631
[3-30] days	Colchicine vs. Placebo	0.79 (0.61; 1.05)	0.1013

Notes: Model based on N = 3456 observations.
Counts of colchicine group with discontinued as in Counts.

For a TTI of 5 days or less, colchicine showed borderline significance with respect to the benefits of administering colchicine within 5 days of an MI. This data suggests that some benefit is still possible administering colchicine 5 days after an MI, having HR 0.68 (0.45 – 1.02) and p=0.063 using the adjusted model.

Effects of time-to-treatment initiation on the secondary and exploratory efficacy endpoints

The effects of colchicine on the secondary and exploratory endpoints are shown in Table 3. The secondary efficacy endpoint consisting of a composite of CV death, cardiac arrest, myocardial infarction or stroke occurred in 3.3% of the patients in the colchicine group and in 6.1% of those in the placebo group when TTI was between days 0 and 3 (HR=0.55; 95% CI, 0.32-0.95, Figure 3B). The exploratory endpoint of all coronary revascularizations, not coronary revascularizations limited to those observed as the primary composite endpoint, including urgent revascularization following unstable angina and hospitalization, occurred in 5.5% of patients in the colchicine group, as compared to 8.7% of those in the

placebo group when TTI was between days 0 and 3 (HR=0.63, 95% CI 0.40-0.97). There were 6 deaths in both study groups when TTI was between days 0 and 3 (HR=1.03, 95% 0.33-3.19).

This TTI analysis of COLCOT shows that early initiation of low-dose colchicine within the first 3 days after MI is associated with a reduction of 48% in the risk of the primary endpoint consisting of a composite of CV death, resuscitated cardiac arrest, MI, stroke, or urgent hospitalization for angina requiring coronary revascularization, in comparison with placebo. This result was due to a lower incidence of MIs, strokes and urgent hospitalizations for angina leading to coronary revascularization. The secondary efficacy endpoint consisting of a composite of CV death, resuscitated cardiac arrest, MI or stroke was also significantly reduced by 45% with early initiation of low-dose colchicine. The benefits were more marked when treatment was initiated within the first 3 days after MI, as compared to between days 4 and 30, supporting the strategy of in-hospital initiation of colchicine in order to improve CV outcomes post-MI.

The main COLCOT results revealed that colchicine reduced the risk of ischemic CV events by 23% in the post-MI setting. Results from the present COLCOT analysis suggest that early suppression of inflammation after MI provides even greater benefits, with a reduction of 48% in the risk of the composite primary endpoint when colchicine was initiated between days 0 and 3. The demonstrated cost-effectiveness of low-dose colchicine also supports its large-scale use after MI. Results of the LoDoCo2 study of patients with stable coronary artery disease complement and further support those of COLCOT in the post-MI setting (see *Nidorf et al. N Engl Med, 2020. and Tong et al. Circulation, 2020)*.

Early initiation of low-dose colchicine after MI greatly reduced the risk of ischemic CV events compared with placebo. These results support in-hospital initiation of adjunctive anti-inflammatory therapy with colchicine for post-MI prevention.

Statistical Analysis

For the COLCOT trial, it was estimated that a sample of approximately 4500 patients undergoing randomization (with 2250 patients in each group) or, in terms of events, a total number of 301 patients with a first positively adjudicated primary end-point event would yield adequate power. The sample-size calculation was based on the primary efficacy end point and assumed a 27% lower risk with colchicine than with placebo, indicated by a hazard ratio of 0.724. With the use of a two-sided test at the 0.05 significance level, the trial would have 80% power if it continued until 301 positively adjudicated primary events occurred in the combined trial groups. The trial design assumed an event rate of 7% in the placebo group at 24 months, an 18-month recruitment period during which patients would be uniformly recruited, a 24-month minimum follow-up period, and a 1% annual rate of loss to follow-up or withdrawal of consent.

The efficacy analyses were conducted with the use of positively adjudicated data and according to the intention-to-treat principle. The primary end point was compared between the two trial groups with the use of a log-rank test, and the hazard ratio from a Cox proportional-hazards model, with a 95% confidence interval, was calculated. A Cox proportional-hazards model with adjustment for important baseline characteristics was also used as prespecified in the protocol.

For the present TTI analysis, the data were centrally analyzed by an independent academic biostatistics center at the Montreal Health Innovations Coordinating Center. The analysis was conducted amongst patients who received at least one dose of the study medication (referred to as the safety population in the main protocol, Figure 1). TTI was defined as the length of time in days between the index MI and the initiation of the study medication, and three specific cutoffs were analyzed (≤ day 3, days 4 to 7, and ≥ day 8). Early initiation of therapy was defined as TTI ≤ 3 days. Baseline characteristics were summarized using counts and percentages for categorical variables and mean ± standard deviation (SD) for continuous variables. For each baseline characteristic, comparisons were made using ANOVA for continuous variables and Chi-Square test for categorical variables according to TTI strata. Analyses of the efficacy endpoints, expressed as time to event, were conducted according to time to treatment initiation. Adjusted hazard ratios (HR) along with 95% confidence intervals (CI) were calculated from stepwise multivariable Cox regression models adjusted for the same covariates that were used in the main analysis of the COLCOT trial (FIG. 2). All statistical tests were two-sided and conducted at the 0.05 significance level. Statistical analyses were performed with the use of SAS software, version 9.4 (SAS Institute).

Examples

The following examples are provided to further illustrate some embodiments of the present invention, but are not intended to limit the scope of the invention; it will be understood by their exemplary nature that other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used.

Example 1: Meta-Analysis of Randomized Controlled Trials (RCTs) of Colchicine for Secondary Prevention of Cardiovascular Disease

Data sources

Medline (PUBMED), EMBASE, and Cochrane central were searched to identify RCTs comparing colchicine to placebo or no colchicine for secondary cardiovascular prevention (inception to September 1, 2020). To maximized sensitivity, citation chasing was performed in Google Scholar, Scopus, and Web of Science. Secondary prevention was defined as patients with clinically manifest or established CAD. Query terms included “colchicine”, “coronary artery disease”, “acute coronary syndrome”, “myocardial

infarction", "cardiovascular disease", "atherosclerosis", and "secondary prevention", either separately or in combination.

Study selection, data extraction, and bias assessment

RCTs that met the following criteria were included in the meta-analysis: 1) compared daily use of low-dose (0.5 mg) colchicine to placebo or no colchicine for secondary cardiovascular prevention; 2) the study reported at least one of the following outcomes: cardiovascular death, MI, stroke, cardiac arrest, or urgent coronary revascularization; 3) other than the trial medication, patients in both arms were treated in accordance to guideline directed medical therapy; 4) the minimum follow-up was at least 1 year; and 5) trials were published in a peer-reviewed scientific journal. Review articles, editorials, meta-analyses, observational studies, and published abstracts were excluded from the present meta-analysis.

Study selection was performed by two independent reviewers, using two levels of screening. At the first level, titles and abstracts of searched studies were screened, followed by review of full texts. The reviewers were not blinded to the title of the journals, authors, or affiliated institutions. Reasons for exclusion were recorded and any discrepancies were discussed until a consensus was achieved.

Using standardized forms, data on study characteristics, patient characteristics, and outcomes were extracted on the intent-to-treat population independently by two reviewers from each eligible study. The Cochrane Collaboration risk-of-bias tool for randomized trials was used to evaluate study quality. The meta-analysis was conducted in accordance to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Outcomes

The primary efficacy endpoint was a composite of cardiovascular death, MI, ischemic stroke, and urgent coronary revascularization. Secondary efficacy endpoints consisted of the components of the primary endpoint, composite of cardiovascular death, MI, and ischemic stroke, as well as deep vein thrombosis or pulmonary embolus, and atrial fibrillation (AF). To maximize consistency in pooling efficacy endpoints among trials, the primary and secondary composite efficacy and stroke endpoints in COLCOT were modified to meet the definition of primary and stroke endpoints of the LoDoCo2 trial. Therefore, adjudicated events of resuscitated cardiac arrest and non-ischemic stroke in COLCOT were excluded from the pooled primary endpoint. The component endpoint of stroke was also restricted to include only ischemic strokes. Incidence rates and hazards ratios (HRs) were calculated for the new primary composite endpoint and ischemic stroke endpoint using individual patient data from COLCOT.

Safety outcomes were limited to serious adverse events (SAEs) and included all-cause and non-cardiovascular mortality, hospitalization for gastrointestinal event, infection, and pneumonia, as well as

diagnosis of cancer. Incidence of SAEs were recalculated for COLCOT using the intent-to-treat population.

Data synthesis and statistical analyses

The DerSimonian and Laird random-effects model was used to calculate the pooled HR and corresponding 95% confidence intervals (CI) for primary and secondary endpoints in the overall trial and in subgroup analyses of the primary endpoint. For safety outcomes, the DerSimonian and Laird random-effects model was used to compute risk ratios (RR) and corresponding 95% CIs. Heterogeneity was evaluated with the Higgins I^2 statistic. I^2 values of <25%, 25-75%, and >75% were considered to indicate low, moderate, and high degrees of heterogeneity, respectively. In subgroup analyses, the Q_b statistic was calculated to determine inconsistency and variability in the treatment effect between strata. Publication and small study bias were assessed with the Egger's regression test and visually by asymmetry in funnel plots. Sensitivity analyses were performed for the primary composite and component outcomes for 1) RCTs in patients with acute coronary syndrome (COLCOT and COPS), and 2) RCTs in patients with stable CAD (LoDoCo and LoDoCo2); and after exclusion of 3) the LoDoCo trial [open-label trial and control group was not randomized to placebo] and 4) the LoDoCo and COPS [increased dose (0.5 mg twice daily) of colchicine prescribed during first month] trials. Statistical analyses were performed using SAS software version 9.4 (*SAS Institute, Cary, North Carolina*) and Stata (Version 16, StataCorp, College Station, Texas).

RESULTS

Literature search results and risk of bias

The search strategy identified 79 studies. After the first- and second-level screening processes, four RCTs were retained in the present meta-analysis. The characteristics of included RCTs are listed in Table 8. The Low-Dose Colchicine (LoDoCo) and Low-Dose Colchicine 2 (LoDoCo2) trials assessed the efficacy of colchicine in patients with stable CAD (defined as clinically stable for ≥6 months) (see, Nidorf et al. *N Engl Med,* 2020. and Tong et al. *Circulation*, 2020). In comparison, the Colchicine Cardiovascular Outcomes Trial (COLCOT) was conducted in patients who had a recent MI (≤30 days) and the Colchicine in Patients with acute coronary Syndrome (COPS) trial enrolled patients with acute coronary syndromes (ACS) (see, Tardif et al. *N Engl J Med.*)

The pooled sample size of enrolled patients was 11,594, of which 5774 patients were randomized to colchicine and 5820 patients were randomized to placebo (or no colchicine, in the LoDoCo trial only). Three trials were designated as "low risk" for overall bias (see, Tardif et al. *N Engl J Med.*, Nidorf et al., *N Engl J Med,* 2020, and Tong et al, *Circulation,* 2020) and 1 trial was marked as being of "some concern" (see, Nirdorf et al., *J Am Coll Cardiol.* 2013; 61: 404-10) in the Cochrane Collaboration risk-of-bias assessment.

Table 8. Study characteristics

	LoDoCo	COLCOT	COPS	LoDoCo2
Year	2013	2019	2020	2020
Study design	Single-blind RCT	Double-blind, placebo controlled RCT	Double-blind, placebo controlled RCT	Double-blind, placebo controlled RCT
Study population	Stable CAD	Recent MI (<30 days)	ACS	Stable CAD
N	532	4,745	795	5,522
Key inclusion criteria	Angiographically proven CAD; clinically stable for <=6 months	MI within 30 days prior; completed planned percutaneous revascularization procedures; treated according to national guidelines that included intensive use of statins	ACS (STEMI/NSTEMI/UA); Evidenced CAD on coronary angiography, managed with either PCI or medical therapy	Evidence of CAD on invasive angiography or computed tomography angiography or a coronary calcium score of ≥400 Agaston units on a coronary-artery calcium scan; clinically stable condition for ≥6 months
Key exclusion criteria	Bypass surgery within 10 years prior	Severe heart failure; left ventricular ejection fraction <35%; stroke within the previous 3 months; type 2 index MI; coronary-bypass surgery either within previous 3 years or planned; severe renal or	CAD requiring surgical revascularization pre-existing long-term colchicine use or immunosuppressant therapy; severe hepatic or renal insufficiency; known active malignancy	Moderate-to-severe renal impairment; severe heart failure; severe valvular disease

		hepatic disease; known cancer		
Median follow-up	24 months	22.6 months	12 months	28.6 months
Primary endpoint	Composite: ACS, out-of-hospital cardiac arrest, or non-cardioembolic ischemic stroke	Composite: CV mortality, resuscitated cardiac arrest, MI, stroke, or urgent hospitalization for angina requiring revascularization	Composite: All-cause mortality, ACS (STEMI/NSTEMI/UA), ischemia-driven revascularization, or non-cardioembolic ischemic stroke	Composite: CV death, MI, ischemic stroke, or ischemia-driven coronary revascularization

*ACS, acute coronary syndrome; CAD, coronary artery disease; CV, cardiovascular; MI, myocardial infarction; RCT, randomized controlled trial; STEMI, ST elevation myocardial infarction; NSTEMI, non-ST elevation myocardial infarction; UA, unstable angina

Patient Characteristics

Baseline patient characteristics for each RCT are summarized in Table 9. The mean age of patients ranged from 60 to 67 years. In all trials, <20% of enrolled patients were women and more than 90% of patients were on concomitant statin therapy. LoDoCo and LoDoCo2 trials had a higher proportion of patients who underwent prior coronary revascularization [percutaneous coronary intervention (PCI) and coronary artery bypass graft (CABG) surgery]. The distribution of all other patient characteristics was relatively similar between trials.

Table 9. Patient characteristics at baseline

	LoDoCo		COLCOT		COPS		LoDoCo2	
	Colchicine N=250	Control N=282	Colchicine N=2366	Placebo N=2379	Colchicine N=396	Placebo N=399	Colchicine N=2762	Placebo N=2760
Age, years (mean ± SD)	67 ± 9.2	66 ± 9.6	60.6 ± 10.7	60.5 ± 10.6	59.7 ±10.2	60.0 ± 10.4	65.8 ± 8.4	65.9 ± 8.7
Women (%)	11.2%	11.0%	19.9%	18.4%	18.7%	22.3%	16.5%	14.1%
Hypertension (%)	Not reported	Not reported	50.1%	52.0%	50.8%	49.9%	51.4%	50.3%
Diabetes (%)	27.6%	32.6%	19.5%	20.9%	18.9%	19.0%	17.8%	18.7%
History of PCI (%)	55.2%	59.9%	16.6%	17.1%	12.9%	12.5%	76.0%	75.3%
History of CABG (%)	15.6%	22.0%	2.9%	3.4%	3.8%	4.8%	11.5%	14.2%
Statin use (%)	94.0%*	96.1%*	98.9%	99.1%	98.2%	99.5%	93.9%	94.0%

*The LoDoCo trial only reported use of high dose statins, not any statin use.

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Clinical efficacy endpoints

Clinical efficacy results from each trial are presented in Table 10. In patients with CAD, the addition of colchicine to standard medical therapy was associated with a statistically significant reduction in the primary composite endpoint of cardiovascular mortality, MI, ischemic stroke, and urgent coronary revascularization, compared to patients on placebo or no colchicine [pooled HR 0.68 (95% CI 0.54-0.81); I^2=37.7%;]. The reduction in cardiovascular events in patients randomized to colchicine was driven by statistically significant reductions in the incidence of MIs [pooled HR 0.62 (95% CI 0.36-0.88); I^2=68.76%], ischemic strokes [pooled HR 0.38 (95% CI 0.13-0.63); I^2=0.0%], and urgent coronary revascularization [pooled HR 0.56 (95% CI 0.30-0.82); I^2=65.1%]. However, there was no statistically significant difference detected for cardiovascular mortality. Sensitivity analyses that pooled the results of COLCOT and LoDoCo2 trials demonstrated similar effect estimates for the primary composite and component outcomes, although magnitude of effect and heterogeneity (I^2) between studies decreased.

Table 10. Study efficacy outcomes

	LoDoCo[11]		**COLCOT[10]**		**COPS[13]**		**LoDoCo2[12]**	
	Colchicine N=250	Control N=282	Colchicine N=2366	Placebo N=2379	Colchicine N=396	Placebo N=399	Colchicine N=2762	Placebo N=2760
Primary composite outcome: Cardiovascular mortality, myocardial infarction, ischemic stroke, urgent revascularization								
Events, N (%)	NA	NA	130 (5.5%)	168 (7.1%)	19 (4.8%)	41 (10.3%)	187 (6.8%)	264 (9.6%)
HR (95% CI)	NA		0.77 (0.61-0.97)		0.47 (0.27-0.82)		0.69 (0.57-0.83)	
Cardiovascular mortality								
Events, N (%)	NA	NA	20 (0.8%)	24 (1.0%)	3 (0.8%)	1 (0.3%)	20 (0.7%)	25 (0.9%)
HR (95% CI)	NA		0.84 (0.46-1.52)		3.09 (0.32-29.71)		0.80 (0.44-1.44)	
Myocardial infarction								
Events, N (%)	4 (1.6%)	14 (5.6%)	89 (3.8%)	98 (4.1%)	11 (3.7%)	22 (5.5%)	83 (3.0%)	116 (4.2%)
HR (95% CI)	0.25 (0.08-0.76)		0.91 (0.68-1.21)		0.52 (0.25-1.07)		0.70 (0.53-0.93)	
Ischemic stroke								

Events, N (%)	1 (0.4%)	4 (1.6%)	4 (0.2%)	16 (0.7%)	2 (0.5%)	6 (1.5%)	16 (0.6%)	24 (0.9%)
HR (95% CI)	0.23 (0.03-2.03)		0.25 (0.08-0.75)		0.34 (0.07-1.70)		0.66 (0.35-1.25)	
Urgent coronary revascularization								
Events, N (%)	NA	NA	25 (1.1%)	50 (2.1%)	3 (0.8%)	12 (3.0%)	135 (4.9%)	177 (6.4%)
HR (95% CI)	NA		0.50 (0.31-0.81)		0.26 (0.07-0.92)		0.75 (0.60-0.94)	

*NA= not applicable. A study result was designed NA when the outcome in the study did not closely match the outcome in the present meta-analysis.

In sensitivity analyses of ACS trials (COLCOT and COPS; N=5540), the primary composite endpoint, MI, and urgent coronary revascularizations were statistically significantly reduced in patients randomized to colchicine compared to placebo. Among patients with stable CAD (LoDoCo and LoDoCo 2; N=6054), the only comparable outcome between trials was MI which was statistically significantly decreased with colchicine compared to placebo.

Although there was no statistically significant difference in the incidence of key secondary endpoints, there was a trend towards reduced AF diagnoses and AF episodes among patients randomized to colchicine [pooled HR 0.86 (95% CI 0.67-1.04); I^2=0.0%].

Visual inspection of funnel plots and the results of the Eggers test indicated there was no significant risk of publication bias for all outcomes (p>0.05 for all) except urgent coronary revascularization (p=0.02).

Subgroup analyses

The statistically significant reduction in the incidence of the primary composite cardiovascular endpoint among patients randomized to colchicine was consistent for most subgroup analyses. There was no significant heterogeneity of the effect of colchicine in the different subgroups studied. Overall, the Q_b statistic suggests that there is minimal variability and inconsistency in the treatment effect between strata in subgroup analyses (p>0.05 for all).

Major adverse events

There was no statistically significant difference between treatment arms for the risk of all-cause [pooled RR 1.04 (95% CI 0.61-1.78); I^2=62.01%] and non-cardiovascular mortality [pooled RR 1.38 (95% CI 0.99-

1.93); I^2=0.0%] (Figure 3). The rates of infections, pneumonias, hospitalizations for gastrointestinal events, and diagnoses of cancer were also not significantly different. For pneumonia, there was a high degree of heterogeneity between trials with opposite directions for the treatment effect (I^2=81.5%;).

In this contemporary meta-analysis of RCTs comparing colchicine to placebo (or no colchicine) for secondary cardiovascular prevention, we found that: 1) treatment with colchicine was associated with a 32% reduction in the incidence of major cardiovascular events; 2) significantly fewer MIs, ischemic strokes, and urgent coronary revascularizations were the primary drivers for the overall decrease in cardiovascular events; 3) the protective treatment effect of colchicine was relatively consistent among most subgroups studied, and 4) colchicine had a favorable safety profile. This is the first comprehensive meta-analysis of RCTs evaluating efficacy and safety of colchicine for secondary cardiovascular prevention in which the endpoints were harmonized across trials. Overall, the synthesis of current evidence in the present meta-analysis indicates that treatment with colchicine for secondary cardiovascular prevention confers beneficial clinical effects.

Secondary prevention of cardiovascular diseases

Although the 4 RCTs investigated the efficacy of colchicine in different segments of the CAD population, including stable CAD, recent MI, and ACS, the results of all trials consistently demonstrated that colchicine was protective against a composite endpoint of cardiovascular mortality, MI, ischemic stroke, and urgent coronary revascularization. The individual trials were not powered to detect statistically significant differences in the components of the primary endpoint. Whereas all trials showed a decrease in urgent coronary revascularizations among patients randomized to colchicine, only COLCOT detected a reduction in ischemic strokes [HR 0.25 (95% CI 0.08-0.75)] and LoDoCo2 a decrease in MIs [HR 0.70 (95% CI 0.53-0.93)]. Pooling HRs from the RCTs revealed that the attenuated risk of major cardiovascular events was attributed to risk reductions of 38%, 62%, and 44% for MIs, ischemic strokes, and urgent coronary revascularization, respectively. Although the 2 largest trials, COLCOT and LoDoCo2, contributed the most weight to analyses, the inclusion of LoDoCo and COPS tended to increase the magnitude of the risk reduction by 4-5%.

Individual trials and pooled results suggest that there is no difference in the incidence of cardiovascular or all-cause mortality with colchicine. These pooled analyses provide reassurance about the safety of colchicine for the outcome of all-cause mortality. Results from both the LoDoCo2 and COPs trials showed a trend towards increased all-cause mortality with colchicine, although neither reached statistical significance [LoDoCo2 HR 1.21 (95% CI 0.86-1.71) and COPS HR 8.20 (95% 1.02-65.61)]. The increased power from pooled analyses demonstrated that the effect of colchicine on all-cause mortality was closer to null or no effect than suggested by individual trials [pooled RR 1.04 (95% CI 0.61-1.78)].

The anti-inflammatory properties of colchicine work through various mechanisms to inhibit the pathogenesis of CAD, and subsequently reduce the incidence of ischemic cardiovascular events. In addition to targeting the NLRP3 inflammasome leading to the reduction of circulating levels of IL-1β and IL-6, colchicine also inhibits cholesterol crystals that promote inflammation and plaque instability in atherosclerosis.

Treatment effects across subgroups

The treatment effect of colchicine was similar with overlapping confidence intervals across age categories (≤65 or >65 years), presence or absence of diabetes and hypertension, and among patients with or without prior coronary revascularization. Although the risk reduction appeared less marked in women compared to men, less than 20% of trial populations were women (1977 women compared to 9617 men). It is, therefore, possible that the lack of a statistically significant reduction in cardiovascular events with colchicine in the subgroup analysis of women reflects limited power. There was no significant heterogeneity between the effects in subgroups and that in the overall population.

Safety

Colchicine has an established safety profile from its centuries of use to treat gout. There was a numerical imbalance in the number of non-cardiovascular deaths between study arms, which did not reach statistical significance. Importantly, there was no effect of colchicine on all-cause mortality. In the COPS study, the increased dose of colchicine during the first month of follow-up might have contributed to higher non-cardiovascular deaths. In addition, 3 of 5 colchicine patients who died from non-cardiovascular causes in COPS had also discontinued colchicine prior to date of death, which may suggest that on-treatment analyses may provide further evidence to the safety of colchicine for non-cardiovascular mortality. Furthermore, the quality of follow-up in COPS was poor, with more than twice the number of patients with incomplete vital status than that of deaths due to inadequate resources (2 part-time healthcare workers following 795 patients). In contrast, the quality of follow-up and rate of vital status ascertainment of the 10,267 patients included in COLCOT and LoDoCo2 were excellent and did not reveal a significant difference in non-cardiovascular mortality between study arms. Finally, the meta-analysis showed no difference in all-cause mortality between groups.

Only the COLCOT trial detected a difference in the rate of pneumonia between patients randomized to colchicine (0.9%) or placebo (0.4%). Pooled analyses of COLCOT and LoDoCo2 did not show an increased risk of pneumonia with colchicine, suggesting this result in the former may have been due to chance. The lack of a difference between groups for infections, hospitalizations, and diagnoses of cancer further validates that colchicine is safe to use in patients with CAD.

As we did not have access to patient-level data from 3 of 4 trials, only modified study-level data was used

for the present meta-analysis. Although meta-analyses based on aggregate patient data continue to be the mainstay of systematic reviews that inform clinical practice, meta-analyses based on individual patient data have several advantages including the ability to verify data, address new questions, and adjust for the same variables across studies. Finally, the small number of eligible studies precluded meta-regression analyses to determine the influence of specific variables or effect modifiers on the association between colchicine and cardiovascular events. Nevertheless, the potential for effect modification was addressed in part by subgroup analyses.

In patients with CAD, the addition of low-dose colchicine to standard medical therapy consistently and significantly reduces the incidence of major cardiovascular events compared to standard medical therapy alone. With the exception of cardiovascular mortality, significant reductions were observed for components of the primary outcome, including MIs, ischemic strokes, and urgent coronary revascularizations.

Example 2: Administration of low-dose colchicine after myocardial infarction

The results of the main COLCOT trial support that the use of colchicine in patients who have had a myocardial infarction in the past 30 days significantly improved their quality of life in several ways. The COLCOT trial was performed as follows. A randomized, double-blind trial involving patients recruited within 30 days after a myocardial infarction was performed. The patients were randomly assigned to receive either low-dose colchicine (0.5 mg once daily) or placebo. A total of 4745 patients were enrolled; 2366 patients were assigned to the colchicine group, and 2379 to the placebo group. Patients were followed for a median of 22.6 months to observe the occurrence of the primary efficacy end point which was a composite of death from cardiovascular causes, resuscitated cardiac arrest, myocardial infarction, stroke, or urgent hospitalization for angina leading to coronary revascularization. The components of the primary end point and safety were also assessed in this study.

In this randomized, double-blind, placebo-controlled, investigator-initiated trial, we assigned patients in a 1:1 ratio to receive either colchicine (at a dose of 0.5 mg once daily) or placebo. The trial protocol, available at NEJM.org, was designed by a trial steering committee.

Trial Population

Adult patients were eligible if they had had a myocardial infarction within 30 days before enrollment, had completed any planned percutaneous revascularization procedures, and were treated according to national guidelines that included the intensive use of statins.

Patients were excluded if they had severe heart failure, a left ventricular ejection fraction of less than

35%, stroke within the previous 3 months, a type 2 index myocardial infarction, coronary-bypass surgery either within the previous 3 years or planned, a history of noncutaneous cancer within the previous 3 years, inflammatory bowel disease or chronic diarrhea, neuromuscular disease or a nontransient creatine kinase level that was greater than three times the upper limit of the normal range (unless due to infarction), clinically significant nontransient hematologic abnormalities, severe renal disease with a serum creatinine level that was greater than two times the upper limit of the normal range; severe hepatic disease, drug or alcohol abuse, current or planned long-term systemic glucocorticoid therapy, or a history of clinically significant sensitivity to colchicine.

Written informed consent was obtained from all the patients before enrollment. Clinical evaluations occurred at 1 month and 3 months after randomization and every 3 months thereafter.

End Points

The primary efficacy end point was a composite of death from cardiovascular causes, resuscitated cardiac arrest, myocardial infarction, stroke, or urgent hospitalization for angina leading to coronary revascularization in a time-to-event analysis.

The secondary end points consisted of the components of the primary efficacy end point; a composite of death from cardiovascular causes, resuscitated cardiac arrest, myocardial infarction, or stroke; and total mortality in time-to-event analyses. Coronary revascularization, hospitalization for heart failure, atrial fibrillation, and deep venous thrombosis or pulmonary embolus were prespecified as exploratory end points in the protocol.

Additional prespecified exploratory end points included the change from baseline to 6 months in the high-sensitivity C-reactive protein level and the change from baseline to 12 months in the white-cell count. The C-reactive protein biomarker substudy was implemented after a protocol amendment and was optional for sites and for patients; 34 sites chose to participate in this substudy.

All serious adverse events were recorded. The only other adverse events recorded were those that were considered to be related to the gastrointestinal system, events that were judged by the investigator to be related to colchicine or placebo, or laboratory abnormalities that had been judged by the investigator to be clinically significant.

Statistical Analysis

In this event-driven trial, it was estimated that a sample of approximately 4500 patients undergoing randomization (with 2250 patients in each group) or, in terms of events, a total number of 301 patients with a first positively adjudicated primary end-point event would yield adequate power. The sample-size calculation

was based on the primary efficacy end point and assumed a 27% lower risk with colchicine than with placebo, indicated by a hazard ratio of 0.724. With the use of a two-sided test at the 0.05 significance level, the trial would have 80% power if it continued until 301 positively adjudicated primary events occurred in the combined trial groups. The trial design assumed an event rate of 7% in the placebo group at 24 months, an 18-month recruitment period during which patients would be uniformly recruited, a 24-month minimum follow-up period, and a 1% annual rate of loss to follow-up or withdrawal of consent.

The efficacy analyses were conducted with the use of positively adjudicated data and according to the intention-to-treat principle. The primary end point was compared between the two trial groups with the use of a log-rank test, and the hazard ratio from a Cox proportional-hazards model, with a 95% confidence interval, was calculated. A Cox proportional-hazards model with adjustment for important baseline characteristics was also used as prespecified in the protocol.

The analysis of the primary end point was repeated in the per-protocol population (i.e., patients without major protocol deviations). Secondary and exploratory end points expressed as time to event were analyzed similarly. The changes from baseline to follow-up were analyzed with the use of an analysis of covariance model with adjustment for baseline value, and estimates of treatment effect are presented with 95% confidence intervals.

The efficacy end points expressed as time to event could be assessed in all patients because the event dates and censoring dates were complete, with the exception of one incomplete event date for atrial fibrillation; therefore, imputation for missing data was not done.

In the analysis of time to event, the following censoring rules were used. For death from any cause and death from cardiovascular causes, data from event-free patients who completed the trial were censored at the date of trial completion, and data from patients who did not complete the trial, such as those who were lost to follow-up or who withdrew consent, were censored at the date of last contact or the date of the assessment of survival status, whichever was later.

For the analysis of death from cardiovascular causes, patients who died from a noncardiovascular cause had their data censored at the time of death.

For all other end points, including the primary end point, the same censoring rules applied, but the survival status was not used because no formal assessment of end points was done at the assessment of survival status.

An analysis of the components of the primary end point with death from noncardiovascular causes as a competing event for death from cardiovascular causes, and with death from any cause as a competing event for the other components, was conducted with the use of the Fine and Gray subdistribution hazard model (Fine JP and Gray RJ, A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc* 1999;94:496-509). No missing data were imputed except for age

To account for the occurrence of multiple primary end-point events within patients, recurrent-event analyses were undertaken with the use of negative binomial regression, Andersen–Gill, and Wei–Lin–Weissfeld models (Rogers JK et al., Analysing recurrent hospitalizations in heart failure: a review of statistical methodology, with application to CHARM-Preserved. *Eur J Heart Fail* 2014;16:33-40; Andersen PK and Gill RD, Cox's regression model for counting processes: a large sample study. *Ann Stat* 1982;10:1100-20; Lin DY and Wei LJ, The robust inference for the proportional hazards model. *J Am Stat Assoc* 1989;84:1074-8; Lin DY et al. Semiparametric regression for the mean and rate functions of recurrent events. *J R Stat Soc* 2000;62:711-30; Wei LJ and Glidden DV, An overview of statistical methods for multiple failure time data in clinical trials. *Stat Med* 1997;16:833-9; Ghosh D, Methods for analysis of multiple events in the presence of death. *Control Clin Trials* 2000;21:115-26; Li QH and Lagakos SW, Use of the Wei-Lin-Weissfeld method for the analysis of a recurring and a terminating event. *Stat Med* 1997;16:925-40; Metcalfe C and Thompson SG, The importance of varying the event generation process in simulation studies of statistical methods for recurrent events. *Stat Med* 2006;25:165-79; Jahn-Eimermacher A, Comparison of the Andersen–Gill model with Poisson and negative binomial regression on recurrent event data. *Comput Stat Data Anal* 2008;52:4989-97).

An interim analysis was performed after 50% of the primary end-point events had been positively adjudicated. The prespecified stopping rule for efficacy was based on the Lan–DeMets procedure with the O'Brien–Fleming alpha-spending function. After review of the interim results, the data and safety monitoring board recommended that the trial should continue as planned.

To account for this interim analysis, the statistical significance level was set to 0.0490 for the final analysis of the primary end point. All other statistical tests were two-sided and conducted at the 0.05 significance level. Statistical analyses were performed with the use of SAS software, version 9.4 (SAS Institute). There was no prespecified plan to adjust for multiple comparisons across the multiple methods that were used to analyze the primary and secondary end points; results of these analyses are reported with point estimates and 95% confidence intervals, without P values. The 95% confidence intervals were not adjusted for multiple comparisons, and inferences drawn from them may not be reproducible. The final amendment to the statistical analysis plan was approved on August 28, 2019, before unblinding of the trial-group assignments occurred.

RESULTS

Patients

Trial enrollment began in December 2015 and was completed in August 2018; the last trial visit was in July 2019. A total of 4745 patients underwent randomization (with 2366 being assigned to the colchicine group and 2379 to the placebo group) and were followed for a median of 22.6 months. At the time of the database lock on August 28, 2019, and unblinding on August 29, 2019, vital status was available for all except 23 patients (99.5%); 89 patients (1.9%) were lost to follow-up, and 30 patients (0.6%) withdrew consent.

The characteristics of the patients at baseline are shown in Table 10.

Table 10. Characteristics of the Patients

Characteristic	Colchicine (N = 2366)	Placebo (N = 2379)
Age — yr	60.6±10.7	60.5±10.6
Female sex — no. (%)	472 (19.9)	437 (18.4)
White race — no./total no. (%)‡	1350/1850 (73.0)	1329/1844 (72.1)
Body-mass index	28.2±4.8	28.4±4.7
Current smoking — no./total no. (%)	708/2366 (29.9)	708/2377 (29.8)
Hypertension — no. (%)	1185 (50.1)	1236 (52.0)
Diabetes — no. (%)	462 (19.5)	497 (20.9)
History of myocardial infarction — no. (%)	370 (15.6)	397 (16.7)
History of PCI — no. (%)	392 (16.6)	406 (17.1)
History of CABG — no. (%)	69 (2.9)	81 (3.4)
History of heart failure — no. (%)	48 (2.0)	42 (1.8)
History of stroke or TIA — no. (%)	55 (2.3)	67 (2.8)
Time from index myocardial infarction to randomization — days	13.4±10.2	13.5±10.1
PCI for index myocardial infarction — no./total no. (%)	2192/2364 (92.7)	2216/2375 (93.3)
Medication use — no. (%)		
Aspirin	2334 (98.6)	2352 (98.9)
Other antiplatelet agent	2310 (97.6)	2337 (98.2)
Statin	2339 (98.9)	2357 (99.1)
Beta-blocker	2116 (89.4)	2101 (88.3)

* Plus–minus values are means ±SD. Data were missing on the following characteristics: age (assessed according to date of birth; see below), for 435 patients (215 in the colchicine group and 220 in the placebo group); body-mass index (the weight in kilograms divided by the square of the height in meters), for 5 (1 and 4 patients, respectively); and information about the index myocardial infarction, for 6 (2 and 4 patients, respectively). Date of birth and race were not required fields because both were considered in some countries to be sensitive data that could allow for the identification of patients. For statistical reporting, missing information regarding the day of birth was replaced by 15, and missing information regarding the month and day of birth was replaced by July 1. CABG denotes coronary-artery bypass graft surgery, PCI percutaneous coronary intervention, and TIA transient ischemic attack.
‡ Race was reported by the patient.

Patients were enrolled a mean of 13.5 days after myocardial infarction. The mean age of the patients was 60.6 years, 19.2% of the patients were women, and 20.2% had diabetes. Most patients (93.0%) underwent percutaneous coronary intervention for their index myocardial infarction. Aspirin, a different antiplatelet agent, and a statin were taken by 98.8%, 97.9% and 99.0% of the patients, respectively.

At the end of the trial, the trial regimen had been discontinued in 18.4% of the patients in the colchicine group and in 18.7% of those in the placebo group. Among the patients who discontinued the trial regimen, the median time of taking the trial drug was 7.1 months (interquartile range, 1.9 to 14.6) in the colchicine group, as compared with 6.1 months (interquartile range, 1.6 to 14.4) in the placebo group. Overall, the median duration of receipt of the trial drug was 19.6 months in the colchicine group and 19.5 months in the placebo group.

Clinical Efficacy End Points

A primary end-point event occurred in 5.5% of the patients in the colchicine group, as compared with 7.1% of those in the placebo group (hazard ratio, 0.77; 95% confidence interval [CI], 0.61 to 0.96; P = 0.02 by the log-rank test). A multivariable Cox regression model with adjustment for baseline covariates yielded a similar result (Table 11).

Table 11. Multivariable Cox Regression Model for Time to First Primary Endpoint.

Effect		Adjusted Hazard Ratio (95% CI)	P Value
Randomized treatment group	Colchicine vs. Placebo	0.78 (0.62-0.98)	0.03
Age at randomization (years)		1.02 (1.01-1.03)	<0.001
History of diabetes	Yes vs. No	1.86 (1.46-2.37)	<0.001
Prior coronary revascularization (PCI or CABG)	Yes vs. No	2.02 (1.58-2.58)	<0.001
Prior heart failure	Yes vs. No	1.81 (1.08-3.04)	0.03

CABG denotes coronary artery bypass graft, and PCI percutaneous coronary intervention. The model was based on 4745 observations. All baseline characteristics that showed an association (P<0.20) with the occurrence of a first positively adjudicated primary endpoint were included in the stepwise multivariable Cox regression. For age at randomization, the hazard ratio is for an increase of one year of age.

In the prespecified per-protocol analysis involving patients who adhered to the protocol, the primary end point occurred in 5.1% of the patients in the colchicine group and in 7.1% of those in the placebo group (hazard ratio, 0.71; 95% CI, 0.56 to 0.90) (Table 12).

Table 12. Rates and Hazard Ratios for the Primary Endpoint and its Components in the Per-Protocol Population†.

Clinical Outcome	Colchicine	Placebo	Hazard Ratio (95% CI)
Per-protocol population	N=2260	N=2270	
Primary endpoint - no. (%)	115 (5.1%)	162 (7.1%)	0.71 (0.56-0.90)
CV death - no. (%)	19 (0.8%)	23 (1.0%)	0.83 (0.45-1.53)
Resuscitated cardiac arrest - no. (%)	5 (0.2%)	5 (0.2%)	1.00 (0.29-3.46)
MI - no. (%)	77 (3.4%)	92 (4.1%)	0.84 (0.62-1.14)
Stroke - no. (%)	5 (0.2%)	19 (0.8%)	0.26 (0.10-0.71)
Urgent hospitalization for angina requiring revascularization - no. (%)	22 (1.0%)	47 (2.1%)	0.47 (0.28-0.78)

CV denotes cardiovascular, and MI myocardial infarction.

†The per-protocol population consisted of patients without major protocol deviations.

Table 13 shows the percentages of patients with events and the hazard ratios for the components of the primary end point, including death from cardiovascular causes (hazard ratio, 0.84; 95% CI, 0.46 to 1.52), resuscitated cardiac arrest (hazard ratio, 0.83; 95% CI, 0.25 to 2.73), myocardial infarction (hazard ratio, 0.91; 95% CI, 0.68 to 1.21), stroke (hazard ratio, 0.26; 95% CI, 0.10 to 0.70), and urgent hospitalization for angina leading to coronary revascularization (hazard ratio, 0.50; 95% CI, 0.31 to 0.81). The hazard ratios remained unchanged in the analysis that took competing events into account.

Table 13. Major Clinical End-points (Intention-to-Treat Population)

End Point	Colchicine (N=2366)	Placebo (N=2379)	Hazard Ratio (95% CI)	P Value
	number (percent)			
Primary composite end point	131 (5.5)	170 (7.1)	0.77 (0.61–0.96)	0.02†
Components of primary end point				
Death from cardiovascular causes	20 (0.8)	24 (1.0)	0.84 (0.46–1.52)	
Resuscitated cardiac arrest	5 (0.2)	6 (0.3)	0.83 (0.25–2.73)	
Myocardial infarction	89 (3.8)	98 (4.1)	0.91 (0.68–1.21)	
Stroke	5 (0.2)	19 (0.8)	0.26 (0.10–0.70)	
Urgent hospitalization for angina leading to revascularization	25 (1.1)	50 (2.1)	0.50 (0.31–0.81)	
Secondary composite end point‡	111 (4.7)	130 (5.5)	0.85 (0.66–1.10)	
Death	43 (1.8)	44 (1.8)	0.98 (0.64–1.49)	
Deep venous thrombosis or pulmonary embolus	10 (0.4)	7 (0.3)	1.43 (0.54–3.75)	
Atrial fibrillation	36 (1.5)	40 (1.7)	0.93 (0.59–1.46)	

* Only the initial event was counted in the analyses of time to first event for the primary composite end point and for the secondary composite end point. In the component analysis, all events (first and subsequent) were counted separately.
† The log-rank test and the multivariable Cox proportional-hazards model including age, history of diabetes, previous coronary revascularization, and previous heart failure yielded similar P values.
‡ The secondary composite end point included death from cardiovascular causes, resuscitated cardiac arrest, myocardial infarction, and stroke.

The secondary efficacy end point consisting of a composite of death from cardiovascular causes, cardiac arrest, myocardial infarction, or stroke occurred in 4.7% of the patients in the colchicine group and in 5.5% of those in the placebo group (hazard ratio, 0.85; 95% CI, 0.66 to 1.10). Data on the primary, secondary, and exploratory efficacy end points are provided in Table 13. Two patients had a first positively adjudicated event of urgent hospitalization for angina leading to coronary revascularization within 14 days after randomization. The median time to this clinical end point was 258 days.

Efficacy results in prespecified subgroups are shown in Table 14. The total number of primary end-point events (first and recurrent) was 154 in the colchicine group and 223 in the placebo group, over periods of 52,949 and 53,060 patient-months of follow-up, respectively. Thus, the primary end-point event rates per 100 patient-months were 0.29 in the colchicine group and 0.42 in the placebo group (rate ratio, 0.66; 95% CI, 0.51 to 0.86) (Table 15).

Table 14. Primary Efficacy Composite Endpoint in Prespecified Subgroups†.

Subgroup	Colchicine	Placebo	Hazard ratio (95% CI)
	no. of patients with event/total no. of patients (%)		
All patients	131/2366 (5.5%)	170/2379 (7.1%)	0.77 (0.61-0.96)
Smoking			
Non-smoker	47/787 (6.0%)	52/797 (6.5%)	0.90 (0.61; 1.34)
Previous smoker	46/871 (5.3%)	77/872 (8.8%)	0.59 (0.41; 0.85)
Active smoker	38/708 (5.4%)	41/708 (5.8%)	0.93 (0.60; 1.44)
History of diabetes			
Yes	40/462 (8.7%)	65/497 (13.1%)	0.65 (0.44; 0.96)
No	91/1904 (4.8%)	105/1882 (5.6%)	0.85 (0.64; 1.13)
History of hypertension			
Yes	83/1185 (7.0%)	112/1236 (9.1%)	0.76 (0.57; 1.01)
No	48/1181 (4.1%)	58/1143 (5.1%)	0.80 (0.54; 1.17)
Prior MI			
Yes	46/370 (12.4%)	47/397 (11.8%)	1.05 (0.70; 1.58)
No	85/1996 (4.3%)	123/1982 (6.2%)	0.68 (0.51; 0.89)
Prior PCI or CABG			
Yes	48/419 (11.5%)	57/447 (12.8%)	0.91 (0.62; 1.34)
No	83/1947 (4.3%)	113/1932 (5.8%)	0.72 (0.54; 0.95)
Prior stroke or TIA			
Yes	8/55 (14.5%)	9/67 (13.4%)	1.09 (0.42; 2.82)
No	123/2311 (5.3%)	161/2312 (7.0%)	0.76 (0.60; 0.96)
Sex‡			
Male	94/1894 (5.0%)	135/1942 (7.0%)	0.70 (0.54; 0.91)
Female	37/472 (7.8%)	35/437 (8.0%)	0.99 (0.63; 1.58)
White blood cell count¶			
Below median	41/660 (6.2%)	46/637 (7.2%)	0.85 (0.56; 1.29)
Above median	34/637 (5.3%)	44/664 (6.6%)	0.80 (0.51; 1.25)

CABG denotes coronary artery bypass graft, MI myocardial infarction, PCI percutaneous coronary intervention, and TIA transient ischemic attack.

†The final amendment to the statistical analysis plan, which listed the subgroups of interest, was approved on August 28, 2019 and unblinding occurred on August 29, 2019.

‡The hazard ratio for the primary endpoint was 0.70 (0.52; 0.93) in men and 0.81 (0.47; 1.41) in women in the per-protocol population.

The median value for total white blood cell count was 8.64 X 10^3/μL.

Table 15. Total (First and Recurrent) Primary Endpoint Events.

Total Primary Endpoint Events		**Colchicine (N=2366)**	**Placebo (N=2379)**	
Number of primary endpoint events per patient	0	2235	2209	
	1	111	132	
	2	18	26	
	3	1	9	
	4	1	3	
Total number of primary endpoint events		154	223	
Total follow-up months		52949	53060	
Rate of primary endpoint events per 100 patient-months		0.29	0.42	
				Hazard Ratio or Rate Ratio (95% CI)
Negative binomial model†				0.66 (0.51; 0.86)
Andersen-Gill model‡				0.69 (0.54; 0.88)
WLW model¶	1st Event			0.77 (0.61; 0.96)
	2nd Event			0.73 (0.48; 1.11)
	3rd Event			0.64 (0.37; 1.10)
	Average			0.77 (0.61; 0.96)

WLW denotes Wei-Lin-Weissfeld method.

†The negative binomial regression model was used to calculate marginal rate ratio.

‡The Andersen-Gill model was used with a robust variance estimator (sandwich estimator) to calculate hazard ratio.

Regarding Table 15, the Wei-Lin-Weissfeld marginal model was used to calculate hazard ratios for the time to the first, second and third event as well as the weighted average of these hazard ratios.

To account for the occurrence of multiple primary endpoint events within patients, recurrent event analyses were undertaken using three statistical approaches as there is no strong consensus as to which method is preferable. First, a negative binomial regression model was used with the number of events as

the outcome and the length of follow-up time in months as an offset term (Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005;352:1685-95). Marginal rate ratio was provided, along with 95% confidence interval. The Andersen and Gill model (Ridker PM, et al., Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med* 2017;377:1119-31) with a robust variance estimator (Ridker PM et al., Low-dose methotrexate for the prevention of atherosclerotic events. *N Engl J Med* 2019;380:752-62; and Ravelli RB et al., Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. *Nature* 2004;428:198-202) was utilized to account for the dependency of within-patient events based on a gap-time approach considering the time since a previous event. The Andersen-Gill model is a simple extension of the Cox model based on all events of all patients and estimates a hazard ratio assuming that the instantaneous risk of experiencing an event is the same irrespective of whether previous events occurred. Results from these two models are often similar (Perico N et al., Colchicine interferes with L-selectin and leukocyte function-associated antigen-1 expression on human T lymphocytes and inhibits T cell activation. *J Am Soc Nephrol* 1996;7:594-601; and Pope RM and Tschopp J, The role of interleukin-1 and the inflammasome in gout: implications for therapy. *Arthritis Rheum* 2007;56:3183-8). Finally, an approach based on the Wei, Lin and Weissfeld marginal model was conducted whereby times from randomization to first, second and subsequent event were modeled with a Cox proportional hazards model that used a covariance matrix estimate for the regression coefficients that accounted for the possible intra-patient correlation (Cerquaglia C et al., Pharmacological and clinical basis of treatment of Familial Mediterranean Fever (FMF) with colchicine or analogues: an update. *Curr Drug Targets Inflamm Allergy* 2005;4:117-24; Imazio M et al., Colchicine in addition to conventional therapy for acute pericarditis: results of the COlchicine for acute PEricarditis (COPE) trial. *Circulation* 2005;112:2012-6; and Nidorf SM et al., Low-dose colchicine for secondary prevention of cardiovascular disease. *J Am Coll Cardiol* 2013;61:404-10).This approach assumes that all patients are at risk for any event since randomization. Marginal hazard ratios for a k^{th} event (i.e. based on time from randomization to k^{th} event), as well as a weighted average of these hazard ratios, were provided along with 95% confidence intervals. It has been argued that this approach preserves the randomization and permits valid treatment effect estimation (Fine JP and Gray RJ, A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc* 1999;94:496-509).

Biomarkers of Inflammation

High-sensitivity C-reactive protein was measured in a subgroup of only 207 patients at the time of randomization and 6 months later, and the median concentration at trial entry was 4.28 mg per liter. The baseline characteristics of these patients were similar to those of the overall population (Table 16), but the small and selected subgroup with these data limits the interpretation of these analyses. The adjusted geometric mean percent changes in the high-sensitivity C-reactive protein level at 6 months after myocardial infarction were −70.0% in the colchicine group and −66.6% in the placebo group, and the

placebo-adjusted geometric mean percent change was −10.1% percentage points in the colchicine group (95% CI, −28.6 to 13.4) (Table 17).

Table 16. Characteristics of the Trial Patients with hs-CRP data values.

Characteristic	Colchicine (N=99)	Placebo (N=108)
Age - years	62.1±9.7	61.2±10.2
Female sex - no. (%)	18 (18.2%)	14 (13.0%)
Caucasian - no. (%)	91 (93.8%)	89 (89.0%)
Body-mass index (kg/m^2)	28.8±4.5	29.1±4.2
Smoking - no. (%)	20 (20.2%)	20 (18.5%)
Hypertension - no. (%)	44 (44.4%)	62 (57.4%)
Diabetes - no. (%)	12 (12.1%)	15 (13.9%)
Prior MI - no. (%)	14 (14.1%)	16 (14.8%)
Prior PCI - no. (%)	24 (24.2%)	21 (19.4%)
Prior CABG - no. (%)	4 (4.0%)	6 (5.6%)
Prior heart failure - no. (%)	4 (4.0%)	1 (0.9%)
Prior stroke/TIA - no. (%)	1 (1.0%)	2 (1.9%)
Index MI to randomization - days	17.0±9.2	15.8±9.8
PCI for index MI - no. (%)	93 (93.9%)	104 (96.3%)
Aspirin use - no. (%)	98 (99.0%)	106 (98.1%)
Other anti-platelet agent - no. (%)	98 (99.0%)	108 (100%)
Statin use - no. (%)	99 (100%)	107 (99.1%)
Beta-blocker - no. (%)	83 (83.8%)	86 (79.6%)

5 CABG denotes coronary artery bypass graft surgery, MI myocardial infarction, PCI percutaneous coronary intervention, and TIA transient ischemic attack.

Table 17. Biomarkers of Inflammation.

Biomarker	Colchicine	Placebo
Hs-C reactive protein (mg/L)	N=99	N=108
Randomization, geometric mean (IQR)†	4.27 (2.12, 7.22)	5.09 (2.45, 11.96)
6 months, geometric mean (IQR)	1.37 (0.75, 2.13)	1.60 (0.90, 2.65)
Adjusted GM percent change (95% CI)‡	-70.0 (-74.6, -64.5)	-66.6 (-71.5, -60.8)
Placebo-adjusted GM percent change (95% CI)¶	-10.1 (-28.6, 13.4)	- -
Total white blood cell count ($10^3/\mu L$)	N=992	N=980
Randomization, geometric mean (IQR)†	8.54 (7.10, 10.40)	8.63 (7.20, 10.70)
12 months, geometric mean (IQR)	6.95 (5.99, 8.30)	7.03 (5.96, 8.48)
Adjusted GM percent change (95% CI)‡	-18.81 (-20.12, -17.47)	-19.02 (-20.46, -17.55)
Placebo-adjusted GM percent change (95% CI)¶	0.26 (-2.15, 2.72)	- -
Circulating lymphocytes ($10^3/\mu L$)		
Randomization, geometric mean (IQR)†	1.79 (1.40, 2.40)	1.79 (1.42, 2.46)
12 months, geometric mean (IQR)	1.83 (1.50, 2.44)	1.82 (1.50, 2.44)
Adjusted GM percent change (95% CI)‡	1.80 (-0.46, 4.11)	0.69 (-1.54, 2.98)
Placebo-adjusted GM percent change (95% CI)¶	1.10 (-2.06, 4.36)	- -
Circulating neutrophils ($10^3/\mu L$)		
Randomization, geometric mean (IQR)†	5.45 (4.36, 7.15)	5.47 (4.30, 7.46)
12 months, geometric mean (IQR)	3.95 (3.27, 5.08)	3.99 (3.34, 5.20)
Adjusted GM percent change (95% CI)‡	-27.63 (-29.48, -25.73)	-27.95 (-29.91, -25.93)
Placebo-adjusted GM percent change (95% CI)¶	0.45 (-3.28, 4.32)	- -

GM denotes geometric mean, HS high-sensitivity, and IQR inter-quartile range.

†The geometric mean was obtained by exponentiating the mean of log-transformed data.

‡ The adjusted geometric mean percent change was obtained by exponentiating the adjusted mean from the analysis of covariance model (based on log-transformed data), then subtracting 1 and multiplying by 100. The bounds of the 95% confidence intervals were obtained similarly.

In Table 17, the placebo-adjusted geometric mean percent change was obtained by exponentiating the adjusted mean difference between groups from the analysis of covariance model (based on log-transformed data), then subtracting 1 and multiplying by 100.

In addition, the C-reactive protein biomarker sub-study was implemented following a protocol amendment and was optional for sites and for patients; 34 sites accepted to participate in this substudy. There were

213 and 208 patients who provided blood samples at baseline and 6 months, respectively. Paired baseline and 6-month hs-CRP values were available in 207 patients. Clinically available white blood cell counts were obtained from 2598 patients at baseline and 1998 patients at 12 months, and paired baseline and 12-month values were available in 1972 patients. Statistical analysis was conducted on the patients who provided both baseline and follow-up data and as these were exploratory analyses, no missing data was imputed.

Information about white-cell counts at baseline and at the 12-month follow-up were also available for a relatively small subgroup of 1972 patients. The adjusted geometric mean percent changes from baseline to 1 year in the total white-cell count were −18.8% in the colchicine group and −19.0% in the placebo group, with no significant difference between groups (0.3% percentage points; 95% CI, −2.2 to 2.7).

Safety and Adverse Events

The incidence of adverse events that were considered to be related to trial drug was 16.0% in the colchicine group and 15.8% in the placebo group, and the overall incidence of serious adverse events was 16.4% and 17.2%, respectively (Table 18).

Table 18. Adverse Events (Safety Population)

Event	Colchicine (N = 2330)	Placebo (N = 2346)	P Value
	number of patients (percent)		
Any related adverse event†	372 (16.0)	371 (15.8)	0.89
Any serious adverse event‡	383 (16.4)	404 (17.2)	0.47
Gastrointestinal adverse event	408 (17.5)	414 (17.6)	0.90
Gastrointestinal serious adverse event	46 (2.0)	36 (1.5)	0.25
Diarrhea adverse event	225 (9.7)	208 (8.9)	0.35
Nausea adverse event	43 (1.8)	24 (1.0)	0.02
Flatulence adverse event	15 (0.6)	5 (0.2)	0.02
Gastrointestinal hemorrhage	7 (0.3)	8 (0.3)	0.56
Infection serious adverse event	51 (2.2)	38 (1.6)	0.15
Pneumonia serious adverse event	21 (0.9)	9 (0.4)	0.03
Septic shock serious adverse event	2 (0.1)	2 (0.1)	0.99
Hospitalization for heart failure	25 (1.1)	17 (0.7)	0.21
Cancer§	43 (1.8)	46 (2.0)	0.77
Anemia	14 (0.6)	10 (0.4)	0.40
Leukopenia	2 (0.1)	3 (0.1)	0.66
Thrombocytopenia	3 (0.1)	7 (0.3)	0.21

* The safety population was defined as patients who took at least one dose of colchicine or placebo. All serious adverse events were recorded, and the only other adverse events recorded were those that were related to the gastrointestinal system, events that were judged by the investigator to be related to colchicine or placebo, or laboratory abnormalities that were judged by the investigator to be clinically significant. This table lists serious adverse events that were present in more than 2% of the patients in either trial group, adverse events that were considered to be related to colchicine or placebo in more than 5% of the patients in either trial group, and any other safety events of special interest. Chi-square tests were conducted to compare the incidence of adverse events between the trial groups.

† These adverse events were considered to be related to colchicine or placebo by the physician in charge of the participant.

‡ There was one serious adverse event of myopathy, which was attributed to high-dose statin therapy (rosuvastatin at a dose of 40 mg daily) by the local investigator and academic sponsor, in a man of short stature (165 cm, 68 kg) with normal renal function in the colchicine group who had received colchicine for 8 days 3 months before the adverse event.

§ Cancers, excluding nonmelanoma skin cancers, occurred in 42 patients (1.8%) in the colchicine group and in 44 (1.9%) in the placebo group.

At least one gastrointestinal adverse event during the double-blind period occurred in 17.5% of the patients in the colchicine group, as compared with 17.6% of those in the placebo group. Diarrhea was reported in 9.7% of the patients in the colchicine group and in 8.9% of those in the placebo group (P = 0.35), and

nausea was more common in the colchicine group than in the placebo group (1.8% vs. 1.0%, P = 0.02). Pneumonia was reported as a serious adverse event in 0.9% of the patients in the colchicine group, as compared with 0.4% of those in the placebo group (P = 0.03).

In COLCOT, the risk of the primary composite efficacy end point of death from cardiovascular causes, resuscitated cardiac arrest, myocardial infarction, stroke, or urgent hospitalization for angina leading to coronary revascularization, as assessed in a time-to-event analysis, was significantly lower among the patients who were randomly assigned to receive 0.5 mg of colchicine once daily than among those who received placebo. This result was due predominantly to a lower incidence of strokes and urgent hospitalizations for angina leading to coronary revascularization.

These results were observed against a background of appropriate medications, which included aspirin, a different antiplatelet agent, and a statin in 98 to 99% of the patients. In addition, percutaneous coronary intervention was performed in 93% of the patients for their index myocardial infarction. The benefits of colchicine with regard to cardiovascular end points in COLCOT were at least as large as those of canakinumab in CANTOS (Ridker PM et al., Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med* 2017;377:1119-31). In the small subgroup of patients with available data, a large (>65%) reduction in the C-reactive protein level occurred over the first 6 months after myocardial infarction in both trial groups in COLCOT, but the difference between the changes in the groups was not significant. These findings must be interpreted cautiously given that this was a small subgroup that was not randomly selected from the full trial sample. A similar observation was made with white-cell counts. The different patient populations involved in the two trials — early after myocardial infarction in COLCOT and stable coronary disease in CANTOS — may also have affected the relationship between biomarkers of inflammation and the effects of treatments on ischemic end points.

The known benefits of colchicine in the treatment of pericarditis were not at play in COLCOT. Postinfarction pericarditis typically occurs within the first few days after the injury, whereas the mean time from the index myocardial infarction to randomization was 13.5 days. There were only two patients with a first positively adjudicated event of urgent hospitalization for angina leading to coronary revascularization within 14 days after randomization, and the median time to this clinical end point was 258 days.

The most common adverse events observed were gastrointestinal. Diarrhea was reported in 9.7% of the patients in the colchicine group and in 8.9% of those in the placebo group, and nausea occurred in 1.8% and 1.0%, respectively. Infection as a serious adverse event was more frequent in the colchicine group than in the placebo group (in 2.2% vs. 1.6% of the patients), and pneumonia as a serious adverse event was also more frequent in the colchicine group (0.9% vs. 0.4%). These differences in the incidence of

infections could be due to the play of chance or could reflect altered immunologic responses.

In contrast to canakinumab (Ridker PM et al., Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med* 2017;377:1119-31), colchicine did not increase the incidence of septic shock in our trial. Infections have previously been described in patients who have attempted suicide by taking an overdose of colchicine (Kocak Z et al., Colchicine intoxication and infection risk: a case report. *J Clin Pharm Ther* 2008;33:451-2). There was no serious adverse event of myopathy linked to colchicine despite the use of statins in 99% of the patients in the trial.

In conclusion, among patients with a recent myocardial infarction, colchicine at a dose of 0.5 mg daily led to a significantly lower percentage of patients with ischemic cardiovascular events than placebo.

Other Embodiments

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope of the claims.

Some embodiments of the invention are within the following numbered paragraphs.

1. A method of treating a patient after having a myocardial infarction (MI), the method comprising initiating the administration of colchicine at a daily low dose to the patient within about 3 days of the MI.

2. A method of treating a patient after having a myocardial infarction (MI), the method comprising initiating the administration of colchicine at a daily low dose to the patient within about 4 days of the MI.

3. The method paragraph 1 or 2, wherein percutaneous coronary intervention was performed for treating the patient's MI.

4. The method of any one of paragraphs 1-3, wherein the patient was prescribed a medication.

5. The method according to paragraph 4, wherein the medication is an antiplatelet agent.

6. The method according to paragraph 4, wherein the medication is aspirin.

7. The method according to paragraph 4, wherein the medication is a statin.

8. The method according to any one of paragraphs 1-7, wherein the patient is at a lower risk of a cardiovascular event, relative to a patient not being administered colchicine.

9. The method according to paragraph 8, wherein the cardiovascular event is an ischemic cardiovascular event.

10. The method according to paragraph 8, wherein the cardiovascular event is cardiovascular death, resuscitated cardiac arrest, myocardial infarction, stroke, or urgent hospitalization for angina requiring coronary revascularization.

11. The method according to any one of paragraphs 1-10, wherein the patient has atherosclerotic coronary artery disease.

12. A method of reducing the risk of a stroke in a patient after having an MI, the method comprising initiating the administration of colchicine at a daily low dose to the patient within about 30 days of the MI.

13. The method according to paragraph 12, wherein the method comprises administering colchicine within 4-7 days of the MI.

14. The method according to paragraph 12, wherein the method comprises administering colchicine within 3 days of the MI.

16. The method according to any one of paragraphs 1-15, wherein the administration of colchicine is initiated upon assessment in (a) an emergency department (ED), (b) the hospital, or (c) a medical office setting.

17. The method according to any one of paragraphs 1-16, wherein the colchicine is in the form of a tablet.

18. The method of paragraph 17, wherein the tablet is coated.

19. The method of paragraph 18, wherein the tablet is film-coated.

20. The method according to any one of paragraphs 1-19, wherein the colchicine is administered at 0.3 to 0.7 mg.

21. The method according to any one of paragraphs 1-20, wherein the colchicine is administered at 0.4 to 0.6 mg

22. The method according to paragraph 21, wherein the colchicine is administered at about 0.5 mg.

23. The method according to any one of paragraph 1-22, wherein the colchicine is administered once, twice or three times a day.

24. The method according to paragraph 23, wherein the colchicine is administered once per day.

25. The method of any one of paragraphs 1-24, wherein the colchicine is administered without pre-loading the patient with colchicine.

26. The method of any one of paragraphs 1-25, wherein the patient is an adult human.

Other embodiments are within the claims.

What is claimed is:

Randomized, n=4745
Placebo, n=2379
Medication never taken, n=33
Safety Population, n=2346
Excluded from the present analysis.
Patients randomized < 30 days after index MI, n=3
No index MI, n= 9
Type 2 MI, n=1
Analyzed within the Placebo group, n=2336
Colchicine, n=2366
Medication never taken, n=36
Safety Population, n=2330
Excluded from the present analysis.
Patients randomized < 30 days after index MI, n=6
No index MI, n=2
Type 2 MI, n=6
Analyzed within the Colchicine group, n=2312

FIG. 1

FIG. 2

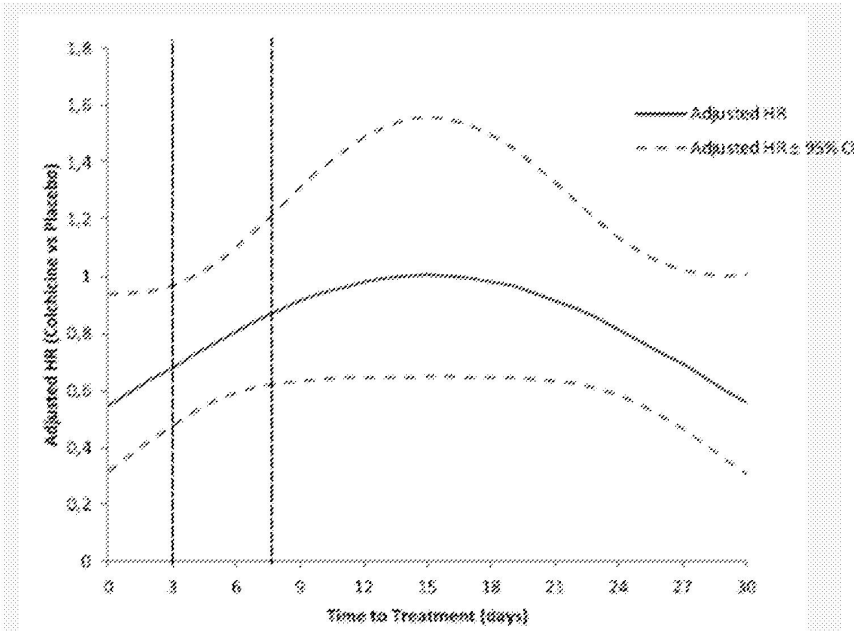

Adjusted HR
Adjusted HR ± 95% CI
Adjusted HR (Colchicine vs Placebo)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
3
6
9
12
15
18
21
24
27
30
Time to Treatment (days)

FIG. 3A

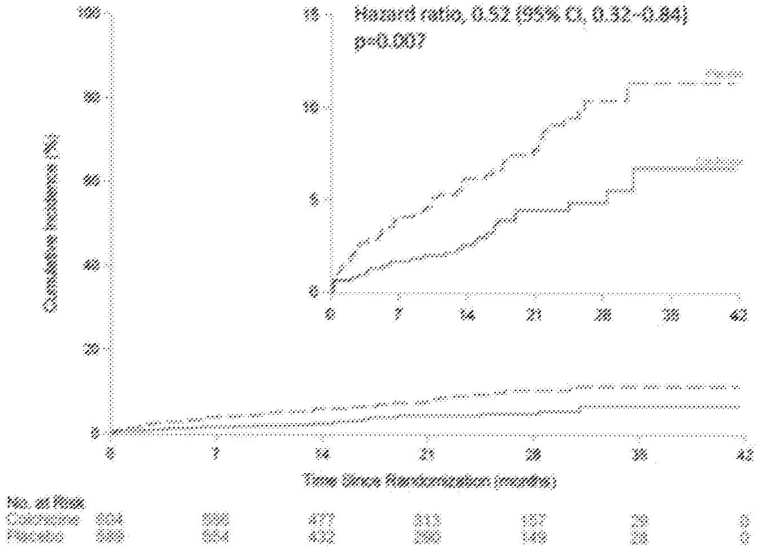
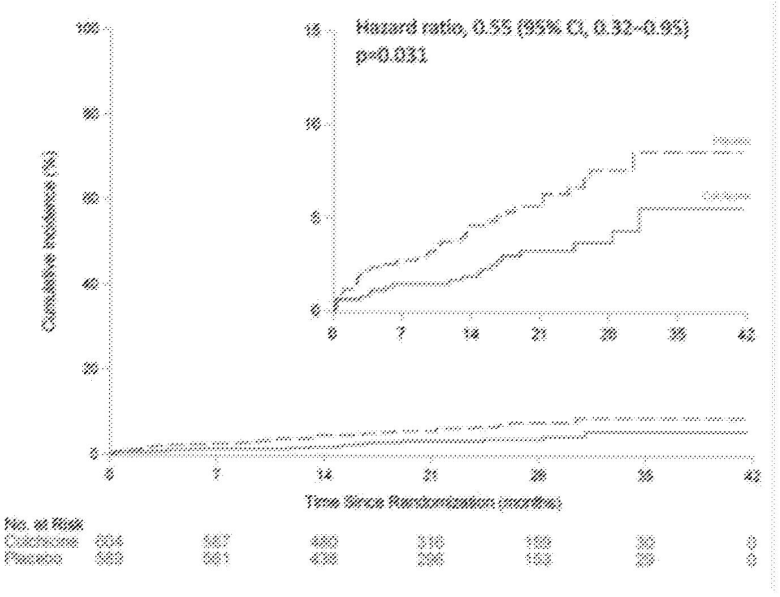

Hazard ratio, 0.52 (95% CI, 0.32–0.84)
p=0.007
Time Since Randomization (months)

FIG. 3B


Hazard ratio, 0.55 (95% CI, 0.32–0.95)
p=0.031
Time Since Randomization (months)

Electronic Patent Application Fee Transmittal	
Application Number:	
Filing Date:	
Title of Invention:	EARLY ADMINISTRATION OF LOW-DOSE COLCHICINE AFTER MYOCARDIAL INFARCTION
First Named Inventor/Applicant Name:	Jean-Claude TARDIF
Filer:	Jan Tittel/Ashley Lombardi
Attorney Docket Number:	50761-029001
Filed as Large Entity	

Filing Fees for Track I Prioritized Examination - Nonprovisional Application under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
UTILITY APPLICATION FILING	1011	1	320	320
UTILITY SEARCH FEE	1111	1	700	700
UTILITY EXAMINATION FEE	1311	1	800	800
REQUEST FOR PRIORITIZED EXAMINATION	1817	1	4200	4200
Pages:				
Claims:				
CLAIMS IN EXCESS OF 20	1202	10	100	1000
Miscellaneous-Filing:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
PUBL. FEE- EARLY, VOLUNTARY, OR NORMAL	1504	1	0	0
PROCESSING FEE, EXCEPT PROV. APPLS.	1830	1	140	140
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**7160**

Electronic Acknowledgement Receipt	
EFS ID:	41114726
Application Number:	17097285
International Application Number:	
Confirmation Number:	3015
Title of Invention:	EARLY ADMINISTRATION OF LOW-DOSE COLCHICINE AFTER MYOCARDIAL INFARCTION
First Named Inventor/Applicant Name:	Jean-Claude TARDIF
Customer Number:	21559
Filer:	Jan Tittel/Leonardo Kwak
Filer Authorized By:	Jan Tittel
Attorney Docket Number:	50761-029001
Receipt Date:	13-NOV-2020
Filing Date:	
Time Stamp:	12:57:26
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$7160
RAM confirmation Number	E2020ACC59547528
Deposit Account	032095
Authorized User	Leonardo Kwak
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.21 (Miscellaneous fees and charges) 37 CFR 1.20 (Post Issuance fees)	

37 CFR 1.19 (Document supply fees)

37 CFR 1.17 (Patent application and reexamination processing fees)

37 CFR 1.16 (National application filing, search, and examination fees)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Application Data Sheet	50761-029001_-_Application_Data_Sheet_Fillable_PDF.pdf	1255617 0a75478c6a47e2c15ec4f8c7fbf3714f61117d53	no	8
Warnings:					
Information:					
2	TrackOne Request	Certification_and_Request_for_Prioritized_Examination.pdf	34203 6afc7a805578cda9043bcbef218abf2e7054421d	no	1
Warnings:					
Information:					
3	Transmittal of New Application	Utility_Patent_Application_Transmittal.pdf	32250 35c6a701182dc9a27e4fec8b388b4c99a9513641	no	1
Warnings:					
Information:					
4		Application.pdf	4933015 b85b8e543c3366a8ebc6c00e9009113e11dbbfc5	yes	50

Multipart Description/PDF files in .zip description

Document Description	Start	End
Abstract	50	50
Claims	48	49
Specification	1	47

Warnings:					
Information:					
5	Drawings-only black and white line drawings	Figures.pdf	811214 e8b7461ad2d2c7e9cd66beca5ff46f265be32443	no	3

Warnings:					
Information:					
6	Fee Worksheet (SB06)	fee-info.pdf	41918 deb660737bd6a28ddb49d4e5b36663b81696e379	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	7108217	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/AIA/14 (02-18)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50761-029001
	Application Number	
Title of Invention	EARLY ADMINISTRATION OF LOW-DOSE COLCHICINE AFTER MYOCARDIAL INFARCTION	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 — Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Jean-Claude		TARDIF	

Residence Information (Select One) US Residency ● Non US Residency Active US Military Service

City	Montreal, QC	Country of Residence [i]	CA

Mailing Address of Inventor:

Address 1	c/o INSTITUT DE CARDIOLOGIE DE MONTREAL		
Address 2	5000, RUE BELANGER EST		
City	Montreal	**State/Province**	QC
Postal Code	H1T 1C8	**Country** [i]	CA

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	21559		
Email Address		Add Email	Remove Email

Application Information:

Title of the Invention	EARLY ADMINISTRATION OF LOW-DOSE COLCHICINE AFTER MYOCARDIAL INFARCTION		
Attorney Docket Number	50761-029001	**Small Entity Status Claimed**	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	3	**Suggested Figure for Publication (if any)**	

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50761-029001
	Application Number	
Title of Invention	EARLY ADMINISTRATION OF LOW-DOSE COLCHICINE AFTER MYOCARDIAL INFARCTION	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	● Customer Number	US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	21559		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Claims benefit of provisional	63/093988	2020-10-20

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50761-029001
	Application Number	
Title of Invention	EARLY ADMINISTRATION OF LOW-DOSE COLCHICINE AFTER MYOCARDIAL INFARCTION	

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Claims benefit of provisional	62/935865	2019-11-15
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.			Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

			Remove
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)
Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.			Add

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50761-029001
	Application Number	
Title of Invention	EARLY ADMINISTRATION OF LOW-DOSE COLCHICINE AFTER MYOCARDIAL INFARCTION	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50761-029001
	Application Number	
Title of Invention	EARLY ADMINISTRATION OF LOW-DOSE COLCHICINE AFTER MYOCARDIAL INFARCTION	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1 Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

● Assignee	Legal Representative under 35 U.S.C. 117	Joint Inventor
Person to whom the inventor is obligated to assign.	Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	INSTITUT DE CARDIOLOGIE DE MONTREAL		
Mailing Address Information For Applicant:			
Address 1	5000, RUE BELANGER EST		
Address 2			
City	Montreal	**State/Province**	QC
Country	CA	Postal Code	H1T 1C8
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

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Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50761-029001
	Application Number	
Title of Invention	EARLY ADMINISTRATION OF LOW-DOSE COLCHICINE AFTER MYOCARDIAL INFARCTION	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add

Signature:

Remove

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Jan N. Tittel/			Date (YYYY-MM-DD)	2020-11-13
First Name	Jan	Last Name	Tittel	Registration Number	52290

Additional Signature may be generated within this form by selecting the Add button. Add

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Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50761-029001
	Application Number	
Title of Invention	EARLY ADMINISTRATION OF LOW-DOSE COLCHICINE AFTER MYOCARDIAL INFARCTION	

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1 The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3 A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent CooperationTreaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Docket No.: 50761-029001
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Utility Application of:
Jean-Claude TARDIF

Application No.: 17/097,285 | Confirmation No.: 3015

Filed: November 13, 2020 | Art Unit: N/A

For: EARLY ADMINISTRATION OF LOW-DOSE COLCHICINE AFTER MYOCARDIAL INFARCTION | Examiner: Not Yet Assigned

TRANSMITTAL FOR THE PAYMENT OF THE SURCHARGE FOR OATH OR DECLARATION

Please charge $140.00 to Deposit Account No. 03-2095 for the fee set forth in 37 C.F.R. § 1.16(f) for filing the inventor's oath or declaration on a date later than the filing date of the application.

Please apply any charges or credits to Deposit Account No. 03-2095.

Dated: November 13, 2020

Respectfully submitted,

/Jan N. Tittel/
Jan N. Tittel, Ph.D.
Registration No.: 52,290
CLARK & ELBING LLP
101 Federal Street
15th Floor
Boston, MA 02110
(617) 428-0200

Electronic Patent Application Fee Transmittal					
Application Number:	17097285				
Filing Date:					
Title of Invention:	EARLY ADMINISTRATION OF LOW-DOSE COLCHICINE AFTER MYOCARDIAL INFARCTION				
First Named Inventor/Applicant Name:	Jean-Claude TARDIF				
Filer:	Jan Tittel/Ashley Lombardi				
Attorney Docket Number:	50761-029001				
Filed as Large Entity					
Filing Fees for Utility under 35 USC 111(a)					
Description		**Fee Code**	**Quantity**	**Amount**	**Sub-Total in USD($)**
Basic Filing:					
Pages:					
Claims:					
Miscellaneous-Filing:					
LATE FILING FEE FOR OATH OR DECLARATION		1051	1	160	160
Petition:					
Patent-Appeals-and-Interference:					
Post-Allowance-and-Post-Issuance:					

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
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Miscellaneous:				
			Total in USD ($)	**160**

Electronic Acknowledgement Receipt	
EFS ID:	41117641
Application Number:	17097285
International Application Number:	
Confirmation Number:	3015
Title of Invention:	EARLY ADMINISTRATION OF LOW-DOSE COLCHICINE AFTER MYOCARDIAL INFARCTION
First Named Inventor/Applicant Name:	Jean-Claude TARDIF
Customer Number:	21559
Filer:	Jan Tittel/Leonardo Kwak
Filer Authorized By:	Jan Tittel
Attorney Docket Number:	50761-029001
Receipt Date:	13-NOV-2020
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Payment was successfully received in RAM	$160
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Authorized User	Leonardo Kwak
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.21 (Miscellaneous fees and charges) 37 CFR 1.20 (Post Issuance fees)	

37 CFR 1.19 (Document supply fees)

37 CFR 1.17 (Patent application and reexamination processing fees)

37 CFR 1.16 (National application filing, search, and examination fees)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	Transmittal_Declaration.pdf	76672 a2ebf356abebc436e58e07f14f29b961597df042	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30177 82374b9d51ab7fc5d8f9ca6bd87cf644ed4c0bfe	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	106849		

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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Used in Lieu of PTO/SB/08a
(Based on PTO 07-09 version)

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 3

Complete if Known	
Application Number	17/097,285
Filing Date	November 13, 2020
First Named Inventor	Jean-Claude TARDIF
Art Unit	1629
Examiner Name	Not Yet Assigned
Attorney Docket Number	50761-029001

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	A*	US-10,265,281	04-23-2019	Nidorf	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]·Number[4]·Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	A	WO-2014/066944-A1	05-08-2014	Heart Care Western Australia Pty Ltd		

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. * CITE NO.: Those application(s) which are marked with an single asterisk (*) next to the Cite No. are not supplied (under 37 CFR 1.98(a)(2)(iii)) because that application was filed after June 30, 2003 or is available in the IFW. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5]Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

IDS Filed November 19, 2020

3024497

PTO/SB/08b (07-09)
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Substitute for form 1449/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				**Application Number**	17/097,285
				Filing Date	November 13, 2020
				First Named Inventor	Jean-Claude TARDIF
(Use as many sheets as necessary)				Art Unit	1629
				Examiner Name	Not Yet Assigned
Sheet	2	of	3	Attorney Docket Number	50761-029001

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	A	Akodad et al., "Interest of colchicine in the treatment of acute myocardial infarct responsible for heart failure in a mouse model," Int J Cardiol. 240:347-353 (2017).	
	B	Bouabdallaoui et al., "Time-to-treatment initiation of colchicine and cardiovascular outcomes after myocardial infarction in the Colchicine Cardiovascular Outcomes Trial (COLCOT)," Eur Heart J. 00:1-8 (2020).	
	C	Deftereos et al., "Anti-Inflammatory Treatment with Colchicine in Acute Myocardial Infarction: A Pilot Study," Circulation 132(15):1395-403 (2015).	
	D	Hennessy et al., "The Low Dose Colchicine after Myocardial Infarction (LoDoCo-MI) study: A pilot randomized placebo controlled trial of colchicine following acute myocardial infarction," Am Heart J. 215:62-69 (2019).	
	E	Newby et al., "Inflammation as a Treatment Target after Acute Myocardial Infarction," N Engl J Med. 381(26):2562-2563 (2019).	
	F	Nidorf et al., "Low-dose colchicine for secondary prevention of cardiovascular disease," J Am Coll Cardiol. 61(4):404-410 (2013).	
	G	Nidorf et al., "Why Colchicine Should Be Considered for Secondary Prevention of Atherosclerosis: An Overview," Clin Ther. 41(1):41-48 (2019).	
	H	Tardif et al., "Efficacy and Safety of Low-Dose Colchicine after Myocardial Infarction," N Engl J Med. 381(26):2497-2505 (2019).	
	I	Tucker et al., "Colchicine as a Novel Therapy for Suppressing Chemokine Production in Patients with an Acute Coronary Syndrome: A Pilot Study," Clin Ther. 41(10):2172-2181 (2019).	
	J	Vaidya et al., "Colchicine Therapy and Plaque Stabilization in Patients with Acute Coronary Syndrome: A CT Coronary Angiography Study," JACC Cardiovasc Imaging. 11(2 PT 2):305-316 (2018).	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet	3	of	3

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Application Number	17/097,285
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Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	K	Vaidya et al., "The Role of Colchicine in Acute Coronary Syndromes," Clin Ther. 41(1):11-20 (2018).	

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*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

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1	Transmittal Letter	Information_Disclosure_Statement.pdf	20021 1c8216a28a249ffb38749493e4477d38695a781d	no	2

Warnings:

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	IDS_Citation_by_Applicant_13_References.pdf	43609 d3a656e980143de7212e22078f632c95ad146d38	no	3
Warnings:					
Information:					
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Docket No.: 50761-029001
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Utility Application of:
Jean-Claude TARDIF

Application No.: 17/097,285 | Confirmation No.: 3015

Filed: November 13, 2020 | Art Unit: 1629

For: EARLY ADMINISTRATION OF LOW-DOSE COLCHICINE AFTER MYOCARDIAL INFARCTION | Examiner: Not Yet Assigned

INFORMATION DISCLOSURE STATEMENT (IDS)

Pursuant to 37 C.F.R. §§ 1.56, 1.97, and 1.98, the attention of the United States Patent and Trademark Office is hereby directed to the references listed on the attached PTO/SB/08. It is respectfully requested that the information be expressly considered during the prosecution of the above-identified application, and that the references be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

This Information Disclosure Statement is filed within three months of the filing date.

In accordance with 37 C.F.R. § 1.98(a)(2)(ii), copies of U.S. patents and U.S. patent application publications are not submitted. Submitted herewith are copies of foreign patents and non-patent literature in accordance with 37 C.F.R. § 1.98(a)(2).

In accordance with 37 C.F.R. § 1.97(g), the filing of this Information Disclosure Statement shall not be construed as a representation that a search has been made. In accordance with 37 C.F.R. § 1.97(h), the filing of this Information Disclosure Statement shall not be construed to be an admission that the information cited in this Information Disclosure Statement is, or is considered to be, material to the patentability as defined in 37 C.F.R. § 1.56(b).

It is submitted that the Information Disclosure Statement is in compliance with 37 C.F.R. § 1.98, and the Examiner is respectfully requested to consider the listed references.

3024496

Please apply any charges or credits to Deposit Account No. 03-2095.

Dated: November 19, 2020

Respectfully submitted,

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WO 2014/066944 A1

(54) **Title**: TREATMENT OR PREVENTION OF CARDIOVASCULAR EVENTS VIA THE ADMINISTRATION OF A COLCHICINE DERIVATIVE

(57) **Abstract**: A method for the treatment or prevention of a cardiovascular event in a subject with atherosclerotic vascular disease comprising the step of: b) administering a therapeutically effective amount of a compound of formula (I), a known colchicine derivative and/or a salt thereof R wherein: R_1, R_2, R_3, R_4, R_9, R_{10}, R_{11} and R_{12} independently represent hydrogen, C_{1-4} alkyl, C_{2-4} alkenyl, C_{3-6} cycloalkyl, halogen, C_{1-4} haloalkyl, nitro, amino, C_{2-4} acylamino, C_{1-4} alkyl or dialkylamino, hydroxyl, C_{1-4}alkoxy, C_{1-4} alkylthio, a group of the formula - $SO_2N(R^x)_2$ or SO_2R^x where R^x is C_{1-4} alkyl,C_{1-4} acyloxy, or optionally substituted phenyl, optionally substituted phenoxy; R_7 and R_8 independently represent hydrogen, C_{1-4} alkyl or C_{1-4} acyl; and R_5', R_5 ", R_6' and R_6" independently represent hydrogen, C_{1-4} alkyl, C_{2-4} alkenyl, C_{3-6} cycloalkyl, halogen, C_{1-4} haloalkyl, nitro, amino, C_{2-4} acylamino, hydroxyl, C_{1-4} alkoxy or C_{1-4} alkylthio a group of the formula -$SO_2N(R^x)_2$ or SO_2R^x where R^x is C_{1-4} alkyl, C_{1-4} acyloxy, or optionally substituted phenyl.

TREATMENT OR PREVENTION OF CARDIOVASCULAR EVENTS VIA THE ADMINISTRATION OF A COLCHICINE DERIVATIVE

TECHNICAL FIELD

[0001] A method for the treatment and/or prevention of cardiovascular events in patients with established atherosclerotic vascular disease.

BACKGROUND ART

[0002] The following discussion of the background art is intended to facilitate an understanding of the present invention only. The discussion is not an acknowledgement or admission that any of the material referred to is or was part of the common general knowledge as at the priority date of the application.

[0003] In patients with atherosclerotic vascular disease, the diseased vessel wall is subject to injurious forces that promote plaque build-up and instability that may lead to coronary occlusion resulting in heart attack, ischemic stroke and sudden death. The response to injury within the diseased vessel is dependent on the architecture and content of atherosclerotic plaques. Lipid-rich plaques with a neo-vascular base are particularly susceptible to the effect of injury, which may leave them vulnerable to neutrophil infiltration. Neutrophils that enter the interstitial space may become activated upon exposure to the plaque contents, inciting an aggressive inflammatory response that may accelerate plaque instability increasing the risk of plaque enlargement and rupture and hence increasing the risk of clinical events.

[0004] Despite routine use of anti-platelet and statin therapy, patients with atherosclerotic vascular disease continue to be at risk of cardiovascular events, possibly because these treatments fail to target some of the inflammatory pathways implicated in the disease.

[0005] A number of additional treatments exist for the prevention or reduction in risk of coronary heart disease, including: antiplatelet agents (besides aspirin), anti-coagulants, angiotensin-converting-enzyme inhibitors (ACEIs); aldosterone receptor antagonists (ARAs); beta-blockers calcium channel blockers and/or nitrates.

[0006] However, many of these treatments are recommended for acute conditions and do not address or provide for a long-term reduction in cardiovascular events in patients with clinically stable atherosclerotic vascular disease.

[0007] It is against this background that the present invention has been developed.

[0008] The present invention seeks to overcome, or at least ameliorate, one or more of the deficiencies of the prior art mentioned above, or to provide the consumer with a useful or commercial choice.

SUMMARY OF INVENTION

[0009] The present invention provides a method for the treatment or prevention of a cardiovascular event in a subject comprising the step of:

administering a therapeutically effective amount of a compound of formula (I), a known colchicine derivative and/or a salt thereof.

[0010] The present invention provides a method for the treatment or prevention of a cardiovascular event in a subject with atherosclerotic vascular disease comprising the step of:

administering a therapeutically effective amount of a compound of formula (I), a known colchicine derivative and/or a salt thereof.

[0011] The present invention further provides a method for the treatment or prevention of a cardiovascular event in a subject with atherosclerotic vascular disease comprising the step of:

administering a therapeutically effective amount of a compound of formula (I), a known colchicine derivative and/or a salt thereof and one or more additional agents selected from the list comprising: a statin and/or an anti-platelet agent.

[0012] Preferably the atherosclerotic vascular disease is a coronary disease. The coronary disease may be stable or unstable.

[0013] The invention further provides a method for the treatment or prevention of a cardiovascular event in a subject comprising the step of:

administering a therapeutically effective amount of a compound of formula (I), a known colchicine derivative and/or a salt thereof

wherein the subject has coronary disease prior to administration of the therapeutically effective amount of a compound of formula (I), a known colchicine derivative and/or a salt thereof. Preferably the coronary disease is a stable coronary disease.

[0014] The present invention provides a method for reducing or preventing cholesterol crystal induced inflammation within atherosclerotic plaques in a subject, comprising the step of

administering a dosage of 0.5 mg or 0.6 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof.

[0015] The invention also provides for the use of a compound of formula (I), a known colchicine derivative and/or a salt thereof in the preparation of a medicament for the treatment or prevention of a cardiovascular event.

[0016] In addition, the invention provides a composition for the prevention or treatment of a cardiovascular event in a subject, the composition comprising a therapeutically effective amount of a compound of formula (I), a known colchicine derivative and/or a salt thereof and one or more pharmaceutically acceptable additives, excipients carriers and/or diluents.

[0017] Preferably, the composition comprises 0.5 mg or 0.6 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof.

[0018] Preferably, the composition further comprises one or more additional agents selected from the list comprising: a statin and/or an anti-platelet agent.

[0019] The present invention provides a regimen for the treatment or prevention of a cardiovascular event in a subject, the regimen comprising the step of:

administering a dosage of 0.5 mg or 0.6 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof once per day.

[0020] The present invention also provides a regimen for reducing or preventing cholesterol crystal induced inflammation within atherosclerotic plaques in a subject, the regimen comprising the step of:

administering a dosage of 0.5 mg or 0.6 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof once or more times per day.

[0021] Preferably, the subject being administered the regimen has atherosclerotic vascular disease, more preferably a coronary disease which may be a stable or unstable coronary disease.

FIGURES

[0022] Figure 1 is a flow chart of the subject enrolment process for Example 1.

[0023] Figure 2 is a graph of the time to first clinical event in each group by treatment.

[0024] Figure 3 is a table of the first time to clinical event across subgroups examined.

[0025] Figure 4 is series of graphs of the time to first clinical event in each group by treatment broken down into specific events.

DETAILED DESCRIPTION OF THE INVENTION

General

[0026] Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. The invention includes all such variation and modifications. The invention also includes all of the steps, features, formulations and compounds referred to or indicated in the specification, individually or collectively and any and all combinations or any two or more of the steps or features.

[0027] Each document, reference, patent application or patent cited in this text is expressly incorporated herein in their entirety by reference, which means that it should be read and considered by the reader as part of this text. That the document, reference, patent application or patent cited in this text is not repeated in this text is merely for reasons of conciseness. Any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention. No admission is made that any of the references constitute prior art or are part of the common general knowledge of those working in the field to which this invention relates.

[0028] The present invention is not to be limited in scope by any of the specific embodiments described herein. These embodiments are intended for the purpose of exemplification only. Functionally equivalent products, formulations and methods are clearly within the scope of the invention as described herein.

[0029] The invention described herein may include one or more range of values (eg. size, displacement and field strength etc). A range of values will be understood to include all values within the range, including the values defining the range, and values adjacent to the range which lead to the same or substantially the same outcome as the values immediately adjacent to that value which defines the boundary to the range.

[0030] Other definitions for selected terms used herein may be found within the detailed description of the invention and apply throughout. Unless otherwise defined, all other scientific and technical terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which the invention belongs. The term "active agent" may mean one active agent, or may encompass two or more active agents.

[0031] Throughout this specification, unless the context requires otherwise, the word "comprise" or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Detailed Description

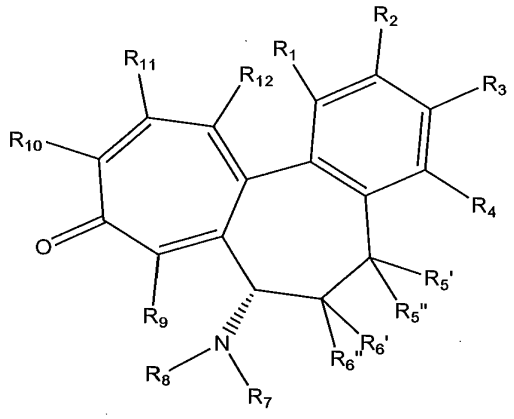
[0032] It is well known that consumption of a range of non-steroidal anti-inflammatory agents, such as Vioxx® (rofecoxib), Celebrex® (celecoxib), ibuprofen, Voltaren® (diclofenac), by patients with a previous history of coronary heart disease or other atherosclerotic vascular disease may lead to a significant increase in the risk of further cardiovascular events such as myocardial infarction. Other anti-inflammatory agents such as cortisone can also increase the risk of adverse effects and cardiovascular events in patients with a previous history of coronary heart disease. Despite the anti-inflammatory effects of these agents and their ability to reduce the markers of inflammation (such as a reduction in the levels of c-reactive protein), they are contra-indicated in the treatment of patients with coronary heart disease or other atherosclerotic vascular disease.

[0033] In atherosclerotic vascular disease, an artery wall thickens as a result of the accumulation of calcium and fatty materials such as cholesterol. It is a syndrome affecting arterial blood vessels, a chronic inflammatory response in the walls of arteries specifically due to atheromatous plaques. Disruption of the plaques may lead to acute coronary syndrome (including ischemic chest pain, acute myocardial infarction, unstable angina); cardiac arrest; and/or stroke such as non-cardio-embolic ischemic stroke.

[0034] Coronary disease and coronary heart disease is a form of atherosclerotic vascular disease caused by plaque building up along the inner walls of the arteries of the heart, which narrows the arteries and reduces blood flow to the heart. Stable coronary diseases are those that occur predictably in intensity, character or frequency at known levels of exertion or other stimuli. Unstable coronary diseases are those that change in intensity, character or frequency.

[0035] The inventors of the present invention have surprisingly found that administration of an anti-inflammatory agent within the group of compounds defined by formula (I) may treat or prevent cardiovascular events in patients with atherosclerotic vascular disease such as coronary heart disease.

Formula (I)



wherein:

R_1, R_2, R_3, R_4, R_9, R_{10}, R_{11} and R_{12} independently represent hydrogen, C_{1-4} alkyl, C_{2-4} alkenyl, C_{3-6} cycloalkyl, halogen, C_{1-4} haloalkyl, nitro, amino, C_{2-4} acylamino, C_{1-4} alkyl or dialkylamino, hydroxyl, C_{1-4} alkoxy, C_{1-4} alkylthio, a group of the formula $-SO_2N(R^x)_2$ or SO_2R^x where R^x is C_{1-4} alkyl, C_{1-4} acyloxy, or optionally substituted phenyl, optionally substituted phenoxy ;

R_7 and R_8 independently represent hydrogen, C_{1-4} alkyl or C_{1-4} acyl; and

R_5', R_5", R_6' and R_6" independently represent hydrogen, C_{1-4} alkyl, C_{2-4} alkenyl, C_{3-6} cycloalkyl, halogen, C_{1-4} haloalkyl, nitro, amino, C_{2-4} acylamino, hydroxyl, C_{1-4} alkoxy or C_{1-4} alkylthio a group of the formula $-SO_2N(R^x)_2$ or SO_2R^x where R^x is C_{1-4} alkyl, C_{1-4} acyloxy, or optionally substituted phenyl.

[0036] In particular, the inventors have discovered that colchicine [N-[(7S)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[α]heptalen-7-yl]acetamide] may treat or prevent cardiovascular events in patients with atherosclerotic vascular disease such as coronary heart disease.

[0037] Certain compounds of formula (I), and methods for their synthesis are described in the following publications, the contents of which are incorporated by reference:

> Kouroupis, Pavlos; Hansen, Hans-Jurgen, *From Colchicine and Some of Its Derivatives to 1,2,3,9,10-Pentamethoxybenzo[a]heptalenes,* Helvetica Chimica Acta; Vol. 78; (1995); p. 1247 – 1277.

Sun, Li; McPhail, Andrew T.; Hamel, Ernest; Lin, Chii M.; Hastie, Susan B.; et al.
Antitumor Agents. 139. Synthesis and Biological Evaluation of Thiocolchicine Analogs 5,6-Dihydro-6(S)-(acyloxy)- and 5,6- Dihydro-6(S)-(aroyloxy)methyl-1,2,3-trimethoxy-9-(methylthio)-8H-cyclohepta[a]naphthalen-8-ones as Novel Cytotoxic and Antimitotic Agents, Journal of Medicinal Chemistry; **Vol.** 36(5); (1993); p. 544 - 551

Kouroupis, Pavlos; Kessler, Jacqueline; Hansen, Hans-Juergen, *10-Alkyl-10-demethylcolchicines,* Helvetica Chimica Acta; Vol. 79; (1996); p. 208 – 212.

Chang, Dong-Jo; Jung, Jong-Wha; An, Hongchan; Suh, Young-Ger; Yoon, Eun-Young; Lee, Geon-Bong; Kim, Soon-Ok; Kim, Wan- Joo; Kim, Young-Myeon, *Design, synthesis and identification of novel colchicine-derived immunosuppressant,* Bioorganic and Medicinal Chemistry Letters; Vol.19(15); (2009); p. 4416 – 4420.

Kozaka, Takashi; Nakagawa-Goto, Kyoko; Shi, Qian; Lai, Chin-Yu; Brossi, Arnold; Lee, Kuo-Hsiung; Hamel, Ernest; Bastow, Kenneth F., *Antitumor agents 273. Design and synthesis of N-alkyl-thiocolchicinoids as potential antitumor agents,* Bioorganic and Medicinal Chemistry Letters; vol. 20(14); (2010); p. 4091 – 4094.

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New C(4)-Functionalized Colchicine Derivatives by a Versatile Multicomponent Electrophilic Aromatic Substitution Helvetica Chimica Acta; Vol. 87(9); (2004); p. 2266 – 2272

Malysheva, Yulia B.; Fedorov, Alexey Yu.; Combes, Sebastien; Allegro, Diane; Peyrot, Vincent; Knochel, Paul;
Gavryushin, Andrei E., *Synthesis and biological evaluation of novel anticancer bivalent colchicine-tubulizine hybrids* Bioorganic and Medicinal Chemistry; Vol. 20(14); (2012); p. 4271 – 4278.

Kouroupis, Pavlos; Linden, Anthony; Hansen, Hans-Juergen, *Synthesis of 4-Acetylcolchicine Helvetica,* Chimica Acta; Vol.79, (1996); p. 203 – 207

Bensel, Nicolas; Lagnoux, David; Niggli, Verena; Wartmann, Markus; Reymond, Jean-Louis
New C(4)-Functionalized Colchicine Derivatives by a Versatile Multicomponent Electrophilic Aromatic Substitution Helvetica Chimica Acta; Vol. 87(9); (2004); p. 2266 – 2272.

Chang, Dong-Jo; Jung, Jong-Wha; An, Hongchan; Suh, Young-Ger; Yoon, Eun-Young; Lee, Geon-Bong; Kim, Soon-Ok; Kim, Wan- Joo; Kim, Young-Myeon, *Design, synthesis and identification of novel colchicine-derived immunosuppressant,* Bioorganic and Medicinal Chemistry Letters; vol. 19; nb. 15; (2009); p. 4416 – 4420.

Hufford, Charles D.; Capraro, Hans-Georg; Brossi, Arnold. *(13)C- and (1)H-NMR. Assignments for Colchicine Derivatives,* Helvetica Chimica Acta; Vol. 63(1); (1980); p. 50 – 56.

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Bartusik, Dorota; Tomanek, Boguslaw; Fallone, Gino; Lattova, Erika; Perreault, Helene; Tuszynski, Jack, *Derivatives of thiocolchicine and its applications to CEM cells treatment using 19F Magnetic Resonance ex vivo* Bioorganic Chemistry; vol. 38; nb. 1; (2010); p. 1 – 6.

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Stefely, Jonathan A.; Miller, Patricia A.; Peterson, Rebecca J.; Moraski, Garrett C.; Miller, Marvin J.; Palchaudhuri, Rahul; Hergenrother, Paul J., *N-((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)arylamide as a new scaffold that provides rapid access to antimicrotubule agents: synthesis and evaluation of antiproliferative activity against select cancer cell lines*, Journal of Medicinal Chemistry; Vol. 53(8); (2010); p. 3389 – 3395.

Kozaka, Takashi; Nakagawa-Goto, Kyoko; Shi, Qian; Lai, Chin-Yu; Brossi, Arnold; Lee, Kuo-Hsiung; Hamel, Ernest; Bastow, Kenneth F. Antitumor agents 273. *Design and synthesis of N-alkyl-thiocolchicinoids as potential antitumor agents*, Bioorganic and Medicinal Chemistry Letters; Vol. 20.(14); (2010); p. 4091 – 4094.

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4-(3-Halo/amino-4,5-dimethoxyphenyl)-5-aryloxazoles and-N-methylimidazoles that are cytotoxic against combretastatin a resistant tumor cells and vascular disrupting in a cisplatin resistant germ cell tumor model
Journal of Medicinal Chemistry, Vol. 53(18); (2010); p. 6595 – 6602.

Nicolaus, Norman; Neudoerfl, Joerg-Martin; Schmalz, Hans-Guenther; Zapke, Janet; Oschkinat, Hartmut; Riesterer, Philipp; Prokop, Aram, *Azides derived from colchicine and their use in library synthesis: A practical entry to new bioactive derivatives of an old natural drug,* ChemMedChem; vol. 5(5) (2010); p. 661 – 665.

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The efficacy of new colchicine derivatives and viability of the T-Lymphoblastoid cells in three-dimensional culture using 19F MRI and HPLC-UV ex vivo, Bioorganic Chemistry; vol. 37(6); (2009); p. 193 – 201.

Chang, Dong-Jo; Jung, Jong-Wha; An, Hongchan; Suh, Young-Ger; Yoon, Eun-Young; Lee, Geon-Bong; Kim, Soon-Ok; Kim, Wan- Joo; Kim, Young-

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MUTUAL PHARMACEUTICAL COMPANY, INC.; MANCHANDA, Rajesh, Thiocolchicine Derivatives, method of making and method of use thereof, Patent: WO2010/138670; (2010); (A2)

[0038] These publications disclose a large number of compounds derived from colchicine, not all of which fall within the scope of formula (I). Compounds outside the scope of formula (I), yet derived from colchicine and described in the aforementioned publications, are collectively referred to herein as "known colchicine derivatives".

[0039] The compounds of formula (I) and the known colchicine derivatives contain at least one asymmetric carbon atom. The methods of the present invention include the use of isolated optical isomers and/or mixtures of such.

[0040] The term C1-4 alkyl as used herein means a straight or branched chain alkyl group containing from 1 to 4 carbon atoms, i.e. methyl, ethyl, isopropyl, n-propyl, s-butyl, isobutyl and n-butyl.

[0041] The term C1-4 haloalkyl means the abovementioned alkyl groups substituted by one or more halogen atoms, e.g. trifluoromethyl.

[0042] The term C1-4 alkoxy and C1-4 alkylthio refer to the abovementioned alkyl groups attached through an oxygen or sulphur atom respectively to the relevant ring.

[0043] The term C2-4 alkenyl refers to groups such as vinyl, allyl and butenyl.

[0044] The term amino indicates a group of formula –NH2 and also substituted amino groups such as mono-C1-4 alkylamino and di-C1-4 alkyl amino groups.

[0045] The term C2-4 acyl amino means an amino group substituted by a C2-4 acyl group such as acetyl.

[0046] The term C1-4 alkanoyl refers to groups such as formyl or acetyl.

[0047] The term C3-8 cycloalkyl means a saturated ring having from 3 to 8 carbon atoms in the ring, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cyclooctyl.

[0048] The term optionally substituted phenyl as used herein means a phenyl group unsubstituted or substituted by one or more groups such as halogen, trifluoromethyl, methyl, methoxy or nitro.

[0049] The term optionally substituted phenoxy as used herein means a phenoxy group unsubstituted or substituted by one or more groups such as halogen, trifluoromethyl, methyl, methoxy or nitro.

[0050] Therefore, the present invention provides a method for the prevention or treatment of a cardiovascular event in a subject comprising the step of: administering a therapeutically effective amount of a compound of formula (I), a known colchicine derivative and/or a salt thereof.

[0051] Preferably, the subject has atherosclerotic vascular disease, more preferably coronary disease, even more preferably clinically stable coronary disease.

[0052] Therefore, the invention further provides a method for the prevention or treatment of a cardiovascular event in a subject with atherosclerotic vascular disease comprising the step of: administering a therapeutically effective amount of a compound of formula (I), a known colchicine derivative and/or a salt thereof.

[0053] The invention further provides a method for the prevention or treatment of a cardiovascular event in a subject comprising the step of: administering a therapeutically effective amount of a compound of formula (I), a known colchicine derivative and/or a salt thereof, wherein the subject has a coronary disease prior to administration of the therapeutically effective amount of a compound of formula (I), a known colchicine derivative and/or a salt thereof. Preferably the coronary disease is a stable coronary disease.

[0054] The method may include the further step of co-administering a second agent for the treatment and/or prevention of a cardiovascular event in a subject. For example, the composition delivered in the method may further include an agent for

anti-platelet therapy. Such anti-platelet therapy agents include: Irreversible cyclooxygenase inhibitors (such as Aspirin); Adenosine diphosphate (ADP) receptor inhibitors (such as Clopidogrel (Plavix®), Prasugrel (Effient®), Ticagrelor (Brilinta®), Ticlopidine (Ticlid®), Cangrelor); Phosphodiesterase inhibitors (such as Cilostazol (Pletal®)); Glycoprotein IIB/IIIA inhibitors (such as Abciximab (ReoPro®), Eptifibatide (Integrilin®), Tirofiban (Aggrastat®)); Adenosine reuptake inhibitors (such as Dipyridamole (Persantine®)); Thromboxane inhibitors including Thromboxane synthase inhibitors and Thromboxane receptor antagonists (such as Terutroban).

[0055] Additionally, the composition delivered in the method may include one or more statins. Statins that may be used in the composition of the present invention include: simvastatin, atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, and rosuvastatin.-

[0056] The additional statin and/or anti-platelet agent may be administered at the same time as the compound of formula (I), a known colchicine derivative and/or a salt thereof, for example as tablets or capsules taken at the same time as each other; within an hour of each other; within 2 hours, 4 hours, 6 hours, 8 hours or 12 hours of each other; or within a day of each other. Alternatively, the additional statin and/or anti-platelet agent may be in the same dosage form (for example the same tablet or capsule) as the compound of formula (I), a known colchicine derivative and/or a salt thereof.

[0057] The invention therefore provides a method for the prevention or treatment of a cardiovascular event in a subject with atherosclerotic vascular disease comprising the step of: administering a therapeutically effective amount of a compound of formula (I), a known colchicine derivative and/or a salt thereof and one or more additional agents selected from the list comprising: a statin or an anti-platelet agent.

[0058] Preferably the atherosclerotic vascular disease is a coronary disease, which may be a clinically stable coronary disease or an unstable coronary disease.

[0059] In one form of the invention, the methods comprise the administration of a compound of formula (I).

[0060] In one form of the invention, the methods comprise the administration of a compound selected from the group of compounds described in one or more of the publications listed above as defining known colchicine derivatives.

[0061] In a preferred form of the invention, the compound of formula (I) has one or more of the following features:

R_1, R_2, R_3, R_4, R_9, R_{10}, R_{11} and R_{12} independently represent hydrogen, C_{1-4} alkyl, C_{2-4} alkenyl, C_{3-6} cycloalkyl, halogen, C_{1-4} haloalkyl, nitro, amino, C_{2-4} acylamino, hydroxyl, C_{1-4} alkoxy, C_{1-4} alkylthio, a group of the formula $-SO_2N(R^x)_2$ or SO_2R^x where R^x is C_{1-4} alky;

R_5', R_5", R_6' and R_6" independently represent hydrogen, C_{1-4} alkyl, C_{2-4} alkenyl, C_{3-6} cycloalkyl, halogen, C_{1-4} haloalkyl, nitro, amino, C_{2-4} acylamino, hydroxyl, C_{1-4} alkoxy or C_{1-4} alkylthio a group of the formula $-SO_2N(R^x)_2$ or SO_2R^x where R^x is C_{1-4} alkyl.

[0062] In a preferred form of the invention, the compound of formula (I) has one or more of the following features:

a. At least one of R_1, R_2 and R_3 is C_{1-4} alkoxy or C_{1-4} alkylthio;

b. At least one of R_7 and R_8 is C_{2-4} acyl;

c. R_{10} is C_{1-4} alkoxy or C_{1-4} alkylthio

[0063] In a preferred form of the invention, the compound of formula (I) has two or more of the above-mentioned features.

[0064] In a preferred form of the invention, the compound of formula (I) has each of the abovementioned features.

[0065] In a more preferred form of the invention, the compound of formula (I) has the following feature:

d. Each of R_4, R_5', R_5", R_6', R_6" R_{11}, R_{12} is H.

[0066] In a more preferred form of the invention, the compound of formula (I) has the following feature:

e. one of R_7 and R_8 is H, and the other is C_{2-4} acyl.

[0067] Thus, in a highly preferred form of the invention, the compound of formula (I) has each of the following features:

a. At least one of R_1, R_2 and R_3 is C_{1-4} alkoxy or C_{1-4} alkylthio;

b. At least one of R_7 and R_8 is C_{2-4} acyl;

c. R_{10} is C_{1-4} alkoxy or C_{1-4} alkylthio;

d. Each of R_4, R_5', R_5", R_6', R_6" R_{11}, R_{12} is independently H;

e. One of R_7 and R_8 is H, and the other C_{2-4} acyl.

[0068] Generally, C_{1-4} alkoxy is preferred over C_{1-4} alkylthio.

[0069] Generally, C_{1-2} alkoxy is preferred over C_{3-4} alkoxy.

[0070] In a preferred form of the invention, the compound of formula (I) is selected from the group of compounds defined by the following features:

R_1, R_2, R_3, and, R_{10}, R_{11} independently represent C_{1-4} alkoxy or C_{1-4} alkylthio;

R_7 and R_8 independently represent hydrogen or C_{2-4} acyl; and

R_4, R_5', R_5", R_6' and R_6" independently represent hydrogen or C_{1-4} alkyl.

[0071] Preferably, R_1, R_2, R_3, and, R_{10}, R_{11} independently represent C_{1-4} alkoxy.

[0072] Preferably still, each of R_1, R_2, R_3, and, R_{10}, R_{11} represent C_{1-4} alkoxy.

[0073] Preferably R_4, R_5', R_5", R_6' and R_6" independently represent hydrogen.

[0074] Preferably still, each of R_4, R_5', R_5", R_6' and R_6" represent hydrogen.

[0075] In a highly preferred form of the invention, the compound of formula (I) has the following features:

each of R_1, R_2, R_3, and, R_{10}, R_{11} represent C_1 alkoxy;

each of R_4, R_5', R_5", R_6' and R_6" represent H;

R_7 represents H; and

R_8 represents C_2 acyl;

such that the compound of formula (I) is colchicine.

[0076] Compounds of formula (I) of the invention are useful in both free base or acid addition salt forms. The acid addition salts are preferably the pharmaceutically acceptable, non-toxic addition salts with suitable acids, such as those with inorganic acids, for example hydrochloric hydrobromic, nitric, sulfuric and phosphoric acids, or with organic acids, such as organic carboxylic acids, for example glycolic, maleic, hydroxymaleic, fumaric, malic, tartaric, citric, salicylic, *o*-acetoxybenzoic, nicotinic or isonicotinic acid, or organic sulfonic acids for example methanesulfonic, ethane sulfonic, 2-hydroxyethanesulfonic, toluene- *p*-sulfonic or naphthalene-2-sulfonic acid.

[0077] Without wishing to be bound by any one theory, we believe the activity of a compound of formula (I), a known colchicine derivative and/or a salt thereof in relation to the prevention of vascular events in patients with coronary disease may be due to the inhibition of neutrophil function, leading to a reduction in the risk of plaque instability and thus the risk of *de novo* vascular events due to disruption of native atherosclerotic plaques in patients with atherosclerotic vascular disease such as coronary disease. For example, the colchicine may be preventing activation and amplification of the [cholesterol] crystal induced inflammation within atherosclerotic plaques and thereby acting to slow the progression of atherosclerosis and promote the stability of atherosclerotic plaques.

[0078] There is therefore provided a method for reducing or preventing cholesterol crystal induced inflammation within atherosclerotic plaques in a subject, comprising the step of

administering a dosage of 0.5 mg or 0.6 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof.

[0079] Preferably the subject with atherosclerotic plaques has atherosclerotic vascular disease, more preferably a stable or unstable coronary disease.

[0080] Preferably the composition administered to reduce or prevent cholesterol crystal induced inflammation within atherosclerotic plaques further comprises one or more additional agents selected from the list comprising: a statin and/or an anti-platelet agent.

[0081] Preferably, the method of treatment of the present invention results in the treatment or prevention of one or more of the following cardiovascular events: acute coronary syndrome (including ischemic chest pain, acute myocardial infarction, unstable angina); cardiac arrest; or stroke such as non-cardio-embolic ischemic stroke.

[0082] Generally, the terms “treat”, "treating" and "treatment" and derivatives used herein have the meaning to affect a subject, tissue or cell to produce a desired pharmacological and/or physiological effect. The treatment may be therapeutic in terms of: preventing progression of the coronary disease or other atherosclerotic vascular disease and cardiovascular events; or causing a partial or complete cure and/or regression of the coronary disease or other atherosclerotic vascular disease and cardiovascular events.

[0083] "Preventing" or "prevention" and derivative terms relate to the partial or complete prevention or at least delaying the onset of the development of coronary disease or other atherosclerotic vascular disease and cardiovascular events or the symptoms thereof in a subject who: has not yet been diagnosed with coronary disease or other atherosclerotic vascular disease or had a cardiovascular event; has had coronary disease and may be in remission and wishes to prevent re-occurrence of the coronary disease and/or cardiovascular event; or has been diagnosed as being at risk of developing coronary disease or other atherosclerotic vascular disease and having a cardiovascular event.

[0084] The invention further provides for the use of a compound of formula (I), a known colchicine derivative and/or a salt thereof in the preparation of a medicament for the treatment or prevention of a cardiovascular event.

[0085] The invention also provides for the use of a therapeutically effective amount of a compound of formula (I), a known colchicine derivative and/or a salt thereof in

the preparation of a medicament for the prevention or treatment of a cardiovascular event in a subject with atherosclerotic vascular disease.

[0086] Preferably the atherosclerotic vascular disease is a clinically stable coronary disease or unstable coronary disease.

[0087] There is also provided the use of a therapeutically effective amount of a compound of formula (I), a known colchicine derivative and/or a salt thereof in the preparation of a medicament for reducing or preventing cholesterol crystal induced inflammation within atherosclerotic plaques in a subject.

[0088] The invention further provides for the use of a therapeutically effective amount of a compound of formula (I) in the preparation of a medicament for the prevention or treatment of a cardiovascular event in a subject.

[0089] In another form of the invention, there is provided the use of a therapeutically effective amount of a compound selected from the group of compounds described in one or more of the publications listed above as defining known colchicine derivatives in the preparation of a medicament for the prevention or treatment of a cardiovascular event in a subject.

[0090] Preferably, the medicament discussed above comprises between 0.01 and 1 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof; between 0.05 and 0.95 mg, between 0.1 and 0.9 mg, between 0.2 and 0.8 mg, or between 0.4 and 0.7 mg. More preferably, it comprises between 0.5 mg and 0.6 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof, most preferably either 0.5 mg or 0.6 mg.

[0091] The present invention further provides a composition for the prevention or treatment of a cardiovascular event in a subject, the composition comprising a therapeutically effective amount of a compound of formula (I), a known colchicine derivative and/or a salt thereof and one or more pharmaceutically acceptable additives, excipients carriers and/or diluents.

[0092] The present invention further provides a composition for reducing or preventing cholesterol crystal induced inflammation within atherosclerotic plaques in

a subject, the composition comprising a therapeutically effective amount of a compound of formula (I), a known colchicine derivative and/or a salt thereof and one or more pharmaceutically acceptable additives, excipients carriers and/or diluents.

[0093] Preferably, the composition comprising a compound of formula (I), a known colchicine derivative and/or a salt thereof comprises between 0.01 and 1 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof; between 0.05 and 0.95 mg, between 0.1 and 0.9 mg, between 0.2 and 0.8 mg, or between 0.4 and 0.7 mg. More preferably, it comprises between 0.5 mg and 0.6 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof, most preferably either 0.5 mg or 0.6 mg.

[0094] The exact amount of the a compound of formula (I), a known colchicine derivative and/or a salt thereof in the composition will of course, depend on the route of delivery, the nature of the coronary heart disease or other atherosclerotic vascular disease, the therapeutically effective amount of colchicine required and the general nature and health of the subject to whom the composition is delivered.

[0095] Preferably the concentration of the a compound of formula (I), a known colchicine derivative and/or a salt thereof in the composition will be of sufficient strength (qs) to provide a therapeutically effective dose to the target cells. Such therapy may be directed to the treatment or prevention of a cardiovascular event in a subject. More preferably, the therapeutically effective dose is directed to the treatment or prevention of a cardiovascular event in a subject who has atherosclerotic vascular disease such as stable or unstable coronary disease.

[0096] The compositions of the present invention, comprising a compound of formula (I), a known colchicine derivative and/or a salt thereof, are preferably adapted to be delivered to the subject in need more than once daily, once daily or less often. For example, if the composition is adapted for oral delivery, the composition is preferably administered once a day. However, it is contemplated that the composition can be administered more frequently than this (for example, two or three times a day) or less often (for example every second day, every third day, once a week). Alternatively, if the composition comprising a compound of formula (I), a known colchicine derivative and/or a salt thereof is in the form of an injectable

formulation, it is preferable that the composition be adapted for delivery once a week, or once a month.

[0097] The compositions may further comprise and one or more additional agents selected from the list comprising: a statin and/or an anti-platelet agent.

[0098] As used herein, the term "therapeutically effective amount" means an amount of a compound of formula (I), a known colchicine derivative and/or a salt thereof effective to yield a desired therapeutic response, for example to prevent or treat a cardiovascular event in a subject. The specific therapeutically effective amount will of course vary with such factors as the particular condition being treated, the physical condition and clinical history of the subject, the type of animal being treated, the duration of the treatment, the nature of concurrent therapy (if any), the specific formulations employed and the structure of the composition.

[0099] The term "pharmaceutically", "physiologically", or "veterinary acceptable" as used herein refers to pharmaceutically active agents, physiologically active agents, veterinary active agents, or inert ingredients which are suitable for use in oral formulations or formulations designed for contact with the skin of animals, including humans, without undue toxicity, incompatibility, instability, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio.

[00100] Additives, excipients carriers and diluents for use in the compositions of the present invention include, without limitation: water, saline, ethanol, dextrose, glycerol, glycerol and polyhydric alcohols, milk protein, vitamins, animal and vegetable oils, polyethylene glycols, lactose, dextrose, sucrose sorbitol, mannitol and other sugars, starches, gum acacia, calcium phosphates, alginate, tragacanth, gelatine, calcium silicate, cellulose and its derivatives such as microcrystalline cellulose and methyl cellulose, polyvinylpyrrolidone, water syrup, methyl and propylhydroxybenzoates, talc, magnesium carbonate, titanium dioxide, magnesium stearate and mineral oil or combinations thereof.

[00101] The compositions can additionally include lubricating agents, dispersion media, pH buffering agents, wetting agents, emulsifying and suspending agents, solvents, preserving agents, sweetening agents or flavouring agents, antifoaming agents, polymers, antioxidants, chelating agents, viscomodulators, tonicifiers,

flavorants, colorants, odorants, opacifiers, suspending agents, binders, fillers, plasticizers, lubricants, absorption-promoting agents and mixtures thereof. Preservatives include antimicrobial, antibacterial and antifungal agents, anti-oxidants, chelating agents and inert gases. The particular selection of constituent that can be included in the compositions described herein will generally depend on the type of preparation.

[00102] In the preferred embodiments, the composition is formulated to enable ready location and retention of the compound of formula (I), a known colchicine derivative and/or a salt thereof in the area required to deliver the therapeutic effect while at the same time not significantly interfering with the compound's efficacy. It will be understood, therefore, that the therapeutically effective composition may be formulated differently based on the area of treatment and how the composition is to be administered.

[00103] There are a range of reference sources for the development of pharmaceutical compositions which may be referred to by the skilled person when developing formulations comprising colchicine, such as "Remington's Pharmaceutical Sciences", 21st Edition (2009), Mack Publishing Company, Easton, Pennsylvania, USA, all the contents of which are incorporated herein.

[00104] A composition comprising a compound of formula (I), a known colchicine derivative and/or a salt thereof may be administered using standard procedures, for example: orally, topically, parenterally, intraorbitally, ophthalmically, intraventricularly, intracranially, intracapsularly, intraspinally, intracisternally, intraperitoneally, buccally, rectally, vaginally, intranasally, or by aerosol administration and/or inhalation spray or via an implanted reservoir.

[00105] In a preferred embodiment the compound of formula (I), a known colchicine derivative and/or a salt thereof is administered internally for the treatment or prevention of a cardiovascular event in a subject. For example, the compound may be delivered by oral routes in the form of a tablet, capsule, liquid dose, gel or powder; by injection e.g. into the blood stream, muscle tissue or directly into an organ such as the heart; by sublingual, buccal, rectal or intravaginal delivery; or by inhalation.

[00106] Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in oral, parenteral, sublingual, buccal, rectal or intravaginal or inhaled compositions may include: ion exchangers; alumina; aluminum stearate; lecithin; self-emulsifying drug delivery systems such as alpha- tocopherol polyethylene glycol 1000 succinate, or other similar polymeric delivery matrices or systems such as nanoparticles; serum proteins such as human serum albumin; buffer substances such as phosphates; glycine; sorbic acid; potassium sorbate; partial glyceride mixtures of saturated vegetable fatty acids; water; salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts; colloidal silica; magnesium trisilicate; polyvinyl pyrrolidone; cellulose-based substances; polyethylene glycol; sodium carboxymethylcellulose; polyacrylates; polyethylene-polyoxypropylene-block polymers; and wool fat.

[00107] The composition of the present invention comprising a compound of formula (I), a known colchicine derivative and/or a salt thereof can further contain one or more additives, provided that they do not detrimentally affect the therapeutic effect afforded by the compound of formula (I), a known colchicine derivative and/or a salt thereof. In one embodiment, the additive is a colorant. In alternative embodiments, the additive is a preservative such as a mould inhibitor or an anti-oxidant, a fragrance, or a stabiliser. The additional additives may also be agents that render the composition an emulsion, a micro-emulsion or a nano-emulsion.

[00108] In a preferred embodiment, the compound of formula (I), a known colchicine derivative and/or a salt thereof is delivered via an oral route. The colchicine may therefore be in the form of a tablet, capsule, gel or liquid composition. If the compound of formula (I), a known colchicine derivative and/or a salt thereof is in the form of a tablet, the tablet may further comprise fillers such as: lactose (milk sugar), microcrystalline cellulose, corn starch, sugars (including sucrose, mannitol, sorbitol, fructose, and dextrose), whey and yeast; and/or binders such as: povidone, xanthan gum and Carbopol. Additional ingredients may be in the form of: disintergrants such as crospovidone, croscarmellose sodium, and gellan gum; coatings such as shellac; lubricants such as magnesium stearate, stearic acid, sodium stearyl fumarate and hydrogenated vegetable oil; colorants such as titanium dioxide and iron oxides;

flavours (in chewable tablets); and plasticizers. Most preferably, the compound of formula (I), a known colchicine derivative and/or a salt thereof is administered in the form of a tablet comprising colchicine, magnesium stearate, lactose, maize starch and povidone. Preferably, the tablet, capsule, gel or liquid composition comprises between 0.01 and 1 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof; between 0.05 and 0.95 mg, between 0.1 and 0.9 mg, between 0.2 and 0.8 mg, or between 0.4 and 0.7 mg. More preferably, it comprises between 0.5 mg and 0.6 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof, most preferably either 0.5 mg or 0.6 mg.

[00109] Alternatively, the compound of formula (I), a known colchicine derivative and/or a salt thereof may be delivered via sublingual, buccal, rectal or intravaginal delivery, for example by administration of a tablet, capsule, gel, powder or spray.

[00110] In another embodiment, the compound of formula (I), a known colchicine derivative and/or a salt thereof is delivered parenterally, preferably by injection, for example subcutaneously or intramuscularly. However, delivery may also be intra-arterially or intraperitoneally for the treatment or prevention of a cardiovascular event in a subject. The composition may be in the form of a sterile injectable preparation, for example, as a sterile injectable suspension for the treatment or prevention of a cardiovascular event in a subject. This suspension may be formulated according to techniques known in the art using suitable dispersing agents, surfactants, and suspending agents (e.g. Tween 80). The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally- acceptable diluent or solvent (e.g. 1, 2-propanediol). Acceptable vehicles and solvents may include mannitol, water, Ringers solution and isotonic sodium chloride solution with buffer. Furthermore, sterile, fixed oils may be employed as a solvent or a suspending medium. For this purpose, any bland fixed oil may be employed including mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives, and natural pharmaceutically acceptable oils, such as polyoxyethylated olive oil or castor oil, may also be used in the preparation of injectables. Preferably, the injectable composition comprises between 0.01 and 1 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof; between 0.05 and 0.95 mg, between 0.1 and 0.9 mg, between

0.2 and 0.8 mg, or between 0.4 and 0.7 mg. More preferably, it comprises between 0.5 mg and 0.6 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof, most preferably either 0.5 mg or 0.6 mg.

[00111] The composition may further comprise a second agent for the treatment and/or prevention of a cardiovascular event in a subject. For example, the composition may further include an agent for in anti-platelet therapy. Such anti-platelet therapy agents include: Irreversible cyclooxygenase inhibitors (such as Aspirin); Adenosine diphosphate (ADP) receptor inhibitors (such as Clopidogrel (Plavix®), Prasugrel (Effient®), Ticagrelor (Brilinta®), Ticlopidine (Ticlid®), Cangrelor); Phosphodiesterase inhibitors (such as Cilostazol (Pletal®)); Glycoprotein IIB/IIIA inhibitors (such as Abciximab (ReoPro®), Eptifibatide (Integrilin®), Tirofiban (Aggrastat®)); Adenosine reuptake inhibitors (such as Dipyridamole (Persantine®)); Thromboxane inhibitors including Thromboxane synthase inhibitors and Thromboxane receptor antagonists (such as Terutroban).,

[00112] Additionally, the composition may include one or more statins. Statins that may be used in the composition of the present invention include: simvastatin, atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, and rosuvastatin.–

[00113] The present invention also provides a regimen for the treatment or prevention of a cardiovascular event in a subject, the regimen comprising the step of:

administering a dosage of 0.5 mg or 0.6 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof once or more per day.

[00114] The present invention also provides a regimen for reducing or preventing cholesterol crystal induced inflammation within atherosclerotic plaques in a subject, the regimen comprising the step of:

administering a dosage of 0.5 mg or 0.6 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof once or more per day.

[00115] Alternatively, the regimen may involve administering the compound of formula (I), a known colchicine derivative and/or a salt thereof every second day, every third day, every fourth day, every fifth day, every sixth day or once a week.

[00116] Preferably, the subject being administered the regimen has atherosclerotic vascular disease, preferably a stable coronary disease.

[00117] The regimen may include the additional step of co-administering one or more additional agents selected from the list comprising: a statin and/or an anti-platelet agent. The one or more additional agents may also be administered once per day.

[00118] Methods for synthesising compounds of formula (I) and/or known colchicine derivatives may be found in the following publications, the contents of which are hereby incorporated by reference.

Substitution at R_1, R_2, R_3, R_4.

1. Synthesis of colchicine derivatives containing an methoxyl:

Chang, Dong-Jo; Jung, Jong-Wha; An, Hongchan; Suh, Young-Ger; Yoon, Eun-Young; Lee, Geon-Bong; Kim, Soon-Ok; Kim, Wan- Joo; Kim, Young-Myeon, *Design, synthesis and identification of novel colchicine-derived immunosuppressant,* Bioorganic and Medicinal Chemistry Letters; vol. 19; nb. 15; (2009); p. 4416 – 4420.

Hufford, Charles D.; Capraro, Hans-Georg; Brossi, Arnold. *(13)C- and (1)H-NMR. Assignments for Colchicine Derivatives,* Helvetica Chimica Acta; Vol. 63(1); (1980); p. 50 – 56.

2. Synthesis of colchicine derivatives having substituent at R_4:

Bensel, Nicolas; Lagnoux, David; Niggli, Verena; Wartmann, Markus; Reymond, Jean-Louis
New C(4)-Functionalized Colchicine Derivatives by a Versatile Multicomponent Electrophilic Aromatic Substitution Helvetica Chimica Acta; Vol. 87(9); (2004); p. 2266 – 2272.

3. Synthesis of colchicine derivative containing a containing an acetyl functional group at R_4:

Kouroupis, Pavlos; Linden, Anthony; Hansen, Hans-Juergen, *Synthesis of 4-Acetylcolchicine Helvetica,* Chimica Acta; Vol.79, (1996); p. 203 – 207.

Substitution at R_8 or R_7

1. Synthesis of colchicine derivatives containing an azide group:

Malysheva, Yulia B.; Fedorov, Alexey Yu.; Combes, Sebastien; Allegro, Diane; Peyrot, Vincent; Knochel, Paul; Gavryushin, Andrei E., *Synthesis and biological*

evaluation of novel anticancer bivalent colchicine-tubulizine hybrids Bioorganic and Medicinal Chemistry; Vol. 20(14); (2012); p. 4271 – 4278.

2. Synthesis of colchicine derivatives containing an amide group:

Bensel, Nicolas; Lagnoux, David; Niggli, Verena; Wartmann, Markus; Reymond, Jean-Louis
New C(4)-Functionalized Colchicine Derivatives by a Versatile Multicomponent Electrophilic Aromatic Substitution Helvetica Chimica Acta; Vol. 87(9); (2004); p. 2266 – 2272

Substitution at R_{10}

1. Synthesis of colchicine derivative containing a thio group.

Kozaka, Takashi; Nakagawa-Goto, Kyoko; Shi, Qian; Lai, Chin-Yu; Brossi, Arnold; Lee, Kuo-Hsiung; Hamel, Ernest; Bastow, Kenneth F., *Antitumor agents 273. Design and synthesis of N-alkyl-thiocolchicinoids as potential antitumor agents,* Bioorganic and Medicinal Chemistry Letters; vol. 20(14); (2010); p. 4091 – 4094.

Danieli, Bruno; Giardini, Alessandra; Lesma, Giordano; Passarella, Daniele; Peretto, Bruno; Sacchetti, Alessandro; Silvani, Alessandra; Pratesi, Graziella; Zunino, Franco, *Thiocolchicine-Podophyllotoxin Conjugates: Dynamic Libraries Based on Disulfide Exchange Reaction*, Journal of Organic Chemistry; Vol. 71: 7; (2006); p. 2848 – 2853.

Quinn, Frank R.; Neiman, Zohar; Beisler, John A. *Toxicity Quantitative Structure-Activity Relationships of Colchicines Journal of Medicinal Chemistry*; Vol. 24(5); (1981); p. 636 - 639

2. Synthesis of colchicine derivative containing an haloalkyl functional group.

Chang, Dong-Jo; Jung, Jong-Wha; An, Hongchan; Suh, Young-Ger; Yoon, Eun-Young; Lee, Geon-Bong; Kim, Soon-Ok; Kim, Wan- Joo; Kim, Young-Myeon, *Design, synthesis and identification of novel colchicine-derived immunosuppressant,* Bioorganic and Medicinal Chemistry Letters; Vol.19(15); (2009); p. 4416 – 4420.

3. Synthesis of colchicine derivative containing an alkoxy group.

Kouroupis, Pavlos; Kessler, Jacqueline; Hansen, Hans-Juergen, *10-Alkyl-10-demethylcolchicines,* Helvetica Chimica Acta; Vol. 79; (1996); p. 208 – 212.

4. Synthesis of colchicine derivative containing an hydroxyl group.

Kouroupis, Pavlos; Hansen, Hans-Jurgen, *From Colchicine and Some of Its Derivatives to 1,2,3,9,10-Pentamethoxybenzo[a]heptalenes,* Helvetica Chimica Acta; Vol. 78; (1995); p. 1247 – 1277.

Sun, Li; McPhail, Andrew T.; Hamel, Ernest; Lin, Chii M.; Hastie, Susan B.; et al.

Antitumor Agents. 139. Synthesis and Biological Evaluation of Thiocolchicine Analogs 5,6-Dihydro-6(S)-(acyloxy)- and 5,6- Dihydro-6(S)-(aroyloxy)methyl-1,2,3-trimethoxy-9-(methylthio)-8H-cyclohepta[a]naphthalen-8-ones as Novel Cytotoxic and Antimitotic Agents, Journal of Medicinal Chemistry; Vol. 36(5); (1993); p. 544 - 551.

EXAMPLES

[00119] The following examples are provided to further illustrate several embodiments of the invention.

Example 1

[00120] A prospective randomized observer blinded end-point trial was conducted to determine whether adding 0.5mg/day of colchicine to standard secondary prevention therapies including aspirin and high dose statins reduces the risk of cardiovascular events in patients with objectively diagnosed and clinically stable coronary disease.

Methods

[00121] Study Conduct and Design: The LoDoCo Trial was conducted under the auspices of the Heart Research Institute of Western Australia. It was designed by the principal investigators, registered with the Australian Clinical Trial Registry [12610000293066], and received ethics approval from the Human Research Ethics Committee at Sir Charles Gairdner Hospital Perth Western Australia in July 2008. There was no external funding source.

[00122] We employed a PROBE [Prospective Randomized, Open, Blinded End-point] design [8] Eligible consenting patients with established coronary disease presenting for routine clinical review were randomized to receive colchicine 0.5mg/day or no colchicine without any other change to their medical therapy. All outcomes were evaluated by an experienced adjudicator blinded to the treatment allocation.

[00123] Study Size and Eligibility: It was planned to recruit a study population that would include 250 patients

randomized to the control group and 250 patients randomized to treatment who were tolerant of colchicine for at least 4 weeks after the date of their randomization.

[00124] Patients were eligible for inclusion if they met each of the following criteria: 1] angiographically proven coronary disease; 2] aged 35 to 85 years; 3] clinically stable for at least 6 months, 4] no major competing co-morbidities or contra-indication to colchicine therapy, 5] considered to be compliant with therapy and attending routine cardiology follow up appointments, and 6] willing to be consented and randomized into the study. Patients with a history of bypass surgery were only eligible if they had undergone bypass surgery more than 10 years before, or had angiographic evidence of graft failure or had undergone stenting since their bypass surgery. All patients signed informed consent before randomization.

[00125] Randomization: The randomization sequence was computer generated, kept concealed from the investigators at all times and was managed by a research assistant who had no involvement in the evaluation or management of study patients. Once the assistant received the consent form, the patients' demographic data were entered into the data base and the investigators and patients were advised in writing of the treatment group to which the patient had been assigned.

[00126] Despite electing to use the lowest dose of colchicine available, it was anticipated that a number of patients would withdraw from therapy early after randomisation due to gastrointestinal side effects. In order to ensure that the requisite number of patients in the treatment arm were actually tolerant of treatment, the protocol allowed for the research assistant to assign a newly recruited patient to treatment if a patient discontinued colchicine due to side effects in the first month. Patients who were intolerant to therapy remained in the study, and were followed in the usual manner and included in the primary intention to treat analysis.

[00127] Intervention: Patients randomized to active treatment were given a prescription for colchicine 0.5 mg daily by their referring cardiologist. The drug was dispensed by their usual chemist, and if

requested, patients were reimbursed for the cost of these scripts. All other treatments were continued as usual.

[00128] Follow-up and definition of clinical outcomes: Patient compliance with treatment and outcome data were collected at routine follow up visits and at the time of any unplanned hospital admission.

[00129] An acute coronary syndrome (ACS) was defined as either [a] Acute Myocardial Infarction (AMI), as evidenced by acute ischemic chest pain associated with a rise in serum troponin above the upper limit of normal [9] or [b] Unstable Angina (UA), as evidenced by a recent acceleration of the patients angina unassociated with a rise in serum troponin but associated with angiographic evidence of a change in the patient's coronary anatomy. (Unstable Angina Braunwald classification types IB and IIB) [10]. The ACS was characterized as being stent-related if there was evidence of significant in-stent stenosis or acute stent thrombosis. Out of Hospital Cardiac Arrest was defined as either a sudden death as evidenced on the patients' death certificate, or a non-fatal out of hospital cardiac arrest, defined as a recovery from sudden collapse associated with documented asystole, ventricular tachycardia or ventricular fibrillation. Non-cardio-embolic ischemic stroke was defined as CT or MRI proven ischemic stroke adjudged by the treating neurologist as not being due to atrial fibrillation or intra-cranial hemorrhage.

[00130] The primary efficacy outcome was the composite, ACS, fatal or non-fatal out of hospital cardiac arrest or non-cardio-embolic ischemic stroke.

[00131] Secondary outcomes were [a] individual components of the primary outcome, and [b] the components of ACS unrelated to stent disease.

[00132] Timelines: The pre-specified study duration was a minimum follow up of two years in all patients. Accordingly the study was closed on May 31, 2012. During May, all living patients were contacted by phone to collect compliance and

outcome data from the last date of follow-up. Final outcome data were available in all patients and no patients were lost to follow up.

[00133] Statistical Power: Assuming that the control group had a combined event rate [ACS, out of hospital cardiac arrest or non cardio-embolic-ischemic stroke] of 8% [11], an accrual interval of 2 years and a follow-up after the accrual interval of 2 years, the planned sample size provided >80% power to detect a hazard ratio of <0.50 based on a two sided significance level of 5%.

[00134] Data analysis: Summary statistics, including mean and standard deviation were calculated for all baseline characteristics by treatment arm. All time to event outcomes were calculated in days by subtracting the date of randomisation from either: (1) the date of event or death; or (2) the trial termination date for those patients not experiencing the defined event.

[00135] As pre-specified, the primary efficacy analysis was based on the intention-to-treat principle. The intention-to-treat analysis included all randomized subjects and all events during the time from randomization to the trial termination. Trial termination date was fixed as May 31, 2012. A secondary pre-specified on-treatment analysis was also performed, based on patients who were both tolerant and compliant to therapy beyond the first month of randomization. All events during the time from randomisation until non-compliance with colchicine treatment regimen were included in this analysis.

[00136] The time-to-first-event for all outcomes is presented using a Kaplan–Meier plot.

[00137] The primary efficacy outcome was analysed using a cox proportional hazards model including treatment group coded as control or colchicine. The secondary outcomes were analysed similarly. In addition, the primary analysis was stratified by gender, age, diagnosis of diabetes, past myocardial infarction, unstable angina, coronary bypass surgery, coronary angioplasty, and therapy with aspirin, clopidogrel or both, high dose statin therapy (defined as a dose of statin equivalent to atorvastatin of 40mg or more), beta blockers, calcium blockers and ACE inhibitors.

Results

[00138] Study Population: Between August 2008 and May 2010, 901 patients with stable coronary disease attending for routine out-patient cardiology review were assessed for eligibility for the study. Of these, 297 [33%] did not meet the entry criteria, 72 [8%] declined to participate and 532 [59%] were enrolled into the study, 250 of whom were randomized to the control group and 282 to treatment. Of those randomized to treatment 32 [11%] reported early intolerance, due to gastrointestinal side effects, and 7 patients subsequently reported that they chose not start therapy (Figure 1). All 532 randomized patients were followed for the duration of the study period which ranged from a minimum of 24 to a maximum of 44 months. Median follow up was 36 months.

[00139] Baseline characteristics according to randomized treatment groups are shown in Table.1. Both groups were well matched for important clinical characteristics although more patients in the colchicine group were taking calcium channel blockers and less were taking beta-blocker therapy. Almost all patients in each group were taking anti-platelet therapy and high dose statin therapy.

Table 1: Study Population

	Control n [%]	**Treatment** n [%]
Total	250	282
Mean Age	67 ± 9.2	66 ± 9.6
Males	222 [89]	251 [89]
Diabetic	69 [28]	92 [33]
Smoker	14 [6]	10 [4]
Past AMI or UA	61 [24]	64 [23]
CABG	39 [16]	62 [22]
PTCA	138 [55]	169 [60]
Aspirin and/or Plavix	235 [94]	262 [93]
DAPT	24 [10]	38 [13]
High Dose Statins	235 [94]	271 [96]
BB[1]	178 [71]	176 [62]
CBB[2]	25 [10]	52 [18]
ACE	150 [60]	155 [55]

[1]$p<0.05$ [2]$p<0.01$ for the comparison of the distributions between treatment and control

Past AMI or UA History of myocardial infarction or unstable angina

CABG	Coronary Artery Bypass Surgery
PTCA	History of Percutaneous Coronary Angioplasty
DAPT	Dual anti-platelet therapy [Aspirin and Clopidogrel]
BB	Beta-blocker therapy,
CCB	Calcium Chanel Blocker therapy
ACE	ACE inhibitor therapy

[00140] Late discontinuations: 30 patients ceased colchicine therapy after a mean period of 2.36 years. Therapy was ceased due to an unrelated inter-current illness in 11 patients, by choice in 5, and for a variety of possible drug related effects in 14 patients [4.9%] as described in Table 2.

Table 2: Withdrawals from therapy

	n [%]
Early Withdrawals	32 [11]
Late Withdrawals*	30 [11]
Unrelated inter-current illness	11 [3.9]
Patient Choice	5 [1.8]
Perceived Side effects	
Intestinal upset	7 [2.5]
Myalgia	2 [0.90]
Myositis	1 [<0.5]
Rash	1 [<0.5]
Alopecia	1 [<0.5]
Itch	1 [<0.5]
Peripheral Neuritis	1 [<0.5]

* Withdrawals after 30 days, Average time to withdrawal 2.36 years

[00141] Outcomes; A primary outcome occurred in 55/532 patients, including 15/282 [5.3%] patients assigned to colchicine treatment, and 40/250 [16%] patients assigned to the control group [hazard ratio 0.33, 95% CI; 0.18-0.59; $p<0.001$, number needed to treat 11]. A sensitivity analysis was performed for the primary outcome, adjusting for the usage of calcium channel blockers and beta blocker therapy. These results were consistent with the primary analysis.

[00142] The time to first clinical event in each group by treatment is shown in Figure 2. The effect of colchicine on the primary outcome was evident early and the benefits of colchicine continued to accrue throughout the follow up period. Results of the primary analysis were consistent in all subgroups examined (Figure 3); There was no evidence of differential treatment effects based on any of the clinical or therapeutic variables.

[00143] The reduction in the primary outcome was largely driven by the reduction in the number of patients presenting with an ACS, [13/282 [4.6%] vs 34/250 [13.4%], hazard ratio 0.33; 95% CI; 0.18-0.63; p<0.001]. Out of hospital cardiac arrest and non-cardio-embolic ischemic stroke were infrequent but were also reduced in the treatment group (Table 3).

Table 3: Primary Outcome and its Components

	Control [n=250] n [%]	Treatment [n=282] n [%]	HR 95% CI	p value
Primary Outcome	40 [16]	15 [5.3]	0.33; 0.18-0.59	<0.001
Components of Primary Outcome				
Acute Coronary Syndrome	34 [13.6]	13 [4.6]	0.33; 0.18-0.63	<0.001
OOH Cardiac Arrest	2 [0.8]	1 [0.35][1]	0.47; 0.04-5.15	0.534
Non-Cardio-Embolic Stroke	4 [1.6]	1 [0.35]	0.23; 0.03-2.03	0.184
Components of ACS				
Stent Related	4 [1.6]	4 [1.4]	NS	
Non-Stent Related	30 [12]	9 [3.2]	0.26; 0.12-0.55	<0.001
Non-Stent Related AMI	14 [5.6]	4 [1.6]	0.25; 0.08-0.76	0.014
Non-Stent Related UA	16 [12]	5 [2.4]	0.27; 0.10-0.75	0.011

OOH Out of Hospital
ACS Acute Coronary Syndrome
AMI Acute Myocardial Infarction
UA Unstable Angina

[1] Non-Fatal

[00144] Of the 47 patients who presented with an ACS, the event was stent related in 8 [17%] [2 in each group had evidence of acute stent thrombosis and 2 in each group had evidence of significant in-stent stenosis]. Further analysis confirmed that patients randomized to treatment were less likely to present with an ACS unrelated to stent disease (9/282 [3.2%] vs 30/250 [12%] hazard ratio 0.26, 95% CI; 0.12-0.55; p<0.001), be it associated with an AMI (4/282 [1.4%] vs 14/250 [5.6%] hazard ratio 0.25, 95% CI; 0.08-0.76; p=0.014) or UA (5/282 [1.8%] vs 16/250 [6.4%] hazard ratio 0.27, 95% CI; 0.10-0.75; p=0.011). (Figure 4, Table 3)

[00145] Of 39 patients randomized to treatment who did not receive therapy beyond the first month due to early intolerance or non-compliance, 4 [10%] presented with an ACS due to acute stent thrombosis (n=1) and UA (n=3). Patients who were both compliant and tolerant to therapy beyond the first month of randomization had significantly fewer events than the control patients (11/243 [4.5%] vs 40/250 [16%] hazard ratio 0.29, 95% CI; 0.15-0.56; p<0.001). The results of all on-treatment analyses were consistent with those based upon the intention to treat analyses (Table 4).

Table 4: Primary Outcome and its Components [On-Treatment Analysis]

	Control [n=250]	**Treatment [n=243]**	**HR 95% CI**	**p value**
		n [%]	n [%]	
Primary Outcome	40 [16]	11 [5.3]	0.29 (0.15, 0.56)	<0.001
Components of Primary Outcome				
Acute Coronary Syndrome	34 [13.6]	9 [4.6]	0.28 (0.13, 0.58)	<0.001

OOH Cardiac Arrest 0.622	2 [0.8]	1 [0.35][1]	0.55 (0.05, 6.03)
Non-Cardio-Embolic Stroke 0.242	4 [1.6]	1 [0.35]	0.27 (0.03, 2.42)
Components of ACS			
Stent Related	4 [1.6]	3 [1.4]	NS
Non-Stent Related <0.001	30 [12]	6 [3.2]	0.21 (0.09, 0.50)
Non-Stent Related AMI 0.033	14 [5.6]	4 [1.6]	0.30 (0.10, 0.91)
Non-Stent Related UA 0.007	16 [12]	2 [2.4]	0.13 (0.03, 0.57)

OOH Out of Hospital
ACS Acute Coronary Syndrome
AMI Acute Myocardial Infarction
UA Unstable Angina

[1] Non-Fatal

[00146] Ten patients in the control group died compared with 4 patients in the colchicine group. Of the 10 controls, 5 died of presumed cardiac cause; 2 following an out-of-hospital cardiac arrest, 2 from cardiogenic shock following myocardial infarction, and 1 following bypass surgery. All 4 patients in the colchicine group died of non-cardiac causes.

[00147] The LoDoCo trial demonstrates that the addition of colchicine 0.5mg/day to standard therapy in patients with stable coronary disease significantly reduces the risk of a cardiovascular event, including an ACS, out of hospital cardiac arrest and non-cardio-embolic ischemic stroke. The benefits of colchicine were achieved on a background of widespread use of effective secondary prevention strategies, including high dose statins, as evidenced by the low event rate in the control group. The effect of adding colchicine became evident early, continued to accrue over time and was largely driven by a reduction in ACS unrelated to stent disease.

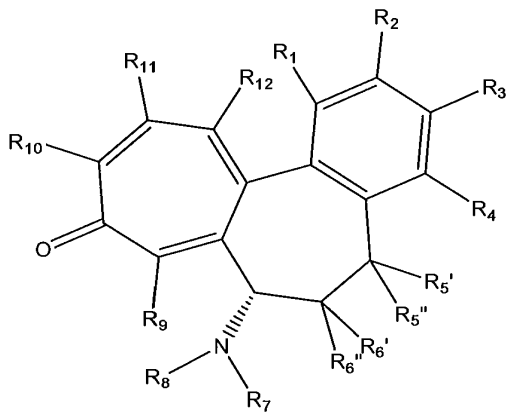
[00148] Numerous variations and modifications will suggest themselves to persons skilled in the relevant art, in addition to those already described, without departing from the basic inventive concepts. All such variations and modifications are to be considered within the scope of the present invention, the nature of which is to be determined from the foregoing description.

CLAIMS

1. A method for the treatment or prevention of a cardiovascular event in a subject with atherosclerotic vascular disease comprising the step of:

a) administering a therapeutically effective amount of a compound of formula (I), a known colchicine derivative and/or a salt thereof

Formula (I)



wherein:

R_1, R_2, R_3, R_4, R_9, R_{10}, R_{11} and R_{12} independently represent hydrogen, C_{1-4} alkyl, C_{2-4} alkenyl, C_{3-6} cycloalkyl, halogen, C_{1-4} haloalkyl, nitro, amino, C_{2-4} acylamino, C_{1-4} alkyl or dialkylamino, hydroxyl, C_{1-4} alkoxy, C_{1-4} alkylthio, a group of the formula $-SO_2N(R^x)_2$ or SO_2R^x where R^x is C_{1-4} alkyl, C_{1-4} acyloxy, or optionally substituted phenyl, optionally substituted phenoxy ;

R_7 and R_8 independently represent hydrogen, C_{1-4} alkyl or C_{1-4} acyl; and

R_5', $R_5"$, R_6' and $R_6"$ independently represent hydrogen, C_{1-4} alkyl, C_{2-4} alkenyl, C_{3-6} cycloalkyl, halogen, C_{1-4} haloalkyl, nitro, amino, C_{2-4} acylamino, hydroxyl, C_{1-4} alkoxy or C_{1-4} alkylthio a group of the formula $-SO_2N(R^x)_2$ or SO_2R^x where R^x is C_{1-4} alkyl, C_{1-4} acyloxy, or optionally substituted phenyl.

2. Use of a compound of formula (I), a known colchicine derivative and/or a salt thereof in the preparation of a medicament for the treatment or prevention of a cardiovascular event in a subject with atherosclerotic vascular disease.

3. A composition for the prevention or treatment of a cardiovascular event in a subject with atherosclerotic vascular disease, the composition comprising a therapeutically effective amount of a compound of formula (I), a known colchicine derivative and/or a salt thereof and one or more pharmaceutically acceptable additives, excipients carriers and/or diluents.

4. The composition of claim 3 comprising between 0.05 and 0.95 mg, between 0.1 and 0.9 mg, between 0.2 and 0.8 mg, or between 0.4 and 0.7 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof.

5. The composition of claim 4 comprising between 0.5 mg and 0.6 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof.

6. The composition of claim 4 comprising 0.5 mg or 0.6 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof.

7. The method of claim 1 wherein the compound of formula (I), a known colchicine derivative and/or a salt thereof is administered once or more per day.

8. A method for reducing or preventing cholesterol crystal induced inflammation within atherosclerotic plaques in a subject, comprising the step of

    administering a dosage of 0.5 mg or 0.6 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof.

9. The method of claim 8 wherein the subject has atherosclerotic vascular disease.

10. A regimen for the treatment or prevention of a cardiovascular event in a subject, the regimen comprising the step of:

    administering a dosage of 0.5 mg or 0.6 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof once per day.

11.A regimen for reducing or preventing cholesterol crystal induced inflammation within atherosclerotic plaques in a subject, the regimen comprising the step of:

administering a dosage of 0.5 mg or 0.6 mg of a compound of formula (I), a known colchicine derivative and/or a salt thereof once per day.

12.The regimen of claim 10 or 11 wherein the subject has atherosclerotic vascular disease.

13.The method of claim 1 or 9, use of claim 2, composition of claim 3 or regimen of claim 12 wherein the atherosclerotic vascular disease is a coronary disease.

14.The method of claim 1 or 8, use of claim 2, composition of claim 3 or regimen of claim 12 wherein the cardiovascular event is chosen from the list comprising: acute coronary syndrome (including ischemic chest pain, acute myocardial infarction, unstable angina); cardiac arrest; or stroke including non-cardio-embolic ischemic stroke.

15.The method of claim 1 or 8, use of claim 2, composition of claim 3 or regimen of claim 10 or 11 wherein the composition or medicament further comprises one or more additional agents selected from the list comprising: a statin and/or an anti-platelet agent.

16.The method, use, composition or regimen of claim 13 wherein the coronary disease is a clinically stable coronary disease.

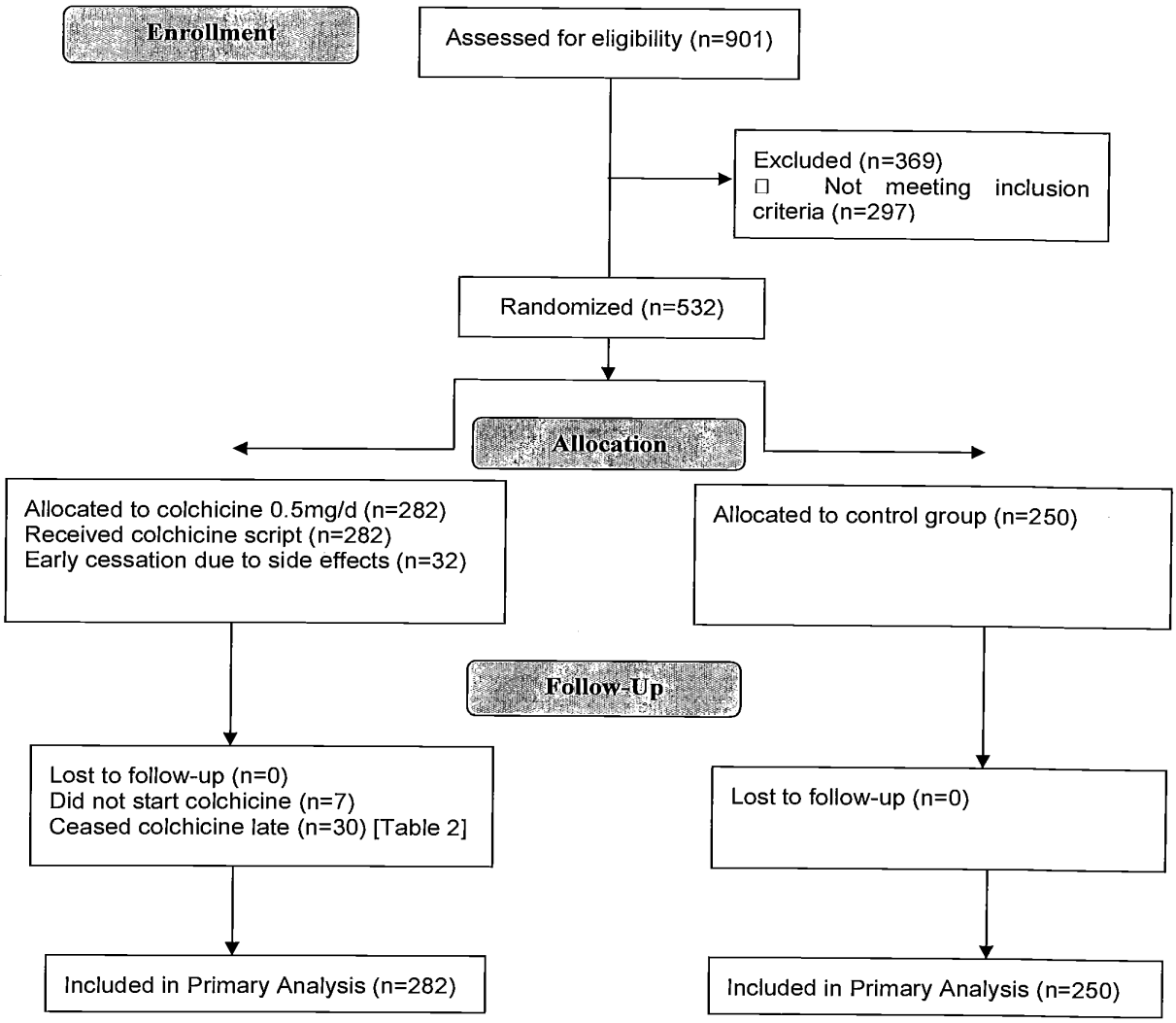
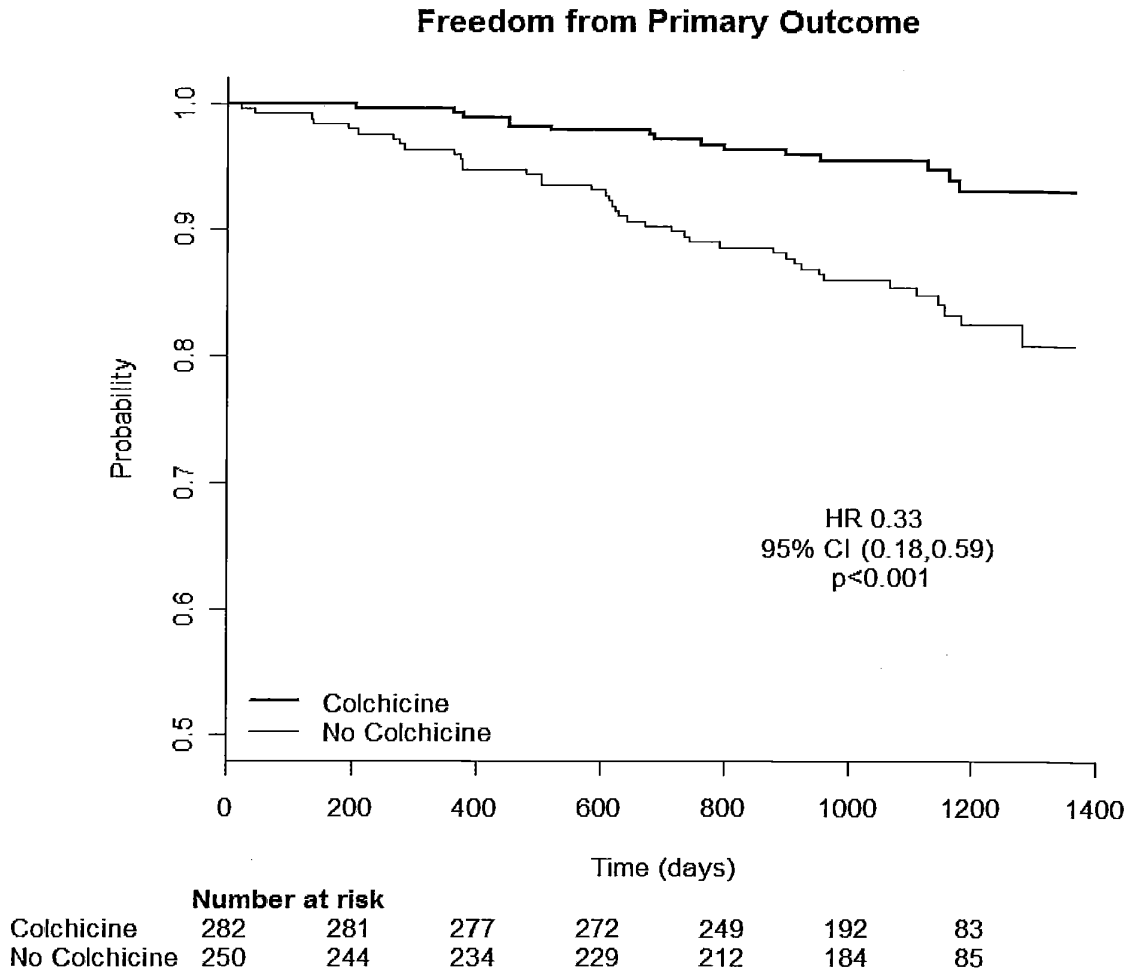

Enrollment
Assessed for eligibility (n=901)
Excluded (n=369)
□ Not meeting inclusion criteria (n=297)
Randomized (n=532)
Allocation
Allocated to colchicine 0.5mg/d (n=282)
Received colchicine script (n=282)
Early cessation due to side effects (n=32)
Allocated to control group (n=250)
Follow-Up
Lost to follow-up (n=0)
Did not start colchicine (n=7)
Ceased colchicine late (n=30) [Table 2]
Lost to follow-up (n=0)
Included in Primary Analysis (n=282)
Included in Primary Analysis (n=250)

FIGURE 1



Number at risk							
Colchicine	282	281	277	272	249	192	83
No Colchicine	250	244	234	229	212	184	85

FIGURE 2

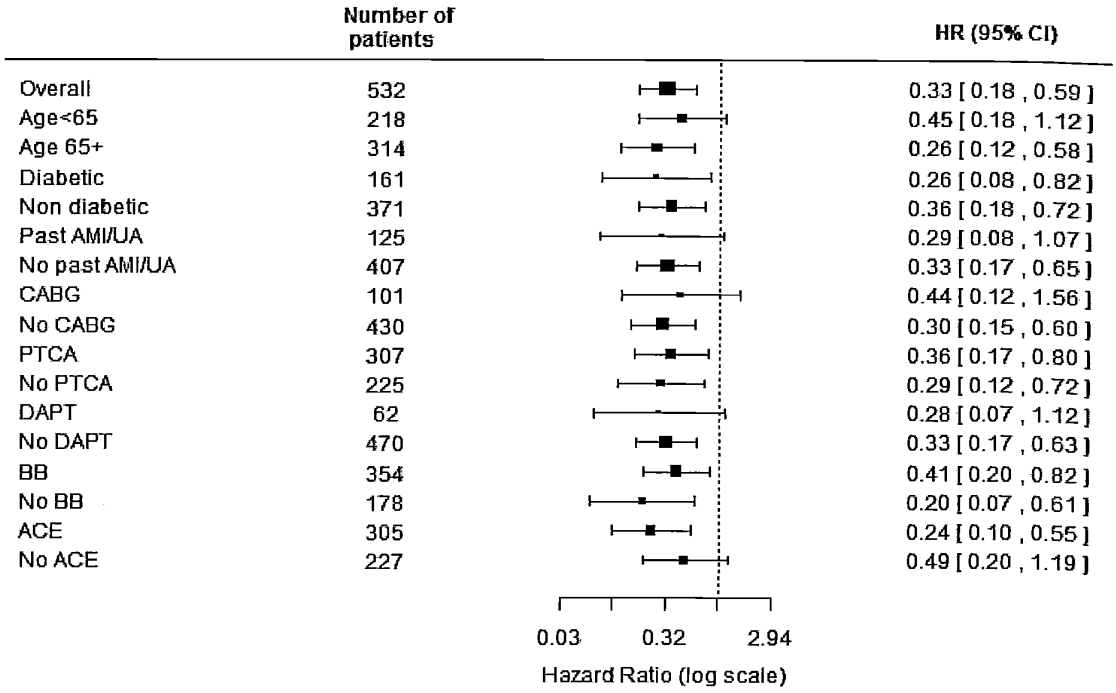

Number of patients
HR (95% CI)
Overall 532 0.33 [0.18 , 0.59]
Age<65 218 0.45 [0.18 , 1.12]
Age 65+ 314 0.26 [0.12 , 0.58]
Diabetic 161 0.26 [0.08 , 0.82]
Non diabetic 371 0.36 [0.18 , 0.72]
Past AMI/UA 125 0.29 [0.08 , 1.07]
No past AMI/UA 407 0.33 [0.17 , 0.65]
CABG 101 0.44 [0.12 , 1.56]
No CABG 430 0.30 [0.15 , 0.60]
PTCA 307 0.36 [0.17 , 0.80]
No PTCA 225 0.29 [0.12 , 0.72]
DAPT 62 0.28 [0.07 , 1.12]
No DAPT 470 0.33 [0.17 , 0.63]
BB 354 0.41 [0.20 , 0.82]
No BB 178 0.20 [0.07 , 0.61]
ACE 305 0.24 [0.10 , 0.55]
No ACE 227 0.49 [0.20 , 1.19]
0.03
0.32
2.94
Hazard Ratio (log scale)

FIGURE 3

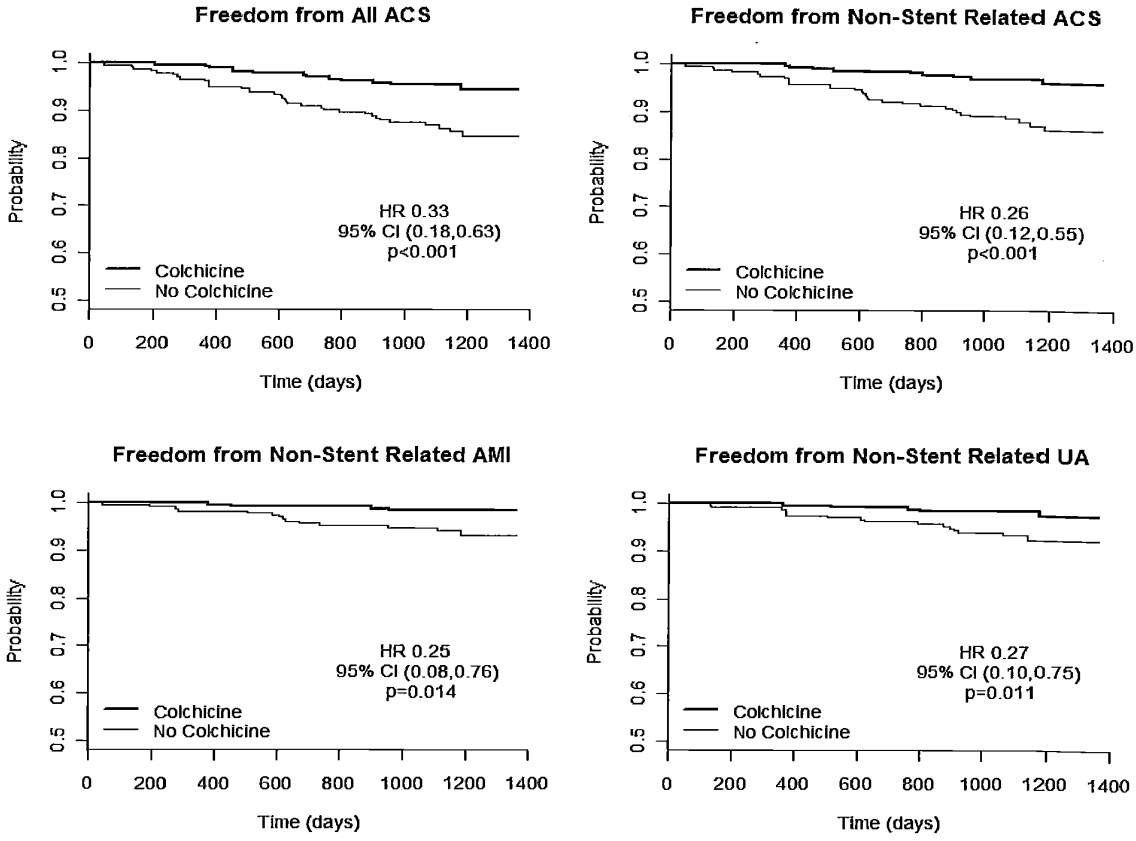

Freedom from All ACS
Probability
1.0
0.9
0.8
0.7
0.6
0.5
HR 0.33
95% CI (0.18,0.63)
p<0.001
Colchicine
No Colchicine
0
200
400
600
800
1000
1200
1400
Time (days)
Freedom from Non-Stent Related ACS
Probability
HR 0.26
95% CI (0.12,0.55)
p<0.001
Colchicine
No Colchicine
Time (days)
Freedom from Non-Stent Related AMI
Probability
HR 0.25
95% CI (0.08,0.76)
p=0.014
Colchicine
No Colchicine
Time (days)
Freedom from Non-Stent Related UA
Probability
HR 0.27
95% CI (0.10,0.75)
p=0.011
Colchicine
No Colchicine
Time (days)

FIGURE 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2013/001261

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/165 (2006.01) A61K 31/122 (2006.01) A61P 9/10 (2006.01)

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)

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)

Chemical Abstracts (STN); EPODOC, WPI, MEDLINE, NPL (EPOQUE): Structure derived from Compound of Formula (I), colchicine, colcrys, colchium, ANG453, ZD6126, cholesterol, atherosclerosis, cardiovascular, coronary, plaque, vascular, ischemia, myocardial, infarction, angina, cardiac, stroke, heart attack, and like terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] 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	Date of mailing of the international search report
16 December 2013	16 December 2013

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Form PCT/ISA/210 (fifth sheet) (July 2009)

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/AU2013/001261**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/131192 A2 (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK) 30 October 2008 Claims 9 to 11; paragraphs [0038] and [0050]	1 to 13 and 15 to 16
X	WO 2007/048004 A2 (CORNELL RESEARCH FOUNDATION, INC.) 26 April 2007 Claims 6, 8; third paragraph page 10, last paragraph page 9 to first paragraph page 10, second paragraph page 8	1 to 7, 10 and 12 to 16
X	WO 2010/046628 A1 (UNIVERSITY OF BRADFORD) 29 April 2010 see page 21 line 31 to page 22 line 16, page 4 line 28 to page 5 line 10	1 to 7, 10 and 12 to 16
X	US 2006/0286108 A1 (BELL, KATHERINE A.) 21 December 2006 Claims 1, 4 and 7	3 to 7
X	US 2011/0190397 A1 (DAVIS, MATTHEW W.) 04 August 2011 Claims 1 and 2	3 to 7

Form PCT/ISA/210 (fifth sheet) (July 2009)

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2013/001261**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2008/131192 A2	30 Oct 2008	None	
WO 2007/048004 A2	26 Apr 2007	WO 2007048004 A2	26 Apr 2007
WO 2010/046628 A1	29 Apr 2010	CN 102143762 A	03 Aug 2011
		EP 2349344 A1	03 Aug 2011
		JP 2012506408 A	15 Mar 2012
		US 2011275554 A1	10 Nov 2011
		WO 2010046628 A1	29 Apr 2010
US 2006/0286108 A1	21 Dec 2006	None	
US 2011/0190397 A1	04 Aug 2011	US 2010305172 A1	02 Dec 2010
		US 7906519 B2	15 Mar 2011
		US 2010305171 A1	02 Dec 2010
		US 7915269 B2	29 Mar 2011
		US 2010179169 A1	15 Jul 2010
		US 7964648 B2	21 Jun 2011
		US 2011190397 A1	04 Aug 2011
		US 8093297 B2	10 Jan 2012
		US 2011275720 A1	10 Nov 2011
		US 8440721 B2	14 May 2013
		US 2012208891 A1	16 Aug 2012
		US 8440722 B2	14 May 2013
		US 2012208881 A1	16 Aug 2012
		US 8445541 B2	21 May 2013
		US 2011039940 A1	17 Feb 2011
		US 2012065992 A1	15 Mar 2012

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

EDITORIALS

Inflammation as a Treatment Target after Acute Myocardial Infarction

L. Kristin Newby, M.D., M.H.S.

Inflammation is widely believed to play a central role in the pathogenesis of atherosclerosis and acute coronary events.[1,2] Although treatments with proven benefits for secondary prevention of coronary artery disease (e.g., statins and aspirin) appear to have salutary effects on inflammation, the identification of an effective treatment specifically targeting inflammation has been elusive.[3] The Canakinumab Antiinflammatory Thrombosis Outcomes Study (CANTOS) provided encouragement that targeting inflammation may be an effective treatment in chronic coronary artery disease. In CANTOS, canakinumab, a monoclonal antibody inhibiting interleukin-1β, reduced the level of high-sensitivity C-reactive protein and resulted in a modestly lower risk of the composite end point of death from cardiovascular causes, myocardial infarction, or stroke than did placebo.[4] Results were driven by a lower risk of nonfatal myocardial infarction, and there was a higher incidence of fatal infections among canakinumab-treated patients than among patients who received placebo, arousing concerns about risk–benefit trade-offs. Subsequently, nonspecific antiinflammatory treatment with methotrexate in the Cardiovascular Inflammation Reduction Trial (CIRT) showed no reduction in the level of high-sensitivity C-reactive protein and no benefit with regard to cardiovascular outcomes,[5] leaving open the question of whether direct intervention on inflammation can improve cardiovascular outcomes.

With this backdrop, Tardif and colleagues[6] now report in the *Journal* the results of the Colchicine Cardiovascular Outcomes Trial (COLCOT), which tested low-dose colchicine, an antiinflammatory agent clinically used to treat gout and pericarditis that works by inhibiting tubulin polymerization and microtubule formation, for secondary prevention after acute myocardial infarction. The investigators randomly assigned 4745 patients (mean age, 61 years) to receive either colchicine (0.5 mg daily) or placebo at a mean of 13.5 days after myocardial infarction; the median follow-up was 22.6 months. Participants had a high prevalence of guideline-recommended treatment: percutaneous coronary intervention for the index myocardial infarction in 93% of the patients, and the use of dual antiplatelet therapy and statins in 98% and 99%, respectively.

The risk of the primary composite end point (death from cardiovascular causes, resuscitated cardiac arrest, myocardial infarction, stroke, or urgent hospitalization for angina leading to coronary revascularization) was lower in the colchicine group than in the placebo group (hazard ratio, 0.77; 95% confidence interval [CI], 0.61 to 0.96; P=0.02), but this result was driven predominantly by lower risks of angina and stroke. A significant effect on death from cardiovascular causes or myocardial infarction was not shown. Overall, the incidence of adverse events or serious adverse events was similar in the two groups; however, some known gastrointestinal side effects of colchicine, including nausea, were more frequent in the colchicine group, as were pneumonias. However, unlike in CANTOS, no

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deaths from infection were reported in COLCOT, and the incidence of septic shock in COLCOT was low and similar in the two trial groups.

Although vital status was known in 99.5% of the patients in COLCOT, 1.9% of the patients were lost to follow-up and 0.6% withdrew consent. Furthermore, nearly 19% of the patients in both treatment groups stopped receiving colchicine or placebo prematurely (median treatment duration among patients discontinuing the trial regimen early, 7.1 months [interquartile range, 1.9 to 14.6] for colchicine and 6.1 months [interquartile range, 1.6 to 14.4] for placebo). This high percentage of patients discontinuing the trial regimen, combined with unknown endpoint status for 2.5% of the patients, could have obscured the true cardiovascular treatment effect or adverse-event profile. Results of a secondary per-protocol efficacy analysis were only modestly more favorable (hazard ratio, 0.71; 95% CI, 0.56 to 0.90), but these findings should be interpreted with caution because numerous biases may be at play in such nonrandomized comparisons; trial interpretation should principally be informed by the primary intention-to-treat analysis. Regardless, the modest benefit driven by a soft end point of hospitalization for angina and revascularization does not support the routine use of colchicine for secondary prevention without a better understanding of the absence of effect on death or myocardial infarction.

An interesting observation in this trial was the lower risk of stroke in the colchicine group than in the placebo group. Similar findings were reported in a systematic review and meta-analysis of four other randomized trials of colchicine.[7] Whether this reflects a unique mechanistic effect of colchicine in the cerebrovasculature or is simply the play of chance is a question for further investigation.

With the report of COLCOT, where does this leave us with inflammation as a therapeutic target? Direct inhibition of interleukin-1β with canakinumab reduced the level of high-sensitivity C-reactive protein as expected but resulted in only a modest composite efficacy benefit driven by myocardial infarction with a worrisome infection risk; nonspecific antiinflammatory treatment with methotrexate had no effect on the high-sensitivity C-reactive protein level or cardiovascular outcomes; and colchicine, which acts through a unique mechanism, did not lower the high-sensitivity C-reactive protein level more than placebo did in the small subgroup of patients with available data but had a modest composite efficacy benefit, albeit driven predominantly by an angina end point. These observations do not lessen the overall importance of inflammation in atherosclerosis, nor should they halt efforts to find more effective antiinflammatory agents with better side-effect profiles. However, they should encourage us to revisit whether inflammation itself is the best treatment target or whether opportunities exist to identify and better understand pathophysiological processes or mediators that incite inflammation and drive atherosclerosis in order to intervene further upstream.

Disclosure forms provided by the author are available with the full text of this editorial at NEJM.org.

From the Division of Cardiology and Duke Clinical Research Institute, Duke University Medical Center, Durham, NC.

This editorial was published on November 16, 2019, at NEJM.org.

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DOI: 10.1056/NEJMe1914878
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Low-Dose Colchicine for Secondary Prevention of Cardiovascular Disease

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Perth, Australia; and Hamilton, Ontario, Canada

Objectives The objective of this study was to determine whether colchicine 0.5 mg/day can reduce the risk of cardiovascular events in patients with clinically stable coronary disease.

Background The presence of activated neutrophils in culprit atherosclerotic plaques of patients with unstable coronary disease raises the possibility that inhibition of neutrophil function with colchicine may reduce the risk of plaque instability and thereby improve clinical outcomes in patients with stable coronary disease.

Methods In a clinical trial with a prospective, randomized, observer-blinded endpoint design, 532 patients with stable coronary disease receiving aspirin and/or clopidogrel (93%) and statins (95%) were randomly assigned colchicine 0.5 mg/day or no colchicine and followed for a median of 3 years. The primary outcome was the composite incidence of acute coronary syndrome, out-of-hospital cardiac arrest, or noncardioembolic ischemic stroke. The primary analysis was by intention-to-treat.

Results The primary outcome occurred in 15 of 282 patients (5.3%) who received colchicine and 40 of 250 patients (16.0%) assigned no colchicine (hazard ratio: 0.33; 95% confidence interval [CI] 0.18 to 0.59; $p < 0.001$; number needed to treat: 11). In a pre-specified secondary on-treatment analysis that excluded 32 patients (11%) assigned to colchicine who withdrew within 30 days due to intestinal intolerance and a further 7 patients (2%) who did not start treatment, the primary outcome occurred in 4.5% versus 16.0% (hazard ratio: 0.29; 95% CI: 0.15 to 0.56; $p < 0.001$).

Conclusions Colchicine 0.5 mg/day administered in addition to statins and other standard secondary prevention therapies appeared effective for the prevention of cardiovascular events in patients with stable coronary disease. (J Am Coll Cardiol 2013;61:404–10) © 2013 by the American College of Cardiology Foundation

Despite routine use of antiplatelet and statin therapy, patients with coronary disease continue to be at risk of cardiovascular events, possibly because these treatments fail to target some of the inflammatory pathways implicated in the disease. The atherosclerotic wall is subject to injurious forces that promote plaque instability. The response to injury within the diseased vessel is dependent on the architecture and content of atherosclerotic plaques (1,2). Lipid-rich plaques with a neovascular base are particularly susceptible to the effect of injury, which may leave them vulnerable to neutrophil infiltration (3). Neutrophils that enter the interstitial space may become activated upon exposure to the plaque contents, inciting an aggressive inflammatory response that may accelerate plaque instability, increasing the risk of plaque enlargement and rupture and hence increasing the risk of clinical events (3).

See page 411

This dynamic sequence of events raises the possibility that inhibition of neutrophil function may lead to a reduction in the risk of plaque instability and thereby reduction in the risk of disease progression. Colchicine has anti-inflammatory properties, including an antitubulin effect that inhibits neutrophil function (4), and this effect is believed to account for the efficacy of colchicine in the prevention of acute manifestations of gout and familial Mediterranean fever (FMF). Patients with FMF receive lifelong colchicine therapy at a dose of 1 to 2 mg/day, which is well tolerated (5,6). Although short-term use of low-dose colchicine has been found to have no effect on

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Manuscript received August 24, 2012; revised manuscript received October 18, 2012, accepted October 23, 2012.

stent-related disease (7), no studies have been performed to determine whether long-term use of low-dose colchicine can be tolerated or can reduce the risk of de novo vascular events caused by disruption of native atherosclerotic plaques in patients with stable coronary disease.

We therefore conducted a prospective, randomized, observer-blinded endpoint (PROBE) trial to determine whether adding colchicine 0.5 mg/day to standard secondary prevention therapies including aspirin and high-dose statins reduces the risk of cardiovascular events in patients with objectively diagnosed and clinically stable coronary disease.

Methods

Study conduct and design. The LoDoCo (Low-Dose Colchicine) trial was conducted under the auspices of the Heart Research Institute of Western Australia. It was designed by the principal investigators, registered with the Australian Clinical Trial Registry (ACTRN 12610000293066), and received ethics approval from the Human Research Ethics Committee at Sir Charles Gairdner Hospital, Perth, Western Australia in July 2008. There was no external funding source.

We employed a PROBE design (8). Eligible consenting patients with established coronary disease presenting for routine clinical review were randomized to receive colchicine 0.5 mg/day or no colchicine without any other changes to their medical therapy. All outcomes were evaluated by an experienced adjudicator blinded to the treatment allocation.

Study size and eligibility. The study population was planned to include 250 patients randomized to the control group and 250 patients randomized to treatment who were tolerant of colchicine for at least 4 weeks after the date of their randomization.

Patients were eligible for inclusion if they met each of the following criteria: 1) angiographically proven coronary disease; 2) age 35 to 85 years; 3) clinically stable for at least 6 months; 4) no major competing comorbidities or contraindication to colchicine therapy; 5) considered to be compliant with therapy and attending routine cardiology follow-up appointments; and 6) willing to provide consent and be randomized into the study. Patients with a history of bypass surgery were only eligible if they had undergone bypass surgery more than 10 years before, had angiographic evidence of graft failure, or had undergone stenting since their bypass surgery. All patients signed informed consent before randomization.

Randomization. The randomization sequence was computer generated, concealed from the investigators at all times, and managed by a research assistant who had no involvement in the evaluation or management of study patients. Once the assistant received the consent form, the patients' demographic data were entered into the database, and the investigators and patients were advised in writing of the treatment group to which the patient had been assigned.

Despite use of the lowest dose of colchicine available, it was anticipated that a number of patients would withdraw from therapy early after randomization because of gastrointestinal side effects. To ensure that the requisite number of patients in the treatment arm were actually tolerant of treatment, the protocol allowed for the research assistant to assign a newly recruited patient to treatment if a patient discontinued colchicine because of side effects in the first month. Patients who were intolerant of therapy remained in the study, were followed in the usual manner, and were included in the primary intent-to-treat analysis.

Abbreviations and Acronyms

ACS = acute coronary syndrome(s)
AMI = acute myocardial infarction
FMF = familial Mediterranean fever
HR = hazard ratio
MI = myocardial infarction
PROBE = prospective, randomized, observer-blinded endpoint
UA = unstable angina

Intervention. Patients randomized to active treatment were given a prescription for colchicine 0.5 mg daily by their referring cardiologist. The drug was dispensed by their usual chemist, and if requested, patients were reimbursed for the cost of these prescriptions. All other treatments were continued as usual.

Follow-up and definition of clinical outcomes. Patient compliance with treatment and outcome data were collected at routine follow-up visits and at the time of any unplanned hospital admissions.

Acute coronary syndrome (ACS) was defined as either: 1) acute myocardial infarction (AMI), as evidenced by acute ischemic chest pain associated with a rise in serum troponin above the upper limit of normal (9); or 2) unstable angina (UA), as evidenced by a recent acceleration of angina unassociated with a rise in serum troponin but associated with angiographic evidence of a change in the patient's coronary anatomy (UA Braunwald classification types IB and IIB) (10). ACS was characterized as being stent related if there was evidence of significant in-stent stenosis or acute stent thrombosis. Out-of-hospital cardiac arrest was defined as: 1) a sudden death as evidenced on the patient's death certificate; or 2) a nonfatal out-of-hospital cardiac arrest, defined as a recovery from sudden collapse associated with documented asystole, ventricular tachycardia, or ventricular fibrillation. Noncardioembolic ischemic stroke was defined as computed tomography—or magnetic resonance imaging—proven ischemic stroke judged by the treating neurologist as not being due to atrial fibrillation or intracranial hemorrhage. The primary efficacy outcome was the composite of ACS, fatal or nonfatal out-of-hospital cardiac arrest, or noncardioembolic ischemic stroke. Secondary outcomes were individual components of the primary outcome and the components of ACS unrelated to stent disease.

Timelines. The pre-specified study duration was a minimum follow-up of 2 years in all patients. Accordingly, the study was closed on May 31, 2012. During May, all living patients were contacted by phone to collect compliance and outcome data from the last date of follow-up. Final outcome

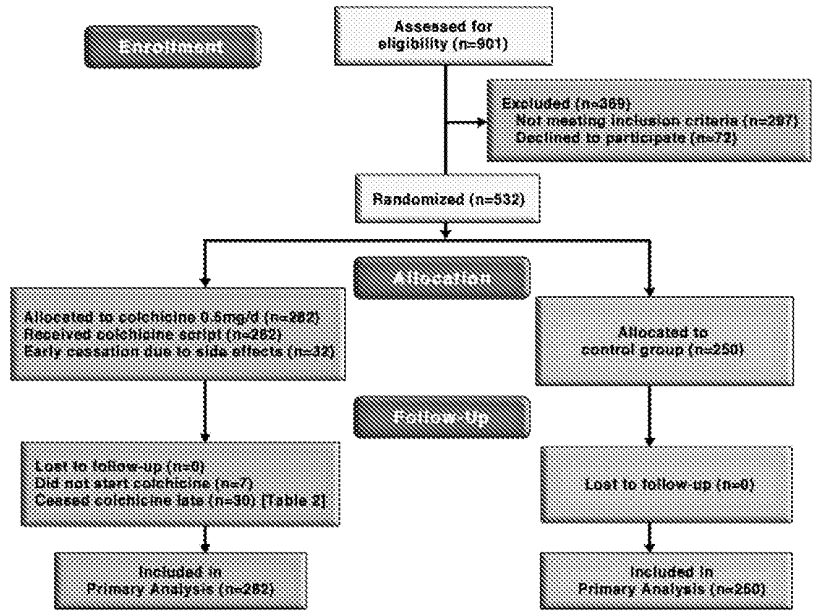


Figure 1 The CONSORT Flow Diagram

The CONSORT diagram showing the number of participants who were randomized, lost to follow-up, and included in the primary data analysis.

data were available for all patients, and no patients were lost to follow-up.

Statistical power. Assuming that the control group had a combined event rate (ACS, out-of-hospital cardiac arrest, or noncardioembolic ischemic stroke) of 8% (11), an accrual interval of 2 years, and a follow-up after the accrual interval of 2 years, the planned sample size provided >80% power to detect a hazard ratio (HR) of ≤0.50 based on a 2-sided significance level of 5%.

Data analysis. Summary statistics, including mean and standard deviation, were calculated for all baseline characteristics by treatment arm. All time-to-event outcomes were calculated in days by subtracting the date of randomization from either the: 1) date of event or death; or 2) trial termination date for those patients not experiencing the defined event.

As pre-specified, the primary efficacy analysis was based on the intent-to-treat principle. The intent-to-treat analysis included all randomized patients and all events during the time from randomization to trial termination. The trial termination date was fixed as May 31, 2012. A secondary pre-specified on-treatment analysis was also performed, based on patients who were both tolerant and compliant to therapy beyond the first month of randomization. All events during the time from randomization until noncompliance with the colchicine treatment regime were included in this analysis.

The time to first event for all outcomes is presented using a Kaplan-Meier plot. The primary efficacy outcome was analyzed using a Cox proportional hazards model including treatment group coded as control or colchicine. The secondary outcomes were analyzed similarly. In addition, the primary analysis was stratified by sex, age, diagnosis of diabetes, past myocardial infarction (MI), UA, coronary bypass surgery, coronary angioplasty, and therapy with aspirin, clopidogrel, or both; high-dose statin therapy (defined as a dose of statin equivalent to atorvastatin 40 mg or more); beta-blockers; calcium blockers; and angiotensin-converting enzyme inhibitors.

Results

Study population. Between August 2008 and May 2010, 901 patients with stable coronary disease attending routine outpatient cardiology appointments were assessed for eligibility for the study. Of these, 297 (33%) did not meet the entry criteria, 72 (8%) declined to participate, and 532 (59%) were enrolled into the study, 250 of whom were randomized to the control group and 282 to treatment. Of those randomized to treatment, 32 (11%) reported early intolerance due to gastrointestinal side effects and 7 patients subsequently reported that they chose to not start therapy (Fig. 1). All 532 randomized patients were followed for the duration of the study period, which ranged from a minimum of 24 to a maximum of 44 months. Median follow-up was 36 months. Baseline characteristics according to randomized treatment group are shown in Table 1. Both groups were well matched for important clinical characteristics, although more patients in the colchicine group were taking

Table 1 Study Population

	Control	Treatment
Total	250	282
Mean age, yrs	67 ± 9.2	66 ± 9.6
Male	222 (89)	251 (89)
Diabetes	69 (28)	92 (33)
Smoker	14 (6)	10 (4)
Past AMI or UA	61 (24)	64 (23)
CABG	39 (16)	62 (22)
PTCA	138 (55)	169 (60)
Aspirin and/or clopidogrel	235 (94)	262 (93)
DAPT	24 (10)	38 (13)
High-dose statin	235 (94)	271 (96)
Beta-blocker*	178 (71)	176 (62)
Calcium channel blocker†	25 (10)	52 (18)
ACE inhibitor	150 (60)	155 (55)

Values are n, mean ± SD, or n (%). *$p < 0.05$. †$p < 0.01$ for the comparison of the distribution between treatment and control.

ACE = angiotensin-converting enzyme; AMI = acute myocardial infarction; CABG = coronary artery bypass surgery; DAPT = dual antiplatelet therapy (aspirin and clopidogrel); PTCA = percutaneous coronary angioplasty; UA = unstable angina.

calcium channel blockers and fewer were on beta-blocker therapy. Almost all patients in each group were on antiplatelet therapy and high-dose statin therapy.

Late discontinuations. Thirty patients ceased colchicine therapy after a mean period of 2.36 years. Therapy was ceased due to an unrelated intercurrent illness in 11 patients, by choice in 5, and for a variety of possible drug-related effects in 14 patients (4.9%) as described in Table 2.

Outcomes. A primary outcome occurred in 55 of 532 patients, including 15 of 282 patients (5.3%) assigned to colchicine treatment and 40 of 250 patients (16%) assigned to the control group (HR: 0.33; 95% confidence interval [CI] 0.18 to 0.59; $p < 0.001$; number needed to treat: 11). A sensitivity analysis was performed for the primary outcome and adjusted for the usage of calcium channel blockers and beta-blocker therapy. These results were consistent with the primary analysis.

The time to first clinical event in each group by treatment is shown in Figure 2. The effect of colchicine on the primary outcome was evident early, and the benefits of colchicine continued to accrue throughout the follow-up period. Results of the primary analysis were consistent in all subgroups examined (Fig. 3). There was no evidence of differential treatment effects based on any of the clinical or therapeutic variables.

Table 2 Withdrawals From Therapy

Early withdrawals	32 (11)
Late withdrawals*	30 (11)
Unrelated intercurrent illness	11 (3.9)
Patient choice	5 (1.8)
Perceived side effects	
Intestinal upset	7 (2.5)
Myalgia	2 (0.90)
Myositis	1 (<0.5)
Rash	1 (<0.5)
Alopecia	1 (<0.5)
Itch	1 (<0.5)
Peripheral neuritis	1 (<0.5)

Values are n (%). *Withdrawals after 30 days; average time to withdrawal was 2.36 years.

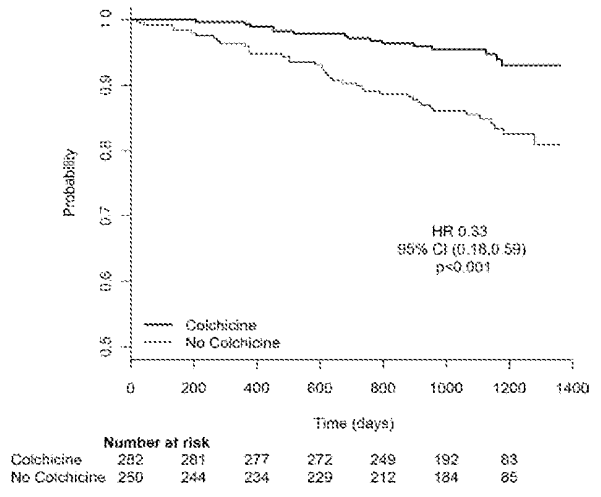


Figure 2 Freedom From the Primary Outcome

Freedom from the primary outcome (acute coronary syndrome, out-of-hospital cardiac arrest, or noncardioembolic ischemic stroke) by treatment. CI = confidence interval; HR = hazard ratio.

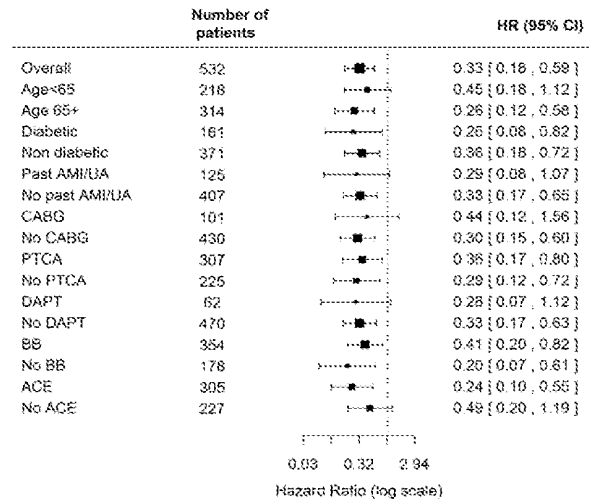


Figure 3 Forest Plot

The hazard ratios and 95% CI of the primary outcome in subgroups defined by patient demographics, past medical history, and concomitant treatments. ACE = angiotensin-converting enzyme inhibitor; AMI = acute myocardial infarction; BB = beta-blocker; CABG = coronary artery bypass graft; DAPT = dual antiplatelet therapy; PTCA = percutaneous coronary angioplasty; UA = unstable angina; other abbreviations as in Figure 2.

Table 3 Primary Outcome and Its Components

	Control (n = 250)	Treatment (n = 282)	HR (95% CI)	p Value
Primary outcome	40 (16)	15 (5.3)	0.33 (0.18–0.59)	<0.001
Components of primary outcome				
Acute coronary syndrome	34 (13.6)	13 (4.6)	0.33 (0.18–0.63)	<0.001
OOH cardiac arrest	2 (0.8)	1 (0.35)*	0.47 (0.04–5.15)	0.534
Noncardioembolic stroke	4 (1.6)	1 (0.35)	0.23 (0.03–2.03)	0.184
Components of ACS				
Stent-related	4 (1.6)	4 (1.4)		NS
Nonstent-related	30 (12)	9 (3.2)	0.26 (0.12–0.55)	<0.001
Nonstent-related AMI	14 (5.6)	4 (1.6)	0.25 (0.08–0.76)	0.014
Nonstent-related UA	16 (12)	5 (2.4)	0.27 (0.10–0.75)	0.011

Values are n (%). *Nonfatal.
ACS = acute coronary syndrome; NS = nonsignificant; OOH = out of hospital; other abbreviations as in Table 1.

The reduction in the primary outcome was largely driven by the reduction in the number of patients presenting with ACS (13 of 282 [4.6%] vs. 34 of 250 [13.4%]; HR: 0.33; 95% CI: 0.18 to 0.63; $p < 0.001$). Out-of-hospital cardiac arrest and noncardioembolic ischemic stroke were infrequent, but their numbers were also reduced in the treatment group (Table 3).

Of the 47 patients who presented with ACS, the event was stent related in 8 (17%; 2 in each group had evidence of acute stent thrombosis, and 2 in each group had evidence of significant in-stent stenosis). Further analysis confirmed that patients randomized to treatment were less likely to present with ACS unrelated to stent disease (9 of 282 [3.2%] vs. 30 of 250 [12%]; HR: 0.26; 95% CI: 0.12 to 0.55; $p < 0.001$), be it associated with AMI (4 of 282 [1.4%] vs. 14 of 250 [5.6%]; HR: 0.25; 95% CI: 0.08 to 0.76; $p = 0.014$) or UA (5 of 282 [1.8%] vs. 16 of 250 [6.4%]; HR: 0.27; 95% CI: 0.10 to 0.75; $p = 0.011$) (Fig. 4, Table 3).

Of 39 patients randomized to treatment who did not receive therapy beyond the first month because of early intolerance or noncompliance, 4 (10%) presented with ACS due to acute stent thrombosis (n = 1) and UA (n = 3). Patients who were both compliant and tolerant to therapy beyond the first month of randomization had significantly fewer events than the control patients (11 of 243 [4.5%] vs. 40 of 250 [16%]; HR: 0.29; 95% CI: 0.15 to 0.56; $p < 0.001$). The results of all on-treatment analyses were consistent with those based on intent-to-treat analyses (Table 4).

Ten patients in the control group died compared with 4 patients in the colchicine group. Of the 10 control patients, 5 died of presumed cardiac cause, 2 following an out-of-hospital cardiac arrest, 2 from cardiogenic shock following AMI, and 1 following bypass surgery. All 4 patients in the colchicine group died of noncardiac causes.

Discussion

The LoDoCo trial demonstrated that the addition of colchicine 0.5 mg/day to standard therapy in patients with stable coronary disease significantly reduced the risk of a cardiovascular event, including ACS, out-of-hospital cardiac arrest, and noncardioembolic ischemic stroke. The benefits of colchicine were achieved on a background of widespread use of effective secondary prevention strategies, including high-dose statins, as evidenced by the low event rate in the control group (11). The effect of adding colchicine became evident early, continued to accrue over time, and was largely driven by a reduction in ACS unrelated to stent disease.

These results are important because they suggest that colchicine, in contrast to its apparent lack of effect in the prevention of stent-related disease (7), may have a role in the prevention of cardiovascular events caused by instability of native atherosclerotic plaques in patients with stable coronary disease, possibly by inhibiting an inflammatory pathway that has been indentified in unstable native coronary plaques (3).

The presence of activated neutrophils in the plaques of patients with unstable coronary syndromes (3) suggested that they may play a key role in the transformation of a stable, to an unstable plaque. Although the mechanism of benefit of colchicine was not the subject of this study, the drug is known to have protean effects that may be responsible for the improved clinical outcome of patients observed in this study, the best documented of which is the inhibition of neutrophil chemotaxis, ingress, and activation within a proinflammatory environment (12) such as may exist in an unstable plaque.

Indirect support for a beneficial effect of colchicine on cardiovascular disease comes from retrospective observations that continuous use of colchicine was associated with a lower than expected risk of AMI in patients with FMF (13) and gout (14), and the demonstration that low-dose colchicine can reduce levels of high-sensitivity C-reactive protein in patients with stable coronary disease (15).

Although most physicians are familiar with the short-term use of colchicine from its widespread use in gout and pericarditis (16), few will have prescribed it continuously to their patients. However, the long-term use of colchicine, at doses of 1 to 2 mg/day, has been well documented to be safe and reasonably well tolerated in patients with FMF (17).

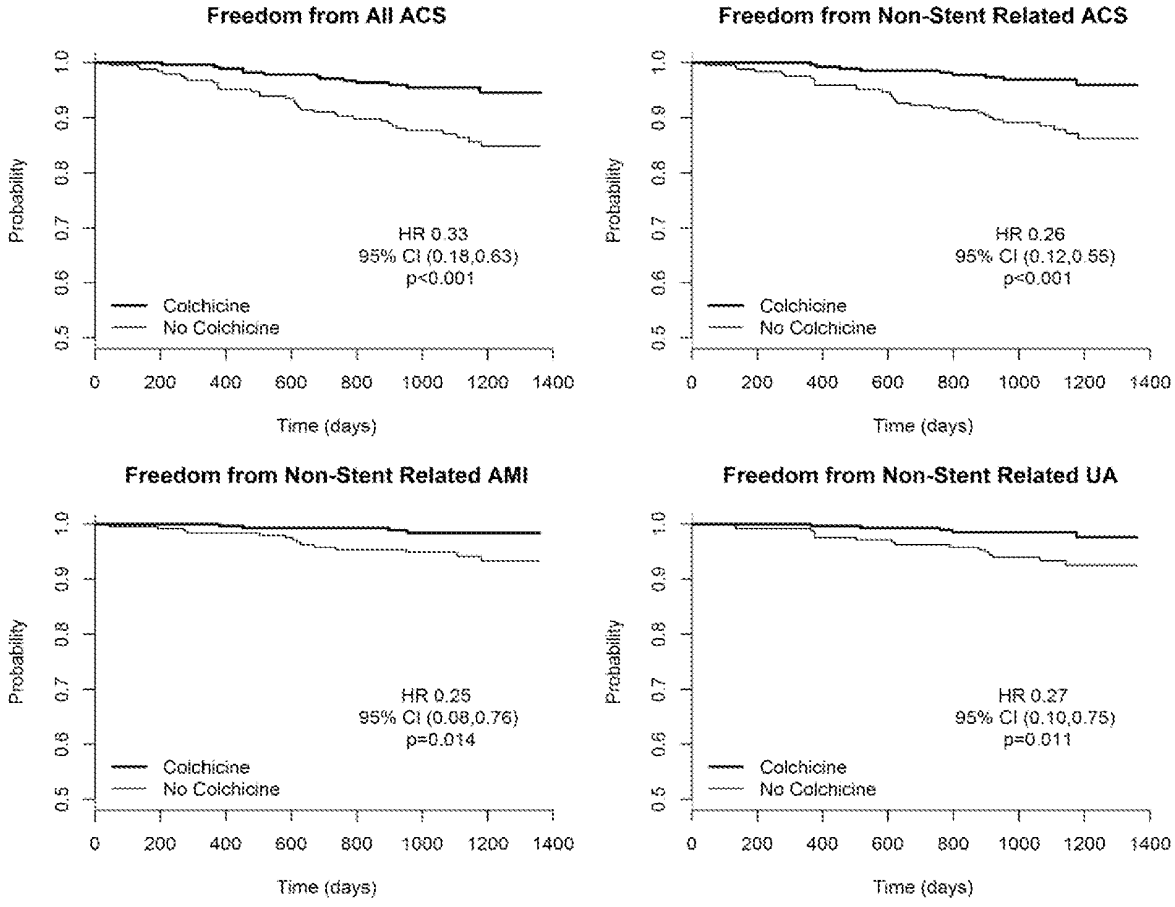


Figure 4 Freedom From Acute Coronary Syndrome

Freedom from acute coronary syndrome (ACS) and components of this outcome (AMI and UA) unrelated to stent disease. Abbreviations as in Figures 2 and 3.

In this study, despite use of the lowest available dose of colchicine, 11% of patients withdrew from therapy early due to intestinal intolerance, and a further 5% ceased therapy late due to a range of possible side effects, indicating that widespread use of the drug may be limited by its side effects. In this regard, it is important to appreciate that combining colchicine with statin therapy has been reported to increase the risk of myalgia and rarely acute rhabdomyolysis in

Table 4 Primary Outcome and Its Components (On-Treatment Analysis)

	Control (n = 250)	Treatment (n = 243)	HR (95% CI)	p Value
Primary outcome	40 (16)	11 (5.3)	0.29 (0.15–0.56)	<0.001
Components of primary outcome				
Acute coronary syndrome	34 (13.6)	9 (4.6)	0.28 (0.13–0.58)	<0.001
OOH cardiac arrest	2 (0.8)	1 (0.35)*	0.55 (0.05–6.03)	0.622
Noncardioembolic stroke	4 (1.6)	1 (0.35)	0.27 (0.03–2.42)	0.242
Components of ACS				
Stent-related	4 (1.6)	3 (1.4)		NS
Nonstent-related	30 (12)	6 (3.2)	0.21 (0.09–0.50)	<0.001
Nonstent-related AMI	14 (5.6)	4 (1.6)	0.30 (0.10–0.91)	0.033
Nonstent-related UA	16 (12)	2 (2.4)	0.13 (0.03–0.57)	0.007

Values are n (%). *Nonfatal.
Abbreviations as in Tables 1 and 3.

patients with renal impairment (18). Further, because colchicine has a narrow therapeutic index, extreme care must be taken to avoid accidental overdoses, which may be fatal. Hence, patients receiving regular colchicine therapy require close clinical supervision.

Despite these caveats, colchicine may be an attractive therapy for secondary prevention of cardiovascular events because it is simple to use and inexpensive, it appears to be highly effective, and long-term use rarely leads to any major irreversible long-term toxicity. Before the drug is adopted clinically, however, it would be important for the findings of this hypothesis-generating study to be confirmed in larger studies of patients with coronary atherosclerosis.

Study limitations. This study has a number of limitations. The use of a PROBE design had the advantages of convenience, reduced cost, and fewer safety concerns; however, it may be subject to outcome ascertainment and reporting bias. Although the decision to allow the research assistant to assign additional patients to treatment if a patient reported early intolerance to therapy had the potential to introduce bias to the randomization process, this was largely avoided because at all times, the investigators remained unaware as to whether a newly recruited patient would be randomized to colchicine or control or would be replacing an intolerant patient. Further, because the treatment groups remained well matched at baseline, the primary events occurred over a 3- to 4-year period after randomization, all randomized patients were followed and included in the primary intent-to-treat analysis, and the results are extremely robust, it is extremely unlikely that our approach materially affected the results or conclusions.

The results of this study were almost entirely driven by an effect of colchicine on nonfatal cardiovascular events that disrupt patients' lives, portend their prognosis, and are responsible for a significant cost burden on the community. Although a favorable trend toward improved mortality was demonstrated, much larger studies are required to confirm whether colchicine may actually reduce the risk of fatal cardiac events. It would be of special interest to examine the value of initiating colchicine in patients recently hospitalized for ACS because they remain at particularly high risk of recurrent events for several months due to disruption of both culprit and nonculprit plaques (19).

Conclusions

In summary, colchicine 0.5 mg/day given in addition to high-dose statins and other standard secondary prevention therapies appeared effective for the prevention of cardiovascular events in patients with stable coronary disease. The major implications of these findings are that it may be possible to reduce the risk of cardiovascular events in patients with stable coronary disease by targeting an inflammatory pathway that has been identified in native unstable atherosclerotic plaques using a low dose of colchicine. Despite the problems with early intolerance, it would appear that colchicine is a worthy candidate drug for future secondary prevention trials in patients with stable coronary disease.

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Key Words: colchicine ▪ secondary prevention ▪ stable coronary disease.

Why Colchicine Should Be Considered for Secondary Prevention of Atherosclerosis: An Overview

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ABSTRACT

Purpose: Colchicine is a widely available, inexpensive drug with a range of antiinflammatory properties that may make it suitable for the secondary prevention of atherosclerosis. This review examines how past and contemporary approaches to antiinflammatory therapy for atherosclerosis have led to a better understanding of the nature of the disease and sets out the reasons why colchicine has the potential to become a cornerstone therapy in its management.

Methods: We performed a literature search using PubMed, the Cochrane library, and clinical trial registries to identify completed and ongoing clinical studies on colchicine in coronary artery disease, and a PubMed search to identify publications on the mechanism of action of colchicine relevant to atherosclerosis.

Findings: A large body of data confirms that inflammation plays a pivotal role in atherosclerosis. The translation of this extensive knowledge into improved clinical outcomes has until recently been elusive. Findings from statin trials support the possibility that targeting inflammation may be beneficial, but this evidence has been inconclusive. Direct inhibition of atherosclerotic inflammation is being explored in current clinical trials. Targeted inhibition of interleukin 1β with canakinumab provided the proof of principle that limiting inflammation can improve outcomes in atherosclerotic vascular disease, but long-term treatment with a monoclonal antibody is unlikely to have widespread uptake. Other approaches using agents with a wider set of targets are being explored. Findings from observational studies suggest that methotrexate may reduce cardiovascular risk in patients with rheumatoid arthritis, but CIRT (Cardiovascular Inflammation Reduction Trial) demonstrated that methotrexate provided no cardiovascular benefit in patients with atherosclerotic vascular disease. Recent demonstration that cholesterol crystals trigger the NLRP3 (nucleotide oligomerization domain–, leucine-rich repeat–, and pyrin domain–containing protein 3) inflammasome and the release of inflammatory cytokines that also drive uric acid crystal–induced inflammation indicates that the multiple actions of colchicine that make it effective in gout may be relevant to preventing inflammation and limiting inflammatory injury in atherosclerosis. The ongoing LoDoCo2 (Low Dose Colchicine2) and COLCOT (Colchicine Cardiovascular Outcomes Trial) trials and several other planned large-scale rigorous trials will determine the long-term tolerability and efficacy of low-dose colchicine for secondary prevention in patients with coronary disease.

Implication: Colchicine holds promise as an important, accessible drug that could be successfully repurposed for the secondary prevention of atherosclerotic cardiovascular disease should its tolerability and cardiovascular benefits be confirmed in ongoing clinical trials. (*Clin Ther.* 2019;41:41–48)
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Keywords: atherosclerosis, cardiovascular disease, colchicine, prevention.

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INTRODUCTION

Colchicine is a widely available, inexpensive drug with a range of antiinflammatory properties that may make it suitable for the secondary prevention of atherosclerosis. This review examines how past and contemporary approaches to antiinflammatory therapy for atherosclerosis have led to a better understanding of the nature of the disease and sets out the reasons why colchicine has the potential to become a cornerstone therapy in its management.

MATERIALS AND METHODS

Using the key terms colchicine, atherosclerosis, coronary artery disease, and prevention, we performed a literature search using PubMed, the Cochrane library, and clinical trial registries to identify completed and ongoing clinical studies on colchicine in coronary artery disease. A PubMed search was also used to identify articles on the mechanism of action of colchicine relevant to atherosclerosis, published between xxx and xxx.

RESULTS

Lessons From Past and Contemporary Approaches to Antiinflammatory Therapy for the Secondary Prevention of Cardiovascular Disease

Over the past 20 years, a large body of data has amassed confirming that inflammation plays a pivotal role in atherosclerosis.[1,2] Three main lines of evidence have supported the concept. First, the work of pathologists Ross in United States[3] and Davies in United Kingdom[3] showed that infiltration of atheroma with inflammatory cells was an invariable part of the initiation and growth of atherosclerotic plaque. Second, Libby[4] demonstrated that enhanced accumulation of inflammatory cells was commonly seen in unstable plaques, suggesting that inflammation was potentially causal as a final step of plaque rupture. Finally, Ridker and others[5,6] demonstrated in multiple cohorts that high-sensitivity C-reactive protein (hs-CRP), a nonspecific biomarker of inflammation produced in the liver in response to interleukin (IL)-6, could be used to identify groups of patients with stable coronary disease who are at high risk for acute coronary events.

The translation of this extensive knowledge into improved clinical outcomes has until recently been elusive. Evidence to support the antiatherosclerotic efficacy of agents including corticosteroids, NSAIDs, and some biologic disease-modifying anti-rheumatic drugs that specifically target a number of inflammatory mediators (eg, tumor necrosis factor α, IL-6, and IL-1) not only failed to demonstrate any clear benefit on cardiovascular outcomes but raised the possibility that targeting such pathways may be associated with an increased risk for adverse off-target events.[7,8]

Indeed, until 5 years ago, only indirect evidence from statin trials continued to support the possibility that directly targeting inflammation in atherosclerosis might truly have merit. Trials of statins in both primary[9] and secondary[10] prevention demonstrated that patents who achieved not only the lowest low-density lipoprotein (LDL) but also the lowest hs-CRP levels on treatment had the lowest cardiovascular risk. However, these data did not conclusively demonstrate that the effect of statins on hs-CRP was truly independent of their low-density lipoprotein–lowering effect.[11]

In order to address this issue, Ridker and others launched 2 large-scale, Phase III, randomized, placebo-controlled trials to test the hypothesis that antiinflammatory therapy could truly improve clinical outcomes in patients with proven coronary artery disease over and above optimal medical therapy, including statins and antiplatelet therapy.

At the time, they chose 2 distinct strategies based on their understanding of the underlying pathophysiology of atherosclerosis. The first trial to launch was CIRT (Cardiovascular Inflammation Reduction Trial)[12] of methotrexate, an effective and generally well-tolerated oral therapy for rheumatoid arthritis (RA), known for its ability to reduce the expression of IL-6. The second to launch was the CANTOS (Anti-inflammatory Therapy with Canakinumab for Atherosclerotic Disease) trial of canakinumab as a highly targeted antiinflammatory strategy with an injectable monoclonal antibody aimed solely at inhibiting the effect of IL-1β, a pivotal cytokine in the inflammatory cascade upstream from IL-6, tumor necrosis factor α, and hs-CRP. IL-1β is the circulating form of IL-1, produced as a precursor (pro–IL-1β) and activated by caspase produced after the activation of NLRP3 (nucleotide oligomerization domain–, leucine-rich repeat–, and pyrin domain–containing protein 3) inflammasome, an innate immune-signaling complex found in proinflammatory macrophages and endothelium that is activated in a broad range of inflammatory diseases, including gout.[13]

The first trial to complete was the CANTOS trial.[14] It examined the effects of 3 doses of canakinumab injected every 3 months in 10,061 patients with a history of acute coronary syndromes and a level of hs-CRP of >2 mg/L.[8] At a median duration of follow-up of 3.7 years, the hazard ratios for the primary outcome (a composite of nonfatal myocardial infarction, nonfatal stroke, and cardiovascular-related death) were 0.93 with the 50-mg dose (P = NS), 0.85 with the 150-mg dose (P = 0.021), and 0.86 with the 300-mg dose (P = 0.031). There was no effect on all-cause mortality. Canakinumab was associated with a small but statistically significant risk for fatal infection (0.31 vs 0.18 events per 100 person-years; P = 0.02). Further analysis demonstrated that patients in the treatment arm whose hs-CRP fell below 2 mg/L at 3 months remained at low risk over the duration of the follow-up period; however, the investigators did not report whether the outcomes in these patients were improved compared with those in patients in the placebo arm of the trial with a level of hs-CRP of <2 mg/L at 3 months.[15]

The CANTOS trial was important as a proof of principle that inhibiting IL-1β can improve clinical outcomes in patients with coronary disease; however, large gaps remain in translating the results of CANTOS to clinical care.[16] Specifically, it was disappointing that the effect on the primary outcome was only modest, that there was no effect on mortality, that there was no clear dose response, and that treatment was associated with a significant risk for fatal infection. In addition, the need for the injection of a monoclonal antibody every 3 months over decades, at an estimated cost of almost $200,000 per annum,[17] were clear signals that canakinumab would never be used in routine clinical practice for secondary prevention in patients with coronary heart disease. Of further relevance is that while canakinumab is widely approved for the treatment of rare periodic fever syndromes in which there is abnormal production of IL-1β, and is approved in Europe for the treatment of active systemic juvenile RA, its demonstrated effects were insufficient to gain approval from the US Food and Drug Administration (FDA) for use in the treatment of flares of gouty arthritis due to tolerability concerns predominantly related to the increased risk for serious infection.[18] More recently still, the FDA rejected an application for canakinumab to be registered for use in the treatment of atherosclerotic vascular disease.[19]

CIRT was the subsequent trial to be reported.[20] It demonstrated that, contrary to observations in largely retrospective, uncontrolled studies in RA, methotrexate provided no cardiovascular benefit in patients with established atherosclerotic vascular disease (without RA). Furthermore, in this population, methotrexate had no effect on circulating levels of IL-1β, IL-6, or CRP.

Both CANTOS and CIRT were pivotal trials that together suggested that the dominant inflammatory pathways involved in atherosclerosis have more in common with those seen in gout than in RA, and this realization has clear therapeutic implications.

Why Colchicine Should Be Considered for the Secondary Prevention of Cardiovascular Disease

In the past decade, new insights into the process of atherosclerosis have emerged. Specifically, there has been significant progress in understanding the inflammatory cascade related to activation of the NLRP3 inflammasome[21] and an increased awareness of the role that cholesterol crystals (CCs) play in the pathogenesis of the disease.[22] Although CCs have been identified in atherosclerotic plaque for over 150 years, their role in the development and progression of atherosclerosis remained largely ignored until recently as the mechanisms by which they can cause inflammatory injury have become clear (Fig. 1).

CCs develop in atherosclerotic plaque in response to changes in the physiochemical environment of the cholesterol-rich core that may be brought about by alterations in temperature, pH, hypoxia, or the addition of cellular debris due to apoptosis. In some circumstances the process of crystallization can be rapid and unchecked, leading to massive expansion and unheralded rupture of the plaque.[23] Most often, CC growth is less dramatic; however, even microscopic CCs have the potential to trigger injury due to their ability to promote an inflammatory response that is akin to, albeit weaker than, that seen in gout. In circumstances in which CCs are not rapidly cleared by resident scavenger cells, the inflammatory process may persist and be sufficiently intense to disrupt the vascular endothelium, leading to atherothrombosis.[24] In most instances, however, CCs are effectively cleared, and in these circumstances,

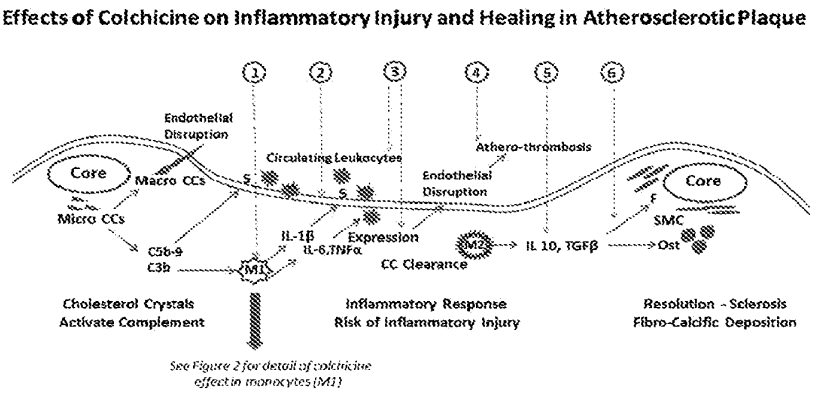


Fig. 1. The formation of cholesterol crystals (CCs) in atherosclerotic plaques may lead to injury. Once formed, microscopic CCs may enlarge to form macroscopic CCs, which can cause sudden unheralded disruption of the endothelium, or they may activate complement to trigger an inflammatory response by promoting the entry of circulating leukocytes into the vascular bed by either directly activating endothelium to produce selectins (S) or by stimulating proinflammatory macrophage (M1) to release interleukin (IL)-1β, which also acts to enhance the recruitment of inflammatory cells into the vascular bed. If the CCs are not rapidly cleared, the inflammatory response can persist, increasing the risk for inflammatory injury that may disrupt the endothelium and lead to atherothrombosis. As CCs are cleared, antiinflammatory macrophages (M2) begin to dominate the milieu and release of antiinflammatory cytokines, including IL-10 and transforming growth factor (TGF)-β, that suppress proinflammatory signals and promote the ingrowth of smooth muscle cells (SMCs), fibrocytes (F), and osteophytes (Ost), leading to the formation of fibrocalcific plaques. Colchicine inhibits the proinflammatory response to CCs by: (1) reducing macrophage expression of IL-1β and the release of various cytokines, including tumor necrosis factor (TNF)-α; (2) reducing endothelial expression of selectins; and (3) inhibiting the actions of circulating leukocytes, including suppressing their ability to release lytic enzymes (eg, matrix metalloproteinase and superoxide). In addition, colchicine: (4) impairs platelet—leukocyte interactions that promote atherothrombosis, and it can promote resolution and healing by (5) stimulating the expression of IL-10 and TGF-β and by (6) limiting the growth of SMCs, fibroblasts, and osteophytes, which if unchecked leads to vascular thickening, deformity, and calcification.

antiinflammatory macrophages begin to dominate the milieu and release antiinflammatory cytokines, including IL-10 and transforming growth factor β, that suppress proinflammatory signals and promote the ingrowth of smooth muscle cells, fibrocytes, and osteophytes, which leads to the formation of fibrocalcific plaques and sclerosis of the vessel wall.

Like uric acid crystals, CCs trigger inflammation by directly activating complement. Factor C3b[25–27] stimulates proinflammatory macrophages to assemble the NLRP3 inflammasome to produce IL-1β,[28,29] which then incites the endothelium to express E-selectin, and factor C5b-9 directly incites endothelial cells to express E-selectin. Hence, 2 independent pathways activated by complement promote the ingress and activation of circulating leukocytes, including neutrophils, into the atherosclerotic bed. This is important as it indicates that selective inhibition of IL-1β may be insufficient for complete dampening of the inflammatory response to CCs because of the ability of C5b-9 to induce the vascular endothelium to express E-selectin and allow continued ingress of circulating leukocytes into the vascular bed. In addition, it is known that crystal-induced inflammation is also driven by mechanisms other than inflammasome, including necroptosis,

necroinflammation, frustrated phagocytosis, granuloma formation, and tissue fibrosis, and that inhibition or genetic deletion of NLRP3 does not affect crystal cytotoxicity in neutrophils.[30] So, while specifically targeting IL-1β may be highly effective in some periodic fevers, it may be less effective in preventing crystal-induced inflammation as seen in gout and atherosclerosis. Indeed, this is a compelling reason why colchicine, with its broader range of cellular effects, may prove to be of greater value in the secondary prevention of cardiovascular disease than is canakinumab.

Colchicine is already widely used and known to be well tolerated and effective for the secondary prevention and treatment of acute gout and familial Mediterranean fever (FMF).[31] Recently it was also demonstrated to be useful in the management of pericarditis and atrial fibrillation.[32] Experience in the treatment of these conditions has confirmed that, aside from early gastrointestinal intolerance, which may limit its use in some patients, colchicine is well tolerated when used continuously, especially at doses of <1 mg/d. The efficacy of colchicine in both gout, in which uric acid crystals activate the same pathways as CCs, and in FMF, in which there is a genetic anomaly that results in overproduction of the NLRP3 inflammasome, speaks clearly to mechanisms by which it may also be of benefit in atherosclerosis.[33]

Effects of Colchicine in Inflammatory Injury and Healing

Within hours of oral ingestion, colchicine is widely distributed and rapidly taken up by a range of cells throughout the body, becoming most highly concentrated in endothelial cells and leukocytes and especially in neutrophils and macrophages. On entry into the intracellular environment, colchicine irreversibly binds to tubulin, thereby interfering with the production of a range of proteins essential for cellular function.[34,35] There are at least 6 separate modes of action of colchicine relevant to atherosclerosis, as summarized in Figs. 1 and 2.

Although there is evidence that regular colchicine treatment results in suppression of the complement classic pathway functional activity in patients with FMF,[36] it is generally believed that the major therapeutic effects of colchicine are related to its ability to alter cellular function. Specifically, in proinflammatory macrophages, colchicine impairs the cellular apparatus required for the assembly of proinflammatory mediators, including matrix metalloproteinase and tumor necrosis factor α. It also affects the assembly of the NLRP3 inflammasome, thereby reducing the release of IL-1β and a range of other interleukins, including IL-6, that are formed in response to danger signals (Fig. 2). In neutrophils, it

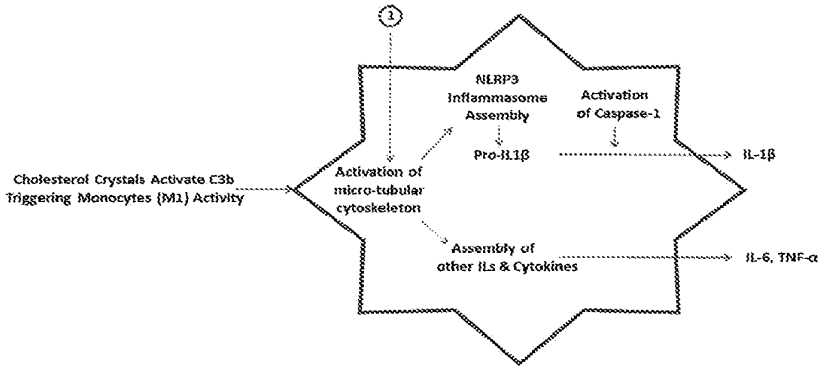


Fig. 2. Detail of the effects of colchicine on monocyte activation. The effects of colchicine on tubulin inhibit the assembly of the NLRP3 (nucleotide oligomerization domain–, leucine-rich repeat–, and pyrin domain–containing protein 3) inflammasome, resulting in upstream inhibition of the interleukin (IL)-1β pathway and the expression of other pivotal proinflammatory cytokines and interleukins. TNF = tumor necrosis factor.

impairs the cellular apparatus required for mobility, adhesion, and the formation and release of cytotoxic substances, including superoxide and a range of proteolytic enzymes. In endothelial cells, colchicine inhibits the production of IL-1β and the expression of E-selectin required for neutrophil adhesion.[35] In addition, colchicine dampens the effects of mast cells[37] and T cells[38,39] that are also pivotal in mediating the response to injury induced by (cholesterol) crystals and can impair the platelet–leukocyte interactions that are important in promoting atherothrombosis.[40] Finally, colchicine can limit the extent of inflammatory injury by promoting antiinflammatory macrophages to release IL-10, TGB- β, and other antiinflammatory cytokines that act to suppress proinflammatory signaling and promote favorable healing by dampening the growth of vascular smooth muscle cells,[41] fibrocytes, and osteophytes, which, if unchecked, may lead to excessive fibrosis, calcification, and deformity of the arterial wall.[42]

Evidence Supporting the Use of Colchicine in Cardiovascular Disease

Recent evidence suggests that the cellular effects of colchicine may translate into clinical benefits in patients with coronary disease. Specifically, it has been demonstrated that colchicine can rapidly and reliably reduce hs-CRP,[43] have favorable effects on the morphology of coronary atherosclerotic plaque,[44] reduce the risk for in-stent stenosis,[45] and reduce the risk for cardiovascular events without tolerability concerns.[46] (A detailed assessment of this evidence is presented in this issue.[47,48])

Of relevance in patients with cardiovascular disease, colchicine 0.5 mg/d does not affect lipid levels, bleeding time, or blood pressure; it is not proarrhythmic; and it has never been reported to cause any clinically important drug interaction when prescribed with the full range of medications commonly used in the treatment of cardiovascular disease. Of the potential drug interactions between colchicine and drugs eliminated via hepatic cytochrome P-450 isozyme 3A4, only the interaction with clarithromycin appears clinically important, and only when colchicine is administered at a dose of >1 mg/d. In addition, colchicine does not directly affect kidney or liver function, and it is well tolerated at low doses in patients with kidney and liver disease.[49,50] Although colchicine has no direct myotoxic effects, myotoxicity has been reported at a dose of >1 mg/d soon after the commencement of statin therapy in patients with moderate renal impairment.[51] Finally, significant bone marrow toxicity and death related to colchicine use have been reported only in the setting of intentional overdose or coadministration with clarithromycin.[52]

The promise of colchicine 0.5 mg/d in patients with coronary disease is currently being explored in several large-scale, rigorous trials, including LoDoCo2 (Low Dose Colchicine2; Australian and New Zealand Clinical Trial Registry identifier: ACTRN12614000093684) in ~5500 patients with stable coronary disease, COLCOT (Colchicine Cardiovascular Outcomes Trial; ClinicalTrials.gov identifier: NCT02551094) in ~4800 patients with recent acute coronary syndromes, and CLEAR-Synergy (Colchicine and Spironolactone in Patients with ST Elevation Myocardial Infarction/ Synergy Stent Registry [OASIS-9]; ClinicalTrials.gov identifier: NCT03048825) in ~4000 patients with ST elevation myocardial infarction undergoing percutaneous coronary intervention.

CONCLUSIONS

Colchicine is a unique, sophisticated agent that has the potential to be successfully repurposed beyond its existing widespread role in the secondary prevention of acute inflammatory flares in gout and FMF. Should ongoing trials confirm its tolerability and benefits in patients with coronary disease, it will likely become a cornerstone therapy in the secondary prevention of cardiovascular disease with the potential to impact the global burden of cardiovascular disease.

Conflicts of Interest

The authors have indicated that they have no conflicts of interest with regard to the content of this article.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinthera.2018.11.016.

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Efficacy and Safety of Low-Dose Colchicine after Myocardial Infarction

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ABSTRACT

BACKGROUND

Experimental and clinical evidence supports the role of inflammation in atherosclerosis and its complications. Colchicine is an orally administered, potent anti-inflammatory medication that is indicated for the treatment of gout and pericarditis.

METHODS

We performed a randomized, double-blind trial involving patients recruited within 30 days after a myocardial infarction. The patients were randomly assigned to receive either low-dose colchicine (0.5 mg once daily) or placebo. The primary efficacy end point was a composite of death from cardiovascular causes, resuscitated cardiac arrest, myocardial infarction, stroke, or urgent hospitalization for angina leading to coronary revascularization. The components of the primary end point and safety were also assessed.

RESULTS

A total of 4745 patients were enrolled; 2366 patients were assigned to the colchicine group, and 2379 to the placebo group. Patients were followed for a median of 22.6 months. The primary end point occurred in 5.5% of the patients in the colchicine group, as compared with 7.1% of those in the placebo group (hazard ratio, 0.77; 95% confidence interval [CI], 0.61 to 0.96; P=0.02). The hazard ratios were 0.84 (95% CI, 0.46 to 1.52) for death from cardiovascular causes, 0.83 (95% CI, 0.25 to 2.73) for resuscitated cardiac arrest, 0.91 (95% CI, 0.68 to 1.21) for myocardial infarction, 0.26 (95% CI, 0.10 to 0.70) for stroke, and 0.50 (95% CI, 0.31 to 0.81) for urgent hospitalization for angina leading to coronary revascularization. Diarrhea was reported in 9.7% of the patients in the colchicine group and in 8.9% of those in the placebo group (P=0.35). Pneumonia was reported as a serious adverse event in 0.9% of the patients in the colchicine group and in 0.4% of those in the placebo group (P=0.03).

CONCLUSIONS

Among patients with a recent myocardial infarction, colchicine at a dose of 0.5 mg daily led to a significantly lower risk of ischemic cardiovascular events than placebo. (Funded by the Government of Quebec and others; COLCOT ClinicalTrials.gov number, NCT02551094.)

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Inflammation appears to play an important role in atherosclerosis.[1] Inhibition of interleukin-1β by the injectable monoclonal antibody canakinumab led to a 15% lower risk of cardiovascular events than was observed with placebo in the Canakinumab Antiinflammatory Thrombosis Outcomes Study (CANTOS) but also led to a slightly higher incidence of fatal infections.[2] In contrast, methotrexate did not affect cardiovascular outcomes or plasma markers of inflammation in the Cardiovascular Inflammation Reduction Trial (CIRT).[3] In light of these differing results and given that canakinumab has not been approved for cardiovascular prevention, the search for a widely used alternative antiinflammatory treatment that may reduce the risk of atherosclerotic events among patients with coronary artery disease continues.

Colchicine is an inexpensive, orally administered, potent antiinflammatory medication that was initially extracted from the autumn crocus and has been used for centuries. Its mechanism of action is through the inhibition of tubulin polymerization and microtubule generation and, possibly, effects on cellular adhesion molecules, inflammatory chemokines, and the inflammasome.[4-6] Colchicine is currently indicated for the treatment of gout, familial Mediterranean fever, and pericarditis.[7,8] In the Low-Dose Colchicine (LoDoCo) trial, patients with stable coronary disease treated with colchicine at a dose of 0.5 mg once daily had fewer cardiovascular events than those not receiving colchicine.[9] However, that trial enrolled only 532 patients and was not placebo-controlled. Because acute coronary syndromes are associated with higher risks of recurrent events and exacerbated inflammation, we conducted the Colchicine Cardiovascular Outcomes Trial (COLCOT) to evaluate the effects of colchicine on cardiovascular outcomes as well as its long-term safety profile in patients who had recently had a myocardial infarction.

METHODS

TRIAL DESIGN AND OVERSIGHT

In this randomized, double-blind, placebo-controlled, investigator-initiated trial, we assigned patients in a 1:1 ratio to receive either colchicine (at a dose of 0.5 mg once daily) or placebo. The trial was funded by the Government of Quebec, the Canadian Institutes of Health Research, and philanthropic foundations. The trial protocol, available with the full text of this article at NEJM.org, was designed by the trial steering committee. The protocol was approved by the institutional review board at each of the 167 centers in the 12 countries that participated in the trial (see the Supplementary Appendix, available at NEJM.org). All trial support activities, including project coordination, medical review, data management, site monitoring, and statistical oversight and analyses, were performed at the Montreal Health Innovations Coordinating Center. Potential trial end-point events were adjudicated by an independent clinical end-point committee composed of experienced cardiologists and neurologists who were unaware of the trial-group assignments. The trial was overseen by a data and safety monitoring board of independent experts. The trial medication and matching placebo were provided by Pharmascience, which had no role in the design or conduct of the trial or in the preparation or review of the manuscript. The first author and the lead statistician (also an author) prepared the first draft of the manuscript, had full access to the trial database, and generated statistical analyses; they also made the decision to submit the manuscript for publication and assume responsibility for the accuracy and completeness of the data and for the fidelity of the trial to the protocol.

TRIAL POPULATION

Adult patients were eligible if they had had a myocardial infarction within 30 days before enrollment, had completed any planned percutaneous revascularization procedures, and were treated according to national guidelines that included the intensive use of statins. Patients were excluded if they had severe heart failure, a left ventricular ejection fraction of less than 35%, stroke within the previous 3 months, a type 2 index myocardial infarction, coronary-bypass surgery either within the previous 3 years or planned, a history of noncutaneous cancer within the previous 3 years, inflammatory bowel disease or chronic diarrhea, neuromuscular disease or a nontransient creatine kinase level that was greater than three times the upper limit of the normal range (unless due to infarction), clinically significant nontransient hematologic abnormalities, severe renal disease with a serum creatinine level that was greater than two times the upper limit of the normal range; severe hepatic disease, drug or alcohol abuse, current or planned long-term systemic glucocorticoid therapy, or a history of clinically significant sen-

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sitivity to colchicine. (Details regarding eligibility criteria are provided in the Supplementary Appendix.)

Written informed consent was obtained from all the patients before enrollment. Clinical evaluations occurred at 1 month and 3 months after randomization and every 3 months thereafter.

END POINTS

The primary efficacy end point was a composite of death from cardiovascular causes, resuscitated cardiac arrest, myocardial infarction, stroke, or urgent hospitalization for angina leading to coronary revascularization in a time-to-event analysis. The secondary end points consisted of the components of the primary efficacy end point; a composite of death from cardiovascular causes, resuscitated cardiac arrest, myocardial infarction, or stroke; and total mortality in time-to-event analyses. Coronary revascularization, hospitalization for heart failure, atrial fibrillation, and deep venous thrombosis or pulmonary embolus were prespecified as exploratory end points in the protocol. Additional prespecified exploratory end points included the change from baseline to 6 months in the high-sensitivity C-reactive protein level and the change from baseline to 12 months in the white-cell count. The C-reactive protein biomarker substudy was implemented after a protocol amendment and was optional for sites and for patients; 34 sites chose to participate in this substudy.

All serious adverse events were recorded. The only other adverse events recorded were those that were considered to be related to the gastrointestinal system, events that were judged by the investigator to be related to colchicine or placebo, or laboratory abnormalities that had been judged by the investigator to be clinically significant.

STATISTICAL ANALYSIS

In this event-driven trial, it was estimated that a sample of approximately 4500 patients undergoing randomization (with 2250 patients in each group) or, in terms of events, a total number of 301 patients with a first positively adjudicated primary end-point event would yield adequate power. The sample-size calculation was based on the primary efficacy end point and assumed a 27% lower risk with colchicine than with placebo, indicated by a hazard ratio of 0.724. With the use of a two-sided test at the 0.05 significance level, the trial would have 80% power if it continued until 301 positively adjudicated primary events occurred in the combined trial groups. The trial design assumed an event rate of 7% in the placebo group at 24 months, an 18-month recruitment period during which patients would be uniformly recruited, a 24-month minimum follow-up period, and a 1% annual rate of loss to follow-up or withdrawal of consent.

The efficacy analyses were conducted with the use of positively adjudicated data and according to the intention-to-treat principle. The primary end point was compared between the two trial groups with the use of a log-rank test, and the hazard ratio, with a 95% confidence interval, was calculated from a Cox proportional-hazards model. A Cox proportional-hazards model with adjustment for important baseline characteristics was also used as prespecified in the protocol. The analysis of the primary end point was repeated in the per-protocol population (i.e., patients without major protocol deviations). Secondary and exploratory end points expressed as time to event were analyzed similarly. The changes from baseline to follow-up were analyzed with the use of an analysis of covariance model with adjustment for baseline value, and estimates of treatment effect are presented with 95% confidence intervals.

The efficacy end points expressed as time to event could be assessed in all patients because the event dates and censoring dates were complete, with the exception of one incomplete event date for atrial fibrillation; therefore, imputation for missing data was not done. In the analysis of time to event, the following censoring rules were used. For death from any cause and death from cardiovascular causes, data from event-free patients who completed the trial were censored at the date of trial completion, and data from patients who did not complete the trial, such as those who were lost to follow-up or who withdrew consent, were censored at the date of last contact or the date of the assessment of survival status, whichever was later. For the analysis of death from cardiovascular causes, patients who died from a noncardiovascular cause had their data censored at the time of death. For all other end points, including the primary end point, the same censoring rules applied, but the survival status was not used because no formal assessment of end points was done at the assessment of survival status. An analysis of the components of the primary end point with death from noncardiovascular causes as a competing event for death from cardio-

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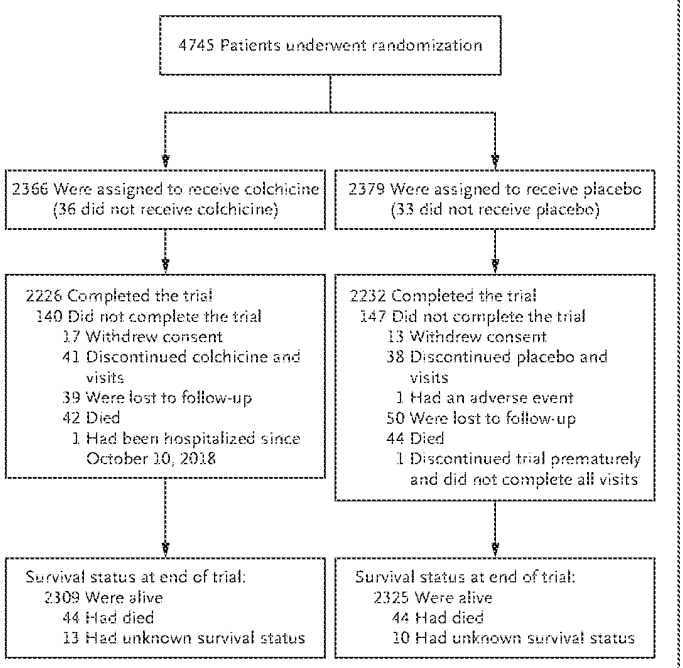


Figure 1. **Randomization and Follow-up of the Patients.**

vascular causes, and with death from any cause as a competing event for the other components, was conducted with the use of the Fine and Gray subdistribution hazard model.[10] No missing data were imputed except for age in cases in which information on the day or the month and day of birth was missing. To account for the occurrence of multiple primary end-point events within patients, recurrent-event analyses were undertaken with the use of negative binomial regression, Andersen–Gill, and Wei–Lin–Weissfeld models.[11-19]

An interim analysis was performed after 50% of the primary end-point events had been positively adjudicated. The prespecified stopping rule for efficacy was based on the Lan–DeMets procedure with the O'Brien–Fleming alpha-spending function. After review of the interim results, the data and safety monitoring board recommended that the trial should continue as planned. To account for this interim analysis, the statistical significance level was set to 0.0490 for the final analysis of the primary end point. All other statistical tests were two-sided and conducted at the 0.05 significance level. Statistical analyses were performed with the use of SAS software, version 9.4 (SAS Institute). There was no prespecified plan to adjust for multiple comparisons across the multiple methods that were used to analyze the primary and secondary end points; results of these analyses are reported with point estimates and 95% confidence intervals, without P values. The 95% confidence intervals were not adjusted for multiple comparisons, and inferences drawn from them may not be reproducible. The final amendment to the statistical analysis plan was approved on August 28, 2019, before unblinding of the trial-group assignments occurred.

RESULTS

PATIENTS

Trial enrollment began in December 2015 and was completed in August 2018; the last trial visit was in July 2019. A total of 4745 patients underwent randomization (with 2366 being assigned to the colchicine group and 2379 to the placebo group) and were followed for a median of 22.6 months. At the time of the database lock on August 28, 2019, and unblinding on August 29, 2019, vital status was available for all except 23 patients (99.5%); 89 patients (1.9%) were lost to follow-up, and 30 patients (0.6%) withdrew consent. Details regarding the disposition of the patients are provided in Figure 1.

The characteristics of the patients at baseline are shown in Table 1. Patients were enrolled a mean of 13.5 days after myocardial infarction. The mean age of the patients was 60.6 years, 19.2% of the patients were women, and 20.2% had diabetes. Most patients (93.0%) underwent percutaneous coronary intervention for their index myocardial infarction. Aspirin, a different antiplatelet agent, and a statin were taken by 98.8%, 97.9% and 99.0% of the patients, respectively.

At the end of the trial, the trial regimen had been discontinued in 18.4% of the patients in the colchicine group and in 18.7% of those in the placebo group. Among the patients who discontinued the trial regimen, the median duration of receipt of the trial drug was 7.1 months (interquartile range, 1.9 to 14.6) in the colchicine group, as compared with 6.1 months (interquartile range, 1.6 to 14.4) in the placebo group. Overall, the median duration of receipt of the trial drug was 19.6 months in the colchicine group and 19.5 months in the placebo group.

CLINICAL EFFICACY END POINTS

A primary end-point event occurred in 5.5% of the patients in the colchicine group, as compared with

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Table 1. **Characteristics of the Patients.***

Characteristic	Colchicine (N = 2366)	Placebo (N = 2379)
Age — yr	60.6±10.7	60.5±10.6
Female sex — no. (%)	472 (19.9)	437 (18.4)
White race — no./total no. (%)†	1350/1850 (73.0)	1329/1844 (72.1)
Body-mass index	28.2±4.8	28.4±4.7
Current smoking — no./total no. (%)	708/2366 (29.9)	708/2377 (29.8)
Hypertension — no. (%)	1185 (50.1)	1236 (52.0)
Diabetes — no. (%)	462 (19.5)	497 (20.9)
History of myocardial infarction — no. (%)	370 (15.6)	397 (16.7)
History of PCI — no. (%)	392 (16.6)	406 (17.1)
History of CABG — no. (%)	69 (2.9)	81 (3.4)
History of heart failure — no. (%)	48 (2.0)	42 (1.8)
History of stroke or TIA — no. (%)	55 (2.3)	67 (2.8)
Time from index myocardial infarction to randomization — days	13.4±10.2	13.5±10.1
PCI for index myocardial infarction — no./total no. (%)	2192/2364 (92.7)	2216/2375 (93.3)
Medication use — no. (%)		
Aspirin	2334 (98.6)	2352 (98.9)
Other antiplatelet agent	2310 (97.6)	2337 (98.2)
Statin	2339 (98.9)	2357 (99.1)
Beta-blocker	2116 (89.4)	2101 (88.3)

* Plus–minus values are means ±SD. Data were missing on the following characteristics: age (assessed according to date of birth; see below) for 435 patients (215 in the colchicine group and 220 in the placebo group), body-mass index (the weight in kilograms divided by the square of the height in meters) for 5 (1 and 4 patients, respectively), and information about the index myocardial infarction for 6 (2 and 4 patients, respectively). Date of birth and race were not required fields because both were considered in some countries to be sensitive data that could allow for the identification of patients. For statistical reporting, missing information regarding the day of birth was replaced by 15, and missing information regarding the month and day of birth was replaced by July 1. CABG denotes coronary-artery bypass graft surgery, PCI percutaneous coronary intervention, and TIA transient ischemic attack.

† Race was reported by the patient.

7.1% of those in the placebo group (hazard ratio, 0.77; 95% confidence interval [CI], 0.61 to 0.96; P = 0.02 by the log-rank test). A multivariable Cox regression model with adjustment for baseline covariates yielded a similar result (Table S1 in the Supplementary Appendix). The event curves that were based on a Kaplan–Meier analysis of the primary efficacy end point are shown in Figure 2. In the prespecified per-protocol analysis involving patients who adhered to the protocol, the primary end point occurred in 5.1% of the patients in the colchicine group and in 7.1% of those in the placebo group (hazard ratio, 0.71; 95% CI, 0.56 to 0.90) (Table S2). Table 2 shows the percentages of patients with events and the hazard ratios for the components of the primary end point, including death from cardiovascular causes (hazard ratio, 0.84; 95% CI, 0.46 to 1.52), resuscitated cardiac arrest (hazard ratio, 0.83; 95% CI, 0.25 to 2.73), myocardial infarction (hazard ratio, 0.91; 95% CI, 0.68 to 1.21), stroke (hazard ratio, 0.26; 95% CI, 0.10 to 0.70), and urgent hospitalization for angina leading to coronary revascularization (hazard ratio, 0.50; 95% CI, 0.31 to 0.81). The hazard ratios remained unchanged in the analysis that took competing events into account.

The secondary efficacy end point consisting of a composite of death from cardiovascular causes, cardiac arrest, myocardial infarction, or stroke occurred in 4.7% of the patients in the colchicine group and in 5.5% of those in the placebo group (hazard ratio, 0.85; 95% CI, 0.66 to 1.10). Data on the primary, secondary, and exploratory efficacy end points are provided in Table 2. Two patients had a first positively adjudicated event of urgent hospitalization for angina leading to coronary revascularization within 14 days after randomization. The median time to this clinical end point was 258 days. Efficacy results in prespecified subgroups are shown in

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Table 2. **Major Clinical End Points (Intention-to-Treat Population).***

End Point	Colchicine (N=2366)	Placebo (N=2379)	Hazard Ratio (95% CI)	P Value
	number (percent)			
Primary composite end point	131 (5.5)	170 (7.1)	0.77 (0.61–0.96)	0.02†
Components of primary end point				
Death from cardiovascular causes	20 (0.8)	24 (1.0)	0.84 (0.46–1.52)	
Resuscitated cardiac arrest	5 (0.2)	6 (0.3)	0.83 (0.25–2.73)	
Myocardial infarction	89 (3.8)	98 (4.1)	0.91 (0.68–1.21)	
Stroke	5 (0.2)	19 (0.8)	0.26 (0.10–0.70)	
Urgent hospitalization for angina leading to revascularization	25 (1.1)	50 (2.1)	0.50 (0.31–0.81)	
Secondary composite end point‡	111 (4.7)	130 (5.5)	0.85 (0.66–1.10)	
Death	43 (1.8)	44 (1.8)	0.98 (0.64–1.49)	
Deep venous thrombosis or pulmonary embolus	10 (0.4)	7 (0.3)	1.43 (0.54–3.75)	
Atrial fibrillation	36 (1.5)	40 (1.7)	0.93 (0.59–1.46)	

* Only the initial event was counted in the analyses of time to first event for the primary composite end point and for the secondary composite end point. In the component analysis, the different types of events were counted separately.
† The log-rank test and the multivariable Cox proportional-hazards model including age, history of diabetes, previous coronary revascularization, and previous heart failure yielded similar P values.
‡ The secondary composite end point included death from cardiovascular causes, resuscitated cardiac arrest, myocardial infarction, and stroke.

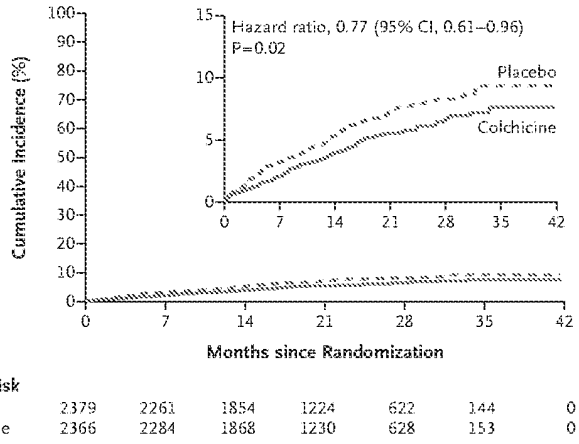


Figure 2. **Cumulative Incidence of Cardiovascular Events (Intention-to-Treat Population).**
Shown are the Kaplan–Meier event curves for the primary efficacy composite end point of death from cardiovascular causes, resuscitated cardiac arrest, myocardial infarction, stroke, or urgent hospitalization for angina leading to coronary revascularization in the colchicine group and the placebo group in a time-to-event analysis. The inset shows the same data on an enlarged y axis.

Table S3. The total number of primary end-point events (first and recurrent) was 154 in the colchicine group and 223 in the placebo group, over periods of 52,949 and 53,060 patient-months of follow-up, respectively. Thus, the primary end-point event rates per 100 patient-months were 0.29 in the colchicine group and 0.42 in the placebo group (rate ratio, 0.66; 95% CI, 0.51 to 0.86) (Table S4).

BIOMARKERS OF INFLAMMATION

High-sensitivity C-reactive protein was measured in a subgroup of only 207 patients at the time of randomization and 6 months later, and the median concentration at trial entry was 4.28 mg per liter. The baseline characteristics of these patients were similar to those of the overall population (Table S5), but the small and selected subgroup with these data limits the interpretation of these analyses. The adjusted geometric mean percent changes in the high-sensitivity C-reactive protein level at 6 months after myocardial infarction were −70.0% in the colchicine group and −66.6% in the placebo group, and the placebo-adjusted

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geometric mean percent change was −10.1 percentage points in the colchicine group (95% CI, −28.6 to 13.4) (Table S6).

Information about white-cell counts at baseline and at the 12-month follow-up were also available for a relatively small subgroup of 1972 patients. The adjusted geometric mean percent changes from baseline to 1 year in the total white-cell count were −18.8% in the colchicine group and −19.0% in the placebo group, with no significant difference between groups (0.3 percentage points; 95% CI, −2.2 to 2.7).

SAFETY AND ADVERSE EVENTS

The incidence of adverse events that were considered to be related to the active drug or placebo was 16.0% in the colchicine group and 15.8% in the placebo group, and the overall incidence of serious adverse events was 16.4% and 17.2%, respectively (Table 3). At least one gastrointestinal adverse event during the double-blind period occurred in 17.5% of the patients in the colchicine group, as compared with 17.6% of those in the placebo group. Diarrhea was reported in 9.7% of the patients in the colchicine group and in 8.9% of those in the placebo group (P=0.35), and nausea was more common in the colchicine group than in the placebo group (1.8% vs. 1.0%, P=0.02). Pneumonia was reported as a serious adverse event in 0.9% of the patients in the colchicine group, as compared with 0.4% of those in the placebo group (P=0.03).

Table 3. **Adverse Events (Safety Population).***

Event	Colchicine (N=2330)	Placebo (N=2346)	P Value
	number of patients (percent)		
Any related adverse event†	372 (16.0)	371 (15.8)	0.89
Adverse events			
Gastrointestinal event	408 (17.5)	414 (17.6)	0.90
Diarrhea	225 (9.7)	208 (8.9)	0.35
Nausea	43 (1.8)	24 (1.0)	0.02
Flatulence	15 (0.6)	5 (0.2)	0.02
Gastrointestinal hemorrhage	7 (0.3)	5 (0.2)	0.56
Anemia	14 (0.6)	10 (0.4)	0.40
Leukopenia	2 (0.1)	3 (0.1)	0.66
Thrombocytopenia	3 (0.1)	7 (0.3)	0.21
Serious adverse events			
Any serious adverse event‡	383 (16.4)	404 (17.2)	0.47
Gastrointestinal event	46 (2.0)	36 (1.5)	0.25
Infection	51 (2.2)	38 (1.6)	0.15
Pneumonia	21 (0.9)	9 (0.4)	0.03
Septic shock	2 (0.1)	2 (0.1)	0.99
Hospitalization for heart failure	25 (1.1)	17 (0.7)	0.21
Cancer§	43 (1.8)	46 (2.0)	0.77

* The safety population was defined as patients who took at least one dose of colchicine or placebo. All serious adverse events were recorded, and the only other adverse events recorded were those that were related to the gastrointestinal system, events that were judged by the investigator to be related to colchicine or placebo, or laboratory abnormalities that were judged by the investigator to be clinically significant. This table lists serious adverse events that were present in more than 2% of the patients in either trial group, adverse events that were considered to be related to colchicine or placebo in more than 5% of the patients in either trial group, and any other safety events of special interest. Chi-square tests were conducted to compare the incidence of adverse events between the trial groups.

† These adverse events were considered to be related to colchicine or placebo by the physician in charge of the participant.

‡ There was one serious adverse event of myopathy, which was attributed to high-dose statin therapy (rosuvastatin at a dose of 40 mg daily) by the local investigator and academic sponsor, in a man of short stature (165 cm, 68 kg) with normal renal function in the colchicine group who had received colchicine for 8 days 3 months before the adverse event.

§ Cancers, excluding nonmelanoma skin cancers, occurred in 42 patients (1.8%) in the colchicine group and in 44 (1.9%) in the placebo group.

DISCUSSION

In COLCOT, the risk of the primary composite efficacy end point of death from cardiovascular causes, resuscitated cardiac arrest, myocardial infarction, stroke, or urgent hospitalization for angina leading to coronary revascularization, as assessed in a time-to-event analysis, was significantly lower among the patients who were randomly assigned to receive 0.5 mg of colchicine once daily than among those who received placebo. This result was due predominantly to a lower incidence of strokes and urgent hospitalizations for angina leading to coronary revascularization.

These results were observed against a background of appropriate medications, which included aspirin, a different antiplatelet agent, and a statin in 98 to 99% of the patients. In addition, percutaneous coronary intervention was performed in 93% of the patients for their index myocardial infarction. The benefits of colchicine with regard to cardiovascular end points in COLCOT were at least as large as those of canakinumab in CANTOS.[2] In the small subgroup of patients with available data, as expected, a large (>65%) reduction in the C-reactive protein level occurred over the first 6 months after myocardial infarc-

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tion in both trial groups in COLCOT, but the difference between the changes in the groups was not significant. These findings must be interpreted cautiously given that this was a small subgroup that was not randomly selected from the full trial sample. A similar observation was made with white-cell counts. The different patient populations involved in the two trials — early after myocardial infarction in COLCOT and stable coronary disease in CANTOS — may also have affected the relationship between biomarkers of inflammation and the effects of treatments on ischemic end points.

The known benefits of colchicine in the treatment of pericarditis were not at play in COLCOT. Postinfarction pericarditis typically occurs within the first few days after the injury, whereas the mean time from the index myocardial infarction to randomization was 13.5 days. There were only two patients with a first positively adjudicated event of urgent hospitalization for angina leading to coronary revascularization within 14 days after randomization, and the median time to this clinical end point was 258 days.

The most common adverse events observed were gastrointestinal. Diarrhea was reported in 9.7% of the patients in the colchicine group and in 8.9% of those in the placebo group, and nausea occurred in 1.8% and 1.0%, respectively. Infection as a serious adverse event was more frequent in the colchicine group than in the placebo group (in 2.2% vs. 1.6% of the patients), and pneumonia as a serious adverse event was also more frequent in the colchicine group (0.9% vs. 0.4%). These differences in the incidence of infections could be due to the play of chance or could reflect altered immunologic responses. In contrast to canakinumab,[2] colchicine did not increase the incidence of septic shock in our trial. Infections have previously been described in patients who have attempted suicide by taking an overdose of colchicine.[20] There was no serious adverse event of myopathy linked to colchicine despite the use of statins in 99% of the patients in the trial.

Our trial has certain limitations. The duration of follow-up was relatively short at approximately 23 months. The risks and benefits of longer-term treatment with colchicine were not evaluated. Although the inclusion of 4745 patients was sufficient for the trial to show a significant benefit with regard to the primary composite efficacy end point, a larger trial could have allowed a better assessment of individual end points and subgroups and the risks associated with colchicine. Finally, our results apply only to patients who have recently had a myocardial infarction.

In conclusion, among patients with a recent myocardial infarction, colchicine at a dose of 0.5 mg daily led to a significantly lower percentage of patients with ischemic cardiovascular events than placebo.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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APPENDIX

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Pilot Study

Colchicine as a Novel Therapy for Suppressing Chemokine Production in Patients With an Acute Coronary Syndrome: A Pilot Study



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ABSTRACT

Purpose: Existing literature reports that colchicine inhibits inflammasome activation and downstream inflammatory cytokine production and stabilizes coronary plaque. However, colchicine's effect on chemokines, which orchestrate multiple atheroinflammatory pathways, is unknown.

Methods: Patients with acute coronary syndrome (ACS) were randomly assigned to colchicine (1.5 mg PO) (n = 12; mean age, 65.2 years) or no treatment (n = 13; mean age, 62.2 years). Blood samples were collected during cardiac catheterization within 24 hours of colchicine administration from the coronary sinus, aortic root, and right atrium. Patients with colchicine-naive stable angina (SAP) (n = 13; mean age, 66.8 years) were additionally sampled. Serum chemokine levels were analyzed with ELISA. In parallel, monocytes from healthy donors were isolated and subjected to colchicine treatment.

Findings: Transcoronary (TC) levels of chemokine ligand 2 (CCL2) and C-X3-C motif chemokine ligand 1 (CX3CL1) were significantly elevated in patients with ACS versus patients with SAP ($P < 0.01$). TC chemokine ligand 5 (CCL5) levels were not significantly ($P = 0.084$) elevated in patients with ACS versus patients with SAP. Colchicine treatment markedly reduced TC levels of CCL2, CCL5, and CX3CL1 in patients with ACS ($P < 0.05$). *In vitro* colchicine suppressed *CCL2* gene expression in stimulated monocytes ($P < 0.05$). Colchicine treatment reduced the intracellular concentration of all 3 chemokines ($P < 0.01$) and impaired monocyte chemotaxis ($P < 0.05$).

Implications: Here, we report for the first time that short-term colchicine therapy significantly reduces the local production of coronary chemokines, in part by attenuating production of these mediators by monocytes. These data provide further evidence of colchicine's beneficial role in patients with ACS. (*Clin Ther.* 2019;41:2172–2181) © 2019 Published by Elsevier Inc.

Keywords: Atherosclerosis, Acute coronary syndrome, Coronary heart disease, Inflammation, Therapeutics.

INTRODUCTION

Chemokines are a class of chemotactic cytokines characterized by their ability to induce cell migration. Beyond their chemotactic function, chemokines are

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involved in a plethora of proatherogenic processes, including cell survival, leukocyte adhesion, foam cell differentiation, and endothelial to mesenchymal transition.[3] Chemokine ligand 2 (CCL2), chemokine ligand 5 (CCL5), and C-X3-C motif chemokine ligand 1 (CX3CL1) are commonly expressed chemokines implicated in atherosclerotic plaque progression and instability. Combined inhibition of CCL2, CCL5, and CX3CL1 axes abolishes atherosclerosis in hypercholesterolemic mice,[3] whereas blockade of a single pathway stunts plaque progression and produces a more stable disease phenotype.[3–5] Furthermore, antagonism of the CCL2 receptor was reported to reduce serum C-reactive protein in humans at high risk of coronary artery disease (CAD).[6] Despite these promising results, there is yet to be a tolerable, effective, and accessible therapeutic option available to favorably modify chemokine expression in humans.

Colchicine is a potent, yet inexpensive, anti-inflammatory medication used commonly in the management of gout and pericarditis. Although colchicine's precise mechanism of action is ill defined, it is known to inhibit microtubule polymerization.[7] As such, colchicine influences numerous cellular processes, including inflammasome function, cytokine release, and phagocytosis.[7–9] Low-dose colchicine treatment was found to reduce the incidence of secondary cardiovascular events and to promote atherosclerotic plaque stability.[10,11]

Given colchicine's purported broad mechanism of action, this pilot study was designed to test its effects on inflammatory chemokines in patients presenting with an acute coronary syndrome (ACS). We hypothesized that (1) the local cardiac production of CCL2, CCL5, and CX3CL1 would be higher in patients with ACS than in patients with stable CAD; (2) colchicine would suppress transcoronary (TC) production of these chemokines; and (3) this reduction in chemokine levels would be due to a direct effect of colchicine on monocyte processes.

PATIENTS AND METHODS

Patient Recruitment

Adult patients indicated for cardiac catheterization at Royal Prince Alfred Hospital (Sydney, NSW, Australia) were recruited for this study. Patients were divided into 2 cohorts, according to clinical presentation (as per the American Heart Association guidelines)[12] as follows: (1) ACS cohort in which patients presented with recent, acute-onset chest pain associated with ECG changes and/or positive cardiac enzymes (creatine kinase or troponin T) and (2) stable CAD cohort in which patients presented with symptomatic chest pain on exertion for a period >3 months or asymptomatic patients with >50% stenosis and positive function tests.

The ACS cohort was randomly assigned, in a 1:1 fashion, to receive either 1 mg, followed by 0.5 mg (1 hour later), of colchicine or no colchicine, 6 to 24 hours before undergoing cardiac catheterization, as per our previous publication.[8] Staff within the catheterization laboratory, but not the patient, were blinded to the assigned treatment. Individuals with stable CAD served as a control cohort and were not treated. Exclusion criteria included (1) cardiogenic shock or hemodynamic instability, (2) known hypersensitivity to colchicine, (3) use of colchicine or other anti-inflammatory medications besides aspirin (ie, corticosteroid drugs); (4) evidence of concurrent inflammatory or infective conditions that may affect serum concentration of acute-phase proteins (ie, rheumatoid arthritis and peripheral vascular disease), (5) use of potent cytochrome p450 inhibitors, (6) moderate renal dysfunction (creatine clearance < 45 mL/min) or hepatic dysfunction (alanine aminotransferase 1.5× upper limit of normal range), and (7) thrombocytopenia or leukopenia.

Demographic and clinical data were recorded. The local Ethics Review Committee approved the study protocol, and all patients gave written informed consent before participating.

Sample Collection

Blood sampling was performed as per our previously published method.[8,13] Briefly, a standard coronary angiogram was performed in all patients. Subsequently, right femoral vein access was obtained with a 5F sheath and a 5F Simmons catheter was advanced into the right atrium. Blood (20 mL) was drawn from the coronary sinus, lower right atrium—inferior vena cava junction (venous), and ascending aorta (arterial). Blood samples were collected in vacuum containers that contained EDTA. Samples were subsequently centrifuged at 1500 rpm at room temperature for 10 minutes; plasma was then immediately collected and stored at −80°C until required for processing. TC concentrations (defined as

coronary sinus concentration minus the central aortic concentration) for all 3 chemokines were calculated.

Cell Culture

Human-derived mononuclear cells (HDMCs) were isolated via elutriation from the buffy coat of healthy individuals. After isolation, HDMCs were seeded in 6-well plates at a density of 3×10^6 cells/well in RPMI. Cells were incubated at 37°C for 2 hours to facilitate adhesion. Subsequently, the medium was replaced with either colchicine (50 nM) or ethanol as a vehicle control in RPMI that contained 10% fetal bovine serum. Cells were incubated for 4 hours before lipopolysaccharide (LPS; 25 ng/mL) stimulation for 4 hours. After this, cells were lyzed by either TRI Reagent (Sigma–Aldrich, Castle Hill, NSW, Australia) or CelLytic (Sigma–Aldrich) for RNA and cellular protein extraction, respectively. All samples were stored at −80°C until required.

Chemokine Quantification

The concentration of plasma and intracellular CCL2, CCL5, and CX3CL1 was analyzed by ELISA (R&D Systems, Minneapolis, Minnesota). The intracellular concentration of the 3 chemokines was assessed by subjecting the cell lysates to bicinchoninic acid analysis and loading equal protein quantities (10 μg) from each sample. ELISAs were performed as per the manufacturer's guidelines.

RNA Isolation and Quantitative Reverse Transcriptase Polymerase Chain Reaction

RNA was isolated from HDMCs with the use of TRI Reagent (Sigma–Aldrich) as per the manufacturer's instructions. RNA was reverse transcribed with the use of the iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, California). Quantitative reverse transcriptase polymerase chain reaction was performed with the use of IQ Sybr mix in a CFX384 thermocycler (Bio-Rad Laboratories). Relative changes in mRNA expression were determined with the $^{\Delta\Delta}$CT method normalized to the housekeeping gene *GAPDH*. The primer sequences used for mRNA amplification are given in Table I.

Migration Assay

Supernatant from HDMCs treated with colchicine (50 nM) or ethanol (vehicle control) and stimulated with LPS (25 ng/mL) for 4 hours was collected and centrifuged at 550 × *g* and 600 μL was added to 12 wells of a 24-well plate. Freshly isolated HDMCs were stained with CellTrace CFSE (Thermo Fisher, Waltham, Massachusetts) as per the manufacturer's instructions. Once stained, 5×10^4 HDMCs in 100 μL of RPMI were added to the inserts (Corning, Tewksbury, Massachusetts; 3-μm pores) of the transwell plate and incubated at 37°C for 1 hour to facilitate adhesion. Thereafter, the inserts were added to the wells that contained the conditioned media and incubated for 2 hours to allow migration. After migration, the transwell inserts were removed, washed 3 times in phosphate-buffered saline, and fixed in 2% formalin for 10 minutes. Once dry, the insert was imaged with the use of the Zeiss Axio Imager Z2, and 3 images of each membrane were obtained with the use of the 10× objective. Migrated cells were counted manually with the use of Image J software (National Institute of Health, Bethesda, Maryland).

Statistical Analysis

Continuous data are reported as mean [SD] or [SEM] when appropriate. Categorial variables are displayed as number and percentage. Proportional differences in categorical data were tested by Pearson χ^2 test. Differences in means of continuous variables were analyzed by 1-way ANOVA or independent *t* test when appropriate. All tests were 2-tailed with the

Table I. Human primer sequences used in gene amplification.

Gene	Forward	Reverse
CCL2	TCATAGCAGCCACCTTCATT	TCGGAGTTTGGGTTTGCTT
CCL5	TCCTCATTGCTACTGCCCT	TTGGCGGTTCTTTCGGGTGA
CX3CL1	TATCTCTGTCGTGGCTGCT	TCCTTGACCCATTGCTCCTT
GAPDH	TTCAACAGCGACACCCACT	TTCCTCTTGTGCTCTTGCT

acceptable type 1 error set at $P < 0.05$. All statistical analyses were performed with SPSS Statistics 25 software (IBM, Armonk, New York), and graphs were produced with GraphPad Prism 7.0 (GraphPad Software, San Diego, California).

RESULTS

Between March 2017 and October 2017, 38 patients were enrolled in the study. This included 25 patients presenting with an ACS and 13 with stable CAD. Of patients within the ACS cohort, 12 were randomly assigned to colchicine, whereas the remaining 13 patients received no treatment. Colchicine treatment was well tolerated, and no adverse reactions were reported.

Baseline Characteristics

Baseline characteristics for the 3 cohorts, untreated ACS, colchicine-treated ACS, and stable CAD, are presented in Table II. Compared with the untreated ACS cohort there were fewer active smokers in the ACS plus colchicine group ($P = 0.035$) and fewer patients currently taking a β-blocker in the stable CAD group ($P = 0.047$). As expected, patients with ACS had significantly higher levels of C-reactive protein and troponin compared with the stable CAD cohort ($P = 0.015$ and $P < 0.001$, respectively).

Patients with ACS Have Increased Tc Chemokine Levels

A significant association was found between TC chemokine levels and clinical presentation. Patients

Table II. Baseline characteristics for study participants.

Characteristic	ACS (n = 13)	ACS + Col (n = 12)	Stable CAD (n = 13)
Age, years	62.2 [12.1]	65.2 [11.1]	66.8 [8.2]
Female	1 (7.7)	3 (25.0)	1 (7.7)
Diabetes	6 (46.2)	5 (41.7)	4 (30.8)
Hypertension	9 (69.2)	9 (75.0)	10 (76.9)
Dyslipidemia	10 (76.9)	8 (66.7)	10 (76.9)
Current smoker	6 (46.2)	1 (8.3)*	4 (30.8)
Previous MI	4 (30.8)	7 (58.3)	2 (15.4)
Previous PCI	4 (30.8)	4 (33.3)	2 (15.4)
Previous CABG	3 (23.1)	1 (8.3)	1 (7.7)
Medications			
Aspirin	13 (100)	12 (100)	12 (92.3)
β-Blockers	10 (76.9)	10 (83.3)	5 (38.5)*
ACE-I	4 (30.8)	8 (66.7)	2 (15.4)
Insulin	0 (0.0)	2 (16.7)	1 (7.7)
Statin	11 (84.6)	9 (75.0)	11 (84.6)
Admission blood value			
Troponin T, μg/L	89 (18–1300)	206.5 (38.5–1083)	7 (4.5–10.5)*
CRP, mg/L	5.2 (2.8–18.9)	2.7 (1.1–19.45)	1.2 (0.7–2.2)*

Data are presented as mean [SD], n (%), or median (IQR).
ACE-I = angiotensin converting enzyme inhibitor; ACS = acute coronary syndrome; CABG = coronary artery bypass graft; CAD = coronary artery disease; Col = colchicine; CRP = C-reactive protein; MI = myocardial infarction; PCI = percutaneous coronary intervention.
*$P < 0.05$ compared with the untreated ACS cohort; χ^2 test used for binary variables; non-normally distributed data (troponin and CRP) were log transformed, and subsequent analysis was performed with an independent t test.

with ACS had significantly higher TC levels of CCL2 and CX3CL1 (12.2 versus −10.0 pg/mL and 73.4 versus −25.0 pg/mL; $P = 0.049$ and $P = 0.007$, respectively) compared with patients with stable CAD. Patients with ACS had elevated levels of TC CCL5, although this did not reach statistical significance (12.6 versus −1.0 ng/mL; $P = 0.16$). When aortic, coronary sinus, and venous levels of the 3 chemokines were compared independently, no significant differences were found (data not shown).

Colchicine Treatment Acutely Suppresses TC Chemokine Levels in Patients with ACS

A single dose of colchicine (1.5 mg) had no significant effect on the concentration of chemokines at the individual sampling sites (data not shown). However, calculation of the TC values revealed that colchicine acutely suppressed TC levels of all 3 chemokines: CCL2 (12.2 versus −12 pg/mL; $P = 0.049$), CCL5 (12.6 versus −16.2 ng/mL; $P = 0.010$), and CX3CL1 (73.4 versus −7.8 pg/mL; $P = 0.016$) as illustrated in Figure 1. Because there were significantly more active smokers in the untreated ACS group, a sensitivity analysis with adjustment for numbers of smokers was performed. This resulted in similar significant differences in chemokine levels between the groups.

Colchicine Suppresses *CCL2* Gene Expression in Activated HDMCs

Colchicine treatment of HDMCs *in vitro*, before LPS stimulation, inhibited *CCL2* gene expression by 59% (4.6 versus 11.1; $P = 0.028$) (Figure 2A). Moreover, colchicine exposure reduced the relative increase in CCL2 intracellular protein concentration associated with LPS stimulation by ~3-fold (0.4 versus 1.3; $P = 0.002$) (Figure 2B).

Unlike CCL2, colchicine treatment had no significant effect on CCL5 or CX3CL1 mRNA expression versus the ethanol-treated control group (data not shown). Despite this, colchicine treatment significantly reduced the relative increase in CCL5 and CX3CL1 intracellular protein concentration associated with LPS stimulation (2.5 versus 9.7; $P = 0.005$; 0.9 versus 1.3; $P = 0.009$, respectively) (data not shown).

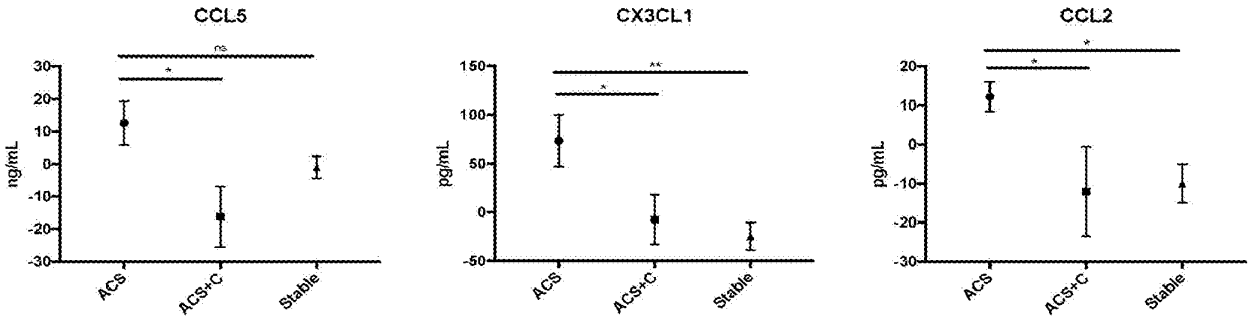


Figure 1. Transcoronary (TC) chemokine levels. During cardiac catherization, 6 to 24 hours after colchicine administration, blood was collected from the coronary sinus and aortic root. The plasma was isolated, chemokine concentrations were analyzed by ELISA, and the TC gradient was calculated (coronary sinus − aortic). *P* values were calculated with a 1-way ANOVA with multiple comparisons; multiplicity was adjusted for by using the Holm–Sidak method. ACS = acute coronary syndrome; ASC+C = acute coronary syndrome plus colchicine; CCL2 = chemokine ligand 2; CCL5 = chemokine ligand 5; CX3CL1 = C-X3-C motif chemokine ligand 1.

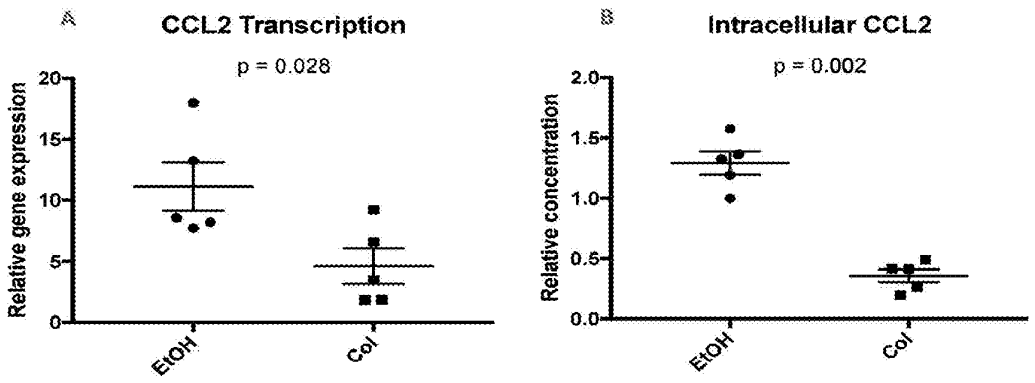


Figure 2. Effect of colchicine (Col) on chemokine ligand 2 (CLL2) expression. Col treatment significantly reduced CCL2 (A) mRNA and (B) intracellular protein levels compared with vehicle (ethanol; EtoH) control. Human-derived mononuclear cells (HDMCs) were isolated from buffy coat and treated with either 50 nM Col (n = 5) or EtOH (n = 5) as a vehicle control for 4 hours. Cells were stimulated with lipopolysaccharide (25 ng/mL) for a further 4 hours before being harvested. The mean difference between treatment groups was analyzed by paired *t* test.

Colchicine Inhibits The Generation of a Functional Chemotactic Gradient

We next used a migration assay to assess the functional consequences of reduced chemokine production. Monocyte migration toward conditioned media generated from colchicine-treated HDMCs was reduced by 46% ($P = 0.035$) compared with the ethanol-treated controls (Figure 3).

DISCUSSION

This pilot study is the first, to our knowledge, that measures TC chemokine levels in patients with ACS and the effect of colchicine on these factors. There are several novel findings. First, patients with ACS have increased TC levels of CCL2 and CX3CL1 compared with patients with stable CAD. Second, a one-off colchicine dose acutely suppressed the TC levels of CCL2, CCL5, and CX3CL1. Finally, colchicine reduced CCL2 expression and chemotaxis in activated HDMCs, suggesting a direct inhibitory effect of colchicine on these cells.

Local Cardiac Production of Chemokines is Elevated in Patients with ACS

Expectedly, these results found that the local cardiac production of CCL2, CCL5, and CX3CL1 is higher in patients with ACS than in patients with stable CAD; however, results for CCL5 were statistically indeterminant. From this trend, a higher powered study might be able to detect a significant difference in the TC levels of CCL5 between these 2 cohorts. As previously reported by our group,[8] venous levels do not track with increased TC production, indicating the importance of measuring the TC gradient to identify local cardiac changes. Although augmented chemokine expression is known to disrupt plaque stability, results of this study are unable to confirm whether the increase in TC chemokine levels were the cause of plaque rupture or consequent to it.

CCL2, CCL5, and CX3CL1 are instrumental in the progression of atherosclerosis and have been suggested to promote instability of atherosclerotic lesions.[14] CCL2 is primarily responsible for inducing monocyte migration into plaque, although it is also involved in regulating matrix metalloproteinase expression; therefore, it directly influences plaque stability.[15,16] In

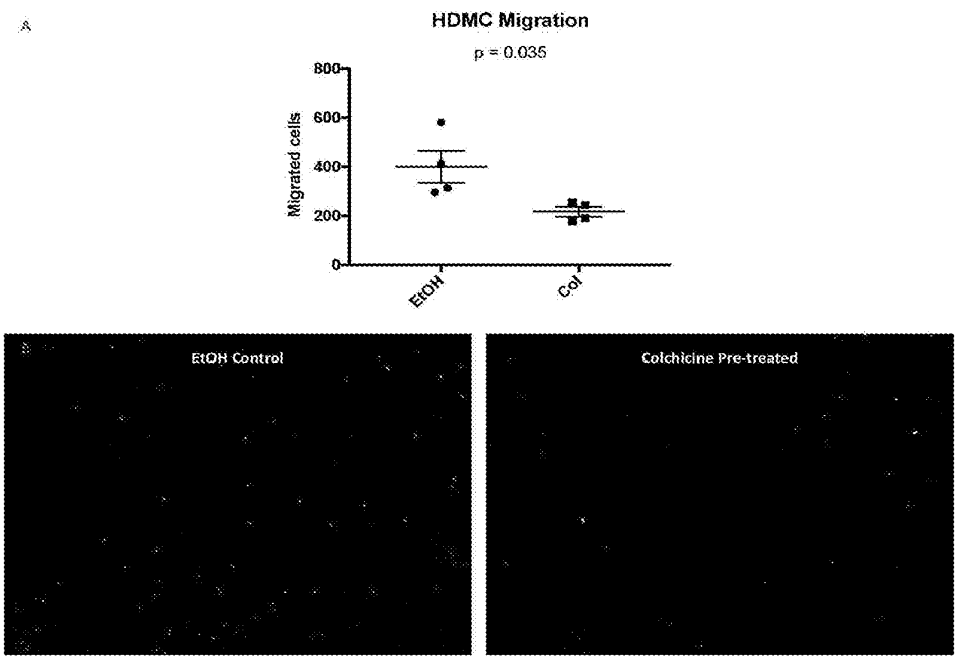


Figure 3. Colchicine (Col) reduced human-derived mononuclear cell (HDMC) migration. (A) Graphical representation of HDMC migration in response to conditioned medium with and without Col. (B) Representative images of carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled HDMCs (green) that have migrated to the underside of the transwell membrane. HDMCs treated with either 50 nM Col or ethanol (EtOH) for 4 hours were stimulated with lipopolysaccharide (25 ng/mL). The supernatant was subsequently collected, centrifuged, and added to a 24-well plate. Freshly isolated CFSE-stained HDMCs were then added to transwell inserts and allowed to migrate toward the conditioned media (n = 4).

mice, CCL2 overexpression accelerates plaque progression[17]; whereas CCL2 blockade stunts progression and limits destabilization of preexisting atherosclerotic lesions.[18] In humans, elevated circulating CCL2 levels after ACS are associated with an increased risk of recurrence and a higher mortality.[19,20] CCL5 induces migration of monocytes, T lymphocytes, and neutrophils toward atherosclerotic lesions. Inhibition of CCL5 signaling attenuates plaque progression and promotes plaque stability in mice.[4] In humans, CCL5 is increased during cardiac ischemia, and elevated CCL5 levels after ACS are associated with progressive atherosclerotic lesions and future cardiovascular adverse events.[21,22] The dual-function chemokine CX3CL1 acts as both a soluble ligand and a membrane-bound adhesion molecule. In atherosclerosis, CX3CL1 is known to facilitate monocyte recruitment to the inflamed endothelium, activate platelets, and promote leukocyte survival.[1,23] Not surprising, increased serum levels of CX3CL1 are associated with an unstable plaque morphology and a higher risk of ACS.[24–26]

Colchicine Reduces TC Chemokine Levels

We found that colchicine administration acutely suppressed the local cardiac production of CCL2, CCL5, and CX3CL1. This reduction in intracardiac chemokine expression has significant therapeutic implications. ACS is most commonly preceded by

plaque rupture and subsequent thrombosis, a process that is driven by the continual recruitment of leukocytes to atherosclerotic lesions. By reducing this chemotactic stimulus, colchicine may increase stability of preformed atherosclerotic lesions and may reduce plaque disruption rates. Encouragingly, the use of colchicine in patients with ACS and patients with CAD has been supported by findings from the LoDoCo trial[10] and the COLPLAST-ACS study[11] which found that colchicine stabilizes coronary plaque and reduces ACS recurrence rates. As such, these current findings combined with previous data from our group indicating suppression of inflammasome-specific cytokines found that colchicine has a broad antiatherogenic mechanism(s) of action.

Colchicine Modulates Chemokine Expression in HDMCs

With the use of an *in vitro* model, we aimed to elucidate the mechanism by which colchicine reduces TC chemokine levels and whether this effect has a functional consequence. Our data found that colchicine regulates HDMC migration, in part by reducing CCL2 expression, a major process that mediates atherogenesis. In line with a previous report from Li et al,[27] this data found that colchicine acts directly on HDMCs to reduce CCL2 mRNA expression. One possible mechanism may be modulation of transcription factor nuclear translocation by disruption of microtubule networks,[28,29] although confirmation of this is beyond the scope of this article. We also found that colchicine reduces CCL5 and CXC3L1 protein levels without affecting transcription; this may be due to interference with post-transcriptional microtubule-dependent packaging and transport of these chemokines, thereby reducing their production. Again, dedicated mechanistic studies are required to confirm these findings. To determine whether the observed reduction in intracellular chemokine expression translated to a reduction in secretion and thus chemotaxis, we used a transwell migration assay. We found that by reducing chemokine production, colchicine inhibits HDMC chemotaxis. This evidence provides a mechanism by which colchicine may improve plaque stability by reducing intraplaque immune cell accumulation. Overall, these data suggest that colchicine suppresses monocyte activation and therefore chemotaxis, as found by the blunted response to LPS stimulation.

This pilot study was designed to identify potential anti-inflammatory mechanisms of colchicine in patients presenting with an ACS, rather than to assess colchicine's efficacy in cardiovascular disease or its effect on plaque stability. As such, this study has 3 important limitations. First, the sample size of this study was relatively small. Consequently, the distribution of baseline characteristics was not entirely equal, and results may be influenced by confounders not identified in this pilot study. Second, we did not measure the effect of colchicine on chemokines in patients with stable CAD. Finally, we only investigated the impact of colchicine on HDMCs, whereas *in vivo* serum chemokine levels are regulated by many cell types. To completely understand colchicine's mechanism of action *in vivo* it would be essential to investigate all relevant cell types, including endothelial cells and macrophages, which also secrete large amounts of chemokines.

CONCLUSION

In summary, these data provide a novel mechanism by which colchicine exerts its anti-inflammatory effects in patients with ACS. These results found that colchicine inhibits chemokine expression and thus monocyte migration. In combination with the existing literature, this evidence highlights the need for further large-scale, outcome-based clinical trials to assess the efficacy of colchicine in cardiovascular disease.

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Bradley Tucker was responsible for drafting and revising the manuscript. Rahul Kurup and Jennifer Barraclough were responsible for patient recruitment and sample collection. Bradley Tucker, Rodney Henriquez, and Sian Cartland performed the laboratory-based experimental procedures. Clare Arnott, Ashish Misra, and Mary Kavurma provided further technical support and aided with data analysis and presentation. Gonzalo Martínez and

Sanjay Patel were responsible for study design and development. Sanjay Patel was the senior author and approved the final manuscript. All authors contributed to editing of the final manuscript.

DISCLOSURES

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Reviews

The Role of Colchicine in Acute Coronary Syndromes



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ABSTRACT

Purpose: Because inflammation is a key process implicated in the pathogenesis of atherosclerosis at all stages, including plaque formation, progression, instability, and rupture, and because colchicine has unique anti-inflammatory properties, this review article summarizes the pathophysiologic mechanisms underpinning inflammation in atherosclerosis and acute coronary syndrome (ACS), outlines anti-inflammatory therapeutic approaches that have been tested thus far, and evaluates the evidence supporting the potential role of colchicine in improving outcomes and reducing cardiovascular morbidity and mortality in patients after ACS.

Methods: PubMed was searched for publications on colchicine and ACSs and atherosclerosis, and www.clinicaltrials.org was searched for completed and ongoing trials of colchicine use in ACSs.

Findings: Despite contemporary optimal medical therapy, patients remain at a high risk of future events after an ACS because of residual inflammation at culprit and nonculprit sites. Several attempts have been made to address this with targeted anti-inflammatory therapies, but until the recent promising results of canakinumab (an anti–interleukin-1β monoclonal antibody), most have failed to find any prognostic benefit in large clinical trials with hard end points. The pathogenic role of neutrophils and monocytes in atheroinflammation is well established, and a fundamental component in this process is the activation of the NOD-like receptor protein 3 inflammasome, a cytosolic multiprotein complex that, when activated by a stress signal such as cholesterol crystals, drives caspase-1–dependent release of 2 key proinflammatory cytokines, which are predictive of future adverse cardiovascular events: interleukin-1β and interleukin-18. Colchicine is a widely available, inexpensive, and well-tolerated medication that, among several anti-inflammatory mechanisms of action, inhibits activation of the NOD-like receptor protein 3 inflammasome complex. A seminal trial has found the beneficial properties of colchicine in reducing adverse cardiovascular events in the stable coronary artery disease population.

Implications: Despite promising results in small prospective observational and randomized trials, there is a need for more evidence evaluating the role of colchicine as a secondary preventive agent after ACSs. (*Clin Ther.* 2019;41:11–20) Crown Copyright © 2018 Published by Elsevier Inc. All rights reserved.

Keywords: acute coronary syndrome, atherosclerosis, colchicine, coronary artery disease, inflammation.

INTRODUCTION

Atherosclerosis and its clinical manifestations, such as coronary artery disease (CAD) causing acute coronary syndrome (ACS), are leading causes of worldwide morbidity and mortality. Contemporary therapy emphasizes modulation of cardiovascular risk factors, lipid-lowering strategies (both dietary and pharmacologic), and antiplatelet medications to prevent thrombosis. However, there is an increasing body of evidence that implicates inflammation as a process central to the pathogenesis of atherosclerotic plaque

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development, instability, and rupture. Patients with an index ACS remain at an increased risk of recurrent cardiovascular events (up to 20% at 3 years),[1,2] in part because of residual coronary inflammation at culprit and nonculprit sites after ACSs,[3,4] despite current guideline-based medical therapy. Recurrent events in these patients are particularly worrisome because they are associated with significantly more morbidity and mortality than the index event.[2]

Statins, which are part of contemporary optimal medical therapy (OMT) after ACSs, are known to confer an anti-inflammatory benefit in addition to their lipid-lowering properties.[5,6] However, there is a now a shift toward developing therapies that specifically target inflammation to further improve outcomes in this high-risk group of patients. Until recently, the key role of inflammation in atherothrombosis remained a hypothesis based on clinical and experimental data, and there was no high-quality evidence that reducing vascular inflammation translates into reduced cardiovascular event rates. The Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS),[7] however, found that the anti-inflammatory canakinumab (at a dose of 150 mg every 3 months) reduced major adverse cardiovascular events (MACEs) in patients with a previous myocardial infarction (MI) and high-sensitivity C-reactive protein (hs-CRP) levels $\geq$2 mg/L. Canakinumab is a monoclonal antibody that specifically targets interleukin (IL)-1β, a key cytokine in the inflammatory cascade that drives the IL-6 signaling pathway. However, limitations to its widespread implementation include a higher rate of fatal infections and a prohibitively high cost. Therefore, there is a crucial need for new therapies in this high-risk patient cohort.

Colchicine is a widely available, tolerable, and inexpensive anti-inflammatory medication that prevents mitosis by inhibiting microtubule polymerization[1,8] but also interferes with several steps in the inflammatory process. It is used extensively in the management of gout, acute and recurrent pericarditis, and familial Mediterranean fever. Recent evidence also supports its role in cardiovascular disease prevention, and it may represent another potentially useful agent for investigating the inflammation hypothesis in atherothrombosis.[9] This review outlines the role of inflammation in ACS and provides an overview of other targeted anti-inflammatory approaches this far. It then details the evidence thus far supporting a role for colchicine as a secondary preventive agent after ACS and comments on trials currently under way to evaluate this.

INFLAMMATION IN ACS AND THE NLRP3 INFLAMMASOME

Extensive evidence supports a pathogenic role for local and systemic inflammation in ACS, and understanding the molecular and cellular mechanisms that drive this process can improve prognostic stratification and allow identification of novel therapeutic targets.[1,4,10] There have been important recent developments in understanding.

Endothelial activation by triggers, such as low-density lipoprotein cholesterol (LDL-C) or hemodynamic strain, promotes the release of proinflammatory cytokines, oxygen-free radicals, and matrix-degrading metalloproteinases, which amplify the local inflammatory process and reduce plaque stability through collagen-mediated weakening of the fibrous cap. Key proinflammatory cytokines play a role in endothelial activation and further inflammatory cell recruitment and activation and potentiate procoagulant properties of endothelial cells and neutrophils. Furthermore, plaque rupture causes blood contact with tissue factor, a potent procoagulant protein also produced by macrophages, which promotes thrombosis.[3,4,10–13]

Polymorphonuclear neutrophils are also increasingly being recognized as important contributors to atheroinflammation and plaque destabilization.[14–17] Neutrophils activated by cholesterol crystals can also expel neutrophil extracellular traps (NETs), which are weblike structures that contain DNA, histones, neutrophil elastase, and myeloperoxidase.[18]

A key player in the innate immune system is the NOD-like receptor protein 3 (NLRP3) inflammasome, a cytosolic multiprotein complex present in myeloid cells, including neutrophils, monocytes, and eosinophils. Activation of the inflammasome is a 2-phase process. Exposure to various stress signal stimuli, such as cholesterol crystals in plaque, is sensed by the NLRP3 receptor, which primes the inflammasome complex, leading to the assembly of NLRP3, the adaptor protein apoptosis–associated specklike protein that contains the caspase recruitment domain, and the effector cysteine protease procaspase-1.[1,19,20] Activation is completed after a second signal (eg, adenosine triphosphate), leading to caspase-1 activation

and secretion of active IL-1β and IL-18. Therefore, exposure of monocytes and neutrophils to stimuli, such as cholesterol crystals, which are found in higher concentrations in unstable plaque (resulting in higher inflammasome activity), triggers the NLRP3 inflammasome. This in turn promotes caspase-1–dependent release of 2 key inflammatory cytokines (IL-1β and IL-18), both of which are predictive of future cardiovascular events and are key mediators in plaque development, progression, and destabilization.

IL-1β is a proinflammatory cytokine involved in the promotion of monocyte and leukocyte adhesion to vascular endothelial cells, growth of vascular smooth muscle cells, and procoagulant activity induction.[7,21,22] The activation of IL-1β also stimulates the downstream IL-6 receptor signaling pathway, which elicits the acute-phase response that drives expression of atherothrombosis mediators, such as fibrinogen and plasminogen activator inhibitor. Patients with ACS also have the highest transcoronary (coronary sinus aortic) gradients of IL-1β, IL-18, and IL-6 compared with those with stable CAD and no CAD, indicating increased local cardiac production due to active atheroinflammation.[23]

Hence, it is clear that the transformation of a silent atherosclerotic lesion to a ruptured unstable plaque causing ACS is critically mediated by activated immune and inflammatory cells that destabilize the lesion and cause subsequent thrombosis and ischemia. However, there is substantial evidence that inflammation associated with ACS is a diffuse pan-coronary process and not confined to a culprit plaque.[4,11,24] A study of 65 patients found widespread activation of neutrophils across the coronary vascular bed in patients with unstable angina, regardless of the location of the culprit lesion.[25] These studies, along with others, support the concept that a widespread coronary inflammatory process is the substrate for individual plaque instability, with thrombosis and ischemia developing at sites where such a process is most intense.[11]

TARGETING INFLAMMATION IN ACS

An appreciation of the role of inflammation in ACS has generated interest in the development of novel anti-inflammatory therapies to alleviate the high rate of recurrent events and adverse prognosis in these patients. However, despite promising early preclinical and mechanistic studies and Phase II trial results, many of these have failed to find benefit in a large, Phase III trial examining hard clinical outcomes.[21,26]

Statins are known to have pleiotropic anti-inflammatory properties that act synergistically with their lipid-lowering properties. The mechanism of action is multifactorial and includes inhibiting T-cell activation, macrophage infiltration, and leucocyte adhesion, as well as reducing reactive oxygen species generation and enhancing endothelial nitric oxide production.[3,4] Several large clinical trials have found that statin therapy reduces hs-CRP, independent of its lipid-lowering effects, translating into improved clinical outcomes.[6,27] The Pravastatin or Atorvastatin Evaluation and Infection Therapy (PROVE-IT) trial[6] randomized 3745 patients with ACS to moderate or intensive lipid-lowering statin therapy and found that patients with a CRP level <2 mg/L after statin therapy had lower event rates (recurrent MI or death due to CAD) than those with higher levels (2.8 vs 3.9 events per 100 person-years, $P = 0.006$). Importantly, this effect of better clinical outcomes was present irrespective of the level of LDL-C achieved on treatment. Furthermore, the cumulative incidence of events at follow-up for patients with CRP levels <2 mg/L but LDL-C levels >1.8 mmol/L was almost identical to those with CRP levels >2 mg/L but LDL-C levels <1.8 mmol/L (3.2 vs 3.1 per 100 person-years), supporting the value of attenuating inflammation after ACS along with lipid lowering. Similarly, the Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT)[28] randomized 18,144 patients stabilized after ACS to simvastatin or ezetimibe/simvastatin and measured LDL-C and hs-CRP at baseline and 1 month after randomization. Again, similar to the PROVE-IT trial, there were an almost identical proportion of patients who met the primary composite end point (cardiovascular death, ACS, coronary revascularization within 30 days, or nonfatal stroke) in the group who achieved hs-CRP levels <2 mg/L but LDL-C levels >1.8 mmol/L and the group with hs-CRP levels >2 mg/L but LDL-C levels <1.8 mmol/L (33.4% vs 33.7% at 7 years). A series of more specific anti-inflammatory agents have been investigated in randomized controlled trials, generally with negative results.

Corticosteroids were investigated as an early logical choice for anti-inflammatory therapy after ACS. A meta-analysis of 11 trials (2646 patients) found a 26% decrease in mortality (odds ratio = 0.74, $P = 0.015$), but sensitivity analyses limited to large studies and randomized trials found a lack of

efficacy.[29] Furthermore, steroids were abandoned after a concerning signal of impaired wall healing after MI, resulting in cardiac rupture.[30]

Nonsteroidal anti-inflammatory drugs were all (except aspirin) found to increase the risk of subsequent MI in patients with CAD and increased mortality (hazard ratio [HR] = 1.63) in patients after MI during a 5-year period.[31]

Pexelizumab was another early example of an anti-inflammatory therapy for ACS. Pexelizumab is a single-chain fragment of a humanized monoclonal antibody that binds to the C5 component of the complement cascade, preventing its cleavage into C5a and C5b-9, which are proinflammatory and activate endothelial cells and leucocytes. On the basis of promising proof-of-concept and early animal studies, 2 Phase II placebo-controlled studies were commenced in patients with ST-elevation MI (STEMI), assessing the effect of pexelizumab after fibrinolytic reperfusion therapy or primary percutaneous coronary intervention (PCI).[21] These studies suggested that pexelizumab may modulate the subacute inflammatory consequences of ischemia and infarct healing. On the basis of this signal, a Phase III trial[32] randomized 5745 patients to placebo or pexelizumab (bolus and infusion), but no difference was found in mortality or the composite end point of death, shock, or heart failure. Because of these results, combined with a subsequent meta-analysis confirming no benefit in patients with STEMI, development and investigation of the drug were discontinued.[21]

Varespladib was also developed to function as a nonspecific inhibitor of secretory phospholipase A2 (PLA2) activity. Secretory PLA2 is an enzyme implicated in atheroinflammation by interacting with tumor necrosis factor α receptor signaling and hydrolyzing LDL-C, resulting in lipid accumulation within macrophages, oxidative stress, and downstream inflammation.[23] In a Phase II randomized trial of 625 patients with ACS, varespladib and atorvastatin significantly reduced LDL-C, hs-CRP, and secretory PLA2 levels at 8 and 24 weeks after ACS compared with placebo and atorvastatin, but there was no difference in MACEs between the 2 groups.[33] In a large, follow-up, randomized, placebo-controlled trial of 5145 patients with ACS, daily treatment with varespladib for 4 months after ACS lowered LDL-C and hs-CRP levels but was associated with a higher rate of the composite primary outcome, resulting in early termination because of potential harm.[34]

Darapladib, on the other hand, is a direct selective inhibitor of lipoprotein-associated PLA2. Lipoprotein-associated PLA2 activity hydrolyzes oxidized LDL-C, which produces pro-inflammatory byproducts and is highly expressed in necrotic cores of vulnerable and ruptured plaque. Moreover, higher lipoprotein-associated PLA2 levels are associated with an increased risk of coronary events.[21,26] After no treatment effect was found in a large, randomized, placebo-controlled trial of 15,828 patients with stable CAD,[35] a similar trial of 13,026 patients with ACS[36] recruited within 30 days of non-STEMI (NSTEMI) or STEMI, with a median follow-up of 2.5 years. Again, no difference was found in the primary composite end point (cardiovascular death, MI, or urgent revascularization) between the darapladib and placebo groups (16.3% vs 15.6%, $P = 0.93$), and no difference was found in all-cause mortality or any of the individual components of the primary end point. Thereafter, lipoprotein-associated PLA2 was abandoned as a therapeutic target to improve cardiovascular outcomes.[21]

Inclacumab is a highly specific human recombinant monoclonal antibody against P-selectin, a cell adhesion molecule that plays a crucial role in leukocyte and platelet recruitment, tethering, and adhesion. Higher levels of P-selectin are found in patients with ACS and are associated with increased rates of cardiovascular mortality.[24] After promising animal models, a Phase II trial of 544 patients with NSTEMI was performed in which patients were randomized to an infusion of placebo, 5 mg/kg of inclacumab, or 20 mg/kg of inclacumab before PCI.[37] The primary outcome was a change in troponin I level from baseline and at 16 and 24 hours. Although no significant difference was found between the placebo and 5-mg/kg group, a difference was found between the placebo and 20-mg/kg group at 24 hours (–23.8%, $P = 0.05$). However, a Phase III trial examining hard clinical outcomes remains to be conducted.

Losmapimod is a selective inhibitor of the p38 intracellular mitogen-activated protein kinase signaling cascade. p38 mitogen-activated protein kinase regulates the transcription of inflammatory cytokines, such as TNF-α, IL-1, IL-6, and IL-8, and potentiates the generation of reactive oxygen species.[24] The Phase II trial randomized 535 patients to losmapimod or placebo within 18 hours of presenting with a NSTEMI and at least 2 hours before PCI.[38] The mean hs-CRP level at 72 hours was lower in the losmapimod group than in the

placebo group ($P = 0.0009$), but no difference was found at 12 weeks. Mean troponin I AUC values also did not differ. However, markers of ventricular function or remodeling suggested benefit, with similar mean B-type natriuretic peptide levels at 72 hours but significantly lower at 12 weeks in the losmapimod cohort ($P = 0.04$). After this, a large, randomized, placebo-controlled Phase III trial was commenced in which 3503 patients hospitalized with an acute MI were randomized to losmapimod or placebo for 12 weeks then followed up for an additional 12 weeks.[39] Although the losmapimod group had significantly lower levels of hs-CRP and B-type natriuretic peptide after drug therapy initiation compared with the placebo group, this did not translate into any difference in the primary composite end point of cardiovascular death, MI, or recurrent ischemia (HR = 1.16, $P = 0.24$); therefore, the trial was not expanded to recruit larger numbers.

Finally, canakinumab has earned significant attention recently as a human monoclonal antibody targeted at IL-1β. A Phase II trial of canakinumab (monthly injections for 4 months) in 556 patients with diabetes and high cardiovascular risk found that IL-1β inhibition with canakinumab significantly reduced plasma levels of IL-6 and hs-CRP, without affecting LDL-C levels, compared with placebo.[40] CANTOS[7] was a large, Phase III, randomized trial that sought to evaluate whether canakinumab on top of OMT could prevent recurrent vascular events in patients with residual inflammatory risk by recruiting 10,061 patients with previous MI and hs-CRP levels ≥2 mg/L. At 48 months, the median reduction in hs-CRP from baseline compared with placebo was 26%, 37%, and 41% in the 50-, 150-, and 300-mg dose groups accordingly, with no significant reduction in lipid levels in any canakinumab groups compared with placebo. At a median follow-up of 3.7 years, in the 150-mg group the event rate for the primary outcome (nonfatal MI, nonfatal stroke, cardiovascular death) was 15% lower than the placebo group (HR = 0.85, $P = 0.021$), which was better than both the 50-mg (HR = 0.93, $P = 0.30$) and 300-mg (HR = 0.86, $P = 0.0031$) groups. The 150-mg cohort also met statistical significance for the secondary outcome, which included the primary end points plus unstable angina leading to urgent revascularization (HR = 0.83, $P = 0.005$). No significant difference was found in all-cause mortality (HR = 0.94, $P = 0.31$) for canakinumab compared with placebo. These results finally affirmed the inflammatory hypothesis in atherothrombosis and have set the stage for a new era of anti-inflammatory cardiovascular therapeutics.[22]

The Cardiovascular Inflammation Reduction Trial is a randomized, double-blinded, placebo-controlled trial that will investigate whether low-dose methotrexate (target dose, 15-20 mg/wk) will reduce rate of MI, stroke, and cardiovascular death among patients with type 2 diabetes mellitus or metabolic syndrome and a documented history of MI or past evidence of multivessel CAD by angiography. It will aim to recruit 7000 patients with a mean follow-up period of 3 to 5 years and is currently under way.[41]

THE ROLE OF COLCHICINE

Colchicine is an anti-inflammatory medication with a well-established adverse effect profile and is widely available and inexpensive. Common (>1%) adverse effects are gastrointestinal (eg, diarrhea, nausea, vomiting), with rarer adverse effects (<0.1%) that include hypersensitivity, hepatitis, myoneuropathy, rhabdomyolysis, and blood dyscrasias. Major interactions are with strong CYP3A4 inhibitors, calcineurin inhibitors, and statins, such as pravastatin, and major precautions are found in patients with renal disease, hepatic disease, and pregnancy.[1,42]

Along with its effect on mitosis by binding to tubulin and promoting microtubule depolymerization, colchicine inhibits the activation of the NLRP3 inflammasome protein complex.[42] There are several purported mechanisms by which colchicine can suppress NLRP3 inflammasome activation, including direct monocyte caspase-1 inhibition, inhibition of the *MEFV* gene preventing inflammasome assembly, inhibition of colocalization of inflammasome cytoplasmic proteins, and inhibition of P2 × 7-mediated pore formation, which is a key step in NLRP3 inflammasome response to adenosine triphosphate .[1,43] Ex vivo monocytes from patients with ACS receiving short-term colchicine (1 mg followed by 0.5 mg 1 hour later) therapy exhibited a marked reduction in intracellular and secreted levels of IL-1β compared with pretreatment levels. There was also a significant reduction in procaspase-1 mRNA levels and secreted caspase-1 protein levels in treated patients, suggesting that short-term colchicine intensely and markedly suppressed monocyte caspase-1 activity, thereby reducing monocyte IL-1β secretion.[43] In addition, in these patients, the same colchicine regimen

significantly reduced transcoronary levels of the key caspase-1–mediated cytokines (IL-1β and IL-18) and downstream IL-6 by 40% to 88% (P = 0.028, 0.032, and 0.032, respectively).[23] These findings are consistent with the concept that local activation of cholesterol crystal-induced inflammation within atherosclerotic plaque drives plaque instability, and that it may be possible to modify future outcomes of patients by blocking NLRP3 inflammasome activation with low-dose colchicine.[44]

Indirect support for a beneficial effect of colchicine on cardiovascular disease comes from 2 retrospective studies that have reported reduced incidence of cardiovascular disease, especially ischemic heart disease, in patients with continuous colchicine use for the treatment of gout and familial Mediterranean fever.[8,42,45,46] Furthermore, a study of 64 patients found that low-dose colchicine (0.5 mg BID) effectively and independently decreased hsCRP levels in patients with stable CAD.[47] The pivotal LoDoCo trial of 532 patients with stable CAD followed up during a median of 3 years found that colchicine 0.5 mg/d administered in addition to high-dose statin therapy and OMT was effective in the prevention of MACEs (HR = 0.33, $P < 0.001$) compared with placebo.[48]

In the setting of plaque rupture, an initial small study followed up 80 patients for 30 days after ACS or acute ischemic stroke and found no difference in hsCRP or platelet aggregation in the group given colchicine (1 mg/d) compared with placebo (Table).[49] However, this study did not specify how soon after the ACS diagnosis colchicine was administered, and by 30 days after the index event, acute inflammatory processes would have subsided even in the control group, rendering these findings difficult to interpret. A trial of 151 patients presenting with STEMI (treated with PCI) randomized them to colchicine (loading dose of 2 mg then 0.5 mg BID) or placebo for 5 days and sought to test the hypothesis that a short course of colchicine could reduce infarct size (Table).[50] The creatinine kinase–MB AUC was nearly half that of the placebo group ($P < 0.001$), indexed cardiac magnetic resonance imaging–defined infarct size was 18.3 mL/1.73 m^2 versus 23.2 mL/1.73 m^2 in the colchicine and placebo groups, respectively ($P = 0.019$), and the relative infarct size as a proportion of left ventricular myocardial volume was 13.0% and 19.8%, respectively ($P = 0.034$). These results suggested a benefit of short-course colchicine in reducing infarct size in patients with STEMI treated with PCI. A smaller study of 44 patients, however, with a follow-up of 1 month, randomized patients with STEMI to colchicine 1 mg/d plus OMT or OMT alone and found no difference in mean peak CRP value during the index hospitalization between the 2 groups (Table).[51]

In a prospective, observational study of 80 patients with ACS allocated in a 1:1 ratio to OMT alone or OMT with colchicine 0.5 mg/d for 12 months, computed tomography coronary angiography was performed at baseline and 12 months (Table).[3] OMT involved risk factor optimization and statin intensification therapy aiming at an LDL-C level <1.8 mmol/L. The primary outcome was change in low attenuation plaque (LAP) volume, and secondary outcomes were change in hsCRP level and change in other computed tomography coronary angiography parameters, including total atheroma volume. LAP has been consistently found in large clinical trials and intracoronary imaging comparison studies to represent a powerful predictor of future ACS and MACEs. A significant mean reduction in LAP volume of 41% and 17% was found in the treatment and control groups, respectively, during the 12-month period, with a significant difference ($P = 0.008$) between the 2 groups. Similarly, there was a significant mean reduction in hsCRP levels in both groups (37% and 15% in the treatment group and controls, respectively) from baseline, and again there was a compelling statistically significant difference ($P < 0.0001$) between the 2 groups. A linear regression and correlation analysis found a highly significant linear relationship ($R^2 = 0.158$, $P < 0.001$) and strong positive correlation ($r = 0.578$) between change in LAP volume and change in hsCRP level. Finally, both groups achieved successful statin intensification therapy after ACS, with a substantial mean reduction in LDL-C of 0.5 and 0.4 mmol/L from a baseline of 2.4 and 2.3 mmol/L in the control (–20%) and treatment (–19%) groups, respectively. However, no difference was found between the 2 groups here, with similar percentage reductions in both groups. A multivariate linear regression analysis of the primary and secondary outcomes retained statistical significance for reduction in LAP volume ($P = 0.04$) and hsCRP level ($P = 0.004$). This study found, for the first time to our knowledge, that regular low-dose colchicine therapy has a substantially more powerful coronary plaque–stabilizing effect than OMT alone. These changes were seen independent of substantial reductions

Table. Main studies evaluating colchicine in ACS.

Study	No. of Patients	Population	Colchicine Regimen	Outcomes
Raju et al[49]	80	ACS or acute ischemic stroke	1 mg/d vs placebo for 30 days	Colchicine did not significantly reduce absolute hs-CRP at 30 days (1.0 vs 1.5 mg/L, $P = 0.22$) or difference in hs-CRP from baseline to 30 days (7.0 vs 7.1 mg/L, $P = 0.64$). No difference in platelet function either using various assays.
Deftereos et al[50]	151	STEMI treated with PCI	1.5 mg immediately, then 0.5 mg 1 hour later, then 0.5 mg BID vs placebo for 5 days	The CK-MB AUC was nearly half that of the placebo group ($P < 0.001$). Indexed CMR-defined infarct size was smaller in the colchicine group (18.3 vs 23.2 mL/1.73 m^2, $P = 0.019$), and the relative infarct size as a proportion of LV myocardial volume was smaller in the colchicine group (13.0% vs 19.8%, $P = 0.034$) compared with placebo.
Akodad et al[51]	44	STEMI treated with PCI	1 mg/d for a month vs no treatment	No significant difference in mean CRP peak value between the colchicine and control groups (29.0 vs 21.9 mg/L, $P = 0.36$) even after adjustment for the culprit artery (27.0 vs 25.0 mg/L, $P = 0.79$).
Martínez et al[23]	83	ACS (m = 40), stable CAD (n = 33), controls (n = 10)	1 mg followed by 0.5 mg within 24 hours before coronary sinus sampling vs no treatment	Transcoronary gradients for IL-1β, IL-18, and IL-6 were highest in patients with ACS ($P = 0.08$, 0.03, and 0.01, respectively), and colchicine administration significantly reduced these gradients by 40%–88% ($P = 0.03$, 0.03, and 0.03, respectively).
Vaidya et al[8]	80	Recent ACS (< 1 month)	0.5 mg/d vs no treatment for a year	Colchicine significantly reduced LAP volume (−41% vs −17%, $P = 0.008$) and hs-CRP (−37% vs −15%, $P < 0.001$) vs controls. Reductions in LDL-C ($P = 0.21$) and total atheroma volume ($P = 0.28$) were comparable in both groups.

ACS = acute coronary syndrome; CAD = coronary artery disease; CK-MB = creatinine kinase–MB; CMR = cardiac magnetic resonance; hs-CRP = high-sensitivity C-reactive protein; IL = interleukin; LAP = low attenuation plaque; LDL-C = low-density lipoprotein cholesterol; LV = left ventricle; PCI = percutaneous coronary intervention; STEMI = ST-elevation myocardial infarction.

in LDL-C during 12 months, which were comparable between the treatment and control groups, representing successful statin intensification therapy in both groups. This process was likely driven by the properties of colchicine, which allow it to attenuate atherosclerosis-associated inflammation in vulnerable plaque, as seen by the proportionally greater reduction in hsCRP level in the treatment group, the lack of effect of colchicine on total atheroma volume, and the strongly positive linear correlation between change in LAP volume and hsCRP level.

Two large randomized trials are upcoming that aim to evaluate the role of colchicine in the setting of an ACS and whose results could be potentially practice changing if they find that colchicine's putative plaque-stabilizing effects translate into improved patient outcomes. The Colchicine Cardiovascular Outcomes Trial aims to recruit 4500 patients with an acute MI within the last 30 days and will compare colchicine 0.5 mg/d for 2 years versus placebo.[3] The primary outcome will be a composite of cardiovascular death, resuscitated cardiac arrest, acute MI, stroke, and hospitalization for angina requiring revascularization. The Colchicine and Spironolactone in Patients with STEMI/SYNERGY Stent Registry trial is not yet under way but will aim to recruit 4000 patients with a STEMI who have undergone PCI and assess outcomes after randomization to colchicine versus placebo and spironolactone versus placebo in a 2×2 factorial design. The primary outcome will be MACEs defined as death, recurrent target vessel MI, stroke, or ischemia-driven target vessel revascularization.

FUTURE DIRECTIONS

The knowledge that inflammation is a critical process in the pathogenesis of atherosclerosis offers new opportunities for the prevention and treatment of CAD and its sequelae of ACS, which result in significant patient morbidity and mortality. During the past several years, multiple therapies have targeted inflammatory pathways involved in ACS and atherosclerosis to attempt to improve patient outcomes, with many failing to translate into therapeutic benefit when tested in large, randomized clinical outcomes trials. Patients who have had an ACS remain at a substantial residual risk of MACEs despite contemporary OMT because of the progression of atherothrombotic disease within and beyond the culprit site, representing an unmet therapeutic need. However, with the advent of CANTOS and several other trials currently under way, the next decade will no doubt define the role of inflammation as a target of cardiovascular risk reduction. Colchicine in particular has recently gained traction in cardiovascular medicine and may have the potential to dampen the inflammatory milieu that causes plaque instability and atherothrombosis, thereby reducing the rate of recurrent cardiovascular events and improving patient outcomes.

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DECLARATION OF INTERESTS

There are no conflicts of interest to declare.

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