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The risk of cancer in statin users : a clinical and genetic approach

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Résumé

Les statines, inhibiteurs de la 3-hydroxy-3-methylglutaryl-coenzyme (HMG-CoA) réductase sont efficaces et largement utilisées dans le traitement des troubles lipidiques, surtout pour l'hypercholestérolémie. Dans plusieurs essais contrôlés randomisés, les statines réduisent significativement le risque d'événements cardiovasculaires tant en termes de morbidité que de mortalité. Une littérature importante s'est développée démontrant une association entre la statine et le cancer, toute type confondue, dans la dernière décennie, sans consensus sur la question de savoir si cette relation existe vraiment. De plus, il n'est pas clair que si cette relation existe réellement, si elle est positive ou négative.

Dû au manque de consensus sur ce sujet, nous avons cherché à étudier l'effet de la statine à forte dose contre faible dose sur l'incidence du cancer et la mortalité par cancer d'un point de vue clinique et génétique. Du point de vue clinique, nous avons utilisé les cohortes TNT et IDEAL, qui visaient à l'origine à examiner l'effet de la statine à forte dose par rapport à la statine à faible dose sur le risque de maladie cardiovasculaire pour la prévention secondaire après un infarctus du myocarde dans un contexte randomisé pour effectuer une analyse post-hoc afin de comparer le risque de cancer ou de décès par cancer entre les utilisateurs de statines à haute et à faible dose. Par la suite, des sous-analyses supplémentaires ont été réalisées en se concentrant exclusivement chez les personnes âgées de 55 ans et plus, les hommes et les femmes. Du point de vue génétique, nous avons réalisé une étude d'association à l'échelle du génome (GWAS) en reliant les données génétiques de la cohorte TNT avec l'incidence du cancer.

Notre étude n'a pas trouvé d'association significative entre la statine à dose plus élevée et le cancer dans l'évaluation clinique. De plus, les résultats du GWAS n'étaient pas en mesure d'identifier une variante génétique fiable associée aux paramètres testés, dont l'incidence de cancer ou la mortalité par cancer. Nous concluons que l'utilisation de la statine à plus haute dose n'était pas associée avec un risque de cancer, ou de mortalité par cancer plus ou moins élevé.

Mots-clés : statines, cancer, cardiologie, pharmacogénomique, cardio-oncologie

Abstract

Statins, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, are efficient and widely used drugs in the treatment of lipid disorders, especially hypercholesterolemia. In several large randomized-controlled trials, statins significantly reduced the risk of cardiovascular events both in terms of morbidity and mortality. Nonetheless, a significant body of literature affirming the association between statin use and cancer has continued to progress in the last decade, with no consensus on whether this relationship truly exists, and if it does, whether it's a positive or negative relationship.

Based on the lack of consensus, we sought to investigate the effect of high-dose vs. low-dose statin on incident cancer and cancer mortality from a clinical and genetic perspective. From the clinical perspective, we relied on data obtained from the TNT and IDEAL cohorts, two randomized-controlled studies that were originally intended to examine the effect of high-dose statin vs. low or usual-dose statin on the risk of cardiovascular disease for secondary prevention after myocardial infarction. We performed post-hoc analysis and evaluated the risk of cancer or cancer mortality between high and low-dose statin users. Additional sub-analyses were performed focusing exclusively on those aged ≥55 years old, men, and women. From the genetic perspective, we performed a genome-wide association study (GWAS) using the TNT cohort with available genetic data on cancer incidence.

Overall, our study failed to find any significant association between higher dose statin and cancer incidence or cancer mortality using clinical data. Furthermore, findings from the GWAS were not able to identify a reliable genetic variant associated with the tested endpoints. We conclude that the use of higher dose statins was not associated with a higher or lower risk of cancer diagnosis or cancer mortality.

Keywords: statins, cancer, cardiology, pharmacogenomics, cardio-oncology

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HMG-CoA: 3-hydroxy-3-methylglutaryl- coenzyme-A

VLDL-cholesterol: very low-density lipoprotein cholesterol

FH: familial hypercholesterolemia

SREBPs: sterol regulatory element binding proteins

NADP: nicotinamide adenine dinucleotide phosphate

NADPH: reduced form of NADP+

HDL: high-density lipoprotein

FDA: Food and Drug Administration

HR: hazard ratio

CI: confidence interval

CTT: Cholesterol Treatment Trialists

CRP: C-reactive protein

SAA: serum amyloid A

RR: relative risks

LFA1: lymphocyte-function associated antigen 1

FPP: farnesysl pyrophosphotate

GGPP: geranylgeranyl pyrophosphotate

IPP: isopentenyl pyrophosphate

RNA: ribonucleic acid

MAP2K1: mitogen activated protein kinase 1

ERK: extracellular regulated kinase

ICAM1: intercellular adhesion molecule 1

RAMQ: Régie de l'Assurance-Maladie du Québec

OR: odds ratio

GWAS: genome-wide association study

CETP: cholesteryl ester transfer protein

SNP: single nucleotide polymorphisms

DNA: deoxyribonucleic acid

CHD: coronary heart disease

AE: adverse events

MAF: minor allele frequencies

À Ludovic et Alexandre.

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Introduction

Statins and Prevention of Cardiovascular Disease

Biological Mechanisms and Historical Background

The pharmaceutical development of the statin class of medicines has transformed the primary and secondary prevention of cardiovascular diseases. Historically, during the early 1950s and late 1960s, many cholesterol-lowering agents were investigated and introduced into clinical settings, including cholestyramine**(1)**, clofibrate**(2)**, plant sterols**(3)**, nicotinic acid**(4)**, neomycin**(5)**, triparanol**(6)**, D-thyroxine**(7)**, and estrogenic hormones**(8)**. Cholestyramine acts by binding bile acids within the intestinal lumen, thereby interfering with their re-absorption and enhancing their fecal excretion. Bile acid synthesis is consequently stimulated, which leads to an increased requirement for cholesterol in the liver, and causes a rise of hepatic 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase activity. Clofibrate's major effect in hyperlipoproteinemia is to reduce very low-density lipoprotein (VLDL)-cholesterol. However, in most patients the cholesterol-lowering effect is small to moderate. Plant sterols act by interfering with the absorption of cholesterol in the intestinal tract, but have no effect on VLDL-cholesterol. Furthermore their effect on low-density lipoprotein (LDL)-cholesterol is variable. Nicotinic acid reduces both cholesterol and triglyceride in humans, with a prominent side effect of cutaneous vasodilation. Neomycin is an effective cholesterol-lowering agent in patients with familial hypercholesterolemia (FH), which acts by precipitating cholesterol within the intestinal tract, thus stopping its absorption. Triparanol inhibits cholesterol synthesis in the final stages of the synthetic pathway. However significant side effects, such as cataracts, occurred; which resulted in its withdrawal from the market. D-thyroxine lowers LDL-cholesterol in both euthyroid and hypothyroid patients. However, long-term use of D-thyroxine was shown to increase mortality in men with arrhythmias, angina pectoris or multiple infarctions led to its discontinuation.**(9)** Estrogens have also been used to treat hyperlipidemia. However, its use is not suitable in men due to their feminizing effects, and how they elevate VLDL and triglycerides.

Unsurprisingly, by the early 1970s, none of the drugs available were considered ideal cholesterol-lowering agents. That being said, the experience with the aforementioned drugs suggested that drug-induced lowering of plasma cholesterol would be viable in the treatment of coronary atherogenesis and heart disease.(10, 11) Beginning in the early 1960s, cholesterol metabolism was largely experimented in animals and human subjects by many investigators. Cholesterol may be derived from the intestinal absorption of dietary cholesterol or from synthesis *de novo* within the body.(12, 13) It was understood that animal cells must regulate their biosynthetic pathways in order to produce the right amounts of end-products, without overproduction. This control is particularly essential in cholesterol homeostatis because cholesterol must be supplied for many cellular functions.(14-16)As such, excess cholesterol must be avoided as it forms solid crystals that kill cells. Excess cholesterol in the bloodstream is also lethal because it deposits in arteries, initiating atherosclerosis.(17)

End-product regulation of cholesterol metabolism is achieved principally through repression of transcription of genes that govern the synthesis of cholesterol and its receptor-medicated uptake from plasma lipoproteins.(18) Cholesterol, as an end-product repressor, is a particular problem as it is an insoluble lipid that resides almost exclusively in cell membranes. So how does the cell sense the level of a membrane-embedded lipid, and how is that information passed on to the nucleus to regulate transcription? Further studies revealed that a novel family of membrane-bound transcription factors called sterol regulatory element binding proteins (SREBPs) that regulate multiple genes involved in cholesterol biosynthesis and uptake mediate cholesterol homeostasis.(19) The SREBPs, which regulate transcription of HMG CoA reductase, also regulate transcription of genes encoding many other enzymes in the cholesterol biosynthetic pathway, including HMG CoA synthase, farnesyl diphosphate synthase, and squalene synthase.(18, 20-22) The SREBPs also regulate the LDL receptor, which supplies cholesterol through receptor-mediated endocytosis. Unexpectedly, the SREBPs were eventually found to modulate the transcription of genes encoding enzymes of fatty acid synthesis and uptake, including acytyl CoA carboxylase, fatty acid synthase, stearoyl CoA desaturase-1, and lipoprotein lipase.(23-26) Therefore, SREBPs coordinate the synthesis of the two major building blocks of membranes, fatty acids, and also, cholesterol.

From a historical perspective, the cholesterol biosynthetic pathway was the first anabolic pathway recognized to undergo end-product feedback suppression. In the early 1950s, Gould et al.(27) incubated liver slices from dogs and rabbits with [^{14}C]acetate and observed that its incorporation into cholesterol was reduced to <2% of the control value when cholesterol had been supplied in the diet. Then in the 1960s, an important enzymatic site for this regulation was noted as the endoplasmic reticulum enzyme (HMG-CoA reductase, which converts HMG CoA to the 6-carbon intermediate, mevalonate.(28, 29) Thus, when cholesterols is added to the diet, cholesterol synthesis is nearly completely suppressed in the liver, is partially suppressed in the intestine, and is low in other body tissues.(13, 30) Feedback suppression of cholesterol synthesis in the liver by dietary cholesterol is mediated through changes in the activity of HMG-CoA reductase.(12, 31) It was thus reasoned that changes in reductase activity are closely related to changes in the overall rate of cholesterol synthesis.(12) And when liver cells become malignant, the control mechanism for cholesterol synthesis is more or less lost.(12) Hence, inhibition of HMG-CoA reductase would represent an effective way of lowering plasma cholesterol in humans.

In 1971, there was a project which initiated the search for microbial metabolites that would inhibit HMG-CoA reductase.(32) The premise of the search was that the suppression of *de novo* cholesterol synthesis in the body by inhibiting HMG-CoA reductase would reduce plasma cholesterol levels in humans. A series of studies showed that a reductase inhibitor, namely mevastatin (or previously known was ML-236B or compactin)(33) had potential. By 1980, the same investigators had shown that mevastatin significantly lowers the levels of LDL-cholesterol in both experimental animals and humans.(34-36) Mevastatin has a hexahydronaphthalene skeleton substituted with a β-hydroxy-δ-lactone moiety, which can be converted into the water-soluble open acid by treatment with alkali.(37) Mevastatin was also shown to inhibit sterol syntheses from both [^{14}C] acetate and [^{14}C]HMG-CoA at nanomolar concentrations but showed no effect on the conversion of [^{3}H]mevalonate into sterols. The obtained results showed mevastatin to be a potent inhibitor of HMG-CoA reductase. Thereafter, the search for additional HMG-CoA reductase inhibitors was continued for another 10 years, leading to the isolation of several compounds of the mevastatin

family.**(38)** The inhibition of HMG-CoA reductase by mevastatin was reversible and competitive with respect to HMG-CoA. Specifically, the K_i value for the acid form was ~1 × 10^{-9} M, while under the same conditions, the K_m for HMG-CoA was ~10^{-5} M.**(37)** It was realized that the affinity of HMG-CoA reductase for compactin is 10,000 fold higher than its affinity for the natural substrate HMG-CoA, providing mevastatin to be a highly potent inhibitor. This mechanism of action by which mevastatin inhibits reductase appeared to be ideal for its development as a drug. Initially, adenosine-2'-monophospho-5'-diphosphoribose, a synthetic nicotinamide adenine dinucleotide phosphate (NADP) analogue was found to be competitive with NADPH in the reaction of HMG-CoA reductase.**(39)** HMG-CoA first binds to the enzyme, followed by the binding of NADPH. Reduction then occurs, with release of NADP, CoA, and mevalonate from the enzyme. These observations seemed to suggest that the lactone portion of the mevastatin molecule is the active center and binds to the HMG binding site of the reductase molecule. The structural similarity between the lactone and HMG portions supports this report. Eventually it was demonstrated that the tight binding of mevastatin is the result of its simultaneous interaction with the HMG binding domain of the enzyme and the adjacent hydrophobic pocket.**(40)** The structural similarity between mevastatin and HMG-CoA and the observed competition by these two molecules helped to further clarify preliminary structure-activity relationships in the inhibition of HMG-CoA reductase. Preliminary studies of the structure-activity relationships suggested an important role for the 3- and 5-hydroxy groups in HMG-CoA reductase inhibition, as activity is abolished by the conversion of either of these hydroxyl groups into the methyl ester.**(32)** The distance between the lactone and decalin ring influences the inhibitory activity which suggests that a certain spatial relationship needs to be maintained between the reactive site (lactone) and the putative binding sites (decalin ring).**(38, 41)**Another essential functional region of mevastatin is its hexahydronaphthalene ring. In 1979, Brown, Dana, and Goldstein**(42)** observed that HMG-CoA reductase activity of cultured mammalian cells is suppressed by LDL, but not by HDL. Later on, they also discovered a cell surface receptor for LDL and elucidated the mechanism by which this receptor mediates feedback control of cholesterol synthesis and HMG-CoA reductase.**(43-45)** These studies served as the grounds in supporting the general idea of developing HMG-CoA reductase inhibitors. In 1985, the Nobel Prize in

Physiology or Medicine was awarded jointly to Brown and Goldstein for their discoveries concerning the regulation of cholesterol metabolism.

While developing cholesterol-lowering agents, the demonstration of efficacy and safety was needed and thus, thoroughly investigated in animal models. In initial investigations, mevastatin was orally given to rats, and plasma lipid levels were measured 3 to 8 hours afterwards. Surprisingly, the feedings of rats with a diet supplemented with 0.1% mevastatin for 7 days caused no changes in plasma cholesterol levels.**(46)** This continued to be the case even when the agent was given to the animals at a dose as high as 500mg/kg for five weeks. Mevastatin was equally ineffective in mice, producing no detectable effects on plasma lipids at 500 mg/kg for five weeks. Mevastatin, when given to rats, inhibited sterol synthesis *in vivo* in the liver for 3 to 8 hours, which showed that the agent was acutely active in rats.**(47)** However, when rats received multiple dose of the drug, hepatic HMG-CoA reductase increased up to 3–10 times compared to controls.**(46)** It became known that rats experienced novel hypercholesterolemia with the administration of nonionic detergent Triton WR-1339.(48) Based on that study, others had then studied and confirmed that the elevated levels of hepatic HMG-CoA reductase were responsible for increased plasma cholesterol.(49-51) In such rat models, the use of mevastatin did result in a slight reduction of plasma cholesterol (-21%), but was still insufficient.(52) As such, the investigators had a hunch that mevastatin should be evaluated within animal models comparable to FH in humans, since in patients with FH, regulation of HMG-CoA reductase is nearly completely lost, resulting in high reductase activity.**(45)**

Commercial eggs contain approximately 300 mg of cholesterol. Based on preliminary analyses, 2/3 of that amount of cholesterol is derived from diet and the remained is supplied by *de novo* synthesis. Thus, authors decided to feed hens that were actively producing eggs a commercial diet supplemented with 0.1% mevastatin for 30 days. Plasma cholesterol was then reduced by as much as 50%, while body weight, diet consumption and egg production were unchanged throughout the experiments.**(53)** This opened up a leeway to conduct experiments in dogs and monkeys. Interestingly, in dogs, mevastatin reduced plasma cholesterol by 30% at a dose of 20mg/kg and as much as 44% at 50mg/kg.**(34)** Ultimately, mevastatin was given to

monkeys for 11 days. The reduction of plasma cholesterol was 21% at a dose of 20mg/kg and 36% at a dose of 50mg/kg.**(35)**

At that point, mevastatin was shown effective in lowering plasma cholesterol in poultry, canine and primate models, but has no effect in rodents. It was then hypothesized that the species differences in mevastatin efficacy was secondary to the ability of certain species to metabolize plasma lipoprotein via hepatic pathways. Mevastatin administration should cause a transient decrease of hepatic cholesterol. In order to meet this deprivation, an increased consumption of plasma cholesterol would occur in hens, dogs, and monkeys, while hepatic HMG-CoA reductase would be elevated in rodents due to their inability to catabolize plasma lipoproteins in the liver. The increase in hepatic reductase, thereby overcoming mevastatin inhibition, is what appears to account for the lack of effectiveness of mevastatin in rodents.**(46)**

Beginning 1976, mevastatin was given at 500 mg/day to a 17-year old patient who had a total cholesterol level of 1000 mg/dl and who had sustained repeated episodes of angina pectoris. Two weeks following treatment with mevastatin, her plasma cholesterol levels were significantly diminished by 20%, but creatinine phosphokinase and transaminases were elevated, and muscular weakness at the proximal part of the extremities comparable to muscular dystrophy was observed.**(36)** The drug was then discontinued due to these adverse effects. By early 1979, several other clinical trials of mevastatin were conducted in patients with severe hypercholesterolemia. These trials were eventually suspended due to the results of a long-term study in which mevastatin was shown to produce severe toxicities in dogs. **(54)** In the early 1980s, data on the LDL-cholesterol-lowering effects of mevastatin in seven patients with FH who received the agent for 24 weeks without serious adverse effects were published.**(55)** LDL-cholesterol was reduced by 29% at a dose of 30 or 60 mg/day and the effect was sustained during the treatment period, with a slight increase in high-density lipoprotein (HDL).**(55)** In a subsequent report, the same authors used the combination of mevastatin and cholestyramine, a bile acid sequestrant in patients with heterozygous FH. LDL-cholesterol significantly decreased by up to 60% without serious adverse effects.**(56)** An

important reduction of plasma cholesterol was also observed in a separate report in patients who received a combination of mevastatin and cholestyramine.**(57)** These studies tremendously enthused the development of other effective cholesterol-lowering agents. For example, other pharmaceutical investigators took an interest in lovastatin (MK-803, mevinolin), discovered in 1978, and replicated the methodological approach conducted with mevastatin. Due to the structural similarity between mevastatin and lovastatin, both agents were hypothesized to have the same biological and pharmacological activities. Beginning the early 1980s, the mechanism of action and efficacy of lovastatin was broadly studied. Eventually it was demonstrated that lovastatin was safe and effective in normal subjects with type II hyperlipoproteinemia.**(58)** Lovastatin significantly lowered plasma levels of total cholesterol and LDL-cholesterol in heterozygous FH in a dose-response relationship.**(59-61)** Within a multi-center trial, lovastatin alone was not sufficient to decrease LDL concentration to desirable levels in patients with FH.**(61)** That being said, high doses of lovastatin produced a substantial reduction of LDL cholesterol in most patients.**(59)** In other subsequent studies, lovastatin was associated with significant reductions of LDL-cholesterol levels in subjects with primary moderate and severe hypercholesterolemia.**(62-64)** The cumulative findings of these trials and the observed safety of lovastatin in experimental studies became the basis for the drug's approval as the first statin agent by the US Food and Drug Administration (FDA) in 1987 and its wide adoption thereafter.**(65)** Other cholesterol-lowering agents were also marketed following the success of lovastatin, including atorvastatin, fluvastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin.

Efficacy of statins

Building on the pioneering work described above, pharmaceutical development of the statin class of medicines has transformed the primary and secondary prevention of cardiovascular disease.**(32)** Statins are small-molecule inhibitors of HMG-CoA reductase, which sits at the apex of a molecular pathway called the *mevalonate* cascade.**(66)** Since the early 1980s, statins have proven to be effective in reducing levels of LDL cholesterol**(67, 68)** and became the first-line option for testing the hypothesis that lowering levels of cholesterol would result not only in a reduction of cholesterol levels, but also lead to lower risks of cardiovascular events, cardiac-related and overall mortality **(Table 1)**. The widespread use of

statin therapy in individuals known to have occlusive vascular disease or considered at higher risk of cardiovascular events for other reasons (e.g. old age, hypertension, diabetes, high cholesterol) has been linked to the diminishing levels of LDL and total cholesterol concentrations in several populations.[69, 70] Moreover, given that statin is frequently given to individuals with elevated LDL cholesterol concentrations, the prevalence of persons with high LDL concentrations has decreased as well. Different statins have different effectiveness strengths, where agents such as atorvastatin and rosuvastatin are able to reduce LDL cholesterol per mg of drug to a greater extent than older agents such as simvastatin or pravastatin.[71, 72] Regardless of the agent used, each doubling of a dose produces an extra reduction of approximately 6% points in LDL cholesterol (e.g. atorvastatin of 40 mg vs. 80 mg results in 43% vs. 49% reductions).

Table 1. Randomized outcomes trials of statin therapy

Design	Participants	Statin	Outcome	Main findings
The West of Scotland Coronary Prevention Study Group (WOSCOPS)(73)				
Randomized double-blind placebo-controlled trial to determine the effect of statin therapy on combined incidence of nonfatal myocardial infarction and death from coronary heart disease.	• 6595 men, aged 45–64 years old with a mean plasma cholesterol level of 272±23 mg per deciliter • All men had hypercholesterolemia with no history of myocardial infarction	Pravastatin 40 mg per day	• Primary endpoint: combined incidence of nonfatal myocardial infarction or death from coronary heart disease. • Secondary endpoint: occurrence of death from coronary heart disease, and nonfatal myocardial infarction.	• Plasma cholesterol levels lowered by 20% and LDL cholesterol levels by 26% among pravastatin users • 248 coronary events (nonfatal myocardial infarction or death from coronary heart disease) occurred in the placebo group vs. 174 in the pravastatin group (RR: 0.69, 95% CI: 0.57–0.83, $P<0.001$)
Scandinavian Simvastatin Survival Study Group (4S)(74)				
Randomized double-blind placebo-controlled trial to determine the effects of statin therapy on overall mortality	• 4444 men and women, aged 35–70 years old with a history of angina pectoris or myocardial infarction and total cholesterol of 5.5–8.0 mmol/l and total triglyceride <2.5 mmol/l	Simvastatin 20–40 mg per day titrated to achieve total cholesterol of 3.0–5.2 mmol/l	• Primary endpoint: overall mortality • Secondary endpoint: time to first major coronary event (coronary death, nonfatal myocardial infarction, resuscitated cardiac arrest and definite silent myocardial infarction	• Mean total cholesterol, LDL cholesterol, and HDL cholesterol lowered by 25%, 35%, and increased by 8%, respectively under simvastatin • 256 deaths occurred in the placebo group compared to 182 in the simvastatin group (RR: 0.70, 95% CI: 0.58–0.85, $P=0.0003$) • 622 major coronary events occurred in the

Design	Participants	Statin	Outcome	Main findings
				placebo vs. 431 events in the simvastatin group (RR:
The Cholesterol and Recurrent Events Trial (CARE)(75)				
Randomized double-blind placebo-controlled trial to determine the effect of statin therapy on combined incidence of fatal coronary event or a nonfatal myocardial infarction.	4159 men and women, aged 21–75 years old, with myocardial infarction who had plasma total cholesterol levels <240 mg/dL and LDL cholesterol levels of 115–174 mg/dL.	Pravastatin 40 mg per day	Primary endpoint: combined incidence of fatal coronary event or a nonfatal myocardial infarction.	• LDL cholesterol level was 28% lower for pravastatin vs. placebo • 274 coronary events were recorded for placebo vs. vs. 212 for pravastatin • Pravastatin was associated with lower incidence of fatal coronary heart disease or confirmed myocardial infarction vs. placebo (OR: 0.76, 95% CI: 0.64–0.91, P=0.003).
The Post Coronary Artery Bypass Graft Trial (Post-CABG)(76)				
Two-by-two factorial design to assign patients	• 1351 men and women, aged 21–74 years old who had LDL cholesterol levels of <200 mg/dL, and ≤2 saphenous-vein coronary bypass grafts placed 1–11 years prior to study • LDL cholesterol levels of 130–175 mg/dL and triglyceride levels <300 mg/dL	Aggressive lowering of LDL cholesterol (40 mg per day of lovastatin) or moderate lowering (2.5 mg per day of lovastatin) with anticoagulation (warfarin) vs. placebo	• Primary endpoint: per-patient percentage of initially patent major grafts that had substantial progression of atherosclerosis (decrease of ≥0.6 mm in lumen diameter) • Clinical outcomes: Death from cardiovascular or unknown causes, nonfatal myocardial infarction, stroke,	Mean LDL cholesterol level of patients who received aggressive treatment ranged from 93–97 mg/dL vs. 132–136 mg/dL for those who received moderate treatment (P<0.001)

Design	Participants	Statin	Outcome	Main findings
			bypass surgery or angioplasty	
Long-term intervention with pravastatin in ischemic disease Study (LIPID)(77)				
Randomized double-blind placebo-controlled trial to determine the effect of statin therapy on mortality from coronary heart disease	9014 men and women aged 31–75 years, with a history of myocardial infarction or hospitalization for unstable angina and initial plasma total cholesterol levels of 155–271 mg/dL	Pravastatin 40 mg per day	• Primary endpoint: Mortality from coronary heart disease. • Secondary endpoint: Overall mortality	• Death from coronary heart disease in 8.3% of the placebo group vs. 6.4% in the pravastatin group (OR: 0.76, 95% CI: 0.65–0.88, $P<0.001$) • Death from any cause in 14.1% of the placebo group vs. 11.0% in the pravastatin group (OR: 0.78, 95% CI: 0.69–0.87, $P<0.001$).
Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TEXCAPS)(78)				
Randomized double-blind placebo-controlled trial to determine the effect of statin therapy on prevention of first acute major coronary event	5608 men and women aged 45–73 years old (55–73 for women) without clinical evident atherosclerotic cardiovascular disease	Lovastatin (20–40 mg per day)	Primary endpoint: First acute major coronary event defined as fatal or nonfatal myocardial infarction, unstable angina, or sudden cardiac death.	183 primary events observed in the placebo vs. 116 in the lovastatin group (RR: 0.63, 95% CI: 0.50–0.79, $P<0.001$)
Effect of rosuvastatin in patients with chronic heart failure (the GISSI-HF trial)(79)				
Randomized double-blind placebo-controlled trial to determine the effect of statin therapy on mortality from cardiovascular disease or any cause.	4574 men and women aged ≥18 years old or older with symptomatic chronic heart failure.	Rosuvastatin 10 mg per day	Primary endpoints: Time to death, and time to death or admission to hospital for cardiovascular reasons	1283 primary events observed in the placebo group vs. 1305 events in the rosuvastatin group (HR: 1.01, 95% CI: 0.91–1.11, $P=0.903$)

Design	Participants	Statin	Outcome	Main findings
The Medical Research Council and the British Heart Foundation (MRC/BHF) Heart Protection Study(80)				
Randomized, double-blind, placebo-controlled trial to determine the effect of statin therapy on overall mortality and fatal or non-fatal vascular events.	20536 men and women aged 40–80 years old with coronary disease, other occlusive arterial disease, or diabetes.	Simvastatin 40 mg per day	Primary endpoint: Mortality and fatal or non-fatal vascular events	• 937 vascular deaths occurred in the placebo group vs. 781 in the simvastatin group (RR: 0.83, 95% CI: 0.75–0.91, $P<0.001$) • 2585 major vascular event was recorded in the placebo group vs. 2033 in the simvastatin group (RR: 0.76, 95% CI: 0.72–0.81, $P<0.001$).
The Lescol(R) Intervention Prevention Study (LIPS)(81)				
Randomized double-blind placebo-controlled trial to determine the effect of statin therapy in reducing major adverse cardiac events	• 1677 men and women aged 18–80 years old with stable or unstable angina or silent ischemia following completion of their first percutaneous coronary intervention • Baseline total cholesterol levels between 135–270 mg/dL with fasting triglycerides <400 mg/dL	Fluvastatin 80 mg per day	Primary endpoint: Time to a major adverse cardiac event, including cardiac death, nonfatal myocardial infarction, or re-intervention procedure.	222 patients experienced ≥1 major adverse cardiac event in the placebo group vs. 181 in the fluvastatin group (RR: 0.78, 95% CI: 0.64–0.95, $P=0.01$)
PROspective Study of Pravastatin in the Elderly at Risk (PROSPER)(82)				
Randomized double-blind placebo-controlled trial to determine the effect of statin therapy on	5804 men and women with pre-existing vascular disease or increased risk due to smoking, hypertension, or diabetes	Pravastatin 40 mg per day	Primary endpoint: composite of coronary death, nonfatal myocardial infarction and fatal or nonfatal	• LDL cholesterol was reduced by 27% with pravastatin • 408 major cardiovascular events occurred in the

Design	Participants	Statin	Outcome	Main findings
reduction of a major cardiovascular event in elderly patients	mellitus		stroke	pravastatin group vs. 473 in the placebo group (HR: 0.85, 95% CI: 0.74–0.97, P=0.014)
The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT)(83)				
Randomized trial to determine the effect of statin therapy on reduction of all-cause mortality compared to usual care.	623 men and women aged ≥55 years old with hypertension and ≥1 additional cardiovascular risk factor.	Pravastatin 40 mg per day	Primary endpoint: all-cause mortality	• Total mortality was similar between the two groups • Nonfatal myocardial infarction and coronary heart disease deaths were 9% lower in the pravastatin group, but not statistically significant
Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT)(84)				
Randomized double-blind placebo-controlled trial to determine the effect of statin therapy on non-fatal myocardial infarction and fatal coronary heart disease.	10305 men and women aged 40–79 years old with hypertension and non-fasting total cholesterol concentrations of ≤6.5 mmol/L	Atorvastatin 10 mg per day	Primary endpoint: non-fatal myocardial infarction and fatal coronary heart disease	• 100 primary events recorded in the atorvastatin group vs. 154 in the placebo group (HR: 0.64, 95% CI: 0.50–0.83, P=0.0005)
Collaborative Atorvastatin Diabetes Study (CARDS)(85)				
Randomized double-blind placebo-controlled trial to determine the effect of statin therapy on reduction of cardiovascular events in	2838 men and women aged 40–75 years old with type 2 diabetes mellitus with no previous history of cardiovascular disease, an LDL-cholesterol level of ≤4.14 mmol/L, fasting	Atorvastatin 10 mg per day	Primary endpoint: time to first occurrence of acute coronary heart disease events, coronary revascularization, or stroke	• 127 primary events were recorded in the placebo group vs. 83 in the atorvastatin group (HR: 0.63, 95% CI: 0.48–0.83, P=0.001). • Trial terminated 2 years

Design	Participants	Statin	Outcome	Main findings
patients with type 2 diabetes mellitus	triglyceride of ≤6.78 mmol/L at one other risk factor for cardiovascular disease			earlier due to benefit
Aggressive Lipid-Lowering Initiation Abates New Cardiac Events (ALLIANCE)(86)				
Randomized trial to determine the effect of statin therapy with a known history of coronary heart disease compared to usual care.	2442 men and women aged >18 years old with known coronary heart disease defined as acute myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass graft surgery, or unstable angina.	• Atorvastatin 10 mg per day. Dose doubled every four weeks until an LDL-C level of <80 mg/dL or a maximum dose of 80 mg per day was reached. • Usual care means patients were maintained on the lipid-lowering program already prescribed.	• Primary endpoint: time to the first occurrence of cardiac death, nonfatal myocardial infarction, resuscitated cardiac arrest, cardiac revascularization, and unstable angina requiring hospitalization • Secondary endpoint: non-cardiac death, peripheral revascularization, hospitalization for congestive heart failure, stroke	• 289 primary events were observed in the atorvastatin group vs. 333 in the usual care group (HR: 0.83, 95% CI: 0.71–0.97, P=0.02) • Levels of LDL-C were reduced more in the atorvastatin vs. usual care group (34.3% vs. 23.3%, P<0.0001)
Atorvastatin Study for Prevention of Coronary Heart Disease Endpoints in non-insulin-dependent diabetes mellitus (ASPEN)(87)				
Randomized double-blind placebo-controlled trial to determine the effect of statin therapy on the occurrence of a major cardiovascular event.	• 2410 men and women aged 40–75 years old with type 2 diabetes mellitus ≥3 years prior to screening. • LDL cholesterol ≤140 mg/dL in those with a history of myocardial	Atorvastatin 10 mg per day	Primary endpoint: cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, recanalization, coronary artery bypass surgery, resuscitated	• 13.7% of patients experienced a primary event in the atorvastatin group vs. 15.0% in the placebo group (HR: 0.90, 95% CI: 0.73–1.12) • Combined endpoint

Design	Participants	Statin	Outcome	Main findings
	infarction or interventional procedure ≥3 months prior, LDL cholesterol ≤160 mg/dL otherwise • Triglyceride levels were required to be ≤600 mg/dL at all visits.		cardiac arrest, and worsening or unstable angina requiring hospitalization	reductions was not statistically significant
Management of Elevated Cholesterol in the Primary Prevention Group of Adult Japanese (MEGA) Study(88)				
Randomized double-blind trial to determine the effect of statin therapy and diet on the first occurrence of coronary heart disease compared to an assigned diet alone.	8214 men and women aged 40–70 years old with hypercholesterolaemia and no history of coronary heart disease or stroke	Pravastatin 10–20 mg per day with diet	Primary endpoint: first occurrence of coronary heart disease	• 66 primary events were recorded in the pravastatin plus diet group vs. 101 in the diet alone group (HR: 0.67, 95% CI: 0.49–0.91, *P*=0.01) • Mean total cholesterol level was reduced by 2.1% and 11.5% in the diet alone vs. pravastatin plus diet groups, respectively
Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER)(89)				
Randomized double-blind placebo-controlled trial to determine the effect of statin therapy on major cardiovascular events	• 17802 men and women aged ≥50 years old (≥60 years old for women) with LDL cholesterol concentration <3.4 mmol/l but high-sensitivity C-reactive protein ≥2mg/l • 41% of patients had metabolic syndrome	Rosuvastatin 20 mg per day	Primary endpoint: occurrence of first major cardiovascular event, including nonfatal myocardial infarction, nonfatal stroke hospitalization for unstable angina, an arterial revascularization procedure or confirmed	142 major cardiovascular events occurred in the rosuvastatin group vs. 251 in the placebo group (HR: 0.56, 95% CI: 0.46–0.69, *P*<0.001) Median LDL cholesterol reduced by 50% in the rosuvastatin group and high-sensitivity C-reactive Protein by 37%

Design	Participants	Statin	Outcome	Main findings
			death from cardiovascular causes	
Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis in Myocardial Infarction 22 (PROVE IT-TIMI 22) trial(90)				
Randomized double blind non-inferiority trial to determine the effect of standard therapy on the combined incidence of a major cardiovascular event or any death compared to intensive therapy	4162 men and women aged ≥18 years old previously hospitalized for an acute coronary syndrome (acute myocardial infarction or high-risk unstable angina)	• Pravastatin 40 mg per day (standard therapy) • Atorvastatin 80 mg per day (intensive therapy)	Primary endpoint: composite incidence of any death, myocardial infarction, unstable angina requiring hospitalization, revascularization, and stroke	• 2-year rates of the primary event were 26.3% in the pravastatin group vs. 22.4% in the atorvastatin group (HR: 0.84, 95% CI: 0.74–0.95, P=0.005) • Intensive therapy was significantly more superior to standard therapy
Aggrastat to Zocor (AtoZ) trial(91)				
Randomized double-blind placebo-controlled trial to determine the effect of statin therapy on a major cardiovascular event	4497 men and women aged 21–80 years old following an acute coronary syndrome event	• Simvastatin 40 mg per day for 1 month followed by 80 mg per day thereafter • Placebo for 4 months followed by simvastatin 20 mg per day	Primary endpoint: composite of cardiovascular event, nonfatal myocardial infarction, readmission for acute coronary syndrome, and stroke	343 patients experienced a primary event in the simvastatin plus placebo group vs. 309 in the simvastatin only group (HR: 0.89, 95% CI: 0.76–1.04, P=0.14)
Treating to New Targets (TNT) trial(92)				
Randomized double-blind trial to compare the effects of low vs. high-dose statin on major cardiovascular events	10001 men and women aged 35–75 years old who had clinical evident coronary heart disease (defined as previous myocardial infarction,	• Atorvastatin 10 mg per day (low-dose) • Atorvastatin 80 mg per day (high-dose	Primary endpoint: composite incidence of coronary heart disease death, nonfatal, nonprocedure related myocardial infarction,	434 primary events occurred in the high-dose group vs. 548 events in the low-dose group (HR: 0.78, 95% CI: 0.69–0.89, P<0.001)

Design	Participants	Statin	Outcome	Main findings
	previous or current angina with objective evidence of atherosclerotic coronary heart disease, and a history of coronary revascularization		resuscitation after cardiac arrest or fatal or nonfatal stroke	
Incremental Decrease in End Points Through Aggressive Lipid Lowering (IDEAL) study(93)				
Randomized open-label blinded endpoint evaluation trial to compare effects of usual-dose statin to high-dose statin therapy on incidence of major coronary events	8888 patients aged <80 years old with a history of acute myocardial infarction	• Simvastatin 20 mg per day (usual-dose) • Atorvastatin 80 mg per day (high-dose)	Primary endpoint: composite occurrence of coronary death, confirmed nonfatal acute myocardial infarction or cardiac arrest with resuscitation	411 primary events occurred in the high-dose group vs. 463 in the usua-dose group (HR: 0.89, 95% CI: 0.78–1.01, *P*=0.07)
Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH)(94)				
Randomized double-blind trial to compare the effect of high-dose vs. usual-dose statin therapy on the risk of a major vascular event	12064 men and women aged 18–80 years old with a history of myocardial infarction	• Simvastatin 80 mg per day (high-dose) • Simvastatin 20 mg per day (usual-dose)	Primary endpoint: a major vascular event defined as coronary death, myocardial infarction, stroke, or arterial revascularization	1477 patients experienced a major vascular events in the high-dose group vs. 1553 in the usual-dose group (RR: 0.94, 95% CI: 0.88–1.01, *P*=0.10)

In an effort to establish the overall success of statins and to follow its progress, the Cholesterol Treatment Trialists' (CTT) Collaboration was created with a pre-specified purpose, which was to assess the effects of lowering LDL cholesterol on atherosclerotic events in different types of patients by conducting meta-analyses of individual patient data from all of the randomized controlled trials of statin therapy with minimum 2 years of therapy with at least 1000 patients.**(95)** Overall, the trials of statin therapy in the primary intervention setting compared to placebo showed an effective 20% proportional reduction in the major vascular event rate per mmol/L LDL cholesterol reduction.**(96)** In the high-dose vs. low-dose trials, the average 0.5 mmol/L (19.3 mg/dL) further reduction in LDL cholesterol resulted in 15% further proportional reduction in the rate of major vascular events.

It has been established that the absolute benefits of using statin therapy depend on the individual's absolute risk of atherosclerotic events and the absolute reduction in LDL cholesterol that can be achieved. In a meta-analysis, CTT showed that 5 years of treatment with a statin therapy that lowers LDL cholesterol by 2 mmol/L (77.2 mg/dL) would be expected to prevent major vascular events in 1000 per 10,000 high-risk patients (10%) and in 500 per 10,000 low-risk patients (5%).**(97)** In some trials, a continued follow-up beyond the end of the study showed that the benefits of statin therapy persisted for many years after the differences in statin use between the randomized groups have stopped.**(98-106)** In terms of coronary mortality, the CTT meta-analyses also showed that a 12% proportional reduction in vascular mortality per mmol/L LDL cholesterol reduction was attributable to 20% proportional reduction in coronary deaths, and an 8% reduction in other cardiac deaths.**(96, 97)**

Statins and the risk of cancer

Pre-clinical studies

Whilst statins inhibit HMG-CoA reductase activity that catalyzes the first rate-limiting step in the mevalonate pathway, and hence are used to treat hypercholesterolaemia**(107)**, HMG-CoA reductase also regulates protein prenylation (farnesylation and geranyl-geranylation) that

facilitates membrane attachment of target proteins involved in cell adhesion, migration and proliferation (e.g. Rho, Rac, Ras).**(108)** Initially, some pre-clinical data in mice showed that statins might be associated with increased risks of liver, forestomach, lung, and thyroid tumors, as well as lymphoma.**(109)** This prompted the development of a whole catalogue of research for the next generation examining the effect of statins on the possible association with cancer.

The plausible mechanisms of how statins can have an effect on cancer can occur through HMG-CoA reductase-dependent or independent pathways. Some of such effects happen through inhibition of HMG-CoA reductase (e.g G-protein activation through geranyl-geranylation). Otherwise, statins can operate by binding directly to lymphocyte-function associated antigen 1 (LFA1). Statins have pleiotropic effects on processes such as angiogenesis and inflammation, and may affect a number of molecular targets and complex signaling pathways. The pleitropic effects of statins result in enhanced risk of some chronic diseases, such as diabetes, age-related macular degeneration, as well as cancer. Statins are thought to exert their potentially beneficial effects in cancer by inhibiting the prenylation of small G-proteins, primary Rho proteins, as a downstream effect of HMG-CoA reductase inhibition.**(110)** Statins also inhibit the formation of downstream lipid isoprenoid intermediates, such as farnesysl pyrophosphotate (FPP) and geranylgeranyl pyrophosphotate (GGPP). Isoprenoids are lipid moieties that are added to proteins, such as G-proteins and its subunits (e.g. Ras, Rho, Rab, Rac, and Rap) during post-translational modification (prenylation), which are necessary to anchor these proteins to the cell membrane. Isoprenoids inhibit HMG-CoA reductase by post-translational downregulation. In normal cells, the reductase undergoes complex feedback regulation at the transcriptional, translational and post-translational levels though the mevalonate pathway. Tumor cells, on the other hand, are resistant to the sterol-mediated feedback of the mevalonate pathway are more sensitive than normal cells to isoprenoid-mediated suppression.**(110-113)**

FPP prenylates Ras (farnesylation). Eventually it was found that GGPP prenylation (geranylgeranylation) of other proteins was a crucial step in the apoptopic, angiogenic and anti-inflammatory effects of statins. Adding GGPP, as well as adding mevalonate, reverses the

desirable effects of statins. Adding FPP doesn't necessarily reverse the effect, despite being the precursor of GGPP, as the restoration requires isopentenyl pyrophosphate (IPP). However, statins block IPP formation upstream of FPP, therefore IPP is not available for converting FPP into GGPP. At the same time it has been seen that adding mevalonate may reverse the effects of statins, as mevalonate cancer restore IPP for the downstream conversion of FPP into GGPP.**(112, 113)**

Previously, it was shown that Rho proteins are important for carcinogenesis.**(114-118)** Specifically, overexpression of RhoA and/or RhoC is associated with poor prognosis in colorectal, breast, bladder, and pancreatic cancers. In ribonucleic acid (RNA) studies, RhoC is the most important isoform in stimulating invasion. RhoA is implicated in epithelial-to-mesenchymal transition (i.e. cancer progression).**(114-116)** Statins can induce apoptosis, a critical component of carcinogenesis, by regulating several signaling pathways including the RAF-mitogen activated protein kinase 1 (MAP2K1)– extracellular regulated kinase (ERK) pathway.**(119)** Statins can also induce apoptosis through the activation of FAS (CD95).**(120)** The effect of statins in cancer models has been revealed in lung, colorectal, breast, and melanoma cancers. For example, statins significantly reduced tumor growth and tumor vascularization in the Lewis lung cancer model.**(121)** Using rat intestine epithelial cells, lovastatin was shown to induce apoptosis by inhibiting geranylgeranylation of the Rho family proteins.**(122)** In 344 rats, pravastatin inhibited colon carcinogenesis induced by the direct-acting carcinogen N-methyl-*N*-nitrosourea.**(123)** Mevastatin inhibited the spread of mouse colon cancer cells that were transplanted into naïve mice, suggesting an anti-metastatic effect with statins.**(124)** In *vitro* studies show that a number of statins inhibit the proliferation of breast cancer cells.**(125-127)** In the same context, statins have shown to induce apoptosis in immortalized breast cancer cell lines through RhoA, which are overexpressed in breast cancer.**(128)** Cerivastatin prevents prenylation of RhoA, causing loss of RhoA from the cellular membrane in breast cancer cells.**(129)** In *vivo* studies showed that lovastatin and simvastatin can decrease tumor formation and inhibit metastasis in mouse mammary tumor models.**(130, 131)** RhoA and RhoC are expressed in human melanoma.**(132)** By inhibiting geranylgeranylation of the Rho family proteins, statins have been shown to induce apoptosis *in vitro* analyses**(133)** and to inhibit invasion *in vivo* studies of human melanoma cells**(134)**.

Concomitantly, other studies have shown that the associations of statins and cancer are not always directly related to the reduction of cholesterol, thereby suggesting HMG-CoA reductase' independent effects. For example, it was previously shown that lovastatin directly binds to the L site of the I domain of the integrin LFA1, which plays an important role in leukocyte migration and T-cell activation.**(135)** Simvastatin and mevastatin were also shown to inhibit LFA1 by binding to the L-site. Blocking the LFA1-intercellular adhesion molecule 1 (ICAM1) interaction may lead to various statins effects on cell adhesion, invasion and inflammation. Recently it was also shown that statins preferentially suppress mutp53-expressing cancer cell growth, and highlights the significance of p53 status in impacting statins' efficacy on cancer therapy.**(136)** In this context it is well established that stabilization of mutant p53 (mutp53) in tumors contributes to malignant progression.**(137)**

Clinical evidence

Although many randomized-controlled trials of single-agent statins for the prevention of cardiovascular disease assessed overall cancer incidence **(Figure 1)** and/or mortality **(Figure 2)**, they were considered as secondary/exploratory endpoints, and therefore not powered to detect a significant difference.

Figure 1. Meta-analysis depicting the effect of statins vs. controls on cancer occurrence using clinical trials.

Study or Subgroup	Statins Events	Total	Controls Events	Total	Weight	Odds Ratio M–H, Random, 95% CI	Year	Odds Ratio M–H, Random, 95% CI
SSSS	57	2221	61	2223	2.2%	0.93 [0.65, 1.35]	1994	
Scotland Coronary Prevention Study Group	116	3302	106	3293	4.0%	1.09 [0.84, 1.43]	1995	
PCABGTI	39	676	35	675	1.3%	1.12 [0.70, 1.79]	1997	
LIPID	379	4512	399	4502	13.4%	0.94 [0.81, 1.09]	1998	
ALLHAT–LLT	378	5170	369	5185	13.0%	1.03 [0.89, 1.19]	2002	
LIPS	46	844	49	833	1.7%	0.92 [0.61, 1.40]	2002	
PROSPER	245	2891	199	2913	7.6%	1.26 [1.04, 1.53]	2002	
MRC/BHF	814	10269	803	10267	28.0%	1.01 [0.92, 1.12]	2002	
ALLIANCE	67	1217	77	1225	2.5%	0.87 [0.62, 1.22]	2004	
German Diabetes and Dialysis	39	619	44	636	1.5%	0.90 [0.58, 1.41]	2005	
MEGA	119	3866	126	3966	4.5%	0.97 [0.75, 1.25]	2006	
CORONA	156	2514	144	2497	5.3%	1.08 [0.86, 1.37]	2007	
JUPITER	298	8901	314	8901	11.1%	0.95 [0.81, 1.11]	2008	
AURORA	107	1389	118	1384	3.9%	0.90 [0.68, 1.18]	2009	
Total (95% CI)		48391		48500	100.0%	1.01 [0.95, 1.06]		
Total events	2860		2844					

Heterogeneity: Tau² = 0.00; Chi² = 9.65, df = 13 (P = 0.72); I² = 0%
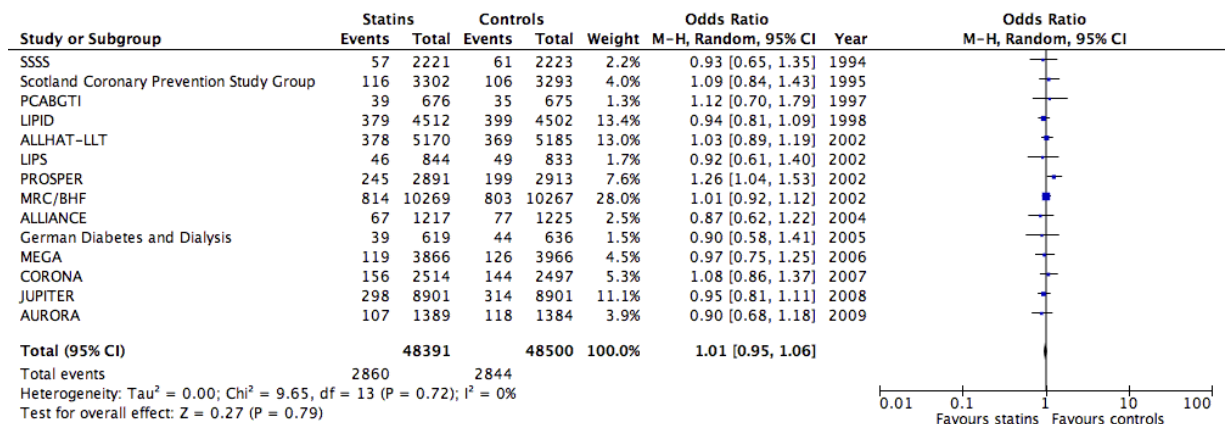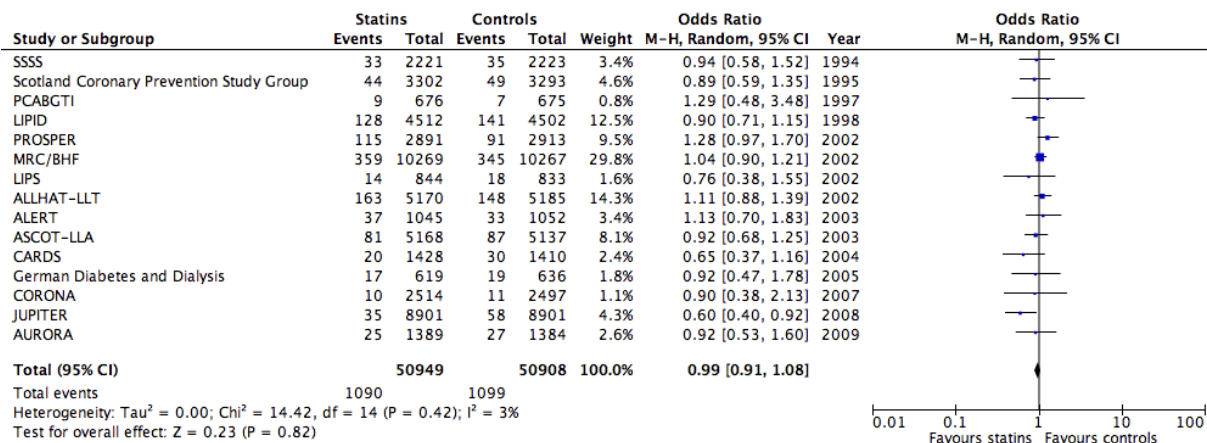Test for overall effect: Z = 0.27 (P = 0.79)

Favours statins   Favours controls

Figure 2. Meta-analysis depicting the effect of statins vs. controls on cancer mortality using clinical trials.

Study or Subgroup	Statins Events	Total	Controls Events	Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
SSSS	33	2221	35	2223	3.4%	0.94 [0.58, 1.52]	1994
Scotland Coronary Prevention Study Group	44	3302	49	3293	4.6%	0.89 [0.59, 1.35]	1995
PCABGTI	9	676	7	675	0.8%	1.29 [0.48, 3.48]	1997
LIPID	128	4512	141	4502	12.5%	0.90 [0.71, 1.15]	1998
PROSPER	115	2891	91	2913	9.5%	1.28 [0.97, 1.70]	2002
MRC/BHF	359	10269	345	10267	29.8%	1.04 [0.90, 1.21]	2002
LIPS	14	844	18	833	1.6%	0.76 [0.38, 1.55]	2002
ALLHAT-LLT	163	5170	148	5185	14.3%	1.11 [0.88, 1.39]	2002
ALERT	37	1045	33	1052	3.4%	1.13 [0.70, 1.83]	2003
ASCOT-LLA	81	5168	87	5137	8.1%	0.92 [0.68, 1.25]	2003
CARDS	20	1428	30	1410	2.4%	0.65 [0.37, 1.16]	2004
German Diabetes and Dialysis	17	619	19	636	1.8%	0.92 [0.47, 1.78]	2005
CORONA	10	2514	11	2497	1.1%	0.90 [0.38, 2.13]	2007
JUPITER	35	8901	58	8901	4.3%	0.60 [0.40, 0.92]	2008
AURORA	25	1389	27	1384	2.6%	0.92 [0.53, 1.60]	2009
Total (95% CI)		50949		50908	100.0%	0.99 [0.91, 1.08]	
Total events	1090		1099				

Heterogeneity: Tau² = 0.00; Chi² = 14.42, df = 14 (P = 0.42); I² = 3%
Test for overall effect: Z = 0.23 (P = 0.82)

Odds Ratio M-H, Random, 95% CI — 0.01 0.1 1 10 100 — Favours statins Favours controls

That being said, the increased risk of cancer and cancer mortality was observed in a few specific instances. For example, data from the first two simvastatin trials showed that non-melanoma skin cancer was more common in the treatment groups.**(74, 80)** Specifically within the randomized trial of cholesterol lowering in 444 patients with coronary heart disease, the Scandinavian Simvastatin Survival Study (4S) found 13 non-melanoma skin cancer cases within the statin group vs. the placebo group (0.6% vs. 0.3%). Similarly, within the Heart Protection Study (HPS), investigators found that simvastatin-treated patients were diagnosed with 243 non-melanoma skin cancers compared to 202 in the placebo-treated group (2.4% vs. 2.0%).

Within a double-blind phase III trial that lasted five years, the Cholesterol and Recurrent Events (CARE) investigators administered either 40 mg or pravastatin per day or placebo to 4159 patients with myocardial infarction who had plasma total cholesterol levels <240 mg per d/L (mean 209) and LDL cholesterol levels between 115–174 mg/dL (mean 139).**(75)** The primary endpoint of the trial was fatal coronary event or a nonfatal myocardial infarction. Overall, the authors found that 274 patients (13%) experienced a primary event in the placebo group vs. 212 (10%) in the pravastatin group (*P*=0.003). Of note, 161 fatal or nonfatal primary cancers were observed in the placebo group vs. 172 in the pravastatin group. These included

colorectal cancer (21 for placebo vs. 12 for pravastatin, respectively, P=0.045), gastrointestinal cancer (15 vs. 14, respectively, P=0.716), liver cancer (1 vs. 0, respectively, P=0.209), lymphoma or leukemia (10 vs. 8, respectively, P=0.538), and melanoma (3 vs. 4, respectively, P=0.763). Notably, breast cancer occurred in 1 patient in the placebo group vs. 12 in the pravastatin group (P=0.002). The investigators of the trial cautioned against over-interpreting these results, and suggested that an anomaly occurred. Subsequently, investigators of the PROspective Study of Pravastatin in the Elderly At Risk (PROSPER) phase III randomized-controlled trial assigned 5804 men and women aged between 70–82 years old with a history of, or risk factors for, vascular disease to pravastatin (40 mg per day, n=2891) or placebo (n=2913).**(82)** Follow-up was 3.2 years on average, with the primary endpoint defined as a composite of coronary death, non-fatal myocardial infarction, and fatal or non-fatal stroke. Pravastatin was associated with a reduced incidence of the primary event (HR: 0.85, 95% CI: 0.74–0.97, P=0.014). However, new cancer diagnoses were significantly more frequent in the pravastatin than placebo groups (HR: 1.25, 95% CI: 1.04–1.51, P=0.020, **Table 2**).

Table 2. Cancer incidence by site, PROSPER(82)

Site	Pravastatin vs. Placebo HR (95% CI)	P
Breast	1.65 (0.78–3.49)	0.19
Gastrointestinal	1.46 (1.00–2.13)	0.05
Renal or genitourinary	1.00 (0.69–1.43)	0.99
Respiratory	1.12 (0.74–1.70)	0.60
Other	1.41 (0.95–2.09)	0.09
Overall	**1.25 (1.04–1.51)**	**0.02**

The Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) trial, a randomized, double-blind trial that recruited 1873 patients with mild-to-moderate asymptomatic aortic stenosis administered either 40 mg of simvastatin plus 10 mg of ezetimibe or placebo daily.**(138)** The primary endpoint was a composite of major cardiovascular events, including death from cardiovascular causes, aortic-valve replacement, nonfatal myocardial infarction, and

hospitalization for unstable angina pectoris, heart failure, coronary-artery bypass grafting, percutaneous coronary intervention, and non-haemorrhagic stroke. The investigators found a higher number of incident cancer cases in the simvastatin-ezetimibe group (11%) than in the placebo group (7.5%, *P*=0.01). Cancer-related mortality was also slightly higher in the treatment group than the control group, although not statistically significant (HR: 1.67, *P*=0.06 with log-rank continuity correction).

Within the JUPITER trial, focusing on patients with LDL cholesterol levels, but high-sensitivity CRP levels (≥2.0 ng per liter), those treated with rosuvastatin had a lower rate of death due to cancer compared to those treated with placebo (0.4% vs. 0.7%, *P*=0.02).(89) However, the authors did not observe any significant difference between the two treatment groups with respect to newly diagnosed cancers.

In a post-hoc analysis assessing the beneficial effects of high- vs. low-dose atorvastatin in women using the Treating to New Targets (TNT) study, Wenger et al.**(139)** found that the risk of non-cardiovascular mortality was significantly higher among women treated with 80 mg of atorvastatin than women treated with 10 mg of atorvastatin daily (HR: 2.38, 95% CI: 1.30–4.37, *P*=0.004), and that this increased mortality rate was predominantly driven by the higher rate of cancer-related deaths for the same groups, respectively (3.6% vs. 1.6%).

Based on the increase in the incidence of cancer among elderly people assigned to pravastatin therapy in the PROSPER trial, Bonovas & Sitaras sought to assess the effect of pravastatin therapy on cancer risk by performing a detailed meta-analysis of randomized-controlled trials.**(140)** Of 12 randomized-controlled trials that met their inclusion criteria, the overall rate of cancer was 7.4% in the pravastatin group (1583 incident cancer cases) and 7.0% in the placebo group (1505 incident cancer cases). In their report, pravastatin was not found to be significantly associated with cancer in the fixed-effect model (RR: 1.06, 95% CI: 0.99–1.13, *P*=0.1) or the random-effect model (RR: 1.06, 95% CI: 0.97–1.14, *P*=0.2). Beyond that, based on over 10,000 cases of incident cancer in the CTT meta-analyses, there were no apparent increased risks, either overall or at any particular site with an average follow-up time of 5 years.**(96, 141)** Within 22 randomized-trials, with a median follow-up of 4.8 years, comprising

a total of 134,537 individual patient records, reducing LDL cholesterol with statin therapy had no effect on incident cancer cases or on death from such cancers in the trials that compared any statin vs. control (cancer incidence: RR: 1.00, 95% CI: 0.96–1.05; cancer mortality: RR: 1.00, 95% CI: 0.93–1.08) or in the trials that compared high- vs. low-dose statin therapy (cancer incidence: RR: 1.00, 95% CI: 0.93–1.07; cancer mortality: RR: 0.93, 95% CI: 0.82–1.06).

Several criticisms were raised by various sources with regard to the meta-analyses performed on statin use and cancer risk across randomized trials. First, physicochemical properties differ across statins, and can be categorized as hydrophilic or lipophilic. This difference has a direct affect on uptake of a particular statin by extrahepatic cells, including malignant cells, which can inhibit cell growth by down-regulating the synthesis of mevalonate.(142) For example, atorvastatin and fluvastatin are generally considered lipophilic, whereas pravastatin is considered hydrophilic. Lipophilic statins have been shown to enter extrahepatic cells, including cancer cells.(127) Second, by pooling cancer sites for all types of statins, one may temper the risk of cancer if and where one exists. Different cancers follow different clinical course and mediators of prognosis vary widely from one phenotype to another. Agglomerating all cancers into one group is largely considered to be problematic. To account for this limitation, the Cholesterol Treatment Trialists' (CTT) collaborators performed a meta-analysis focusing specifically on cancer, and sites of cancer across 27 randomized trials of statins. No statistically significant effect was observed for statin use and specific cancers.(141) Third, statin trials have limited follow-up durations for the purpose of assessing incidence of cancer. and cancer death. For example, it is not surprising that for some cancers, such as that of prostate, there is an extended latency time between the initiation of the cancer and clinical detection. Although the median age at diagnosis is around 65 years old for prostate cancer, autopsy studies indicate that prostatic intraepithelial neoplasia, a premalignant precursor, and histologic prostate cancer is apparent in the third and fourth decade of a man's life.(143) It is noteworthy that a few of the randomized-controlled statin trials performed post-hoc analyses by extending the follow-up beyond the scheduled study treatment period for up to 15 years with no evidence that any effects on incident cancer or cancer death became manifest.**(98-106)** Ultimately, none of the statin clinical trials were designed to address cancer incidence or mortality. Various considerations in relation to this limitation need to be acknowledged, such

as the lack of distinction between recurring vs. a newly diagnosed cancer, the absence of cancer screening prior to study start, and unknown familial history with respect to cancer.

In summary, there are a few instances where higher incident cancer cases or cancer deaths were reported within randomized settings. One study showed a lower cancer-related death in the statin group. In consideration of the limitations, the overall effect size obtained from pooled post-hoc analyses does not appear to suggest any significant relationship between statin use and cancer. Sub-group analyses of such pooled-analyses revealed similar non-effect.

Secondary data evidence

The persisting debate on statins and cancer may be attributed to the numerous observational studies that have reported significant associations between statins and cancer using secondary data. Large population-based cohorts have been used to examine the association. Notably, Nielsen et al.[144] assessed mortality among patients from the entire Danish population diagnosed with cancer between years 1995 and 2007 and compared those who had used statins regularly prior to their cancer diagnosis (n=18,721) to those who had never used statins (n=27,7204). The authors conducted a nested matched analysis (1:3) for sex, age at cancer diagnosis, year of diagnosis, as well as a propensity-score analysis and additional adjustment for the area code of the provider. Their findings suggest that regular statin users were less likely to die of any cause (HR: 0.86, 95% CI: 0.83–0.89, *P*=0.01) and of cancer (HR: 0.85, 95% CI: 0.81–0.87, *P*<0.001) relative to their never-statin users counterparts.

Blais et al.[145] also using a nested case-control study design relied on the administrative health databases of the *Régie de l'Assurance-Maladie du Québec* (RAMQ) to examine the association between statin use and cancer incidence. Between 1988 and 1994, the authors selected 6,721 beneficiaries aged ≥65 years old during a follow-up of 2.7 years, who were free of cancer one year prior to study entry. Statin users were less likely than bile acid-binding resin users (controls) to be diagnosed with any cancer (RR: 0.72, 95% CI: 0.57–0.92). The effect of statins on incidence of specific cancer sites was variable (**Table 3**).

Table 3. Effect of statins on incidence of specific cancer sites adapted from Blais et al.(145)

Cancer sites	Statins vs. Non-statins Adjusted RR (95% CI)*
Skin	0.81 (0.47–1.39)
Prostate	0.74 (0.36–1.51)
Lung	0.94 (0.43–2.05)
Breast	0.67 (0.33–1.38)
Colon	0.83 (0.37–1.89)
Bladder and Kidney	0.43 (0.16–1.13)
Uterus	0.30 (0.11–0.81)
Lymphoma	2.17 (0.38–12.36)
All other cancers	0.61 (0.43–0.85)

Rate Ratios were adjusted for age, sex, previous cancer, year of cohort entry, use of fibric acids, use of other lipid-reducing agents, and comorbidities.

Similar studies ensued. Graaf et al.(146) wanted to test the risk of incident cancers between statin users and users of other cardiovascular medications within a matched case-control study design using the PHARMO database. Between 1985 and 1998, 3129 cancer cases were matched 16976 controls. Statin users were less likely to be diagnosed with cancer (odds ratio [OR]: 0.80, 95% CI: 0.66–0.96) compared to users of other cardiovascular medications. This trend persisted in sensitivity analyses focusing on usage for >4 years (OR: 0.64, 95% CI: 0.44–0.93) and a minimum of 1350 defined daily doses taken (OR: 0.60, 95% CI: 0.40–0.91). Friis et al.(147) relying on the Prescription Database of North Jutland County and the Danish Cancer Registry compared overall and site-specific cancer incidence among 12251 statin users (≥2 prescriptions) with cancer incidence among non-users and users of other lipid-lowering drugs (n=1257) between years 1989 and 2002. The authors found that the risk of overall cancer among statin users were lower compared to nonusers (RR: 0.86, 95% CI: 0.78–0.95) and compared to other lipid-lowering users (RR: 0.73, 95% CI: 0.55–0.98). Site-specific analyses were not significant.

In contrast, Kaye & Jick(148) found that within the General Practice Research Database, statin use was not associated with 13 cancers (RR: 1.0, 95% CI: 0.9–1.2). However, the authors did

observe that long-term statin utilization (≥5 years) was associated with a slightly increased risk of colon (RR: 3.5, 95% CI: 1.1–10.9) and rectal (RR: 4.2, 95% CI: 1.0–16.6) cancers. The observed increased risk between statin use and colon/rectal cancer was subsequently reversed in a large population-based matched case-control study.(149) Specifically the Molecular Epidemiology of Colorectal Cancer Study found that long-term use of statins (≥5 years) significantly reduced the risk of colorectal cancer (OR: 0.50, 95% CI: 0.40–0.63) compared to non-users. This association remained significant after adjusting for the use or non-use of aspirin or other nonsteroidal anti-inflammatory drugs, physical activity status, hypercholesterolemia, family history of colorectal cancer, ethnic group, and level of vegetable consumption (OR: 0.53, 95% CI: 0.38–0.74). Within a comprehensive meta-analysis of 19 studies totaling over 1.5 million patients, Bonovas et al.(150) found no evidence of an association between statin use and risk of colorectal cancer in randomized-controlled trials (RR: 0.95, 95% CI: 0.80–1.13, k=6) or among cohort studies (RR: 0.96, 95% CI: 0.84–1.11, k=3). However, the authors did observe that statin use was associated with a slight reduction in the risk of colorectal cancer among case-control studies (RR: 0.91, 95% CI: 0.87–0.96, k=9).

Since the CARE trial suggested that statin use may be related to an increased risk of breast cancer, many epidemiological studies sought to explore the association. Beck et al.(151) using a Saskatchewan population health services database identified women with ≥1 prescription of statin from years 1989 to 1997. Following an age and sex-mated non-exposed group with a mean follow-up of 4.2 years, the authors found the risks of breast cancer in those aged >55 years old was higher for statin users (RR: 1.15, 95% CI: 0.97–1.37). An interaction with hormonal therapy was also observed, where they found that for subjects aged >55 years old with hormone replacement therapy exposure times of more than 6 years (i.e. ≥37 prescriptions), statin use was associated with an increased rate of breast cancer (RR: 2.04, 95% CI: 1.20–3.46). While largely speculative, the near doubling of risk may be related to hormonal therapy. In a multicenter prospective cohort study comprising of community-based clinical centers, Cauley et al.(152) sought to test the hypothesis that the use of lipid-lowering drugs may be linked with breast cancer in older women (mean age: 77 years old). In contrast with the previous report, statin users were found to be less likely to be diagnosed with breast cancer than those using other lipid-lowering drugs (RR: 0.28, 95% CI: 0.09–0.86) and

nonusers (RR: 0.37, 95% CI: 0.14–0.99) in adjusted analyses. Coogan et al.(153) assessed the relationship between statin use and the risk of breast cancer in the hospital-based Case-Control Surveillance Study of Drugs and Serious Illnesses. The authors found that among more than 1000 breast cancer cases and matched clinic controls, statin users were more likely, although not statistically significant, to develop invasive breast cancer than non-statin users (RR: 1.20, 95% CI: 0.70–2.0). The lack of a significant association was also observed in a population-based case-control study that comprised of female residents within Washington counties.(154) Cases were identified from the Cancer Surveillance System diagnosed with a primary invasive breast cancer aged between 65–79 years old between years 1997 and 1999. Controls consisted of 1007 women without breast cancer who were randomly chosen from the same source. Statin users were not more likely than nonusers to be diagnosed with invasive breast cancer (OR: 0.90, 95% CI: 0.70–1.20). The effect of long-term use of statins (>5 years) was also without any statistical significance. The pooled weight of the evidence from observational studies indicates that statin use does not increase (or protect against) the risk of breast cancer (fixed effect model RR: 1.03, 95% CI: 0.93–1.14; random effect model RR: 1.02, 95% CI: 0.89–1.18).(155)

The extent of the investigation on the use of statins and prostate cancer has also been quite widespread. Using an ongoing prospectively collected data of the Health Professionals Follow-up Study (HPFS)(156), the investigators evaluated the effects of statins and other cholesterol-reducing drugs on prostate cancer risk. Overall, there was no significant reduction with use of statin and prostate cancer risk. However, a sub-analysis showed that statin users were less likely to harbour an aggressive disease phenotype than nonusers (RR: 0.60, 95% CI: 0.36–1.00). In a case-control study using the Veterans Affairs system(157), statin utilization was inversely associated with prostate cancer risk (OR: 0.38, 95% CI: 0.21–0.69) and high-grade prostate cancer (OR: 0.25, 95% CI: 0.11–0.53). Within a large population-based database relying on electronic records, Yu et al.(158) examined the use of statins following a prostate cancer diagnosis and its effect on cancer-related and overall mortality. Post-diagnosis use of statins was found to be associated with lower risks of prostate cancer mortality (HR: 0.76, 95% CI: 0.66–0.88) and overall mortality (HR: 0.86, 95% CI: 0.78–0.95). The protective effect was even stronger amidst pre-diagnosis statin users.

Inflammation, cardiovascular disease, and cancer

In the last two decades, a few studies have sequentially shown that biomarkers of inflammation, such as high-sensitivity C-reactive protein (CRP) can predict heart disease, independent of traditional cardiovascular risk factors. This led to subsequent studies which appeared to suggest that statin therapy may also contain anti-inflammatory properties in addition to its lipid lowering functions**(159, 160)**, and that the risk of heart disease may be further reduced by targeting inflammation itself. One of the first of such evidence to emerge was a nested case-control study by Ridker et al.**(161)** who compared CRP and serum amyloid A (SAA) levels, established inflammation biomarkers, amongst 391 participants' pre-randomization blood samples collected from the Cholesterol and Recurrent Events (CARE) trial who subsequently developed recurrent nonfatal myocardial infarction or a fatal coronary event to a group of participants who remained free of these events during follow-up. In stratified analyses, the association between inflammation and risk of coronary events was significant among those randomized to placebo (relative risks [RR]: 2.11, *P*=0.048) but was attenuated and non-significant among those randomized to pravastatin (RR: 1.29, *P*=0.5). This raised the possibility that statin therapy may be clinically effective in persons with elevated CRP levels without hyperlipidemia.**(162)**

Subsequently, several investigators demonstrated that the beneficial outcomes after statin therapy relate to both a reduction in cholesterol level and inflammation inhibition, through independent pathways.**(162-165)** Ultimately, Ridker et al.**(163)** showed that patients with low CRP levels after statin therapy had better clinical outcomes (i.e. recurrent myocardial infarction or death from coronary causes) than those with higher CRP levels; independent of LDL cholesterol among 3745 patients with acute coronary syndromes treated with atorvastatin (intensive treatment) or pravastatin (moderate treatment). The same group of authors used those findings to develop the JUPITER trial, which was a randomized placebo-controlled multicenter study that was planned based on the knowledge that as much as half of all myocardial infarctions and strokes occur among otherwise healthy men and women with levels of LDL cholesterol that were below then-guideline thresholds for treatment.(89) Thus,

recruitment focused on healthy persons with levels of LDL cholesterol below the thresholds, but with elevated levels of high-sensitivity CRP. As the investigators hypothesized, rosuvastatin effectively reduced LDL cholesterol levels by 50% and high-sensitivity CRP by 37% compared to placebo, thereby confirming the anti-inflammatory properties that statins bear in addition to its cholesterol lowering abilities. Overall the use of rosuvastatin during the trial significantly reduced the risk of a first major cardiovascular event, defined as nonfatal myocardial infarction, nonfatal stroke, hospitalization for unstable angina, an arterial revascularization procedure, or confirmed death from cardiovascular causes (HR: 0.56, 95% CI: 0.46–0.69, *P*<0.001).

Based on the JUPITER trial's findings, the authors postulated that one can reduce the occurrence of cardiovascular events by reducing vascular inflammation in the absence of lipid lowering. It was under this premise that the Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) trial was designed, which specifically sough to assess the effectiveness of canakinumab, a monoclonal antibody targeting the IL-1β innate immunity pathway, for the prevention of recurring cardiovascular disease in patients with a history of myocardial infarction and high-sensitivity CRP levels.(166) IL-1β inhibition has been previously shown to lower IL-6 and high-sensitivity CRP levels, without reducing LCL-cholesterol levels. This randomized, double-blind, placebo-controlled trial recruited 10,061 patients who were randomly assigned to canakinumab (50, 150, or 300 mg) or placebo. Patients treated with 150-mg (HR: 0.85, 95% CI: 0.74–0.98, *P*=0.021) and 300-mg (HR: 0.86, 95% CI: 0.75–0.99, *P*=0.031) were significantly less likely to experience an occurrence of a major cardiovascular event compared to placebo.

Investigators from the CANTOS trial hypothesized that canakinumab might also reduce the incidence of cancer given the role of IL-1β in promoting tumor invasiveness, growth, and metastatic spread. In consequence, they tested their hypothesis in a post-hoc analysis and found that those treated with interleukin-1β inhibition had a lower incidence of lung cancer and lung cancer mortality than those treated with placebo among 10,061 patients with atherosclerosis who had had a myocardial infarction, and who were previously free of a cancer diagnosis. Specifically, relative to placebo, 150 mg of canakinumab (HR: 0.61, 95% CI: 0.39–

0.97, *P*=0.034) and 300 mg of canakinumab (HR: 0.33, 95% CI: 0.18–0.59, *P*<0.001) resulted in a statistically significantly lower risk of incident lung cancer.

By formally confirming the fundamental role that IL-1β plays in atherosclerotic progression, the CANTOS trial confirmed that its direct inhibition is effective and safe. In addition, it also showed that by targeting the anti-inflammatory pathway, there was a lower incidence of lung cancers, and by that opened the floodgates to many future possibilities. Although the authors of the CANTOS trial themselves acknowledge that canakinumab was unlikely to have had direct effects on oncogenesis and the development of new lung cancers, it is possible that canakinumab slowed down the rate of progression, invasiveness, and metastatic spread of undiagnosed lung cancers at trial entry. This biological explanation would be in line with previous pre-clinical studies that linked cytokines such as interleukin-1β and angiogenesis and tumor growth, as well as tumor invasiveness in malignant cells. The hypothesis that fits this theoretical paradigm goes back to the time of Virchow, where inflammation was first linked to cancer.(167)

The unanticipated relationship found between canakinumab and incidence of lung cancer evokes much interest when considering that statins themselves contain anti-inflammatory properties. Some types of statins, such as rosuvastatin, are particularly effective at reducing levels of high-sensitivity CRP (JUPITER). The biological mechanisms underlying inflammation, cardiovascular disease, and eventually cancer are currently unknown. That said, the evidence indicates that inflammation can have a direct impact on atherosclerosis and simultaneously cancer, independent of cholesterol pathways. Going forward, the foundation on which inflammation, and its inhibition is the key connecting atherosclerotic progression and cancer has been established, investigators may seek to assess how other anti-inflammatory agents may result in similar findings as CANTOS in the settings of cardiology and oncology.

The role of pharmacogenomics studies.

Pharmacogenomics and cardiovascular disease

Genomic approaches have provided a valuable asset to help unravel the complexity of cardiovascular disease in the last decades.**(168)** In particular, pharmacogenomics has helped facilitate the identification of biomarkers that can help physicians with drug selection, dose, and treatment duration, and to some extent, avert adverse effects related to treatment. But perhaps most importantly, pharmacogenomics has provided insights into the biological mechanisms of drug action, which has contributed to novel therapeutic agents.

With regards to our research question on the relationship between statin and cancer risk, we believe that a genetic study can broaden our understanding for two principal reasons:

First, the confirmation of a relationship between statin use and cancer has yet to be made. The lack of a clear-cut relationship, with conflicting reports on the connection between statin and cancer, is could be indicative of individual variation in response to statins, supporting the value for a pharmacogenomics investigation;

Second, it was recently found that the reduced risk of cardiovascular disease via anti-inflammatory pathways may directly, or indirectly, have an impact on cancer incidence and/or progression.**(169)** This suggests that much of the underlying biological mechanisms relating inflammation, cancer, and cardiovascular disease are not entirely understood yet.

Given these considerations, three different scenarios may unfold following the hypothesis-free genome-wide association study (GWAS) approach proposed in the present research project:

1. *To identify a subset of patients with certain genetic variant(s) who will have a protective effect on cancer incidence and/or prognosis following statin use:*

   An obvious example of this type of scenario is the dal-OUTCOMES study, which is a randomized trial of 15,871 individuals who had had a recent acute coronary syndrome and recruited to receive the cholesteryl ester transfer protein (CETP) inhibitor dalcetrapib or placebo.**(170)** At the end of the study, the investigators of the trial found that dalcetrapib treatment did not improve their primary efficacy endpoint, defined as a composite of death from coronary heart disease, nonfatal myocardial infarction, ischemic stroke, unstable angina, or cardiac arrest with resuscitation compared to placebo (HR: 1.04, 95% CI: 0.93–1.16, P=0.52). Instead of completely discarding CETP inhibitor dalcetrapib as a treatment option, a GWAS was performed to identify genetic modulators of dalcetrapib response. In

that report, the authors found an association at the *ADCY9* locus where homozygotes for the 'A' allele at the lead SNP, rs1967309, which represents nearly 17% of Europeans, had a 39% risk reduction of the composite primary endpoint compared to placebo.**(171)** What this meant was that a number of patients with this specific genetic phenotype would benefit from the drug. Based on those findings, the dal-GenE trial was formed and is now recruiting patients. The trial seeks to test dalcetrapib compared to placebo amongst individuals with acute coronary syndrome patients on the reduction of cardiovascular events in a select genetic population defined by the rs1967309 "AA" genotype. It is possible that genetic variants would be responsible for statin-induced risk of cancer progression or metastasis.

2. *To identify a subset of patients based on a genetic variant who are more susceptible of developing cancer or having their disease worsened following statin use;*

   In this regard, the use of GWAS for evaluating those more at risk of experiencing a severe adverse event has frequently been adopted. For example, numerous reports have suggested that utilization of statins portends to higher risk of muscle-related adverse reactions, ranging from non-specific myalgias to rhabdomyolisis.**(172)** Such severe adverse effects can lead to treatment discontinuation, thereby increasing patients' risks of coronary events. Many investigators have attempted to drive their research towards identifying genetic predictors of statin-induced myopathy.**(173)** Those studies helped identify a gene that has been consistently associated with statin-induced myopathy in genetic studies (*SLCO1B1*), encoding the organic anion transporting polypeptide 1B1 responsible for hepatic statin uptake.**(174)** Similarly, a GWAS may help identify a gene that is associated with statin-induced cancer incidence or mortality.

3. *We may better understand the biological pathways that underlie or link cardiovascular disease and cancer:*

   One valuable deliverable that can be obtained from GWAS is the discovery of variants of genes involved in specific pathways, which can help to identify new biological mechanisms and become novel drug targets. Although not in the context of cancer, proof of principle for this has been provided with the development of evolocumab, a PCSK9 antibody. Essentially,

PCSK9 was discovered in 2003 where a report highlighted its role in cholesterol regulation.**(175)** Two missense gain of function PCSK9 mutations causing autosomal dominant hypercholesterolemia were discovered in a study of French families, thereby validating its role in lipid disorders.**(176)** Thereafter, a sequencing study among 128 African-American persons with low plasma LDL concentration levels identified two nonsense loss-of-function PCSK9 mutations that were associated with a 40% risk reduction in plasma LDL concentration levels.**(177)** A follow-up of the effect of these mutations in patients within the Atherosclerotic Risk in communities (ARIC) study showed that the 85 carriers of the loss-of-function mutations had a 88% risk reduction in coronary heart disease compared to non-carriers.**(178)** In a large meta-analysis composed of 66,698 individuals, those with PCSK9 protein variant R46L had decreased LDL- concentration levels of 13% and reductions ischemic heart disease of 30% compared to non-carriers.**(179)** The cumulative evidence demonstrated that inhibition of PCSK9 lowers LDL concentration levels and the risk of coronary artery disease, which prompted the development of therapeutic PCSK9 inhibitors for the treatment of coronary artery disease. Specifically, the Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) trial showed that evolocumab added to background statin therapy further reduced LDL-c to a median of 30 mg/dl in patients with baseline atherosclerotic disease and significantly lowered the risk of cardiovascular events compared to statin therapy alone.(180) This example perfectly highlights how improved understanding of cardiovascular pathophysiology through genetic discoveries provides new opportunities for drug development. The improved understanding of biological mechanisms through pharmacogenomics studies can potentially bridge the knowledge gap with respect to statins, its anti-inflammatory properties, and cell cycle. Once the mechanism is understood, then new therapies can be developed to intervene in the pathway.

Future directions

The apparent relationship between inflammation and cancer remains poorly understood. If genetic damage drives carcinogenesis, what is the role of inflammation under this paradigm? The CANTOS trial has provided a platform for research scientists to re-circulate these questions. Previously, epidemiological studies have consistently shown that

chronic use of aspirin, as well as other non-steroidal anti-inflammatory drugs is associated with a reduced risk of mortality from colorectal and lung cancers.(181, 182) Other reports have also shown that men with prostate cancer or colorectal cancer using statins seem to benefit from a survival advantage relative to non-statin users. (183, 184) Since statins also operate under anti-inflammatory principles, the protective effect of statin use amongst patients with cancer may be related to that. It would be unlikely that statins can actually prevent cancer. The authors from the CANTOS trial also caution against the belief that canakinumab may have resulted in the prevention of lung cancer. However, there is plausible reason to hypothesize that statins are somehow able to slow cancer growth, although this remains to be further studied, given the short follow-up duration of cardiovascular clinical trials for the purpose of assessing cancer-related endpoints. Whether a long-term, sustained use of anti-inflammatory drugs for cardiovascular disease can potentially change the course of cancer development and progression in some patients remains a difficult, yet open question. A study focusing on high-responders of anti-inflammatory agents recruiting patients that are genetically-identified for the prevention of cardiovascular disease and/or cancer may be conceivable in the future.

Hypothesis and objectives

Statins reduce LDL cholesterol levels and are vastly used to prevent coronary heart disease. Cholesterol is an essential structural component of mammalian cell membranes and is fundamental for cellular proliferation. Statins inhibit the production of endogenous cholesterol and block protein prenylation. Therefore, it has been postulated that statins may influence cell proliferation and migration. To date, there is controversy with respect to the association between statins and the risk of cancer. Some argue that cancer risk reduction may be triggered via reductions in inflammation, neovascular formation, and cell proliferation through statins(185), or in contrast, statins can inhibit selenoprotein synthesis and decrease natural killer cell function, which in turn may enhance cancer risk(186).

Primary objective:

Our primary objective was to explore the association between statin use and cancer incidence from a clinical and genetic perspective.

From the *clinical perspective,* we will rely on data from the Treating to New Targets (TNT) study, a parallel-group study that randomized 10,001 patients without any survival-limiting disease to double-blind treatment to either high-dose statin (atorvastatin at 80 mg) or low-dose statin (atorvastatin at 10 mg)(92), as well as the Incremental Decrease in End Points Through Aggressive Lipid Lowering (IDEAL) study, a prospective randomized open-label blinded endpoint evaluation trial of 8,888 patients treated with high-dose statin (atorvastatin at 80 mg) or usual-dose statin (simvastatin at 20 mg)(93). In this part of the analyses, our *hypothesis* is that individuals taking high-dose statins would demonstrate a protective effect with respect to cancer diagnosis during the study period than their low-dose statin counterparts.

From the *genetic perspective,* we will rely on a subset of participants to the TNT study who consented to genetic research using deoxyribonucleic acid (DNA) samples from nearly 6,000 study participants. A genome-wide genotyping experiment on all 6,000 DNA samples from TNT using the Illumina MEGA chip, which includes 1.7 million single nucleotide

polymorphisms (SNPs) were completed. Given the incomplete understanding of pathophysiological mechanisms between statin and cancer, we will adopt a hypothesis-free GWAS approach to assess whether any genetic variant in the genome can contribute to cancer risk association in statin users. In this part of the analyses, our goal is to identify genetic associations that are in detectable range amongst a statin-user population with the hypothesis that carriers of identified genetic factors amongst high-dose statins users would have a protective risk of cancer diagnosis during the study period compared to low/usual-dose statin users.

Secondary objective:

Our secondary objective was to explore the association between statin use and cancer mortality from a clinical perspective. Given the limited number of cancer deaths available in our data set, we did not plan to explore this association from the genetic perspective.

Sub-analyses:

In sub-analyses, we further compared cancer incidence and cancer mortality in high-dose vs. low-dose statins by stratifying the population according to patient age (≥55 years old), men, and women.

Exploratory analyses:

In exploratory analyses, we also abstracted information on baseline statin users (i.e. those who were already taking statins prior to trial enrolment) and considered it as an additional variable. Finally, we also performed competing-risk regression analysis and defined the risk of any death as a competing endpoint of interest. Specifically, the risk of succumbing to death during the study period would 'prevent' the participant from experiencing a cancer diagnosis, hence, death of any cause represents a competing event to being diagnosed with cancer.(187)

Methodology

Section I: Variable definitions and reliability/validity discussion.

Primary independent variable: Within the TNT study, exposure was defined as high-dose (80 mg) or low-dose (10 mg) atorvastatin per day, which represented the study's primary intervention. There are two important aspects to consider. First, the design of the protocol was that prior to the randomization process, all patients had to undergo a 'wash-out' period (up to 8 weeks) for the purpose of having all patients have LDL cholesterol levels consistent with then-guidelines for the treatment of stable coronary heart disease (CHD). At completion of this wash-out period, all 15,464 individuals entered the open-label run-in period and received 10 mg of atorvastatin per day. By this end of this phase, a total of 70 patients (out of 5,461) were excluded due to non-compliance with treatment. Second, 5,006 and 4,995 patients were randomized to receive low-dose and high-dose atorvastatin, respectively. However, during the study, respectively 1,486 and 1,257 patients crossed-over from the low-dose group to the high-dose group, and from the high-dose group to the low-dose group. Unfortunately, the information on timing of the switch and exact dosing was not reliable. To address the potential bias this may have introduced to our findings, we repeated all our analyses in the per-protocol population, which includes only patients who received the treatment that was originally allocated to them. It is noteworthy that in these sensitivity analyses, our results failed to reveal any differences with those of the primary analyses for high-dose vs. low-dose statin use and cancer. It may be because that restricting our analyses to the per-protocol population, which drops patients who do not follow the protocol, does little to address the aforementioned foresight, and that and that the exercise itself, as some have previously desmonstrated, is a futile activity in the context of a randomized trial.(188)

Within IDEAL, the primary independent variable of interest was high-dose atorvastatin (80 mg) or usual-dose simvastatin (20 mg) per day, which represented the study's primary intervention. In comparison to the TNT study, the study design of the IDEAL trial did not include a 'wash-out' period, where treatment compliance was tested. Notably, some patients in the usual-dose group may have crossed-over to the high-dose arm, or vice-versa. Additionally,

it was also permitted for patients to stop their treatment and take a different statin during the study. In consequence, sensitivity analyses using the per-protocol cohort were also performed using IDEAL.

Primary endpoints: For the purpose of our analysis, we considered the occurrence of a cancer diagnosis [1], incident cancer [2], and cancer mortality [3] as endpoints:

[1] The occurrence of a cancer diagnosis may include individuals who had cancer prior to trial enrolment or an incident cancer (i.e. individuals who did not report a cancer diagnosis prior to trial enrolment), which was the first measured endpoint. [2] Second, we also examined incident cancer diagnoses in individuals free of cancer diagnosis prior to study start. In both cases, since neither TNT or IDEAL were designed for the purpose of examining cancer risk, we defined cancer that occurred during the study by using events from the 'adverse events' (AE) records and cancer that occurred prior to study start using baseline medical history questionnaires and baseline medication questionnaires data **(Figure 1).**

Figure 3.   Visual timeline of cancer assignations for the TNT and IDEAL

| Previous Cancer | Study start (Day 1) | Cancer during study period | Died of Cancer |

TNT (n=304)
- 63 had a cancer dx from the AE file prior to study start
- 247 were taking a cancer-related medication at baseline

Some patients may have a record in the AE file and in the medication file; hence numbers may not add up
*
IDEAL (n=609)
- 424 had a cancer dx from the history file
- 185 were taking a cancer-related medication at baseline

Primary analyses:
TNT (n=10001); IDEAL (n=8888)
*
Secondary analyses (after excl. those with cancer at baseline):
TNT (n=9697);
IDEAL (n=8279)

TNT (n=1384)
- 1158 had a new cancer diagnosis or died of cancer after study start
- 226 had a cancer diagnosis or died of cancer after study start although they already had cancer at study start
*
IDEAL (n=781)
- 669 had a new cancer diagnosis or died of cancer after study start
- 112 had a cancer diagnosis or died of cancer after study start although they already had cancer at study start

TNT
160 died of cancer;
IDEAL
211 died of cancer.

In defining patients who had cancer prior to study start: Using baseline medical history questionnaires, a total of 63 and 424 patients in TNT and IDEAL had a cancer diagnosis prior to study start, respectively. Using records of medication, a total of 247 and 185 patients in TNT and IDEAL were taking anti-cancer medication prior to study start, respectively. By linking this information to the main file, this resulted in 226 and 112 patients in TNT and IDEAL who had a cancer prior to study start, respectively. From a technical point of view, the numbers obtained from medical history and medication questionnaires do not add up because some patients had a record in both data source.

In defining patients who had cancer following study start: Using records of AEs, a total of 1,384 (13.8%) and 781 (8.8%) patients in TNT and IDEAL had a cancer occurrence after the study start, respectively. The labels associated with a cancer diagnosis are depicted in the **Appendix 1.**

To summarize, 226 out of 1384 individuals had a cancer diagnosis prior to study start within TNT; and 112 out of 781 individuals had a cancer diagnosis prior to study start within IDEAL.

As such, cancer incidence (i.e. new onset of cancer) was recorded in 1158 (1384 minus 226) patients within TNT and 669 (781 minus 112) within IDEAL. This means that 11.6% (1158/10001) of patients who participated in TNT and 7.5% (669/8888) of patients who participated in IDEAL had a new diagnosis of cancer following study start. While there was no formal assessment in confirming that this methodology was the standard procedure, records of AEs are rigorously maintained and thoroughly verified by the sponsor (here the pharmaceutical industry) and the research coordinators.

[3] The third endpoint examined was death from cancer. This endpoint was an adjudicated endpoint within both the TNT and IDEAL trials, whereby a panel of medical experts determined the cause of death of participants. Overall, 160 (1.6%) and 211 (2.4%) patients died due to cancer within the TNT and IDEAL studies, respectively. These numbers are corroborated in other reports.(92, 93, 189)

Secondary endpoints: We also had to account and tabulate for the occurrence of a major cardiovascular event, defined as death from coronary heart disease, nonfatal non-procedure-related myocardial infarction, resuscitation after cardiac arrest, or fatal or nonfatal stroke for TNT; and a major coronary event, defined as coronary death, hospitalization for nonfatal acute myocardial infarction, or cardiac arrest with resuscitation for IDEAL for the purpose of competing-risk analyses (see description in methods below).

Follow-up information: Within the study, we tabulated several follow-up times: time from randomization (index date) until last follow-up [1], time from randomization until cancer [2], time from randomization until cancer mortality [3], and time from randomization until the occurrence of a secondary endpoint [4].

[1] Time from randomization until last follow-up was defined as the time a patient is randomized prior to therapy initiation until death from any cause or last follow-up (study end) for both TNT and IDEAL. [2] Time from randomization until a cancer diagnosis was tabulated by first taking each patient who had an eventual cancer diagnosis and its corresponding date within the AE file. If a patient had more than one cancer record, then only the first cancer record occurrence was used. For some patients within the TNT study, the time of a first cancer

diagnosis after study start had to be imputed due to a missing day, month, or year. For all patients with a missing day, it was set to the first of each month; for all patients with a missing month, it was set to June; for all patients with a missing year, it was set to the median time from randomization to a cancer diagnosis in all other patients without a missing year (+1.1 year from date of randomization). The number of patients who had to have their date of cancer diagnosis imputed is described in the **Appendix 2.** Within IDEAL, no dates of cancer diagnosis were missing; and hence, no imputation was necessary. [3] The time from randomization until cancer mortality was distinctively coded within both the TNT and IDEAL databases, as cancer death was an adjudicated endpoint. [4] Finally, we also coded the time from randomization until our definition of secondary endpoints for both the TNT and IDEAL trials. This endpoint was available within the databases, as it represented the primary endpoints of interest in the original trials. One must acknowledge an important bias related to interval-censoring prevalent in clinical trials where the precise date of an event is actually unknown as the events are reported during scheduled visits only. Sensitivity analyses may be conducted to overcome such bias(190, 191), however, given that for both TNT and IDEAL visits were frequent, the error associated with interval-censoring was assumed to be negligible.

Definition of previous statin use: Given that both the TNT and IDEAL trials were targeted for individuals in the secondary prevention setting, i.e. patients with a previous myocardial infarction, previous or current angina with objective evidence of atherosclerotic coronary heart disease, and a history of coronary revascularization, it was highly likely that some patients recruited were previous statin users. We sought to account for this confounding and tabulated previous statin usage for both trials. Within TNT, we created an algorithm to parse through the list of medications and the associated dates of all patients. Search words were 'atorvastatin', 'simvastatin', 'cerivastatin', 'fluvastatin', 'pravastatin', and 'lovastatin'. In order to obtain some sense of how accurate our algorithm was at capturing previous statin use for all patients enrolled in the TNT trial, we used the same algorithm to code baseline aspirin use and compared its rate with what had been reported by TNT investigators.(192) To improve external validity we had another investigator run the algorithm independently. The original TNT study reported an aspirin use rate of 88%, and our algorithm found a rate of 91% and 92%, respectively by two separate investigators. Within the IDEAL study, baseline statin use

was readily coded as a binary variable (yes or no), and according to types of statins (i.e. atorvastatin, yes or no).

Other covariates: In additional iterations of our analyses, we further adjusted for age (dichotomized <55 and ≥55 years old) and sex (men, women). These variables were readily coded within the databases. We chose to perform additional adjustment for age groups because a non-linear relationship was observed between age and risk of cancer in these cohorts. Further analyses using the minimum *P*-value approach as described by Mazumbar and Glassman(193) revealed that the ideal age cut-off with respect to cancer was 55 years old. The choice to stratify our analyses according to sex was based on existing literature, which suggested an increased risk between statin use and breast cancer in women.(75)

Section II: Data cleaning steps used within the trial and for the purpose of the project.

Clinical data:

In planning for our analyses, we prepared a few independent datasets to run our analyses: the overall cohort as reported per original publication (n=10,001 for TNT, n=8,888 for IDEAL), only patients without a cancer diagnosis prior to study start (n=9,697 for TNT, n=8,279 for IDEAL), only men (n=8,099 for TNT, n=7,187 for IDEAL), women (n=1,902 for TNT, n=1,701 for IDEAL), those aged ≥55 years old (n=7,309 for TNT, 6,744 for IDEAL), and the per-protocol populations (n=7,258 for TNT, n=7,218 for IDEAL).

Genetic data:

Genotyping

Genome-wide genotyping was performed using 200ng of genomic DNA in GLP-environment at the Beaulieu-Saucier Pharmacogenomics Centre (Montreal, Canada). The Illumina Infinium Multi-Ethnic Global Array (MEGA) Consortium v1 BeadChip (Illumina, San Diego, CA)

including 1,705,969 genomic markers were used and processed according to the manufacturer's specifications. BeadChips were analyzed using Illumina's Beeline v1.0.37.0 with the data manifest MEGA_Consortium_15063755_B2 using the manufacturer's cluster file HapMap_MEGA_2015. Genotype data files were produced in three instalments of comparable size.

GWAS

PyGenClean (194) version 1.4 and PLINK (195) version 1.07 were used for the quality checks (QC) and genetic data cleanup process. 6163 TNT samples were genotyped, 436 samples were excluded for lack of completion (<98% call rate), 157 for data discordance (discordant duplicates, unexpected twins, sex-check problems), one individual from 20 related pairs, and 403 samples were excluded from the Caucasian cluster based on MDS components including the genotypes of HapMap CEU, JPT-CHB, and YRI data analysed using k-nearest neighbour with a threshold of 1.9σ in PyGenClean (v1.7.1). The MDS analysis was repeated excluding the HapMap samples, and the first 10 principal components were retained as adjustment covariates in the GWAS. Genome-wide imputation was performed using IMPUTE2 (v2.3.2) (196) and phasing with SHAPEIT2 (v.2r790) (197). Strand alignment was solved by flipping non A/T and C/G SNPs and ambiguous A/T and C/G SNPs were were imputed. Imputation was based on 1,058,670 genetic variants using the phased 1000 Genomes reference data phase 3 released on October 2014 including all populations. The pseudo-autosomal regions on the X chromosome were imputed separately from the rest of the chromosome. Internal cross-validation using IMPUTE2 provided a mean genotype concordance of 99.6%. Missing genotypes of genotyped SNPs were also imputed. A total of 11,692,729 genetic variants with imputation probability of 0.90 or greater and completion rate of 98% or greater were obtained, leaving 4,973,854 genetic variants with minor allele frequency (MAF) greater than 5% were used for the GWAS.

Section III: Analyses

Clinical:

Primary analysis: First we focused on all patients who participated in the TNT and IDEAL studies. Descriptive analysis consisted of examining patient and clinical characteristics at randomization between treatment groups using a chi-square test for categorical variables and a *t*-test for continuous variables. Since this was a clinical trial, all baseline descriptives between the two treatment groups were well-balanced, as previously described.(92, 93, 189, 198) The risk of cancer diagnosis and incident cancer between high- and low-dose statin was evaluated using univariable Cox regression analysis. In additional analyses, we adjusted for age (continuous variable), sex, and previous utilization of statins prior to study entry. Furthermore, sub-analyses were conducted by repeating the aforementioned steps exclusively amongst those aged ≥55 years old, men, and women. Subsequently, we repeated our analyses by examining the risk of cancer mortality in the entire population, and stratified according to age (≥55 years old) and sex. Finally, in order to examine the risk of incident cancer, we restricted our analyses to cancer-free patients. In an effort to reconcile our findings obtained from both the TNT and IDEAL databases, we performed a pooled-analysis of the findings from the iterations mentioned above. The inverse-variance meta-analysis method was used to pool results from the TNT and IDEAL studies. Reviewer Manager (RevMan), version 5.3 (Cochrane Collaboration, London, UK) was used to carry out the weighted averages reported as HRs with 95% CI and a DerSimonian and Laird random-effects model. A *P*-value less than or equal to 0.05 was considered as a statistically significant result.

Exploratory analysis: In the event that a patient experiences any death (other than cancer mortality) that would prevent him or her from experiencing our primary endpoints of interest (here, cancer diagnosis or cancer or cancer mortality), we relied on competing-risk regression analysis, as described by Fine and Gray(187), instead of the traditional Cox regression analysis. In those analyses, we compared the risk of incident cancer and cancer mortality by adjusting for the competing event, as defined per secondary event. This set of analysis was

eventually omitted from the final article due to space constraints, and because it did not add anything novel to our main findings.

GWAS:

We assessed whether genetic variants were associated with new onset of cancer in statin-users using a hypothesis-free GWAS. Details of this process are elaborated in the supplementary materials. Genetic variants with allele frequencies >5% were analysed using Cox proportional hazards regression modeling with the program Genipe v1.2 using 5,119 Caucasian patients with complete clinical data for analysis. The genetic models included adjustment for sex, age, treatment arm, and 10 principal components to account for genetic ancestry. Statistical tests performed on the genetic data were two-sided and adjusted to account for the multiple testing of SNPs using the standard Bonferroni corrected significance threshold of 5.0×10^{-8}, for $\alpha = 0.05$. This standard assumes a million independent variants in the human genome.

Additional analyses were performed in pre-specified subgroup analyses for patients aged ≥55 years old (n=3,669), in men (n=4,041), and women (n=928) separately. These steps were repeated for the analyses in the high dose 80mg atorvastatin arm only (n=2,463) and in high-dose users aged ≥55 years old (n=1,839), men (n=2,019), and women (n=444). We also evaluated genetic variants for cancer mortality in all available patients (n=5,119). However, no sub-analyses were performed for this endpoint due to the limited number of events (n=62). The GWAS in all patients using both high and low dose treatment arms had 80% power to detect a SNP of minor allele frequencies (MAF) = 0.30 for an additive genetic model associated with a 48% increase risk of cancer incidence.

Section IV: Strengths and limitations of the used data.

The evident advantage in using data obtained from randomized-controlled trials is that patient and clinical characteristics are balanced between the main intervention group, which was high-dose and low-dose statin treatment. As opposed to population-based studies, we were not limited by indication bias. The other main advantage was that we had access to genomic

information of patients enrolled in TNT and we were able to perform, for the first time, a genomic analysis of the effect of statin on cancer using high-quality data. Finally, it is noteworthy that both the TNT and IDEAL studies have a reasonably adequate follow-up time (median: 4.9 years for TNT and 4.8 years for IDEAL). That being said, the duration of follow-ups for the two trials is still considered limited for the purpose of assessing cancer-related endpoints.

The first main limitation of the data used was that the cancer diagnoses obtained from the adverse event records were not adjudicated by a group of oncologists. However, as the reliability and validity of the primary endpoint is not readily verifiable, it becomes difficult for future studies to corroborate our results, or to generate additional hypotheses using the same database, as the methodologies may differ slightly and produce different rates. The other limitations pertain to the lack of a control group that did not receive any statins ever. It is noteworthy that both populations in the TNT and IDEAL trials were individuals who previously had a coronary heart disease event, which means that many of them will have taken statins for a certain period of time already before enrolling in the study. In this context, our question is more related to whether a higher dose of statins is associated with cancer compared to a lower dose, and not whether statins users vs. non-users have more or less likely to be diagnosed with cancer, as such we rely on the hypothesis of a dose-related effect of statins on cancer risk. Third, the identification of patients previously treated with statins was limited by a lack of a formal variable within the TNT cohort. Instead we had to abstract the information ourselves by searching through an exhaustive list of medications and baseline conditions. In the same way, there is a chance that some patients may have had cancer but did not have a record for it. Furthermore, some patients may have had asymptomatic cancer prior to study start, which would falsely increase the rate of incident cancers. Fourth, there is the debate of how representative the recruited population is of a randomized-controlled trial in relation to the general population. For example, the proportions of male gender and white race participants were 80% and 94% within the TNT trial, respectively. This may undermine the use of statins in women and non-white race individuals where heart diseases are highly prevalent, such as Hispanics and African-Americans. That said, others have argued that representativeness should not become the primary preoccupation of researchers.(199) The

dilemma becomes, however, that while population-based cohorts are naturally more generalizable than a randomized cohort; there is the problem that the indication for statins is never clear in the former. In some cases, patients take statins for preventive reasons, despite not harboring any cardiovascular-related disease. Such preventive use can be associated with increased doctor visits and more rigorous precaution with one's health (e.g. better eating habits, less likely to smoke), which may reduce the odds of cancer diagnoses for a number of other reasons, thereby creating a false protective relationship between statin and cancer. Finally, although genomic data was available for a large group of patients, we could have benefitted from an even larger sample size with respect to some sub-group analyses, such as those stratified by men and women.

Results (Clinical Perspective)

Article

Absence of cancer risk with high-dose versus low-dose statin in TNT and IDEAL

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Abstract (150 words)

Anti-inflammatory therapy for atherosclerotic disease was associated with reduced risk of incident lung cancer in an exploratory analysis. Statins, a cholesterol-lowering medication for prevention of coronary heart events and cardiovascular mortality, have shown to hold anti-inflammatory functions. Our objective was to examine the impact of high-dose statin therapy on the risk of cancer and cancer mortality in a pooled analysis of the Treating to New Targets (TNT, n=10,001) and the Incremental Decrease in End Points Through Aggressive lipid Lowering (IDEAL, n=8888) studies. The primary outcomes were cancer diagnosis and cancer mortality. Secondary outcome was cancer incidence in cancer-free patients. Our pooled analyses showed that the risk of cancer diagnosis (HR: 1.03, 95% CI: 0.95–1.12, *P*=0.5), cancer mortality (HR: 0.99, 95% CI: 0.78–1.26, *P*=0.9), and incident cancer (HR: 1.05, 95% CI: 0.96–1.15, *P*=0.3) was not statistically significantly different between high-dose vs. low/usual-dose statins in the pooled analyses.

Brief Communication

Recently, investigators from the Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) trial[1] performed an exploratory analysis and revealed that patients randomized to canakinumab, an anti-inflammatory interleukin-1B inhibitor, were significantly less likely to be diagnosed with incident lung cancer (150 mg: hazard ratio [HR]: 0.61, 95% confidence interval [CI]: 0.39–0.97, 300 mg: HR: 0.33, 95% CI: 0.18–0.59) than their placebo counterparts.[2] In addition, the risk of any fatal cancers was also significantly lower for those treated with the highest dose of canakinumab (300 mg: HR: 0.49, 95% CI: 0.31–0.75) suggesting that the apparent benefit of the therapy may extend beyond lung cancer.

By principle, statins, an effective cholesterol-lowering medication for prevention of cardiovascular disease and mortality, also bearing anti-inflammatory properties[3], may then also be associated with a lower risk of incident cancer or cancer death. Indeed, several epidemiological studies have previously suggested that use of statins, although variable, may be associated with the risk of cancer.[4-11] Occasionally, previous randomized trials on statins and the prevention of cardiovascular disease have also noted irregular rates of cancer amongst statin users.[12-17]

In this context, our primary objective was to assess the risk of cancer and cancer mortality between high-dose vs. low-dose statin users by pooling data from the Treating to New Targets (TNT)[18] and Incremental Decrease in Endpoints Through Aggressive Lipid lowering (IDEAL)[19] trials. Our secondary objective included the comparison of incident cancers in participants without cancer at study initiation. In sub-analyses, we further examined patient age, sex, and previous statin use as additional moderators of the

impact of statins on cancer. Our hypothesis stated that high-dose statin results in a protective effect on cancer and cancer death.

The design, rationale, and outcomes of the TNT and IDEAL trials have been previously described.[18-21] In primary analyses, we examined cancer diagnosis and cancer mortality in all patients that participated in the TNT (n=10,001) and IDEAL (n=8,888) trials. In secondary analyses, we examined incident cancer in cancer-free patients. The definition of cancer status for both trials is detailed in the **Appendix**. Sub-populations in secondary and exploratory analyses focused on those aged ≥55 years old, men, and women. The risk of cancer between high- and low-dose statin was evaluated using Cox regression analysis. In an effort to summarize the results, we performed a pooled-analysis of the findings observed from both TNT and IDEAL by using the inverse-variance methodology provided by Reviewer Manager (RevMan, version 5.3) via a DerSimonian and Laird random-effects model.

Our results showed that the risk of and incident cancer (HR_{pooled}: 1.05, 95% CI: 0.96–1.15, P=0.3) was not statistically significantly different between high-dose vs. low/usual-dose statins in the pooled analyses (Table 1). Additional results focusing on cancer diagnosis (HR_{pooled}: 1.03, 95% CI: 0.95–1.12, P=0.5) and cancer mortality (HR_{pooled}: 0.99, 95% CI: 0.78–1.26, P=0.9), as well as those obtained within sub-analyses also failed to reveal any significant effect (data not shown).

By lowering cholesterol with 3-hydroxy-3-methyl-coenzyme A (HMG CoA) reductase inhibitors, several randomized-controlled trials demonstrate that coronary event rates were significantly reduced in both primary and secondary settings compared to

placebo.[22, 23] Subsequently, the possibility has been raised that statins may possess functions beyond merely lowering cholesterol.[3] Amongst its repertoire of the speculated activities, it was suspected that statins might also hold anti-inflammatory properties. Concomitantly, chronic inflammation and inflammatory processes are known to play a critical role in the pathogenesis of atherosclerosis, where markers of inflammation (e.g. C-reactive protein) have shown to be highly predictive of cardiovascular events.[24-26] In parallel, the inadequate resolution of inflammation has also been postulated to harbour a major role in tumor growth, progression, and the risk of metastases.[27-29]

In light of the recent results linking canakinumab, an anti-inflammatory therapy, and lung cancer, we sought to revisit the topic using data from the TNT and IDEAL studies. In a pooled population of 18,888 individuals, our post-hoc analyses indicated a lack of relationship between high-dose statin users with respect to cancer, cancer death, and incident cancer.

Since the trial was aimed at examining statins and its efficacy for prevention of cardiovascular disease, our current study's primary endpoint of interest (i.e. cancer) was partially captured from the trials' reported adverse events, which can be variable and incomplete. It is possible that some patients had asymptomatic cancer, which remained undetected, and cancer information was at times unspecific and likely variable between study sites. Although patients with life-limiting disease were excluded upfront; patients were not specifically screened for cancers. It should be mentioned that the TNT study protocol allowed for patients to take other types of statins during the trial period, which may have neutralized the 'low-dose' group. Finally, given the study designs and indications of TNT and IDEAL, we could not test the treated vs. non-treated hypothesis.

Although our group was not able to confirm our proposed hypothesis, formulated based on the positive findings conducted by the CANTOS investigators on canakinumab and lung cancer, it should not deter investigators in the future to explore how the anti-inflammatory properties of statins may have an impact on cancer. Specifically, as different statins have differing degrees of anti-inflammatory properties, it is possible that different statins will produce different findings. Furthermore, although many malignancies arise in areas of chronic inflammation, not all do. Future studies may thus explore the possibilities of cancer-specific incidence. Finally, a pharmacogenomics evaluation may provide additional insights and future perspectives in understanding the underlying biological mechanisms of interactions between inflammation, atherosclerosis, and tumor microenvironment, and identify mediators of such interactions.

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Table 1. Pooled Cox regression analyses for prediction of incident cancer in the TNT and IDEAL studies, and stratified according to men, women, and those aged ≥55 years old.

	Incident cancer HR (95% CI)	P
All patients (n=17976)	1.05 (0.96–1.15)	0.3
Male (n=14612)	1.06 (0.95–1.17)	0.3
Female (n=3435)	1.04 (0.83–1.29)	0.8
≥55 years old (n=14900)	1.05 (0.95–1.16)	0.3

HR: hazard ratio, CI: confidence interval.

Appendix 1. Definition of cancer status per TNT and IDEAL.

Primary endpoints: For the purpose of our analysis, we considered the occurrence of a cancer diagnosis [1], and cancer mortality [2] as endpoints.

[1] An occurrence of a cancer diagnosis may mean any occurrence of cancer, where it includes individuals who had cancer prior to trial enrolment [A] or an incident cancer (i.e. individuals who did not have a cancer diagnosis prior to trial enrolment) [B]. In both cases, since neither TNT or IDEAL were designed for the purpose of examining cancer risk, we defined cancer that occurred during the study by using records from the 'adverse events' (AE) records and cancer that occurred prior to study start using baseline medical history questionnaires and baseline medication questionnaires data.

Figure 1. Visual timeline of cancer assignation for the TNT and IDEAL.

In defining patients who had cancer prior to study start: Using baseline medical history questionnaires, a total of 63 and 424 patients in TNT and IDEAL had a cancer diagnosis prior to study start, respectively. Using records of medication, a total of 247 and 185 patients in TNT and IDEAL were taking anti-cancer medication prior to study start, respectively. By linking this information to the main file, this resulted in 226 and 112 patients in TNT and IDEAL who had a cancer prior to study start, respectively. From a technical point of view, the numbers obtained from medical history and medication questionnaires do not add up because some patients had a record in both data source.

In defining patients who had cancer following study start: Using records of AEs, a total of 1,384 (13.8%) and 781 (8.8%) patients in TNT and IDEAL had a cancer occurrence after the study start, respectively. The labels associated with a cancer diagnosis are listed below:

Gastrointestinal carcinoma

TNT:

IDEAL:

Bladder carcinoma/Bladder cancer

Bone cancer

Breast carcinoma/breast cancer

Skin carcinoma/skin melanoma

Carcinoma/tumor

Carcinoma of larynx

Carcinoma of lung

Carcinoma of mouth

Endometrial carcinoma

Ovarian cancer

Prostatic carcinoma

Sarcoma

Thyroid carcinoma

Myeloma

Bladder Carcinoma

Bone Sarcoma

Brain Neoplasm Malignant

Breast Carcinoma

Breast Neoplasm Malignant Female

Breast Neoplasm Malignant Male

Carcinoma

Cervix Carcinoma

Cervix Carcinoma In situ

Colon Carcinoma

Endometrial carcinoma

Esophageal Carcinoma

Hepatoma

Gastrointestinal carcinoma

Gastric Carcinoma

Carcinoma of the Larynx

Larynx Neoplasm Malignant

Carcinoma of Lung

Lymphoma Malignant

Melanoma Malignant

Myeloma

Neoplasm Malignant Aggravated

Pancreas Neoplasm Malignant

Pharynx Neoplasm Malignant

Prostatic carcinoma

Rectal Carcinoma

Renal Carcinoma

Sarcoma

Seminoma

Skin melanoma

Skin Neoplasm Malignant

Thyroid carcinoma

Thyroid   Neoplasm   Malignant

To summarize, 226 out of 1384 individuals had a cancer diagnosis prior to study start within TNT; and 112 out of 781 individuals had a cancer diagnosis prior to study start within IDEAL. As such, cancer incidence (i.e. new onset of cancer) was recorded in 1158 (1384 minus 226) patients within TNT and 669 (781 minus 112) within IDEAL. This means that 11.6% (1158/10001) of patients who participated in TNT and 7.5% (669/8888) of patients who participated in IDEAL had a new diagnosis of cancer following study start. While there was no formal assessment in confirming that this methodology was the standard procedure, records of AEs are rigorously maintained and thoroughly verified by the sponsor (here the pharmaceutical industry) and the research coordinators.

[2] Death from cancer was an adjudicated endpoint within both the TNT and IDEAL trials, whereby a panel of medical experts determined the cause of death of participants. Overall, 160 (1.6%) and 211 (2.4%) patients died due to cancer within the TNT and IDEAL studies, respectively. These numbers are corroborated in other reports.[1-3]

Follow-up information: Within the study, we tabulated several follow-up times: time from randomization (index date) until last follow-up [1], time from randomization until cancer [2], time from randomization until cancer mortality [3], and time from randomization until the occurrence of a secondary endpoint [4].

[1] Time from randomization until last follow-up was defined as the time a patient is randomized prior to therapy initiation until death from any cause or last follow-up (study end) for both TNT and IDEAL. [2] Time from randomization until a cancer diagnosis was tabulated by first taking each patient who had an eventual cancer diagnosis and its corresponding date within the AE file. If a patient had more than one cancer record, then the first cancer record was taken. [3] The time from randomization until cancer mortality was distinctively coded within both the TNT and IDEAL databases, as cancer death was an adjudicated endpoint. [4]

Definition of previous statin use: Given that both the TNT and IDEAL trials were targeted for individuals in the secondary prevention setting, i.e. patients with a previous myocardial infarction, previous or current angina with objective evidence of atherosclerotic coronary heart

disease, and a history of coronary revascularization, it was highly likely that some patients recruited were previous statin users. We sought to account for this confounding and tabulated previous statin usage for both trials. Within TNT, we created an algorithm to parse through the list of medications and the associated dates of all patients. Search words were 'atorvastatin', 'simvastatin', 'cerivastatin', 'fluvastatin', 'pravastatin', and 'lovastatin'. In order to obtain some sense of how accurate our algorithm was at capturing previous statin use for all patients enrolled in the TNT trial, we used the same algorithm to code baseline aspirin use and compared its rate with what had been reported by TNT investigators.[4] To improve external validity we had another investigator run the algorithm independently. The original TNT study reported an aspirin use rate of 88%, and our algorithm found a rate of 91% and 92%, respectively by two separate investigators. Within the IDEAL study, baseline statin use was readily coded as a binary variable (yes or no), and according to types of statins (i.e. atorvastatin, yes or no).

References

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Results (Genetic Analysis)

We assessed whether genetic variants were associated with new onset of cancer in statin-users using a hypothesis-free GWAS.

The genetic models included adjustment for sex, age, treatment arm, and 10 principal components to account for genetic ancestry. Statistical tests performed on the genetic data were two-sided and adjusted to account for the multiple testing of SNPs using a significance threshold of 5.0×10^{-8}.

Additional analyses were performed in pre-specified subgroup analyses for patients aged ≥55 years old (n=3,669), in men (n=4,041), and women (n=928) separately. These steps were repeated for the analyses in the high-dose 80mg atorvastatin arm only (n=2,463) and in high-dose users aged ≥55 years old (n=1,839), men (n=2,019), and women (n=444). We also evaluated genetic variants for cancer mortality in all available patients (n=5,119). However, no sub-analyses were performed for this endpoint due to the limited number of events (n=62).

We performed a GWAS using Cox proportional hazards to test for genetic factors associated with on-statin cancer risk in the TNT population free of cancer at baseline, with adjustment for age, sex, treatment arm and genetic ancestry, as well as in subgroups of those aged ≥55 years old, and in men and women separately. We did not find significant results below the genome-wide threshold ($P < 5.0 \times 10^{-8}$, **Table 4**).

Table 4. Summary results from the GWAS analyses showing per-allele hazard ratio for time to new onset of cancer. Single nucleotide polymorphisms with P-value $\leq 1.0 \times 10^{-6}$ in the GWAS for SNPs or the GWAS for SNP*treatment arm interaction are reported.

SNP	Gene	Location	MAF	Chr	Risk Allele	Reference Allele	HR (95% CI) for high- vs. low-dose atorvastatin	P	HR (95% CI) for the SNP*	P	HR (95% CI) for SNP*treatment interaction**	P
All patients												
rs115509517§	ZNF608, LOC101927421	Intergenic	0.097	5	T	G	1.05 (0.90–1.23)	5.35×10^{-1}	1.52 (1.29–1.79)	7.83×10^{-7}	1.12 (0.80–1.56)	5.11×10^{-1}
rs6897935§	ZNF608, LOC101927421	Intergenic	0.098	5	T	G	1.05 (0.90–1.23)	5.01×10^{-1}	1.51 (1.28–1.79)	8.99×10^{-7}	1.15 (0.83–1.60)	4.07×10^{-1}
rs73304713§	ZNF608, LOC101927421	Intergenic	0.098	5	G	A	1.06 (0.91–1.24)	4.76×10^{-1}	1.52 (1.28–1.79)	8.89×10^{-7}	1.16 (0.83–1.61)	2.97×10^{-1}
Patients aged ≥55 years old												
rs10145958§	NOVA1, LOC101927062	Intergenic	0.169	14	G	A	1.05 (0.89–1.24)	5.72×10^{-1}	1.44 (1.25–1.67)	7.76×10^{-7}	0.84 (0.63–1.12)	2.33×10^{-1}
rs139889241§	NOVA1, LOC101927062	Intergenic	0.169	14	A	G	1.05 (0.89–1.23)	5.90×10^{-1}	1.45 (1.25–1.67)	7.38×10^{-7}	0.84 (0.63–1.12)	2.30×10^{-1}
rs2207563§	NOVA1, LOC101927062	Intergenic	0.169	14	T	C	1.05 (0.89–1.24)	5.63×10^{-1}	1.45 (1.25–1.67)	6.63×10^{-7}	0.84 (0.63–1.12)	2.36×10^{-1}
rs56151002§	NOVA1, LOC101927062	Intergenic	0.168	14	A	T	1.05 (0.89–1.24)	5.62×10^{-1}	1.46 (1.26–1.69)	3.99×10^{-7}	0.83 (0.62–1.11)	2.01×10^{-1}
rs61989674§	NOVA1, LOC101927062	Intergenic	0.168	14	A	G	1.05 (0.89–1.24)	5.38×10^{-1}	1.46 (1.26–1.69)	4.40×10^{-7}	0.83 (0.62–1.10)	1.93×10^{-1}
All patients (with interaction between genotype and treatment)												
rs2661790	SOX5	Intronic	0.410	12	T	C	1.04 (0.89–1.22)	5.96×10^{-1}	0.89 (0.79–0.99)	3.81×10^{-2}	1.88 (1.49–2.37)	1.09×10^{-7}
Patients aged ≥55 years old (with interaction between genotype and treatment)												
rs2661790	SOX5	Intronic	0.407	12	T	C	1.05 (0.89–1.25)	5.52×10^{-1}	0.86 (0.76–0.97)	1.67×10^{-2}	1.91 (1.49–2.45)	3.94×10^{-7}
Men (with interaction between genotype and treatment)												
12:129696385:A	LOC101927735	Intronic	0.488	12	T	G	1.08 (0.91–1.30)	3.77×10^{-1}	1.06 (0.93–1.20)	3.90×10^{-1}	1.93 (1.50–2.48)	2.87×10^{-7}
rs1013741	LOC101927735	Intronic	0.487	12	A	C	1.06 (0.88–1.26)	5.53×10^{-1}	1.05 (0.92–1.18)	4.82×10^{-1}	1.90 (1.48–2.43)	4.68×10^{-7}

*Adjusted for components 1–10, age, sex (except when model was sex-stratified), and treatment group (high-dose atorvastatin vs. low-dose atorvastatin).

**Adjusted for components 1–10, age, sex (except when model was sex-stratified), treatment arm, and an interaction term between treatment group (high-dose atorvastatin vs. low-dose atorvastatin) and the SNP.

§Imputed SNPs

Bold: Statistically significant at the threshold of $P<5e-08$

SNP: single-nucleotide polymorphism, Chr: chromosome, HR: hazard ratio, CI: confidence interval

We also tested the interaction term between treatment and genotypes in the pre-specified groups, but we did not find any *P*-values below the significance threshold. Additional analyses were repeated among patients treated with high-dose atorvastatin only. However, no *P*-values below the significance threshold could be detected (**Table 5**).

Table 5. Summary results from the GWAS analyses showing per-allele hazard ratio for time to new onset of cancer in high-dose atorvastatin users (80 mg). Single nucleotide polymorphisms with *P*-value $\leq 1.0 \times 10^{-6}$ in the GWAS for SNPs are reported.

SNP	Gene	Location	MAF	Chr	Risk Allele	Reference Allele	HR (95% CI) for the SNP*	P
All patients								
rs57671180§	LOC286370, LINC01508	Intergenic	0.139	9	G	A	1.65 (1.36–2.01)	6.13×10^{-7}
Patients aged ≥55 years old								
JHU_4.102655669	FLJ20021, BANK1	Intergenic	0.053	4	T	C	2.12 (1.57–2.86)	9.89×10^{-7}
rs4744117	LOC286370, LINC01508	Intergenic	0.358	9	A	G	1.54 (1.30–1.81)	2.81×10^{-7}
rs10481711§	LOC286370, LINC01508	Intergenic	0.360	9	T	G	1.54 (1.31–1.82)	2.58×10^{-7}
rs57671180§	LOC286370, LINC01508	Intergenic	0.139	9	G	A	1.69 (1.37–2.08)	8.86×10^{-7}
rs77572351	LINC00595, ZMIZ1-AS1	Intergenic	0.127	10	C	T	1.78 (1.43–2.22)	3.05×10^{-7}
rs111318030§	MIR1297, MIR5007	Intergenic	0.058	13	C	G	2.03 (1.54–2.69)	6.00×10^{-7}
rs4885046§	MIR1297, MIR5007	Intergenic	0.058	13	C	G	2.05 (1.55–2.71)	4.19×10^{-7}
rs4885047§	MIR1297, MIR5007	Intergenic	0.058	13	A	T	2.03 (1.54–2.69)	5.82×10^{-7}
rs9536718§	MIR1297, MIR5007	Intergenic	0.058	13	A	G	2.03 (1.54–2.69)	5.76×10^{-7}
rs2152753§	MIR1297, MIR5007	Intergenic	0.059	13	A	G	2.02 (1.54–2.68)	6.36×10^{-7}
rs9527204§	MIR1297, MIR5007	Intergenic	0.059	13	T	C	2.03 (1.54–2.68)	6.19×10^{-7}
rs10507587§	MIR1297, MIR5007	Intergenic	0.058	13	A	G	2.03 (1.54–2.68)	6.40×10^{-7}
rs4885052§	MIR1297, MIR5007	Intergenic	0.058	13	T	C	2.03 (1.53–2.68)	6.50×10^{-7}
rs9527206§	MIR1297, MIR5007	Intergenic	0.058	13	T	C	2.03 (1.53–2.68)	6.51×10^{-7}
rs1336992§	MIR1297, MIR5007	Intergenic	0.058	13	A	C	2.06 (1.56–2.72)	3.81×10^{-7}
rs9316718§	MIR1297, MIR5007	Intergenic	0.058	13	A	G	2.03 (1.53–2.68)	6.54×10^{-7}
rs8181888§	MIR1297, MIR5007	Intergenic	0.059	13	C	T	2.03 (1.53–2.68)	6.71×10^{-7}
rs58332706§	MIR1297, MIR5007	Intergenic	0.059	13	A	AC	2.03 (1.53–2.68)	6.71×10^{-7}
rs9569031§	MIR1297, MIR5007	Intergenic	0.059	13	A	G	2.03 (1.53–2.68)	6.71×10^{-7}
rs9569032§	MIR1297, MIR5007	Intergenic	0.059	13	C	T	2.03 (1.53–2.68)	2.68×10^{-7}
rs7332800§	MIR1297, MIR5007	Intergenic	0.058	13	A	G	2.03 (1.53–2.68)	6.56×10^{-7}
rs145707799§	MIR1297, MIR5007	Intergenic	0.059	13	T	TACTGTTAGA	2.01 (1.52–2.66)	8.45×10^{-7}
rs9527216§	MIR1297, MIR5007	Intergenic	0.061	13	C	T	1.99 (1.52–2.62)	7.21×10^{-7}
rs10872941§	LOC644919	ncRNA_intronic	0.116	14	T	C	1.71 (1.38–2.12)	9.25×10^{-7}
rs72676079§	LOC644919	ncRNA_intronic	0.127	14	T	G	1.67 (1.36–2.05)	9.65×10^{-7}
rs17111437§	LOC644919	ncRNA_intronic	0.127	14	C	T	1.67 (1.36–2.05)	9.66×10^{-7}
rs17111444§	LOC644919	ncRNA_intronic	0.131	14	C	T	1.66 (1.36–2.04)	9.02×10^{-7}
Men								
rs738499	TEF	Intronic	0.286	22	G	T	1.57 (1.32–1.88)	7.54×10^{-7}
rs5758321§	TEF	Intronic	0.283	22	T	C	1.58 (1.32–1.89)	6.36×10^{-7}

rs2273071[§]	TEF	Intronic	0.288	22	T	C		1.59 (1.33–1.90)	3.71×10^{-7}
rs132903[§]	TEF, TOB2	Intergenic	0.284	22	A	G		1.59 (1.33–1.91)	3.98×10^{-7}
rs36100462[§]	TEF, TOB2	Intergenic	0.279	22	C	T		1.58 (1.32–1.90)	7.59×10^{-7}
Women									
rs60784956[§]	MIR1255B1, MIR4801	Intergenic	0.101	4	T	C		3.63 (2.23–5.92)	2.33×10^{-7}
rs34173117[§]	MIR1255B1, MIR4801	Intergenic	0.101	4	CT	C		3.64 (2.23–5.94)	2.15×10^{-7}
rs80168265[§]	MIR1255B1, MIR4801	Intergenic	0.099	4	T	TACTC		3.66 (2.25–5.96)	1.87×10^{-7}
rs61796494[§]	MIR1255B1, MIR4801	Intergenic	0.099	4	G	A		3.66 (2.25–5.96)	1.87×10^{-7}
rs73237313[§]	MIR1255B1, MIR4801	Intergenic	0.099	4	C	T		3.66 (2.25–5.96)	1.86×10^{-7}
rs73237314[§]	MIR1255B1, MIR4801	Intergenic	0.099	4	G	A		3.66 (2.25–5.96)	1.86×10^{-7}
rs74815180[§]	MIR1255B1, MIR4801	Intergenic	0.101	4	A	G		3.21 (2.02–5.10)	8.17×10^{-7}
rs141350675[§]	MIR1255B1, MIR4801	Intergenic	0.101	4	T	C		3.21 (2.02–5.10)	8.14×10^{-7}
rs76891207[§]	MIR1255B1, MIR4801	Intergenic	0.101	4	T	C		3.21 (2.02–5.10)	8.14×10^{-7}
rs57547264[§]	MIR1255B1, MIR4801	Intergenic	0.101	4	T	C		3.21 (2.02–5.10)	8.12×10^{-7}
rs56184231[§]	MIR1255B1, MIR4801	Intergenic	0.101	4	C	T		3.21 (2.02–5.10)	8.15×10^{-7}
rs56285674[§]	MIR1255B1, MIR4801	Intergenic	0.101	4	G	A		3.21 (2.02–5.10)	8.15×10^{-7}
rs2375963[§]	MIR1255B1, MIR4801	Intergenic	0.101	4	A	G		3.21 (2.02–5.09)	8.16×10^{-7}
rs55689515[§]	MIR1255B1, MIR4801	Intergenic	0.101	4	T	C		3.21 (2.02–5.09)	8.19×10^{-7}
rs61796532[§]	MIR1255B1, MIR4801	Intergenic	0.101	4	G	A		3.21 (2.02–5.09)	8.19×10^{-7}
rs117329860[§]	SYT4, LINC01478	Intergenic	0.078	18	C	T		3.46 (2.12–5.66)	7.20×10^{-7}
rs78928980[§]	SYT4, LINC01478	Intergenic	0.078	18	A	G		3.40 (2.09–5.53)	8.31×10^{-7}

*Adjusted for components 1–10, age, and sex (except when model was sex-stratified).

[§]Imputed SNPs

SNP: single-nucleotide polymorphism, Chr: chromosome, HR: hazard ratio, CI: confidence interval

Finally we sought to explore the genetic variants for prediction of cancer mortality in all patients by adjusting for high- vs. low-dose atorvastatin, age, sex, and genetic ancestry. No *P*-value passed the significance threshold. The best *P*-value for genetic association with mortality was detected with SNP rs75967297 on chromosome 13 between genes *LINC00423* and *KL* ($HR_{allelic}$: 2.61, 95% CI: 1.80–3.79, *P*=3.87×10^{-7}, MAF= 0.48) using 5119 patients including 62 cancer deaths.

In summary, in all the GWAS performed, we could not find any genetic factors that were associated with cancer incidence or cancer mortality in patients receiving atorvastatin. A meta-GWAS analysis using the IDEAL study population may eventually be valuable. Due to the abundance of negative results following the GWAS, we chose to omit them from the article that was submitted for peer-review journal publication.

Discussion

Public health perspective

In addition to our clinical and genetic perspectives, it was also important to consider how our assessment of whether statins are associated with cancer or cancer death may have an impact on the general population. Statins are a prevalent drug that is prescribed extensively in the general population within both the primary and secondary settings. Had statins been found to increase the risk of cancer, this would have resulted in important safety and public health implications. Our results indicated a negative association. From the public health perspective, several points may be noteworthy to consider based on our results.

Our study consistently showed a lack of effect of high-dose statin vs. low-dose statin and the risk of cancer or cancer mortality. Our GWAS, the first ever performed on the relationship between statin use and cancer, was also systematically unable to detect a variant that was significantly associated with incident cancer. These findings provide important information on the safety of statin utilization for the prevention of cardiovascular-related events. Serious public health consequences following misleading claims of statin therapy and its safety have previously been reported. For example, researchers at the Picker Institute conducted interviews and focus groups with patients, general physicians, and cardiologists, and also piloted online surveys on the impact of exaggerated side-effects as portrayed in the media of statin therapy in the United Kingdom.(200) The authors found that ensuing the bad press coverage on statins, there was increased reticence among doctors to prescribe statins, as well as reduced compliance by patients to pursue or continue statin therapy. Patients who stopped their treatment altogether were also most likely to cite 'concerns about side-effects' as the reason cited for not wanting to take statins following an initial consultation. Of the general practitioners and cardiologists who were surveyed, many stated that they felt less confident discussing statins with patients as a result of negative media coverage. It is presumed that physicians and patients are generally wary of side effects with a medication. The under-utilization of statin therapy(201) in patients at risk of heart attacks and strokes have been

attributed to some perceptions that the general public has in regard to statin therapy and its safety concerns. In the Prospective Urban Rural Epidemiological (PURE) study, only 15% of surveyed-individuals were taking statins. Experts have cautioned against the under-recognition that observational studies and case reports have limitations on reliability and validity of data.(172)

Future clinical perspective

In our study, higher-dose statin use was not found to be significantly associated with a lower risk of cancer compared to lower- or usual-dose statins in the pooled analyses using the TNT and IDEAL cohorts. Our hypothesis-free approach of the GWAS of the TNT cohort also failed to reveal any significant genetic variant associated with the risk of cancer. For nearly two decades, the debate on whether statins can increase or decrease the risk of cancer incidence has not been settled. Consistent with our findings, post-hoc analyses of other randomized-controlled trials regarding statins and the risk of cancer were not conclusive. Several meta-analyses that examined the pooled-effect of statins vs. placebo using data from randomized trials failed to observe a significant effect, including a dose-response relationship. In contrast, many epidemiological studies have observed a significant and somewhat meaningful effect between statin users and cancer. These lower level evidence-based reports succinctly purport that statin use is linked with cancer. Unfortunately, their credibility is lessened as different studies reporting different effect sizes, not to mention opposing direction of the effect (risk or protection). In addition, observational studies are notoriously limited by their lack of cause-and-effect inference. Nonetheless, these reports persist, and hence, so do the lack of agreement.

Arguably, the variable results of the purported associations, or lack of, between statins and cancer may be related to the study type.(202) Population-based studies can be exposed to treatment selection biases and present more heterogeneous patient populations. In addition, non-captured characteristics of statin and non-statin users (e.g. exercise, eating habits, smoking habits, alcohol)(203) that are known to be associated with cancer risks may confound

results of population-based nature. As noted by experts, it may be that statins *per se* are not to blame, but that something related to statin use is. In a recent study, the authors prospectively followed 1081 patients with a baseline myocardial infarction without any history of cancer between years 2002 and 2010.(204) The risk of developing cancer in patients who then developed heart failure was significantly higher than non-heart failure patients, even after adjusting for age, sex, and comorbidities (HR: 1.71, 95% CI: 1.07 to 2.73). The authors postulated that patients with cardiovascular disease conditions are naturally followed more intensely than the average population, which could increase their chances of being diagnosed with cancers. This may have been the case in our study as well, where within the TNT population previous statin users were shown to be more likely to harbour cancer than never-users. However, similar to the pitfalls of population-based studies, the real explanation is hard to uncover given that this information was retrospectively obtained from patients at study initiation. In particular, we only had documentation of prior statin use without complete information on duration, doses or indications.

Nonetheless, we believe that the reliance on genetics in future studies will be able to paint a better picture of underlying biological mechanisms that relate inflammation to cardiology and cancer. Specifically, the exploratory analysis using data from a randomized-controlled phase III trial, namely CANTOS, showed that those randomized to receive canakinumab, an anti-inflammatory therapy, were significantly less likely to have incident lung cancer compared to the control group. Studies confirming or exploring the mechanistic and therapeutic threads between cardiology and oncology are anticipated in the near future. Currently, the relationship revolving inflammation, atherosclerotic disease, and cancer remains poorly understood. In a Mendelian randomization, the authors tested the hypothesis that genetically elevated levels of CRP because of polymorphisms in the CRP gene can cause an increased risk of cancer in the general population. To test that hypothesis, they examined whether four common SNPs in the CRP gene that are associated with altered plasma CRP levels are causally associated with an increased risk of cancer. For a doubling of the plasma CRP level, the risk of cancer was 9% higher (HR: 1.09, 95% CI: 1.03–1.14). However, the estimated odds of cancer associated with a genetically-induced doubling in CRP level was not significant (OR: 0.94, 95% CI: 0.81–1.08). These results suggest that elevated CRP levels, an established marker of inflammation,

do not cause cancer *per se*. However, the possibility that inflammation itself could lead to cancer has not been excluded.(205) Furthermore, as part of another Mendelian randomization study, a genetic risk score for SNPs that lower 3-hydroxy-3-methyglutaryl-CoA reductase expression, and therefore mimic the effects of statin therapy to reduce cholesterol levels, was associated with causal reduction in risk of colorectal cancer, supporting possible on-target benefits of statin therapy.(206) It is plausible that statins, bearing anti-inflammatory properties themselves, can also impact some types of cancer under an alternate biological mechanism that is currently unclear. It is also possible that this mechanism is invoked given a specific genetic damage for a sub-population. It would be anticipated that future pharmacogenomics studies, in adjunct to prospective trials, may provide additional insights and ultimately enlighten the unending discussion on statins and cancer.

Strengths and Limitations

The advantage of using data obtained from two clinical trials on statins is that unlike observational data sources, there is a more stringent definition of statin use. That said, similar to population-based data, the participants recruited within the two studies had previously been treated with statins, for an undetermined period of time. The main advantage of having a randomized-controlled trial dataset is that the patient and disease characteristics of the intervention and control groups are well-balanced. Therefore any effect, or non-effect that will be detected between the two examined groups (high vs. low-dose statin) will be less likely due to baseline differences between the two groups. That being said, many important risk factors that would enhance the risk of cancer are not accounted for (e.g. family history). The most interesting aspect of our analysis was the availability of genetic data of participants within the TNT trial. This allowed us to conduct a first-ever GWAS on the effect of high-dose vs. low-dose statin and cancer. Although our results were consistently negative from both the clinical and genetic perspectives, such findings may be considered positive from the public health perspective.

The main limitation of the data used was that it was a dose-response study as opposed to a statin vs. non-statin study on the risk of cancer. Other limitations include the fact that our primary endpoint (i.e. cancer incidence) was not an adjudicated endpoint, which limits reliability and validity. This is because the clinical trial was not designed for the evaluation of cancer-related endpoints. Several other issues revolve around this, including the date of when cancer was first recorded, which we had to impute on several occasions due to missing data. What's more is that since cancer was not a monitored endpoint, it's possible that many patients would have been diagnosed with an asymptomatic cancer (i.e. false negatives) had they been tested. Furthermore, we do not have the stage of the cancer. The reality is that it is very difficult to reliably evaluate the risk of cancer following statin therapy in most existing databases. The only way to truly answer that question would be through a randomized-controlled trial. However, that is unlikely to happen given cost and ethical issues.

Conclusions

Our objective was to assess the effect of more vs. less statins on cancer incidence and cancer mortality in a post-hoc analysis of two previous randomized-controlled studies, namely the TNT and IDEAL trials. By pooling the populations from two trials we had a total of 18,889 patients. In addition, we sought for the first time to perform a hypothesis-free GWAS where genotyping was available for patients who consented to participate in the TNT study. While our study was equipped with an adequate follow-up time and larger sample size, our post-hoc clinical analyses did not detect a significant effect between high-dose statin with cancer incidence or cancer mortality compared to low/usual-dose statins. Sub-analyses according to patient age and sex showed similar results. In an effort to identify a genetic profile that may inform prevention or stratify patients, we then performed a GWAS using genetic data that was available for patients enrolled in the TNT study. Consistent with the clinical segment of our analyses, our GWAS results also failed to find genetic variants associated with cancer outcomes in statin users of the TNT clinical trial.

Despite these results, our knowledge on the underlying biological mechanisms with regard to statins and cancer is clearly limited. Previous studies have indicated that inflammation does not appear to cause cancer directly. Yet, a recent study has revealed an interesting association between canakinumab, an anti-inflammatory therapy via interleukin-1B inhibitor aimed at patients with atherosclerosis who had a previous myocardial infarction, and incident lung cancer. To parallel this observation with statins, which also hold anti-inflammatory effects, a recent Mendelian randomization study mimicking the effects of statins suggest that a causal relationship between statin use and reduced risk of colorectal cancer may be possible. These data warrant considerations of future trials of statins for certain types of cancer prevention and treatment.

Our own future efforts will be aimed at improving the statistical power of our GWAS and repeat our analyses, and possibly performing additional analyses for specific types of cancers.

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Appendix 1. Definitions used to capture cancer

TNT:

Bladder carcinoma/Bladder cancer

Bone cancer

Breast carcinoma/breast cancer

Skin carcinoma/skin melanoma

Carcinoma/tumor

Carcinoma of larynx

Carcinoma of lung

Carcinoma of mouth

Endometrial carcinoma

Ovarian cancer

Prostatic carcinoma

Sarcoma

Thyroid carcinoma

Myeloma

Gastrointestinal carcinoma

IDEAL:

Bladder Carcinoma

Bone Sarcoma

Brain Neoplasm Malignant

Breast Carcinoma

Breast Neoplasm Malignant Female

Breast Neoplasm Malignant Male

Carcinoma

Cervix Carcinoma

Cervix Carcinoma In situ

Colon Carcinoma

Endometrial carcinoma

Esophageal Carcinoma

Hepatoma

Gastrointestinal carcinoma

Gastric Carcinoma

Carcinoma of the Larynx

Larynx Neoplasm Malignant

Carcinoma of Lung

Lymphoma Malignant

Melanoma Malignant

Myeloma

Neoplasm Malignant Aggravated

Pancreas Neoplasm Malignant

Pharynx Neoplasm Malignant

Prostatic carcinoma

Rectal Carcinoma

Renal Carcinoma

Sarcoma

Seminoma

Skin melanoma

Skin Neoplasm Malignant

Thyroid carcinoma

Thyroid Neoplasm Malignant

Appendix 2. Number of patients with imputed time to cancer.

TNT:

Bladder carcinoma/Bladder cancer: 20

Bone cancer: 4

Breast carcinoma/breast cancer: 25

Skin carcinoma/skin melanoma: 175

Carcinoma/tumor: 211

Carcinoma of larynx: 1

Carcinoma of lung: 30

Carcinoma of mouth: 2

Endometrial carcinoma: 0

Ovarian cancer: 1

Prostatic carcinoma: 48

Sarcoma: 0

Thyroid carcinoma: 1

Myeloma: 3

Gastrointestinal carcinoma: 32

Total: 553