STATINS AND CANCER PREVENTION

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Abstract | Randomized controlled trials for preventing cardiovascular disease indicated that statins had provocative and unexpected benefits for reducing colorectal cancer and melanoma. These findings have led to the intensive study of statins in cancer prevention, including recent, large population-based studies showing statin-associated reductions in overall, colorectal and prostate cancer. Understanding the complex cellular effects (for example, on angiogenesis and inflammation) and the underlying molecular mechanisms of statins (for example, 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase-dependent processes that involve geranylgeranylation of Rho proteins, and HMG-CoA-independent processes that involve lymphocyte-function-associated antigen 1) will advance the development of molecularly targeted agents for preventing cancer. This understanding might also help the development of drugs for other ageing-related diseases with interrelated molecular pathways.

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Statins potently reduce CHOLESTEROL levels and decrease the incidence of cardiovascular and cerebrovascular events. The remarkable prevention of CARDIOVASCULAR DISEASE (CVD) and the relative safety of statins have led to their widespread use and their recent conversion from prescription to over-the-counter status in the United Kingdom. Statins are small-molecule inhibitors of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase (also known as HMGCR), which sits at the apex of a molecular pathway called the MEVALONATE cascade. As well as reducing cholesterol levels, statins can inhibit other products and reactions in the mevalonate pathway, including the generation of mevalonate and downstream isoprenoids. Isoprenoids are long, hydrophobic molecules — for example, farnesyl and geranylgeranyl groups — that attach to various proteins such as members of the Ras/Rho superfamily. This attachment allows them to anchor to cell membranes and perform their normal functions. Inhibition of this hydrophobic modification of signalling proteins by statins has significant effects on cell growth *in vitro*. Epidemiological data indicate that these *in vitro* effects might be more than just laboratory phenomena and might contribute to the prevention of human cancers.

Statins first attracted interest for cancer prevention as an unexpected result of safety monitoring in large RANDOMIZED CONTROLLED TRIALS (RCTs) of statins and other lipid-lowering agents (for example, fibrates, nicotinic acid and cholestyramine) in preventing CVD. This monitoring was implemented because the RCTs showed consistent increases in statin-associated non-CVD mortality[1]. Preclinical data in rodents indicated that statins might increase the incidences of, primarily, liver, forestomach, lung and thyroid tumours, and lymphoma[2]. Observational data also indicated that levels of cholesterol inversely correlated with cancer risk and cancer mortality[1]. Therefore, the CVD-prevention RCTs began monitoring cancer as a possible adverse drug effect.

The RCT safety results indicated that statins did not increase overall cancer incidence or cancer mortality (non-CVD mortality associated with cholesterol-reducing drugs seemed to result mainly from accidents[1]). Indeed, statins were associated with provocative reductions in colorectal cancer and melanoma, but possibly increased the occurrence of breast cancer. The adverse breast cancer finding (from a secondary analysis involving a very small number of cases) has been overturned by subsequent intensive clinical and

Summary

- Statins function in the mevalonate pathway as small-molecule inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which lowers cholesterol. These agents are effective in preventing cardiovascular disease (CVD), largely because of this effect.
- Large randomized controlled trials (RCTs) that analysed the effects of statins and other lipid-lowering agents (for example, fibrates, nicotinic acid and cholestyramine) to prevent CVD included safety monitoring to address whether statins increased cancer incidence and cancer mortality. Ironically, these results were the first to suggest that statins can prevent cancer.
- In addition to the HMG-CoA-dependent effects, statins have important cholesterol/HMG-CoA-independent effects, such as effects on lymphocyte-function-associated antigen 1 (LFA1), that are thought to contribute to potential cancer prevention.
- Important work in preclinical models of colorectal and breast cancer, and melanoma, indicates that statin anticancer effects involve the inhibition of geranylgeranylation, primarily of Rho proteins.
- Secondary results of the CVD RCTs, as well as observational and preclinical studies, indicate that statins have a strong potential for preventing colorectal cancer and melanoma.
- The beneficial effects of statins on inhibiting carcinogenesis could involve their effects on important disease pathways including inflammation, immunomodulation and angiogenesis.
- Statins are broad-spectrum agents. Current research is revealing important new statin targets (such as LFA1, Rho isoforms, and post-prenylation enzymes) leading to the development of more-specifically targeted agents for cancer prevention.

OBSERVATIONAL STUDIES and by preclinical data, most of which indicate that statins have potentially beneficial effects on breast cancer. The RCT monitoring ultimately focused statin research on cancer prevention, which seems promising for colorectal, prostate, breast and skin (melanoma) cancer. This has been highlighted by two recent and large POPULATION-BASED STUDIES that show statin-associated reductions in the risk of colorectal cancer or advanced prostate cancer. Moreover, the RCT monitoring demonstrated that statins have a very favourable overall safety profile for long-term use in cancer prevention.

The effects of statins can occur through HMG-CoA reductase-dependent or HMG-CoA reductase-independent pathways. Some effects require the inhibition of HMG-CoA reductase (and therefore, for example, of G-protein activation through geranylgeranylation), or statins can function through other mechanisms such as binding directly to lymphocyte-function-associated antigen 1 (LFA1). Statins have pleiotropic, or seemingly unrelated, distinct effects on processes such as ANGIOGENESIS and inflammation, and also affect a number of novel molecular targets and complex signalling pathways. Because of these pleiotropic effects, statins potentially have activity in a number of chronic human diseases (for example, NEURODEGENERATION, AGE-RELATED MACULAR DEGENERATION, OSTEOPOROSIS, CVD and cancer) by targeting their common and distinct molecular pathways. This potential of statins to improve the control of several important chronic diseases has important public health implications.

CHOLESTEROL
A lipid ringed sterol used by the body for the production of hormones, vitamin D and cell membranes; high levels in the blood stream are a marker for heart disease.

CARDIOVASCULAR DISEASE
Disease caused by atherosclerosis of the coronary arteries.

MEVALONATE
A fatty acid formed from HMG-CoA by HMG-CoA reductase, and an essential intermediate in the biosynthesis of cholesterol or geranylgeranyl pyrophosphate, which leads to the isoprenylation of the small G-proteins.

RANDOMIZED CONTROLLED TRIAL
A study of individuals who are randomized to a therapy, which is used to evaluate the effect of a therapy versus a placebo. Resistant to bias from unmeasured risk factors as these should be distributed equally in both groups.

The preclinical, epidemiological and clinical studies of statins in cancer prevention will be reviewed here. These studies have focused primarily on colorectal, breast and prostate cancer, and melanoma, and indicate that statins are one of the most promising classes of agents currently available for testing in cancer prevention.

Statins and the mevalonate pathway

Statins reduce serum cholesterol levels by competitively inhibiting HMG-CoA reductase, the rate-limiting enzyme in the synthesis of mevalonate (the fatty acid intermediate in cholesterol biosynthesis) (FIG. 1). However, the efficacy of statins in reducing cardiac events seems to exceed the degree of cholesterol reduction and extends to patients with normal cholesterol levels[3,4], leading investigators to question whether statins have beneficial effects beyond reducing cholesterol[5]. HMG-CoA reductase is bound approximately 1,000 times more effectively by statins with open-ring structures[6] (the structures and properties of different statins are described in BOX 1) than by its natural substrate, HMG-CoA. Statins are generally thought to exert many of their effects in cancer by inhibiting the prenylation of small G-proteins, primarily Rho proteins, as a downstream effect of HMG-CoA reductase inhibition[7].

By inhibiting the biosynthesis of mevalonate, statins also inhibit the formation of downstream lipid isoprenoid intermediates such as farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). The isoprenoids are lipid moieties that are added to various proteins, including G-proteins and the G-protein subunits RAS, RHO, RAB, RAC and RAP, during post-translational modification (prenylation) and anchor these proteins to the cell membrane. Isoprenoids suppress HMG-CoA reductase by post-translational downregulation. In normal cells, the reductase is subject to complex feedback regulation at the transcriptional, translational and post-translational levels by both sterol and non-sterol products of the mevalonate pathway. Tumour cells, however, are resistant to sterol-mediated feedback and are more sensitive than normal cells to isoprenoid-mediated suppression[7–10].

Post-translational prenylation by FPP or GGPP is essential for G-protein function. FPP prenylates RAS (farnesylation), which was perhaps the most important target of interest in the early study of statin effects on carcinogenesis. The most relevant studies to date, however, indicate that GGPP prenylation (geranylgeranylation) of other proteins, including the Rho proteins, is the crucial step in the apoptotic, angiogenic and inflammatory effects of statins, as well as other important cellular effects of statins. Adding GGPP generally reverses desirable statin effects, as does adding mevalonate, whereas adding FPP generally either does not reverse the effects or does so to a lesser degree. The FPP add-back experiments pinpoint the primary influence of geranylgeranylation and de-emphasize the influence of farnesylation on statin effects.

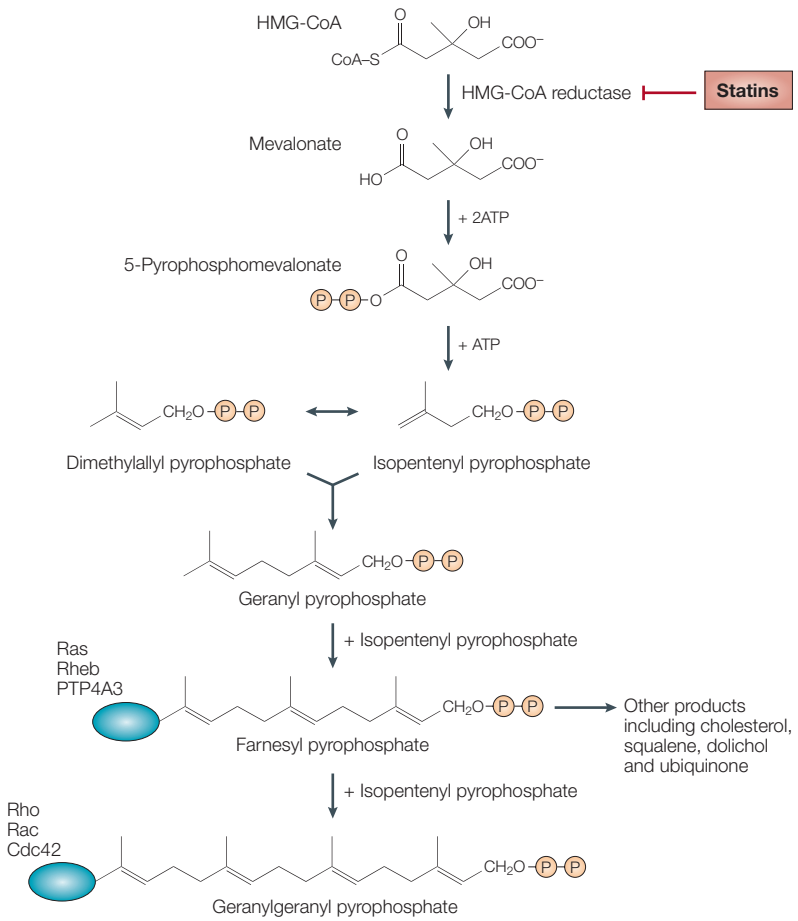


Figure 1 | **Mevalonate pathway.** Statins inhibit the conversion of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonate. Molecules of ATP are then used to phosphorylate mevalonate to pyrophosphomevalonate, which is then converted to isopentenyl pyrophosphate (IPP). IPP can be reversibly converted to dimethylallyl pyrophosphate (DMAPP). IPP and DMAPP can then be combined to form the 10-carbon isoprenoid geranyl pyrophosphate (GPP). Additional IPPs can be added to produce farnesyl pyrophosphate (FPP), the 15-carbon isoprenoid, and geranylgeranyl pyrophosphate (GGPP), the 20-carbon isoprenoid. Inhibition of this pathway by statins prevents the formation of both mevalonate and its downstream product, IPP. This inhibition can be reversed completely with mevalonate. Supplementation with FPP will restore farnesylation but not geranylgeranylation, as IPP is not available to convert FPP into GGPP. Supplementation with GGPP will only restore geranylgeranylation. Several other branches of this pathway can convert FPP into various other products, including cholesterol. In general, FPP helps prenylate proteins in the Ras, Rheb, and PTP4A3 families, whereas GGPP helps prenylate proteins in the Rho, Rac and Cdc42 families. A few G-proteins (including RHOB and NRAS) can be either farnesylated or geranylgeranylated.

Even though FPP is the immediate precursor of GGPP (FIG. 1), adding FPP generally does not restore GGPP (and therefore reverse statin activity) because this restoration requires isopentenyl pyrophosphate (IPP). Statins block IPP formation upstream of FPP, so IPP is not available for converting FPP into GGPP. Adding mevalonate can reverse the effects of statins because mevalonate can restore IPP for the downstream conversion of FPP into GGPP[9,10].

Other emerging data also indicate the importance of Rho proteins (BOX 2) in carcinogenesis[11–15] (FIG. 2). Overexpression of RHOA and/or RHOC (but not RHOB) is associated with poor prognosis in colorectal cancer, as well as breast, bladder, pancreas and other cancers. The RHOA and RHOC isoforms have high sequence similarity, and can therefore be difficult to differentiate from one another. Recent short interfering (si) RNA studies indicate that RHOC is the most important isoform in stimulating invasion; RHOA has also been implicated in epithelial-to-mesenchymal transition, an important event in cancer progression[11,13]. RHOB is unusual among prenylated proteins in that it can be farnesylated or geranylgeranylated, and can have differential (enhancing or suppressive) effects on carcinogenesis that might relate to the nature of its prenylation[15].

The intensive study of the functional mechanisms of statins is just beginning. Many proteins besides the Rho proteins are geranylgeranylated, and the ability of statins to inhibit these proteins could be important to the pleiotropic effects of these drugs. Notwithstanding the add-back data discussed at the beginning of this section, RAS might also contribute to the effects of statins — for example, through cross-talk with Rho-mediated signalling pathways[11,13,14,16] (BOX 2). The *in vitro* mechanistic studies of statins have been criticized for often using high concentrations of statins (1–200 micromolar) because maximal statin concentrations in serum with standard anti-cholesterol dosing are 10–200 nanomolar[6,7,17]. Some *in vitro* anti-proliferative and pro-apoptotic effects have been reproduced *ex vivo* in serum from statin-treated patients[18]. Other effects (for example, on LIPID RAFTS or the proteasome) have not yet been shown to occur at the lower range of statin concentrations. However, mechanistic studies with high doses of statins can be used to identify targets that can be used to develop other, more specific drugs that will function at clinically achievable doses and therefore have potential future clinical relevance.

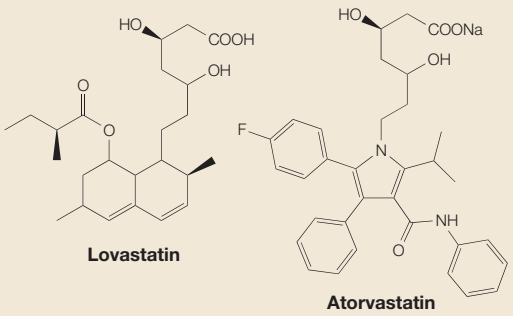
HMG-CoA reductase-independent effects

Many of the effects of statins are not clearly related to the reduction of cholesterol, leading to the search for HMG-CoA reductase-independent effects of statins. Lovastatin directly binds to the L (lovastatin) site in the I (inserted) domain of the integrin LFA1, which has an important role in leukocyte migration and T-cell activation[19]. Binding of lovastatin to the LFA1 I-domain induces a conformational change in LFA1 and inhibits the interaction of LFA1 with intercellular-adhesion molecule 1 (ICAM1) through an allosteric mechanism. LFA1 with a mutant I-domain locked into its active, ligand-binding domain is resistant to lovastatin. Therefore, lovastatin inhibits the function of LFA1 by stabilizing the receptor in an inactive conformation. Simvastatin and mevastatin also inhibit LFA1 by binding to the L-site. Blocking the LFA1–ICAM1 interaction could contribute to the effects of statins on cell-adhesion, invasion and inflammation. Recent data showed that simvastatin can induce apoptosis in Epstein–Barr virus (EBV)-transformed lymphoblastoid cell lines (the induction depended on simvastatin binding to the I-domain of LFA1) and can delay the development of EBV-associated lymphomas in SEVERE COMBINED IMMUNODEFICIENT MICE

OBSERVATIONAL STUDIES
Studies in which data on risk factors and disease outcomes are collected to detect associations between risk factors and disease. Individuals are not randomized, and choose their own exposure to risk factors, so bias can occur and might be undetected.

Box 1 | **Properties and structures of statins**

Statins are derived from fungi (for example, lovastatin (shown below), simvastatin, pravastatin and mevastatin) or are made synthetically (for example, atorvastatin (shown below) and fluvastatin). All available statins, except pravastatin, are lipophilic. Recent data has indicated that this is crucial to statin anticancer activity. Different statins have different oral absorption and bioavailability profiles[6]. For example, the bioavailability of lovastatin is < 5%, whereas that of atorvastatin is ~40%. All statins have a side chain with either an open-ring (acid) or a closed-ring (lactone) structure, which is an inactive prodrug that is converted to the active form, β-hydroxy-acid, by carboxyesterases in the liver and plasma. Most statins (for example, lovastatin, simvastatin and atorvastatin) are metabolized (oxidatively) by CYP3A4, although CYP2C9 also has a role. Some data indicate that certain statin prodrug forms (which do not inhibit 3-hydroxy-3-methylglutaryl coenzyme-A reductase) can inhibit the proteasome. Lovastatin specifically inhibits lymphocyte-function-associated antigen 1 (LFA1) (as do simvastatin and mevastatin) by binding to the L (lovastatin) site of the I (inserted) domain of LFA1.



(SCID mice)[20]. These findings have important implications for the prevention of EBV-associated lymphomas in immunocompromised people.

Statins reportedly also target the protein-degradation machinery, specifically the proteasome. This targeting can apparently be independent of the inhibition of HMG-CoA reductase because a closed-ring statin, which does not inhibit HMG-CoA reductase (BOX 1), inhibits the proteasome[21]. Inhibition of the proteasome could account for the effects of statins on the cyclin-dependent kinase inhibitors (CDKIs) p21 (also known as CDKN1A) and p27 (also known as CDKN1B), although other mechanisms have been reported[22].

Statin effects on disease-associated pathways

Inflammation and immunomodulation. Inflammation has a crucial role in the pathogenesis of many chronic human diseases, including atherogenesis, carcinogenesis and neurodegenerative diseases. The beneficial effects of statins on CVD have been linked to their anti-inflammatory and immunomodulatory effects on adhesion (for example, those involving LFA1, ICAM1, vascular CAM1 (VCAM1) and E-selectin), inflammatory mediators (for example, CD40, interleukin (IL)-1β, IL-6, tumour-necrosis factor-α (TNFα), major histocompatibility complex II (MHC-II), T-helper (T_H) 1 and T_H2 cytokines, and C-REACTIVE PROTEIN (CRP)), and other pathways. Statins can affect the leukocyte adhesion cascade by HMG-CoA reductase-independent and HMG-CoA reductase-dependent mechanisms. Statins can block LFA1 expression and reduce ICAM1 expression in some systems, and these effects can be reversed by adding GGPP or mevalonate, indicating dependence on HMG-CoA reductase. Nuclear factor of κB (NF-κB), which is activated by the Rho proteins, has a key role in the transcriptional regulation of certain cytokines, chemokines and adhesion molecules, as well as the important inflammatory promoter CRP. Statins suppress CRP at the transcriptional level, and CRP can upregulate ICAM1 and VCAM1 in endothelial cells. Suppression of CRP correlates with the beneficial effects of statins on CVD in RCTs. However, levels of CRP and modulation of these levels by statins does not correlate significantly with levels of low-density lipoprotein (LDL) cholesterol[5,23].

Statins can modulate the differentiation of T lymphocytes *in vitro* and *in vivo*, producing a shift from a pro-inflammatory T_H1 profile to an anti-inflammatory T_H2 profile. Atorvastatin can induce the phosphorylation of signal transducer and activator of transcription 6 (STAT6) and secretion of T_H2 cytokines, and inhibit STAT4 phosphorylation and secretion of T_H1 cytokines. Activation of NF-κB, which promotes T_H1 development, can also be inhibited by atorvastatin[24–26]. Recent data indicate that statin immunomodulatory effects can involve MHC-I antigens[27]. At high concentrations *in vitro*, statins also interfere with lymphocyte function by depleting membrane cholesterol and disrupting the integrity of lipid rafts[5].

Angiogenesis. Statins can inhibit angiogenesis in some chronic human diseases (for example, age-related macular degeneration) and promote it in others (for example, CVD). This duality is complex and related to the organ site, cell type, disease process, and possibly to statin dose or concentration. Statins significantly reduced tumour growth and tumour vascularization in the Lewis lung cancer model[28]. Certain preclinical studies have led to the speculation that statins are pro-angiogenic at low doses but anti-angiogenic at high doses[28], raising concern that clinically relevant doses might enhance tumour-associated angiogenesis. A recent study of ischaemia and cancer in the same animal, however, indicated that doses that augmented blood flow to the ischaemic tissue did not increase blood flow or capillary density in implanted colon tumours, the growth of which was substantially retarded[29]. Recent data indicate that clinically relevant (low) doses of statins activate AKT[30] and krüppel-like

POPULATION-BASED STUDY
A type of observational study in which the entire population of a geographical area (county, state or country) is studied for risk factors, and disease outcomes are recorded as they occur, to detect associations between risk factors and the incidence of disease. Minimizes selection bias.

ANGIOGENESIS
The generation of new blood vessels, particularly arterial supply vessels. Can occur after trauma, ischaemic (lack of oxygen) injury or during the growth of a tumour.

NEURODEGENERATION
A group of neurological diseases, affecting the central nervous system, that involve the loss of neurons. These diseases include Alzheimer disease and Parkinson disease.

AGE-RELATED MACULAR DEGENERATION
A disease that blurs the central, high-resolution vision of the eye by damaging the macula. It is the main cause of central vision loss in Americans who are 50 or more years old.

OSTEOPOROSIS
A condition that is characterized by a decrease in bone mass as well as by decreased bone density and increased risk of bone fracture.

Box 2 | Cellular and molecular effects of G-proteins

Certain Rho proteins are implicated in cytoskeleton organization and the motility, migration, adhesion, invasiveness and metastatic potential of cancer cells[11–15]. Although Rho mutants have not been identified in human tumours, Rho proteins are overexpressed in various human cancers and are typically geranylgeranylated. In addition, overexpression of the RAC1B splice variant of RAC1 has been reported to contribute to carcinogenesis[16]. Overexpression of RHOA or RHOC is associated with invasion and metastasis of certain cancers, and a dominant-negative mutant of RHOA blocks growth factor-induced cancer cell invasion. Statins can inhibit epidermal growth factor-induced cancer cell invasion by preventing geranylgeranylation of RHOA. Ras proteins are important signalling molecules and are mutated in approximately 30% of all human cancers. Ras proteins are typically farnesylated (not geranylgeranylated), which allows them to localize to the inner surface of the cell membrane, and this farnesylation is required for transformation by Ras. There is substantial crosstalk between the Rho, Ras and Rac pathways[11–15].

factor 2 (KLF2)[31], and this correlates with increasing nitric oxide production and angiogenesis. However, higher doses (>100 nanomolar) inhibit protein prenylation, cell growth and migration, and angiogenesis[32–35]. The effects of statins on AKT are reversible with the addition of mevalonate, and the effects on KLF2 are reversible with the addition of mevalonate and GGPP, but not FPP. Although real, the opposing effects of statins on angiogenesis in ischaemia and cancer are only beginning to be understood at the molecular level.

Apoptosis and proliferation. Resistance to apoptosis is a hallmark of carcinogenesis, and the induction of apoptosis has been an important focal point in the development of preventive drugs. Statins can induce apoptosis by regulating several signalling pathways, including the RAF–mitogen activated protein kinase kinase 1 (MAP2K1, also known as MEK)–extracellular regulated kinase (ERK) pathway[36]. Induction of apoptosis by lovastatin in NIH3T3 cells can be reversed by dominant-active RHOA. Data indicate that RHOA regulates the expression of the anti-apoptotic protein BCL2. The overexpression of RHOA prevented, at least in part, the downregulation of BCL2 expression by statins. Downregulation of BCL2 and ERK1/ERK2 by statins is reversed by GGPP. Statins can also induce apoptosis through the activation of FAS (CD95)[37]. Statins can inhibit proliferation by downregulating CDK2 activity and upregulating the expression of p21 and p27, and these effects are reported to be either HMG-CoA-dependent or HMG-CoA-independent in different systems[21,22]. These effects can occur at serum levels that are achieved in humans who are taking standard statin doses, as shown in studies with serum from subjects treated with 40 mg day^{-1} of fluvastatin to cultured human vascular smooth muscle cells (at 15% volume for volume); increased apoptosis was induced in concert with decreasing levels of expression of BCL2 (REF. 18). Evidence shows that statins induce chromatin condensation and DNA laddering[38,39], and the activation of caspases in association with statin-induced apoptosis has also been documented[40,41].

Statins can inhibit cellular proliferation through the induction of G1/S-arrest[42,43] and/or G2/M-arrest[44,45] in numerous cell lines. It seems that the effects of statins on proliferation and apoptosis are independent of each other, although both can occur in the same cell line at different concentrations[18]. Statins seem to induce apoptosis and inhibit proliferation to a greater degree in malignant than in non-malignant cells[7,36], possibly because of the increased expression of HMG-CoA reductase and a greater requirement for mevalonate-derived isoprenoids in tumour as opposed to normal cells[46].

Statin effects in cancer models

Colorectal cancer models. It was initially thought that statins could prevent colorectal cancer because they suppressed farnesylation and so inhibited the activation of RAS, which is an important event in the development of colorectal cancer. However, early experiments found that statins inhibit growth and induce apoptosis in colorectal cancer cell lines regardless of the mutational status of RAS[22]. RHOA is overexpressed in colorectal cancer and is associated with angiogenesis, invasion and a poor prognosis. A dominant-negative RHOA mutant blocked integrin $\alpha_6\beta_4$-induced and leptin-induced colorectal cancer invasion[47]. Statin-induced apoptosis could be reversed by GGPP, but not by FPP, in various colorectal cancer cell lines, indicating that statins act at least in part through the Rho proteins. This argument is supported by data from spontaneously immortalized rat intestinal epithelial cells, which show that lovastatin induces apoptosis by inhibiting geranylgeranylation of the Rho family proteins[48]. Inflammation is another important factor in the development of colorectal cancer[49], and statin anti-inflammatory effects might be another avenue for preventing colorectal cancer, particularly that associated with inflammatory-bowel-disease-associated colorectal cancer.

The study of statins in animals includes several different colorectal cancer mouse-model studies, which consistently found that statins reduced tumour incidence by 30–67%. In F344 rats, pravastatin significantly inhibited colon carcinogenesis induced by the direct-acting carcinogen *N*-methyl-*N*-nitrosourea[50]. Studies in a model of rat colon cancer induced by AZOXYMETHANE have produced several informative results[51]. Lovastatin reduced the initiation of ABERRANT CRYPT FOCI, and this effect was prevented by adding back GGPP but not FPP. The naturally occurring isoprenoids farnesol and lanosterol, which are feedback inhibitors of HMG-CoA reductase, can reduce aberrant crypt foci formation[52]. Squalene and PERILLYL ALCOHOL also have significant preventive activity. Mevastatin inhibited the spread of mouse colon cancer cells that were transplanted into naive mice, indicating that statins might also have an anti-metastatic effect[53]. Although statins have been shown to have preventive effects in these models, no reports of the effects of statins in the genetically predisposed mouse models of colorectal cancer, such as the $APC^{Min/+}$ mouse, have been published to date.

LIPID RAFTS
Cholesterol-rich areas of the cell membrane.

SEVERE COMBINED IMMUNODEFICIENT MOUSE (SCID mouse). Mice with this defect in their immune system do not have B cells or T cells. Therefore, they can accept tumour cells from another species without rejection.

C-REACTIVE PROTEIN (CRP). An inflammatory mediator produced by the liver in response to pro-inflammatory signals. Raised levels of CRP correlate with cardiovascular disease risk and are thought to indicate instability of inflamed atherosclerotic plaques.

AZOXYMETHANE
A potent carcinogen that is used to induce colon cancer in rats and mice. Treatment with azoxymethane activates the epidermal growth factor receptor and stimulates the synthesis of transforming growth factor-α.

ABERRANT CRYPT FOCI
A pre-cancerous change that represents early clonal precursors of colorectal neoplasia; presumed to precede microadenomas.

PERILLYL ALCOHOL
A naturally occurring isoprenoid.

Limited laboratory data support the hypothesis that statins in combination with NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs) will be beneficial in colorectal cancer prevention. The CYCLOOXYGENASE 2 INHIBITORS (COX2 inhibitors) celecoxib and lovastatin had dose-dependent synergistic activity in inducing apoptosis in human HT-29 colon cancer cells associated with induction of caspase 3 (REFS 54,55). Similar *in vitro* results of lovastatin plus sulindac occurred in two colon cancer cell lines. An azoxymethane rat model of chemically induced carcinogenesis showed that lovastatin was synergistic with sulindac in reducing the total number of aberrant crypt foci[51,54]. Recent work in this rat model showed that low-dose atorvastatin combined with low-dose celecoxib suppressed the development of invasive and non-invasive adenocarcinomas of the colon by 95%. The low-dose combination was more effective than a high dose of either agent alone[56]. This result is particularly relevant in light of the recent findings that celecoxib and other NSAIDs can increase the risk of CVD[57].

Breast cancer models. A number of statins (especially hydrophobic statins) inhibit the *in vitro* proliferation of breast cancer cells (including oestrogen receptor-negative cells and cells with activated Ras or ERBB2 pathways)[21,58,59]. RHOA-like proteins are overexpressed in breast cancer cells and their levels increase with histological grade and proliferation index in tumour samples[60]. Statins induce apoptosis in immortalized breast cancer cell lines and apparently do so through RHOA. Cerivastatin prevents prenylation of RHOA, causing the loss of RHOA from the cellular membrane in breast cancer cells. These effects correlated with the inhibition of downstream focal adhesion kinase, AKT and ROCK (Rho-associated, coiled-coil-containing protein kinase) pathways, inactivation of β-catenin activity, inhibition of NF-κB transcriptional activity, and increased expression of the CDKI p21 and G1/S arrest. All of these effects were reversible with GGPP but not FPP[61]. Furthermore, a pan inhibitor of RHOA, RHOB, and RHOC (C3 EXOENZYME)[62], or a dominant-negative RHOA, produced effects similar to those of cerivastatin[63].

There is strong evidence that statins influence the migration of breast cancer cells and that this effect can involve hydroxylated cholesterol (oxysterol), which is derived from the mevalonate pathway and is reported to modulate the concentration of both intracellular cholesterol and sphingomyelin in several cell types. Oxysterol derived from OSTEOBLASTS seems to induce migration of MCF-7 cells in soft agarose, but this effect is inhibited by the RHOA-associated kinase inhibitor Y-27632 and by the HMG-CoA reductase inhibitor mevinolin[64]. Stimulation of breast cancer progression by the hyaluronan receptor CD44 in a human metastatic breast cancer cell line required RHOA and involved downstream effects on the activity of phosphatidylinositol 3-kinase (PI3K) and the phosphorylation of AKT. These effects could be reversed by a dominant-negative form of RHOA-associated kinase[65].

Breast cancer and neuroblastoma cell line studies indicate that inhibiting the proteasome might be a HMG-CoA reductase-independent pathway of statin activity[21,66]. The closed-ring (prodrug) form of lovastatin and the proteasome inhibitor lactacystin inhibited proteasome activity in MDA-MB-157 breast cancer cells, which was associated with stabilization of p21 and p27 (REF. 21). These effects have been questioned in other model systems in which data indicate that some open-ring statins can inhibit proteasome activity and some closed-ring statins can stimulate it[67,68].

In vivo studies in mouse mammary tumour models demonstrated that lovastatin and simvastatin can decrease tumour formation and inhibit metastasis[69,70]. The plant isoprenoids β-ionone and geraniol inhibit rodent mammary tumour development. Simvastatin was recently shown to inhibit ERBB2-dependent breast cancer growth *in vivo* at clinically relevant doses[71].

Melanoma models. RHOA and RHOC are widely expressed in human melanoma and are implicated in the establishment of metastasis[72]. Inhibiting geranylgeranylation of the Rho family proteins is an

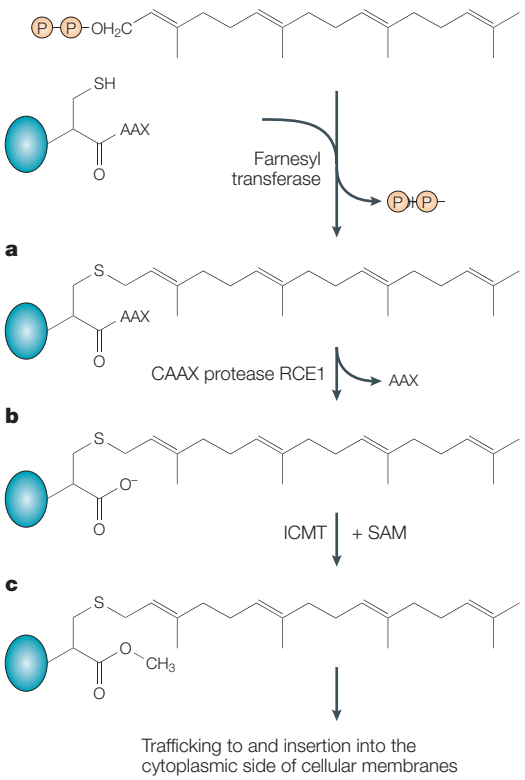


Figure 2 | **Prenylation pathway, as illustrated by the prenylation of the Rho family of small G-proteins.** **a** | Rho, which has the carboxy-terminal cysteine-aliphatic-aliphatic-X (CAAX) motif, is combined with FPP at the cysteine residue by farnesyl transferase. **b** | The CAAX protease, RAS-converting enzyme 1 (RCE1), then cleaves off the AAX tripeptide from the carboxy terminus of the protein at the endoplasmic reticulum membrane. **c** | The isoprenylcysteine carboxyl methyltransferase (ICMT) enzyme then adds a carboxy methyl group from S-adenosylmethionine (SAM) to the prenylated cysteine residue. Rho, with its newly-attached hydrophobic anchor, then moves to the appropriate cellular membrane, remaining in the cytoplasmic compartment.

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS
Include aspirin, ibuprofen, celecoxib, and many others. Associated with the decreased occurence of colon cancer and colon polyps.

CYCLOOXYGENASE 2 INHIBITORS
Drugs that specifically inhibit the cyclooxygenase 2 enzyme (for example, rofecoxib), which have been associated with decreased colon polyps cancer. A subset of NSAIDs.

C3 EXOENZYME
ADP-ribosyltransferase isolated from *Clostridium botulinum*. Substrates include the Rho proteins. ADP-ribosylation inactivates these signalling proteins.

OSTEOBLASTS
Cells originating in the bone marrow that generate new bone. These cells are stimulated by statins, which might explain the decreased risk of fracture in statin users.

TPRAS
A transgenic mouse model that contains a mutated human T24 *HRAS* gene driven by a 2.5 kb promoter region from the mouse tyrosinase gene that enables expression within melanocytes.

ISOPRENYLATION
Post-translational covalent addition of a farnesyl (15-mer) or a geranylgeranyl (20-mer) moiety to proteins that lack transmembrane domains, which allows them to localize to membranes and perform their usual function.

RELATIVE RISK
A measure of the comparative risk of developing a disease or condition. Statistically, relative risk is the chance that a person receiving an exposure (statins) will develop a condition (cancer) compared with the chance that a non-exposed person will develop the same condition.

HYPERCHOLESTEROLAEMIA
Increased cholesterol in the blood, associated with heart disease and stroke.

important mechanism by which statins induce *in vitro* apoptosis[73] and inhibit *in vivo* invasion and metastasis[74] of human melanoma cells. RHOC is important for the migration of melanoma cells, and atorvastatin can inhibit RHOC-augmented transcription. Inhibiting Rho proteins using C3 exoenzyme caused differentiation in B16 melanoma cells, indicating that inhibition of Rho is required for cAMP-induced differentiation. By contrast, a constitutively active Rho protein or an overexpressed Rho kinase prevented differentiation[75]. Inhibiting the downstream Rho-associated kinase (with a specific kinase inhibitor) had an anti-invasive effect in B16 melanoma cells and an anti-metastatic effect (superior to that of paclitaxel) in C57BL/6 mice injected with B16 cells. Lovastatin reduced membrane-associated Rho proteins and metastasis in the B16F10 mouse melanoma model[76,77].

Although statins have not yet been studied in animal models of melanoma prevention, work in a TPRAS mouse model indicates that inhibition of HMG-CoA reductase and ISOPRENYLATION might be important in preventing melanoma. Cutaneous melanoma can be induced in TPRAS transgenic mice by topical 7,12-dimethylbenz[a]anthracene, or by ultraviolet irradiation. Perillyl alcohol, which can inhibit the isoprenylation of small G-proteins and the activation of downstream targets such as MAPK and AKT in melanoma *in vitro*, delayed the appearance of tumours and produced a 25–35% reduction in 7,12-dimethylbenz[a]anthracene-induced melanoma in TPRAS mice[78].

Observational studies in humans

Three of the four most important prospective prescription or medical-record database studies of statins[79–83] (TABLE 1) showed significant statin-associated reductions (from 14–28%) in overall cancer incidence. A case–control study based on the large Dutch PHARMO database involved 3,129 cancer cases and 16,976 controls (Dutch residents without cancer), and compared the risk of incident cancer between people who were treated with statins and those who were treated with other cardiovascular medications. The cases were patients with an incident cancer, and the controls were matched on gender, year of birth, geographical region, duration of follow-up and index cancer date. Statin use was associated with a significant (20%) reduction in the overall risk of cancer (a RELATIVE RISK of 0.8; confidence interval (CI), 0.66–0.96), which was further reduced with larger cumulative doeses and a longer duration of use. PHARMO case–control analyses also showed non-significant reductions in the incidence of colorectal, skin and prostate cancer[80]. These PHARMO findings are consistent with those of two other important prospective observational studies, presented in TABLE 1.

Another case–control study used the large British General Practice Research Database (GPRD) and did not find that statin use was associated with a significant reduction in overall, breast, colon or skin cancer risks. However, the GPRD data did indicate a marginally increased risk for colorectal cancer associated with statin use for more than 60 months (REF. 81). A study of breast cancer in the GPRD found that HYPERCHOLESTEROLAEMIA and the use of the fibric acid derivative bezafibrate was associated with increased breast cancer risk, whereas statin use was associated with no increased risk[82]. Other GPRD nested case–control data indicate that statins are associated with a non-significant 2.5-fold increase in the risk of developing melanoma[81].

Nested case–control studies using the large Quebec Administrative Health Database found that statin use significantly reduced the overall incidence of cancer by 28% compared with using bile-acid-binding resins (also used to treat hypercholesterolaemia). Non-significant but cancer-specific findings of these case–control studies included reduced risks of developing colorectal, breast and skin cancer (melanoma and non-melanoma combined) associated with statin use[79].

There are several important observational studies of statins besides the large prospective database

Table 1 | **Important prospective observational studies of statins and the risk of developing cancer**

Study	Population	All cancers*	Breast cancer*	Colorectal cancer*	Melanoma*	Prostate cancer*	References
Blais *et al.*	Quebec Administrative Health Database: 542 cases; 5,420 controls	0.72 (0.57–0.92)‡	0.67 (0.33–1.8)	0.83 (0.37–1.89)	0.81 (0.47–1.39) (all skin cancer)	0.74 (0.36–1.51)	79
Graaf *et al.*	Dutch Database of 8 Cities: 3,129 cases; 16,976 controls	0.80 (0.66–0.96)‡	1.07 (0.65–1.74)	0.87 (0.48–1.57) (Colon) 0.48 (0.16–1.48) (Rectum)	0.63 (0.22–1.84) (all skin cancer)	0.37 (0.11–1.25)	80
Kaye & Jick	UK General Practice Research Database: 3,244 cases; 14,844 controls	1.0 (0.9–1.2)	0.9 (0.6–1.3)	1.0 (0.6–1.7)	2.5 (0.8–7.3)	1.3 (1.0–1.9)	81,82
Friis *et al.*	Population-Based Danish Cohort Study: 334,754 residents; 12,251 statin users	0.86 (0.78–0.95)‡	1.02 (0.76–1.36)	0.85 (0.65–1.11)	Not available	0.87 (0.61–1.23)	83

*Indicates that results are shown as odds ratio (95% confidence interval). ‡Indicates a statistically significant effect or difference.

studies outlined above and in TABLE 1. A recent large study (n = 34,438 men) of the effects of statins and other cholesterol-reducing drugs on prostate cancer risk was conducted within the ongoing prospective cohort of the Health Professionals Follow-up Study[84]. There was no overall reduction in prostate cancer risk, but a provocative analysis of the extent of the disease showed a significant (46%) reduction in advanced prostate cancer risk (compared with non-drug users), and the risk decreased with increasing duration of use ($p = 0.008$). The risk reduction was even stronger (66%) for metastatic and fatal disease. Although the study involved drugs other than statins, the strongest risk reductions occurred towards the end of study, when 90% of the participants who were using drugs to reduce cholesterol levels were taking statins for this purpose. This study evaluated different stages of disease and indicates that the mixed prostate results cited in TABLE 1 are owing to evaluations of non-stage-specific prostate cancer within the listed studies. A recent case–control study conducted in the Veterans Affairs system has shown that statin use is significantly inversely associated with overall prostate cancer risk and is strongly inversely associated with high-grade prostate cancer; these reductions increased with prolonged statin use[85]. Another Veterans Affairs case–control study also showed a significant, duration dependent, inverse association of statin use with prostate cancer risk[86]. These positive epidemiological results are supported by positive, albeit limited, preclinical studies of statins in prostate carcinogenesis[87].

The Molecular Epidemiology of Colorectal Cancer (MECC) study is a large population-based, case–control study in northern Israel involving 1,953 colorectal cancer cases (diagnosed between 1998 and 2004) and 2,015 population-based controls matched for age, gender, ethnicity and clinic. Using statins for at least 5 years was associated with a significant (47%) reduction in the risk for developing colorectal cancer after adjustments for multiple factors, including ethnicity, family history, NSAID use and hypercholesterolaemia[88]. This reduction was specifically associated with statins because MECC analyses of bezafibrate, which reduces cholesterol through effects on peroxisome proliferator-activated receptor-α and does not inhibit HMG-CoA reductase, showed no association with a reduction in the risk of developing colorectal cancer. The association of statins with reduced colorectal cancer remained significant after an adjustment for NSAID use, which was also associated with significantly reduced colorectal cancer and remained so after an adjustment for statin use. These data show a strong inverse association between colorectal cancer and long-term statin use, which is consistent with the pre-clinical data showing biological plausibility, the other observational data (described above) indicating a protective effect of statins, and evidence from a secondary analysis of a statin RCT, discussed below. On the other hand, the short-term exposure to statins was not associated with colorectal adenoma risk in a pooled analysis of secondary data from three adenoma chemoprevention trials[89].

Secondary data from an early RCT of pravastatin in prevention of CVD, the Cholesterol and Recurrent Events (CARE) trial (discussed below), indicated an increase in breast cancer associated with statin use. This led to several epidemiological studies of the association of statins with breast cancer risk. The weight of the evidence from the observational studies indicates that statins do not increase breast cancer risk (some studies indicate a protective statin effect), but the data are somewhat mixed. One large cohort study in Saskatchewan, Canada, was conducted from 1989 to 1997 and involved 13,592 statin users and 53,880 non-users[90]. Women using statins for ≥4 years had a significantly (74%) reduced risk of developing breast cancer. Breast cancer risk in all statin users was increased non-significantly by 9%, which was driven by a non-significant (15%) increased risk in post-menopausal women. A recent multicentre United States cohort study involving 7,528 Caucasian women[91] (adjusted for age and body weight) found that statin users had a significantly (72%) reduced risk of developing breast cancer (compared with non-users). Women who used other lipid-lowering drugs also had a significantly reduced risk of developing breast cancer compared with non-users. A hospital-based study of more than 1,000 breast cancer cases and matched clinic controls[92] found a 1.2-fold (95% CI 0.7–2.0) increased risk of developing invasive breast cancer in statin users. A recent case–control study of statins and breast cancer risk was conducted in post-menopausal women (975 cases and 1,007 controls) from three counties of the state of Washington. There was no increased overall breast cancer risk for statin users and a beneficial, non-significant trend was detected for long-term (>5 years) statin users[93].

Other studies of statins and specific cancers include a pilot study using data from computerized pharmacy and diagnosis databases of the Veterans Affairs medical system. Statin use was less in 328 melanoma cases than in 2,000 controls (18% versus 30%), giving statin users a significant (approximately 50%) reduction in the risk of melanoma[94]. Other recent case–control studies have reported that statin use is associated with a reduction in the risk of developing lung, pancreatic and oesophageal cancer and non-Hodgkin lymphoma[95–98]. The study by Friis *et al.* (TABLE 1) also found a slight, statistically non-significant (16%) increase in the risk of developing liver cancer[83] that needs to be considered further in the context of a carcinogenic effect and hepatoxicity in animal studies[2]. A large European multicentre case–control study of lymphoma, Epilymph, recently reported that statin use was associated with a 40% reduction in the risk of developing B-cell or T-cell lymphoma. This study included 2,362 cases and 2,469 controls from both hospital-based and population-based study centres, and reported an ODDS RATIO of 0.6 (95% CI 0.4–0.6) after adjustment for age, sex, country, use of aspirin and other NSAIDs, smoking and educational background[99].

ODDS RATIO
The odds ratio is a way of comparing whether the probability of a certain event is the same for two groups, and is calculated using a 2×2 table. An odds ratio of one implies that an event is equally likely in both groups. An odds ratio greater than one implies that an event is more likely in the first group. An odds ratio less than one implies that the event is less likely in the first group.

META-ANALYSIS
A statistical practice of combining the results of a number of studies to overcome the problem of reduced statistical power in studies with small sample sizes; analysing the results from a group of studies can allow a more accurate estimation of effects.

The interpretation of observational studies, whether indicating beneficial, neutral or harmful effects, must be tempered by considering the multiplicity of biases to which these studies are prone. Without random allocation of treatment, observational studies are vulnerable to potential imbalances between characteristics of statin users and those of non-users based on physician selection in prescribing statins and differences between patients who elect to take statins and those who do not. It is also possible that a higher proportion of statin users (than non-users) follow preventive health practices — for example, cholesterol screening, which led to their use of statins, and cancer screening, which would lead to higher cancer detection rates.

The overall data from observational studies indicate that statins are associated with a reduced risk of developing cancer. The primary endpoint of each of the four studies listed in TABLE 1 was the diagnosis of any malignancy, with specific cancers studied as secondary endpoints (selectively illustrated in the table). These studies are too small to evaluate statins and the risks of developing individual cancers, but the primary endpoint from three of these four studies indicates a protective effect of statins for all malignancy. Cancer-specific studies, such as the MECC and Epilymph, will be required to assess the preventive potential of statins for site-specific cancers. In addition, the observational studies that have been published to date will benefit from a formal META-ANALYSIS to account for the limitations of testing multiple comparisons of secondary endpoints in pharmacological databases, and to carefully consider the specific statins that were used. For example, the studies by Graaf *et al.*[80] and Friis *et al.*[83] are heavily weighted by the use of simvastatin, which accounted for most prescriptions in both studies, and other statins might not have equal preventive potential[100].

Randomized controlled trials

Several large RCTs of single-agent statins for the prevention of CVD included secondary endpoints of overall cancer incidence and mortality and the incidence of certain specific cancers[101–107] (TABLE 2). None of these RCTs found a significant difference in overall cancer incidence or mortality between the statin and placebo groups, but there were significant findings with respect

Table 2 | **The effects of statins on cancer (secondary analysis) in randomized clinical trials**

Study and statin (reference)	Cohort	Cancer mortality*	Mean duration (years)	Dose (mg day^{-1})	Age range (years)	% Who were women	Total cancer incidence*	Breast cancer incidence*	Colorectal cancer incidence*	Melanoma incidence*
4S; simvastatin (101)	4,444 with CHD. Treated: 2,221; placebo: 2,223	85; 100	5.2	20–40	35–70	19	227; 248	7; 5	25; 32	9; 7
WOSCOPS; pravastatin (102)	6,595 men with increased cholesterol. Treated: 3,302; placebo: 3,293	44; 49	4.8	40	45–64	0	116; 106	NA	31; 30	NA
CARE; pravastatin (103)	4,159 post-MI. Treated: 2,081; placebo: 2,078	49; 45	4.8	40	21–75	14	172; 161	12; 1‡	12; 21	4; 3
LIPID; pravastatin (104)	9,014 with CHD. Treated: 4,512; placebo: 4,502	128; 141	5.6	40	31–75	17	379; 399	NA	Odds ratio = 0.89 (confidence interval 0.63–1.24)	NA
AFCAPS; lovastatin (105)	6,605 with normal cholesterol. Treated: 3,304; placebo: 3,301	NA	5.3	20–40	45–73	15	252; 259	13; 9	25; 20	14; 27‡
HPS; simvastatin (106)	20,536 with CHD, PVD or DM. Treated: 10,269; placebo: 10,267	3.5%; 3.4%	5.0	40	40–80	25	7.9%; 7.8%	38; 51	NA	NA
PROSPER; pravastatin (107)	5,804 elderly at high risk of CVD. Treated: 2,891; placebo: 2,913	4.0%; 3.1%	3.2	40	70–82	52	8.4%; 6.8%	18; 11	NA	NA

*Indicates that results are shown as occurrence in treated group; occurrence in placebo group. ‡Indicates a statistically significant effect or difference. 4S, Scandinavian Simvastatin Survival Study; AFCAPS, Air Force Coronary Atherosclerosis Prevention Study; CARE, Cholesterol and Recurrent Events; CHD, coronary heart disease; CVD, cardiovascular disease; DM, diabetes mellitus; HPS, Heart Protection Study; LIPID, Long-Term Intervention with Pravastatin in Ischaemic Disease; MI, myocardial infarction; NA, not available; PVD, peripheral vascular disease; WOSCOPS, West of Scotland Coronary Prevention Study.

to the incidences of melanoma and breast cancer. The Air Force Coronary Atherosclerosis Prevention Study (AFCAPS) of lovastatin versus placebo in 6,605 men and women with normal cholesterol levels found a significant secondary reduction in melanoma incidence in statin users (14 melanomas) versus placebo users (27 melanomas) ($p = 0.04$)[105]. The CARE trial of pravastatin (to prevent CVD) in 4,159 patients with average cholesterol levels provided the alarming secondary result of an increase in breast cancers among post-menopausal women who received 4 or more years of pravastatin (12 cancers) versus placebo (1 cancer) ($p = 0.002$)[103].

The CARE investigators suggested that their breast cancer finding might be a statistical anomaly as the breast cancer rate in the placebo arm was much less than would be expected in this population[4]. Furthermore, 3 of the 12 cancers in the statin arm were recurrences, 1 was non-invasive, and 1 occurred within 6 weeks of starting statin therapy. Other RCTs found no significant secondary increases in breast cancer associated with statins. For example, the large-scale Heart Protection Study[106] showed a non-significantly reduced risk of breast cancer in its statin arm (TABLE 2). Other findings of CARE included a reduced incidence of colorectal cancer in the statin (12 occurrences) versus placebo arm (21 occurrences) ($p = 0.11$) and no statin-associated reduction in melanoma (versus placebo), which resulted from the only direct assessment of melanoma in a CVD RCT besides that of AFCAPS.

The CVD-oriented designs of these RCTs substantially limited their ability to assess cancer outcomes. They had relatively small sample sizes and short follow-up periods, which resulted in very small numbers of overall or specific cancer cases. These RCTs also had short durations of statin treatment (TABLE 2), which severely limited the ability of even the RCT meta-analyses (with larger numbers of cancer cases) to detect cancer preventive effects or risk reductions, which can take years to materialize.

Nevertheless, summary analyses of the RCT data provide reassuring evidence that statins are not associated with an overall increased risk of developing cancer[106-110] and support observational evidence that statins significantly reduce all-cause mortality[111]. No RCT of statins has had cancer as the primary endpoint, and the analysis of secondary endpoints in CVD prevention trials is vulnerable to sampling variation. Therefore, the statistically significant findings of differences in individual cancers (for example, the increase in breast cancer and decrease in melanoma shown in TABLE 2) need to be interpreted cautiously. Meta-analysis provides a systematic, explicit approach to measuring the effects of statins on cancer, but even this approach has limited sensitivity for detecting preventive or carcinogenic potential at specific cancer sites. Therefore, a consensus of evidence regarding statins and cancer will probably continue to rely on a combination of observational and interventional studies with longer follow-up.

Future directions

The pleiotropic effects of statins are related to their interactions with diverse signalling pathways and targets[5–7,10,112–117]. These signalling pathways involve crucial tumorigenic processes (such as inflammation), which interact in complex ways and can be dependent or independent of HMG-CoA reductase. Preclinical research studies on statins are identifying new non-selective statin targets, such as the Rho proteins and LFA1, that provide a basis for the development of new targeted anticancer drugs.

Statins can inhibit the function of the Rho proteins by inhibiting geranylgeranylation. The Rho isoforms can now be targeted selectively with RNA interference (RNAi) therapies. Preclinical (*in vitro* and *in vivo*) breast cancer models show that targeting RHOA and RHOC isoforms can be more effective than targeting geranylgeranyl transferase or using statins, possibly because RNAi does not inhibit RHOB, which is implicated in tumour suppression[11,63,118]. Rho proteins might be just the tip of a geranylgeranylated protein iceberg in the HMG-CoA pathway (FIG. 1) that have crucial functions in cancer and other human diseases[116]. For example, statin research in virology has discovered a new geranylgeranylated protein involved in hepatitis C that could become a target for preventing hepatitis C-related liver cancer[119]. Related studies have identified post-prenylation enzymes (FIG. 2) as novel targets in neoplasia (for example, colorectal cancer and melanoma)[120,121]. Studies of the crystal structure of the LFA1 I-domain and lovastatin have allowed selective inhibitors of LFA1 (LFA703, LFA878) to be derived from lovastatin. These derivatives inhibit LFA1 more selectively and potently than lovastatin does, and do not affect HMG-CoA reductase[122].

The favourable interactions between statins and NSAIDs are another promising future direction in, for example, colorectal cancer prevention. NSAIDs are associated with a 30–50% reduction in colorectal cancer risk and mortality in many epidemiological studies, and statins were associated with an almost 50% colorectal cancer risk reduction in the MECC study[88]. NSAIDs also have a clinical record of suppressing sporadic and familial adenomatous colorectal polyps. Preclinical *in vitro* and *in vivo* data from colorectal cancer models indicate the synergistic activity of various combinations of NSAIDs and statins[51,54–56]. For example, atorvastatin plus celecoxib was more effective at low doses than a high dose of either agent alone. The potential importance of these combined agent data is emphasized by recent data showing that celecoxib and other NSAIDs are associated with an increased risk of CVD mortality[57,123]. The combination could allow reduced NSAID doses while countering the detrimental effects of NSAIDs with beneficial effects of statins on CVD risk. Similar results involving EGFR (epidermal growth factor receptor) show that statins inhibit EGF-induced RHOA translocation and cancer-cell invasion, inhibit EGFR autophosphorylation and have synergistic anticancer activity with EGFR inhibitors[12,124].

Future large-scale (phase III) trials of statins in cancer prevention might be difficult to conduct, as

statin use for preventing CVD is highly recommended and growing in people (generally older) who will have an increased cancer risk[5,125]. Therefore, rigorous analyses within large prospective cohort studies, such as the recent analysis of statins and prostate cancer risk within the Heath Professionals Follow-up Study[84], might be the best future avenue for understanding the full impact of statins on public health. Relatively small phase I and II statin trials will also be important for providing biological plausibility (for example, identifying effects on intraepithelial neoplasia or molecular biomarkers) for observational results. Statin research, however, is identifying many potential selective-targeting approaches with novel agents that might enter future large-scale clinical testing.

Concluding remarks

Statins have an established record of human safety and efficacy in CVD prevention and show promise for cancer prevention in observational, preclinical and certain aspects of randomized controlled studies. The preclinical study of statin effects in HMG-CoA reductase-dependent and HMG-CoA reductase-independent pathways is helping develop selective-targeting approaches for preventing cancer and several other ageing-related diseases (for example, neurodegenerative disorders). This pleiotropic aspect of statins indicates the broad impact that these agents are having on public health[125–127], which should be defined by ongoing and future well-designed observational studies of cancer (and other diseases) within large prospective cohorts.

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Competing interests statement

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