



ANTICANCER AGENTS

Effects of statins and farnesyltransferase inhibitors on the development and progression of cancer

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KEYWORDS

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Summary Statins (3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors – HMG-CoA reductase inhibitors) have been approved for the treatment of lipid disorders. Recently, *in vivo* studies with experimental animals and *in vitro* studies indicated a possible role for statins in the treatment of malignancies. Inhibition of the enzyme HMG-CoA reductase results in decreased farnesylation and geranylgeranylation of several proteins essential for cellular proliferation and survival. Inhibition of Ras farnesylation was originally thought to be the mechanism that mediates statin-induced effects in cancer. Consequently, specific inhibitors of the enzyme farnesyltransferase (FTIs) were developed. Currently, the mechanisms that mediate statin- and FTI-induced antitumour effects are questioned. It remains unclear which proteins and signal transduction cascades are involved. This review focuses on the effects and possible therapeutic application of statins and FTIs. Antitumour properties such as induction of growth arrest and apoptosis, inhibition of metastasis and inhibition of angiogenesis are discussed. Furthermore, the mechanisms of statin- and farnesyltransferase inhibitor-induced effects and the involvement of a number of cellular components (such as farnesylated and geranylgeranylated proteins, the mitogen-activated protein kinase signalling pathway, the phosphoinositide 3'-kinase signalling pathway, and cell cycle regulatory proteins) are reviewed. In addition,

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clinical and epidemiological data with respect to statins and farnesyltransferase inhibitors are summarised. We propose that inhibitors of the mevalonate pathway are particularly effective when administered in combination with other drugs. Therefore, the mechanisms and effects of combined therapy of statins or farnesyltransferase inhibitors with chemotherapeutics, biphosphonates, non-steroidal anti-inflammatory drugs, specific inhibitors of geranylgeranyltransferase and inhibitors of tyrosine kinase activity are discussed.

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Non-standard abbreviations

| | | | |
|-------------------|------------------------------------------------|---------|-----------------------------------------------|
| AML | acute myeloid leukaemia | IGF-1R | insulin-like growth factor-1 receptor |
| bFGF | basic fibroblast growth factor | IPP | isopentenylpyrophosphate |
| Erk | extracellular signal-regulated kinase | MAPK | mitogen-activated protein kinase |
| Cdk | cyclin-dependent kinase | MMP | matrix metalloproteinase |
| COX-2 | cyclo-oxygenase-2 | Myr-Ras | myristilated Ras |
| EGF | epidermal growth factor | NOS | nitric oxide synthase |
| FTI | farnesyltransferase inhibitor | u-PA | urokinase-type plasminogen activator |
| FOH | farnesol | u-PAR | urokinase-type plasminogen activator receptor |
| FPP | farnesylpyrophosphate | PDGF | platelet-derived growth factor |
| GGOH | geranylgeraniol | PI3K | phosphoinositide 3'-kinase |
| GGPP | geranylgeranylpyrophosphate | PKB | protein kinase B |
| HMG-CoA reductase | 3-hydroxy-3-methylglutarylcoenzyme A reductase | Rb | retinoblastoma gene product |

Introduction

3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (HMG-CoA reductase inhibitors or "statins") are efficient and widely used drugs in the treatment of lipid disorders, especially hypercholesterolemia. The statin family comprises of lovastatin, simvastatin, atorvastatin, fluvastatin, and pravastatin. Cerivastatin has been withdrawn from the US market in 2001. Although statins are approved only for the treatment of lipid disorders, there is increasing evidence that they may be useful in the treatment of other conditions, such as Alzheimer's disease¹⁻³ and osteoporosis.⁴ Additionally, the potential of statins in the treatment of cancer has been investigated extensively in the last decade.

The mechanism of action of this class of drugs is considered to be inhibition of HMG-CoA reductase, which is an enzyme upstream in the mevalonate biosynthetic pathway (Fig. 1). HMG-CoA reductase catalyses the conversion of HMG-CoA into mevalonate.⁵ An important intermediate of the mevalonate pathway is farnesylpyrophosphate (FPP; C₁₅), an unsaturated carbon chain. Mevalonate can be converted into FPP in a number of steps (Fig. 2). FPP is

a precursor of several products of the mevalonate pathway, such as cholesterol, heme A, dolichols, and ubiquinones. In addition, geranylgeranylpyrophosphate (GGPP; C₂₀) can be synthesised from FPP. Both FPP and GGPP are essential for the activation of a variety of intracellular proteins. In this activation step, the farnesyl or geranylgeranylmoieties are coupled to the protein, resulting in a farnesylated or geranylgeranylated protein. These reactions are catalysed by farnesyltransferase and geranylgeranyltransferase, respectively. This type of protein activation is referred to as (iso)prenylation.

Several proteins involved in signalling are dependent on prenylation for their activity, such as Ras, nuclear lamins, transducin γ , rhodopsin kinase, Rho, and all of the remaining heterotrimeric G proteins and small G proteins.^{6,7} The farnesylated Ras protein has been most extensively studied. It has an important function in cell growth and differentiation.⁵ By inhibition of HMG-CoA reductase, statins can block the mevalonate pathway and consequently, Ras activation. Since mutated Ras has been detected in approximately 30% of human tumours, aberrant Ras function is thought to play a role in carcinogenesis.⁸ Therefore, the therapeutic potentials of statins in the treatment of cancer

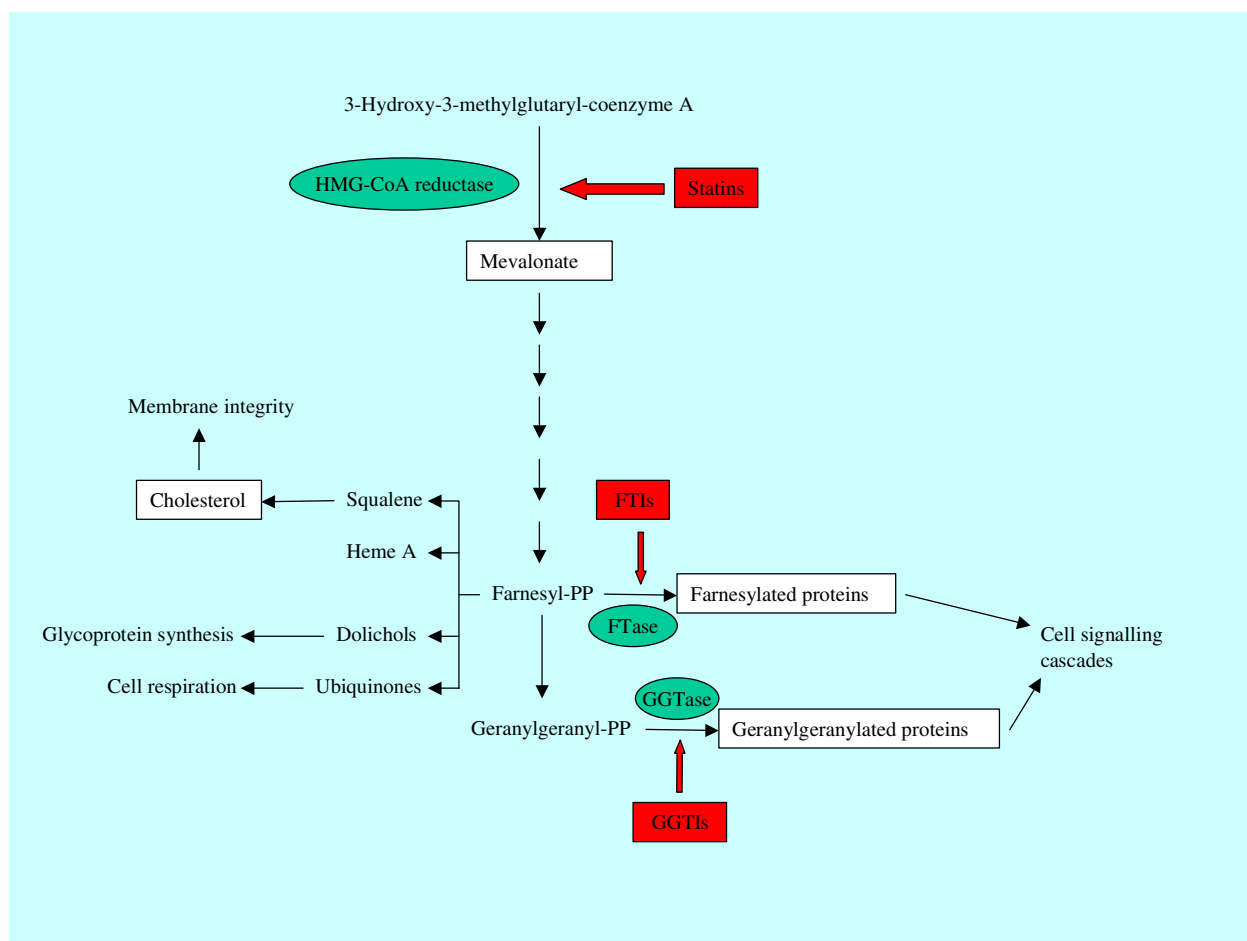


Figure 1 Overview of the mevalonate pathway and targets for inhibition by statins, farnesyltransferase inhibitors, and geranylgeranyltransferase inhibitors.

has been studied extensively in the last decade. Furthermore, the antitumour properties of specific inhibitors of farnesyltransferase (FTIs) and geranylgeranyltransferase (GGTIs) have been investigated. Most research has been focussed on statins and FTIs rather than GGTIs. Although inhibition of Ras farnesylation was originally considered to be the mechanism responsible for possible antitumour properties of statins and FTIs, there is increasing evidence that other mechanisms are involved as well.

This review focuses on the potential therapeutic application of statins in cancer. Antitumour properties of statins and the mechanisms involved, such as induction of growth arrest and apoptosis, and inhibition of metastasis and angiogenesis, are discussed and an overview of clinical and epidemiological data on the incidence of cancer in users of statins are presented. Additionally, therapeutic effects of FTIs in cancer are reviewed and combinational therapeutic use of statins and FTIs with other agents is discussed.

Antitumour properties of statins

Growth arrest and apoptosis

In vitro studies on various cell lines have shown that statins have growth inhibitory potentials, either by induction of G1-arrest,^{9–17} G2/M-arrest^{12,18} or cell death.^{11,15,17,19–30} Since chromatin condensation and DNA laddering have been observed, statin-induced cell death is considered to occur via apoptosis.^{15,22–24,26,27,30,31} These phenomena are both time- and dose-dependent.^{9,25,32,33} In an ex vivo experiment, serum of fluvastatin-treated patients was added to human vascular smooth muscle cells. An antiproliferative effect was detected after the addition of 1.5% serum, whereas a proapoptotic effect was only observed at higher serum concentrations.³⁰ Additionally, cerivastatin induced G1-arrest in breast cancer cells, but signs of apoptosis were not observed.³⁴ These data suggest that

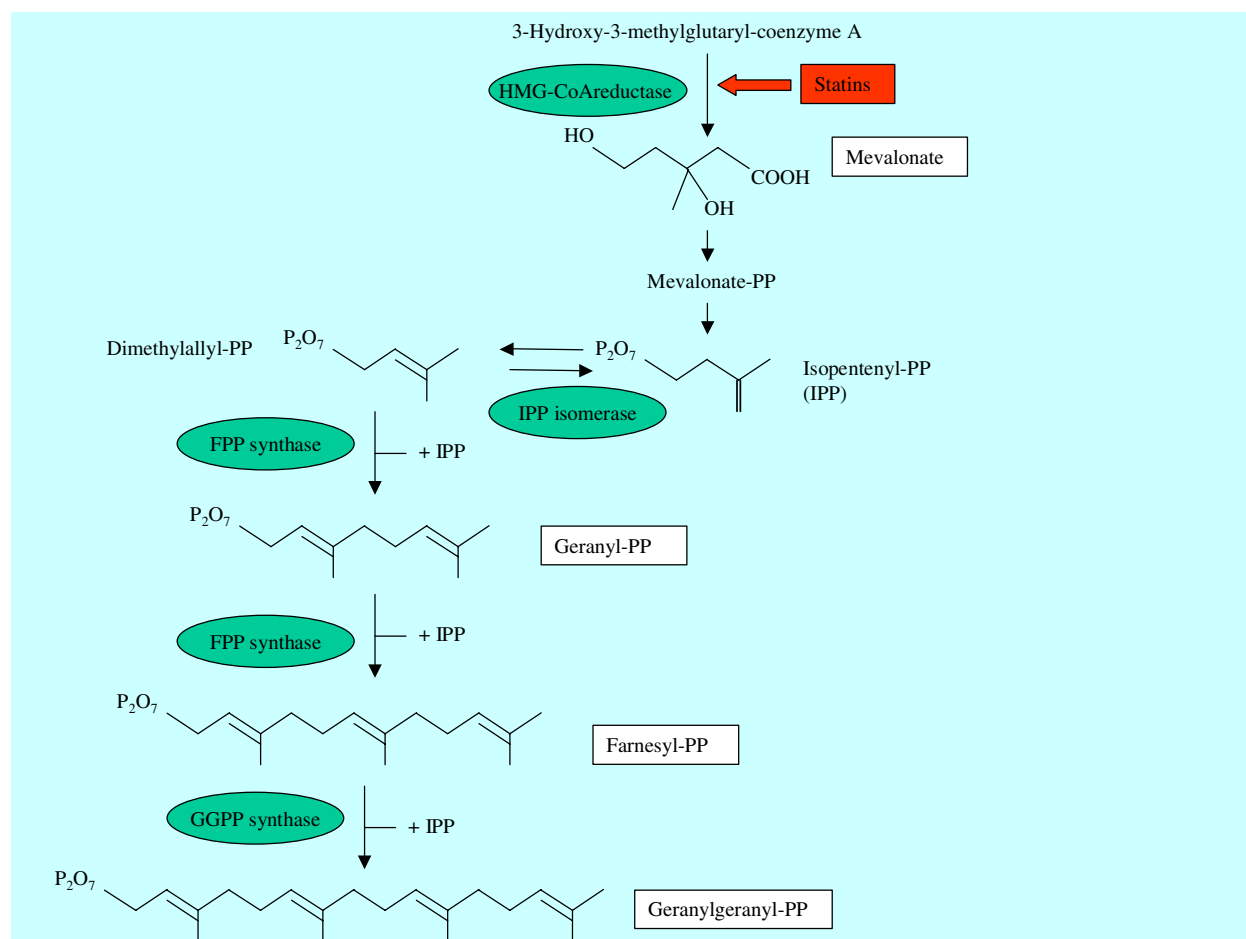


Figure 2 The biosynthetic pathway of farnesylpyrophosphate (farnesyl-PP) and geranylgeranylpyrophosphate (geranylgeranyl-PP) synthesis from 3-hydroxy-3-methylglutaryl-coenzyme A.

statin-induced effects on proliferation and apoptosis are either independent³⁰ or dependent on the statin concentration.

Growth arrest and apoptosis occur *in vitro* at lovastatin concentrations ranging from 0.1 to 100 μM depending on the cell line applied. A phase I trial revealed that administration of lovastatin in doses from 2 to 25 mg/kg daily results in drug plasma concentrations ranging between 0.1 and 3.9 μM .³⁵ These findings indicate that at least for some cell lines, lovastatin-induced antiproliferative and proapoptotic effects are within the therapeutically achievable dose ranges of lovastatin. However, lovastatin monotherapy may not be sufficient to inhibit proliferation and induce apoptosis in cell lines that require lovastatin concentrations higher than 3.9 μM .

Wong et al.³⁶ showed that different statins were not equipotent in inducing apoptosis. In acute myeloid leukemic (AML) cell lines, cerivastatin was at least 10 times more potent than other statins in the induction of apoptosis. In addition, leukemic

cells from patients with AML that were weakly responsive to lovastatin, were significantly more responsive to equimolar concentrations of cerivastatin. It was shown previously that statins can be divided into three groups with respect to their antiproliferative properties. The inhibitory potency of simvastatin, lovastatin, fluvastatin and atorvastatin is in the same order of magnitude, whereas pravastatin is significantly less potent, and cerivastatin is more potent.³⁷

In vivo experiments in laboratory animals confirmed statin-induced inhibition of tumour growth. Maltese et al.³⁸ have shown that treatment with lovastatin results in inhibition of tumour growth in mice. They observed that in untreated animals, HMG-CoA reductase activity correlates with neuroblastoma tumour growth. Furthermore, lovastatin inhibited growth of EJ3-derived tumours in mice³⁹ and suppressed development of colorectal tumours in rats.⁴⁰ Administration of pravastatin inhibited the incidence of chemically-induced colon tumours in rats⁴¹ and mice.⁴² In rats, local application of

simvastatin to gliomas effectively inhibited tumour growth.⁴³ These *in vitro* and *in vivo* data indicate that statins may inhibit primary tumour growth by induction of growth arrest and apoptosis.

Metastasis

During the metastatic process, cells detach from the primary tumour, enter and exit the circulation, and start growing in a secondary site. Protease activity, invasion of the basement membrane, cell migration, angiogenesis and attachment to and detachment from the extracellular matrix are considered to be essential processes in metastasis.⁴⁴ Recently, statins were shown to have anti-metastatic properties in *in vitro* experiments. Reduced gene expression of matrix metalloproteinase-9 (MMP-9) was detected after lovastatin treatment of NIH-3T3 cells, resulting in decreased MMP-9 activity.⁴⁵ Furthermore, cerivastatin suppressed expression of urokinase-type plasminogen activator (u-PA), u-PA receptor (u-PAR), and MMP-9 in monocytes.⁴⁶ In addition, reduced monocyte MMP-9 secretion was observed after simvastatin treatment as well.⁴⁷ However, MMP-2 and MMP-9 activity and uPA secretion were not reduced after lovastatin treatment of F3II mammary carcinoma cells.⁴⁸

Furthermore, lovastatin reduced invasiveness of lymphoma cells,⁴⁹ human glioma cells,⁵⁰ melanoma cells⁵¹ and NIH-3T3 cells⁴⁵ in matrigel. Fluvastatin blocked invasiveness of pancreatic cancer cells, colon cancer cells and breast cancer cells.⁵² Invasiveness of a highly metastatic breast cancer cell line was reduced by cerivastatin.³⁴ Moreover, migration of a F3II mammary carcinoma cell line⁴⁸ and a melanoma cell line⁵¹ was inhibited by lovastatin. Similarly, simvastatin and cerivastatin reduced migration of human monocytic THP-1 cells⁴⁷ and endothelial cells,⁵³ respectively. In addition, lovastatin treatment resulted in reduced ability of lymphoma cells,⁴⁹ F3II mammary carcinoma cells⁴⁸ and melanoma cells⁵¹ to attach to an extracellular matrix.

The results of these *in vitro* experiments suggest that statins may reduce metastatic processes. This was confirmed in *in vivo* experiments. Lovastatin treatment resulted in reduction of metastatic dissemination to the lungs in mice with established tumours of F3II mammary carcinoma cells.⁴⁸ Moreover, colon-derived MCA-26 cancer cells induced less liver metastasis in lovastatin-treated mice, as compared with saline-treated mice.⁵¹ Although primary tumour growth was not affected in lymphoma-bearing rats, the number

of lymph node metastases was decreased significantly by lovastatin treatment.⁴⁹ Similarly, lovastatin and simvastatin treatment decreased experimental lung metastasis in laboratory animals inoculated with B16F10 melanoma cells and BN472 mammary adenocarcinoma cells, respectively.^{51,54} Again, a significant effect on primary tumour growth was not observed.⁵¹ Fluvastatin treatment of mice injected with pancreatic tumour cells resulted in a marked reduction in the number and size of metastatic nodules in the liver.⁵⁵ These *in vitro* and *in vivo* data suggest that statins may reduce metastatic processes in patients with cancer.

Angiogenesis

Neovascularisation is thought to be important for both primary tumour growth and metastasis.⁴⁴ In a search for mechanisms that may explain the non-lipid-related anti-atherosclerotic properties of statins, *in vitro*^{56–59} and *ex vivo*⁶⁰ experiments have shown that in smooth muscle vascular cells and vascular endothelial cells, statins promote apoptosis, whereas an inhibitory effect on cell migration and proliferation was observed.^{37,53,57,58,60–62} Since endothelial cell migration and proliferation are crucial steps in angiogenesis, the effects of statins on angiogenesis have been investigated. Statins were reported to have antiangiogenic effects as well as proangiogenic effects. Cerivastatin and simvastatin were demonstrated to inhibit capillary tube formation in a human microvascular endothelial cell line^{53,63} and *in vivo* angiogenesis was shown to be inhibited in matrigel⁶⁴ and chick chorioallantoic membrane models.^{63,64} In mice injected with Ras-3T3 cancer cells, lovastatin potentiated the inhibitory effect of TNF- α on tumour growth and vascularisation.⁶⁵ In an *in vivo* Lewis lung cancer model, cerivastatin decreased tumour growth and vascularisation by 59% and 51%, respectively.⁶⁶

However, Kureishi et al.⁶⁷ found an increase in revascularisation induced by simvastatin treatment in ischemic limbs of normocholesterolemic rabbits. A biphasic effect of cerivastatin and atorvastatin on angiogenesis was demonstrated in human dermal microvascular endothelial cells⁶⁶ and human umbilical vein endothelial cells.⁶⁸ Relatively high doses of these statins inhibit angiogenesis, whereas low doses of these statins had opposite effects and increase angiogenesis.⁶⁶ Based on these findings, long-term simvastatin treatment was suggested to promote vascularisation of developing tumours.^{69,70}

Park et al.⁶³ suggest that differences in time course, cell type and the current confusion on the difference between angiogenesis and arteriogenesis may explain different findings on the effects of statins on angiogenesis. Statin-induced effects on angiogenesis were linked with increased activity of endothelial nitric oxide synthase (eNOS).^{67,71} The scaffolding protein caveolin-1, a negative regulator of eNOS, was shown to be down-regulated by statin therapy.⁷² The effect of statins on eNOS/caveolin interactions depends on the type of endothelial cells. It was shown that statin treatment resulted in NO-mediated proangiogenic effects only in cells with low caveolin expression.⁷² Differences in caveolin expression may explain in part why the effect of statins is not similar in different cell lines.

These preclinical data indicate that statins induce growth arrest and apoptosis, inhibit secretion of proteolytic enzymes, reduce invasiveness and inhibit angiogenesis. These effects contribute to reduction of tumour growth and metastasis in preclinical models, suggesting that statins may be useful in anticancer therapy.

Mechanisms of statin-induced cytostasis and cytotoxicity

Cholesterol- and dolichol-mediated effects

The mechanisms of statin-induced effects on cell proliferation and induction of apoptosis are not yet fully understood. Several products of the mevalonate pathway, such as cholesterol, dolichol, ubiquinone, and isoprenylated proteins, have been evaluated as possible key compounds in the mechanism of statin-induced cytostasis. Although inhibition of protein prenylation has been generally accepted as an important mechanism for statin-induced effects, other intermediates of the mevalonate pathway have been suggested as critical compounds in statin effects as well. Siperstein and Fagan^{73,74} observed a lack of negative feedback control in cholesterol synthesis in hepatomas and proposed that there was a relation between loss of cholesterol synthesis control and carcinogenesis. Several observations support this hypothesis. Impairment or complete loss of the cholesterol negative feedback has been observed in various types of cancer cells.⁷⁵ Furthermore, cancer cells seem to require increased levels of cholesterol and cholesterol precursors.⁷⁶ In laboratory animals, downregulation of the levels of

cholesterol by diet or drugs resulted in decreased tumour growth⁷⁷ and survival was significantly better in animals with low cholesterol plasma levels.⁷⁸

Recently, it was shown that statin-induced neuronal cell death could be prevented by treatment with cholesterol, indicating that the viability of these neuronal cells is dependent on cholesterol levels rather than non-sterol isoprenoids.⁷⁹ However, cholesterol was not effective in prevention of statin-induced growth arrest or cell death in various other cell lines.^{23,32,50,80} Non-sterol isoprenoids may not be crucial for survival of neuronal cells because in contrast with most cells, neuronal cells do not divide. The lack of DNA synthesis might provide an explanation for the discrepancy between neuronal cells and most other cell types.⁷⁹ Furthermore, neuronal cells require higher amounts of cholesterol for membrane synthesis as compared to other cell types. The higher cholesterol requirement may provide an alternative explanation for decreased viability of neuronal cells caused by statin-induced cholesterol depletion.⁷⁹

These data indicate that downregulation of cholesterol levels may contribute to inhibition of tumour growth, but cholesterol synthesis is not a key target of statins in relation to cell proliferation and/or cell death.

Another possible mechanism of statin-induced growth inhibition and apoptosis may be inhibition of dolichol synthesis.⁸¹ Dolichyl phosphate has a role in *N*-linked glycosylation of membrane proteins. It was shown that breast cancer cells were blocked in G1 by inhibition of HMG-CoA reductase and by specific inhibition of *N*-linked glycosylation by tunicamycin.⁸² Furthermore, tunicamycin decreased survival of Ewing's sarcoma cells.⁸³ Moreover, *N*-linked glycosylation was shown to be downregulated in lovastatin-treated cells. Particularly, *N*-linked glycosylation of insulin-like growth factor-1 receptor (IGF-1R) was decreased in association with decreased DNA synthesis.^{84,85} Although DNA synthesis was restored by addition of mevalonate, administration of both mevalonate and tunicamycin B prevented initiation of DNA synthesis.⁸⁴ Addition of exogenous dolichyl phosphate upregulated IGF-1R expression in correlation with induction of DNA synthesis.⁸⁵ These data suggest that inhibition of *N*-linked glycosylation contributes to statin-induced effects. However, dolichyl phosphate was found to be unable to reverse statin-induced effects on cell growth in other studies³² and therefore, this mechanism is probably not a critical step in statin-induced growth inhibition.

Role of isoprenylated proteins

Several *in vitro* studies have shown that simultaneous addition of a statin and mevalonate prevented statin-induced apoptosis and G1-arrest.^{9,17,22–27,32,33,80,86–88} Furthermore, mevalonate is able to rescue statin-pretreated cells from apoptosis and G1 arrest when administered within a critical time window.^{17,33} These results indicate that mevalonate or mevalonate derivatives are key targets for statin-induced effects on cell proliferation.

In order to further explore biochemical mechanisms of growth inhibition, FPP and GGPP have been added to statin-treated cells. The structure and biosynthetic pathway of FPP and GGPP are shown in Fig. 2. GGPP is able to prevent statin-induced apoptosis, whereas FPP has no or only partially preventive effects.^{32,89–92} Furthermore, addition of farnesol (FOH) or FPP to statin-treated cells did not overcome growth inhibition,^{9–11,34,93} morphological changes⁵² or invasiveness.⁵⁵ In contrast, the addition of geranylgeraniol (GGOH) or GGPP induced exponential growth,^{9,10,34,93} prevented morphological changes⁵² and restored invasiveness.⁵⁵ FOH and GGOH are precursors of FPP and GGPP, respectively. These data suggest a role for geranylgeranylated proteins, rather than

farnesylated proteins, in statin-induced morphological changes and growth arrest. In contrast, other authors reported that addition of either FOH or FPP prevented morphological changes¹¹ and abrogated lovastatin-induced cytotoxicity,^{11,22,94} suggesting a role for farnesylated proteins as well. Nevertheless, it seems that GGPP has distinct effects on cancer cells, but so far few studies have been performed to establish whether inhibition of isoprenylation with GGTIs has therapeutic effects in cancer.

Ras and Rho proteins

Several studies indicate that statin-induced cytostasis and cytotoxicity depends on the ability to interfere with receptor signalling. Isoprenylated proteins have a role in signal transduction cascades downstream of membrane receptors. Generally, prenylation of a protein results in translocation from the cytosol to a membrane. In Fig. 3, farnesylation of Ras and subsequent plasma membrane translocation are shown. Only membrane-associated Ras is able to interact with membrane receptors, resulting in activation of downstream signalling cascades. Therefore, the Ras protein can only be active after farnesylation.

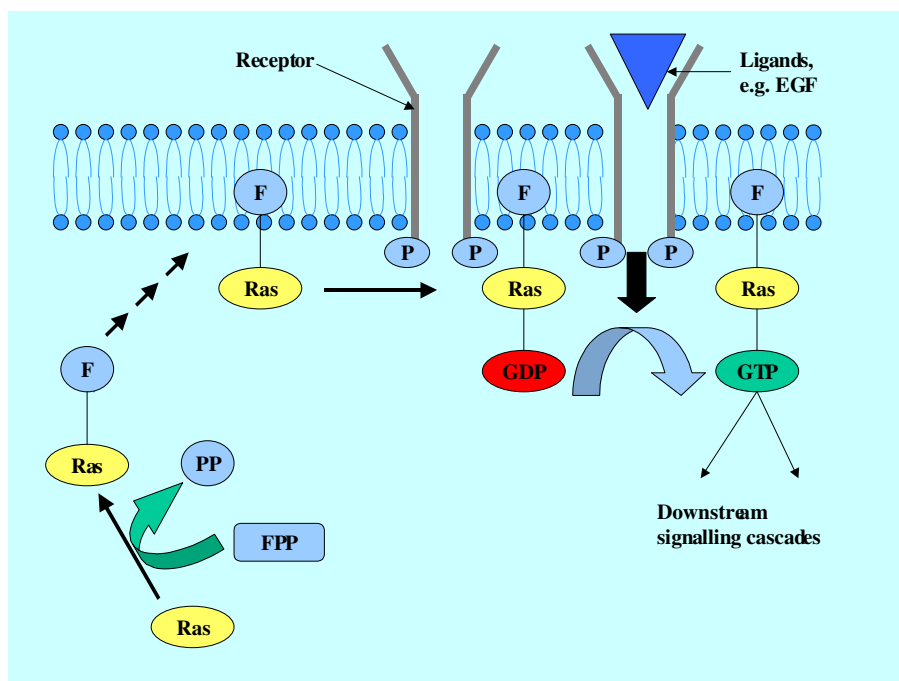


Figure 3 Farnesylation of Ras. FPP, farnesylpyrophosphate; F, farnesyl-moiety; EGF, epidermal growth factor. After farnesylation, Ras is targeted to the plasma membrane. After membrane association, Ras can be activated by membrane receptors. Binding of appropriate ligands to the receptor tyrosine kinase results in an interaction between inactive GDP-associated Ras and the membrane receptor. Subsequently, GDP is exchanged for GTP and signalling cascades downstream of Ras are activated.

Farnesylated Ras proteins are associated with mitogenic signal transduction in response to growth factor stimulation,⁹⁵ whereas proteins of the Rho subfamily, such as Rho, Rac1, and Cdc42, regulate signal transduction from receptors in the membrane in a variety of cellular events related to cell morphology, cell adhesion, cell motility, cell growth and cancer cell metastasis.^{96,97} RhoA and RhoC are posttranslationally modified by geranylgeranylation, whereas RhoB can be farnesylated and geranylgeranylated.^{98,99}

Several authors suggested that Ras protein is a critical target for statins. In mesothelioma cells, lovastatin treatment resulted in decreased membrane-associated Ras, whereas cytosolic Ras protein levels increased. Mesothelioma cell lines that were more sensitive to lovastatin treatment showed more Ras translocation from membrane to cytosol as compared with less sensitive cell lines.²² Statin-induced Ras translocation was confirmed in promyelocytic HL-60 cells²³ and monocytic cells.⁴⁶ In human smooth muscle cells, simvastatin inhibited posttranslational processing of the Ras protein. Additionally, DNA synthesis induced by stimulators of the Ras/mitogen-activated protein kinase (MAPK) pathway was inhibited by simvastatin.³⁷ Chang et al.¹⁰⁰ observed that sensitivity of NIH-3T3 cells to lovastatin treatment was increased by transfection with Ras oncogenes. Cells transfected with dominant-negative Ras, showed reversed susceptibility to lovastatin. Furthermore, invasiveness of NIH-3T3 cells was decreased by lovastatin treatment concomitantly with a decreased membrane localisation of Ras proteins. Moreover, specific inhibition of farnesyltransferase downregulated invasiveness as well.⁴⁵ These results indicate a role of farnesylation of Ras in statin-induced effects.

There is mounting evidence that Ras is not the key compound that explains the antiproliferative and proapoptotic effects of statins. Declue et al.¹⁰¹ showed that NIH-3T3 cells transformed with myristylated Ras (myr-Ras) were equally sensitive to lovastatin-induced growth inhibition as Ras-transfected cells. Since myr-Ras does not depend on farnesylation for appropriate membrane localisation and function, this result indicates that lovastatin-induced growth inhibition is not solely dependent on Ras. In addition, cerivastatin-induced translocation of Ras from the membrane to the cytosol was only partially inhibited and required a longer period of time than the effects on proliferation and invasiveness.³⁴ Although lovastatin induced growth arrest and apoptosis in NIH-3T3 cells, Ras protein was not translocated.¹⁰² This was confirmed in mammary carcinoma cells,

where lovastatin did not induce significant effects in subcellular distribution patterns of Ras proteins.⁴⁸

Since addition of either GGPP or GGOH reversed statin-induced effects, proteins of the geranylgeranylated Rho subfamily were investigated as possible targets. In vascular smooth muscle cells, pravastatin induced G1-arrest, apoptosis and Rho translocation from the membrane to the cytosol.⁹² Similarly, pravastatin induced redistribution of Rho proteins during neuronal cell death.⁹¹ Fluvastatin reduced the membrane RhoA fraction, whereas the cytosolic fraction increased. Furthermore, microinjection of activated RhoA prevented lovastatin-induced morphological changes in Swiss 3T3 fibroblasts¹⁰³ and direct inactivation of Rho by *Clostridium difficile* toxin B induced morphological changes and apoptosis that were identical to that induced by lovastatin in intestinal epithelial cells.¹⁰⁴ Likewise, specific inhibition of Rho by exoenzyme C3 reduced EGF-induced invasiveness of pancreatic cancer cells, similar to fluvastatin.^{52,55} In THP-1 monocytes, migration was reduced by treatment with either simvastatin or C3.⁴⁷ These data suggest a role for geranylgeranylated Rho proteins in statin-induced effects.

Most authors consider inhibition of protein prenylation and consequent loss of function as the mechanism that causes statin-induced effects on proliferation and apoptosis, but a conflicting mechanism has been suggested by Agarwal et al.¹⁰⁴ These authors sustain a role for geranylgeranylated Rho proteins in statin-induced effects. However, they observed in intestinal epithelial cells that GGPP completely prevented lovastatin-induced cytoskeletal changes and apoptosis, whereas Rho translocation to the membrane was only partially restored. Likewise, the protein synthesis inhibitor cycloheximide prevented lovastatin-induced effects without restoring Rho membrane translocation. These findings indicate that restoration of membrane translocation of Rho is not necessary for prevention of lovastatin-induced morphological changes and apoptosis. It is suggested that the protein synthesis inhibitor cycloheximide prevents accumulation of a protein necessary for lovastatin-induced effects. This protein may inactivate RhoA.¹⁰⁴ Recently, farnesylated RhoB and geranylgeranylated RhoB were reported to be potent suppressors of human tumour growth. Moreover, specific inhibitors of geranylgeranyltransferase and farnesyltransferase were reported to increase RhoB expression.¹⁰⁵ Based on these findings, Agarwal et al. proposed that an imbalance between the relative amounts

of RhoA and RhoB is a potential mechanism of lovastatin-induced apoptosis.¹⁰⁴

Ras and Rho proteins have been investigated extensively and have been shown to be involved in statin-induced effects. However, data on the exact role of these proteins are conflicting, possibly due to differences in cell lines and experimental conditions. Furthermore, the involvement of other prenylated proteins can not be excluded yet.

The MAPK and PI3K pathway

The MAPK pathway is a well-studied downstream effector pathway of the Ras protein, involved in cell cycle progression and proliferation (Fig. 4). Briefly, the serine-threonine kinase Raf-1 is activated by Ras. Subsequently, Raf-1 phosphorylates two MAPK kinases (MEKs), which in turn phosphorylate mitogen-activated protein kinases (MAPKs; also known as extracellular signal-regulated kinases, Erks). Activated MAPKs translocate to the nu-

cleus and activate transcription of several factors involved in cell proliferation.

Since the Ras protein was originally considered to be a major target of statin therapy, its downstream MAPK signalling pathway was investigated. Evidence for the involvement of MAPK in statin-induced effects is somewhat conflicting. In human pancreatic cancer cells, treatment with either lovastatin or fluvastatin inhibited proliferation whereas MAPK phosphorylation was unaffected.⁵⁵ In arterial smooth muscle cells, treatment with lovastatin in a concentration which almost completely abolished basic fibroblast growth factor (bFGF)-induced mitogenesis only partially inhibited bFGF-induced phosphorylation of p42 APK.¹⁰⁶ Furthermore, pravastatin induced apoptosis in vascular smooth muscle cells, whereas MAPK was unaffected.²⁰ In contrast, lovastatin reduced proliferation and induced apoptosis in human astrocytomas. This was accompanied by decreased MAPK activation.¹⁰⁷ In Rat-1 fibroblasts and human aortic endothelial cells, statin treatment resulted in

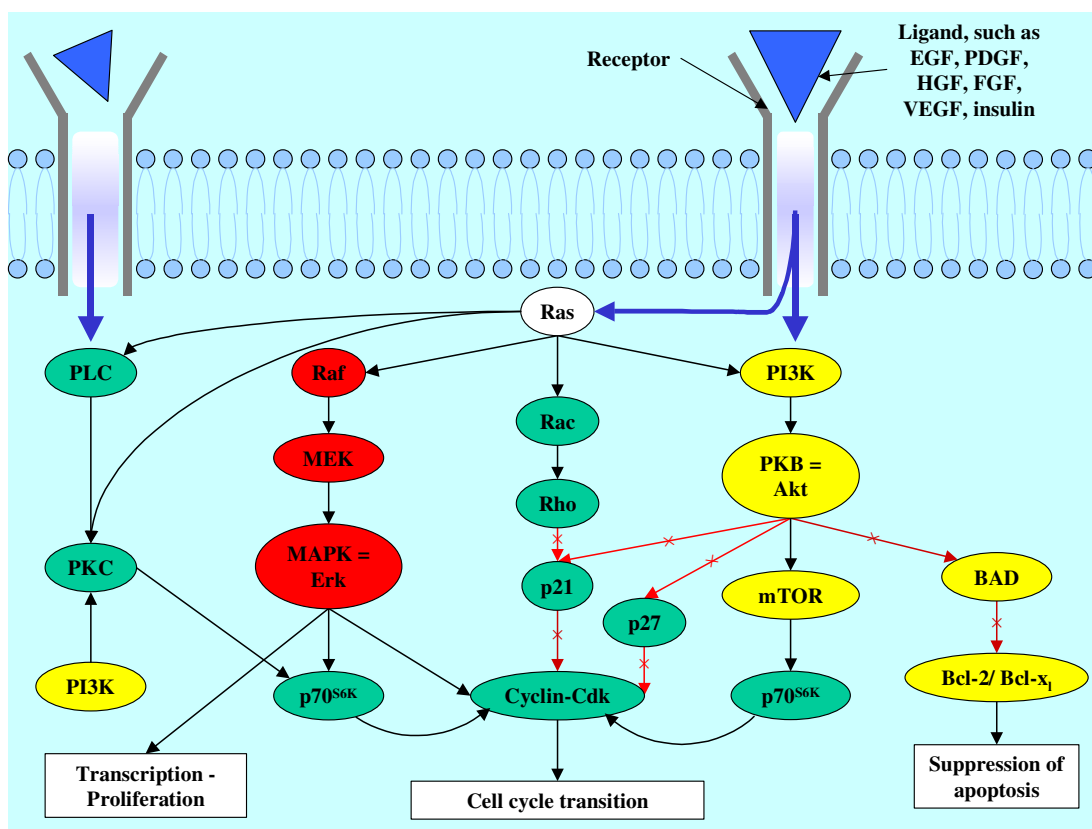


Figure 4 Intracellular signal transduction cascades. PI3K, phosphoinositide 3'-kinase; PLC, phospholipase C; PKC, protein kinase C; PKB, protein kinase B; BAD, pro-apoptotic protein of Bcl-2 family; mTOR, mammalian target of rapamycin; MAPK, mitogen-activated protein kinase; MEK, MAPK kinase; cyclin-Cdk, complex of cyclin and cyclin-dependent kinase; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; →: stimulation; →*: inhibition.

decreased MAPK phosphorylation.^{108,109} In addition, specific inhibition of the MAPK pathway resulted in decreased cellular growth of 3T3 cells and *ras*-transformed BALB 3T3 cells.¹¹⁰

There is mounting evidence that phosphoinositide 3'-kinase (PI3K) is involved in statin-induced effects on proliferation and induction of apoptosis (Fig. 4). Downstream targets of PI3K are protein kinase B (PKB or Akt) and p70^{S6K}. The PI3K/Akt pathway promotes survival by affecting the Bcl-2 protein family. Activated Akt suppresses apoptosis by phosphorylation of the proapoptotic protein BAD, disabling heterodimerisation of BAD with antiapoptotic proteins Bcl-2 and Bcl-XL.^{111,112} P70^{S6K} mediates activation of the 40S ribosomal protein S6, which is important for cell cycle progression.

Lovastatin inhibited PI3K activity in L6 myoblasts and PDGF-induced PI3K activity in vascular smooth muscle cells.^{113,114} In Rat-1 fibroblasts, insulin-induced PI3K activity was inhibited.^{109,115,116} Furthermore, lovastatin treatment resulted in decreased PDGF-induced PI3K activity, whereas the amount of PI3K protein was unaffected. The levels of tyrosine-phosphorylated PDGFR bound to PI3-kinase after PDGF stimulation was reduced after treatment with lovastatin, suggesting that lovastatin either affected PDGFR autophosphorylation or expression of PDGFR.¹¹⁵ Moreover, pravastatin decreased both PI3K activity and DNA synthesis²⁰ and specific inhibitors of PI3K activity induced cell death with morphological changes that are similar to those caused by simvastatin.^{20,117} Particularly, simvastatin inhibited activity of PI3K when it was Ras-bound, suggesting that inhibition of Ras prenylation was crucial.¹¹⁷ It was shown earlier that PI3K is a direct target of Ras.¹¹⁸

Proteins of the Bcl-2 family have been shown to be affected by statin treatment. Overexpression of the antiapoptotic Bcl-2 protein suppresses statin-induced apoptosis in Ras-transformed NIH/3T3 cells, human malignant glioma cells, and the acute myeloid leukaemic cell line AML-5.^{21,100,119} In addition, statin treatment resulted in downregulation of Bcl-2 protein in HT29 colon cancer cells, vascular smooth muscle cells, myeloid leukemic cells, and immortalised rat brain neuroblasts.^{89,120–122} The proapoptotic Bax protein is upregulated in HT29 colon cancer cells and rat cortical neurons.^{89,91} These data demonstrate that statin treatment results in disruption of the balance between proapoptotic and antiapoptotic members of the Bcl-2 family. It remains to be determined whether disruption of this balance is caused by statin-induced inhibition of the PI3K pathway.

These results show that the effects of statins on signalling cascades, such as MAPK and PI3K, are not unambiguous. This can be explained by two observations. Nguyen et al.¹²³ concluded that the requirement of specific signalling pathways for malignant transformation is dependent on the type of cells and the nature of the oncogenes. Moreover, regulation of the MAPK and PI3K pathways is rather complex. It was shown that these pathways cross-talk at the level of Raf-1 and Akt.^{124–126} The PI3K signalling cascade has a role in regulation of the Erk pathway, both positively and negatively. This regulation depends on the nature and strength of the incoming signal.¹²⁷ These findings indicate that activation of signalling pathways varies in different malignant cell types. This may explain the unambiguous effects of statins on these pathways.

Involvement of proteins that regulate the cell cycle

Transition through the cell cycle is controlled by the activity of cell cycle checkpoint proteins, such as cyclins, cyclin-dependent kinases (Cdks), and Cdk inhibitors. The activity of Cdk-cyclin complexes is necessary for phosphorylation of the retinoblastoma (Rb) gene product, resulting in the release of transcription factor E₂F, which is required for transition through S, G₂, and M phases (Fig. 5; Ref. 128). The activity of cyclin D₁-Cdk4/6 and cyclin E-Cdk2 complexes is particularly important for passage through the G₁ phase.¹²⁹ Cdk inhibitors downregulate activity of

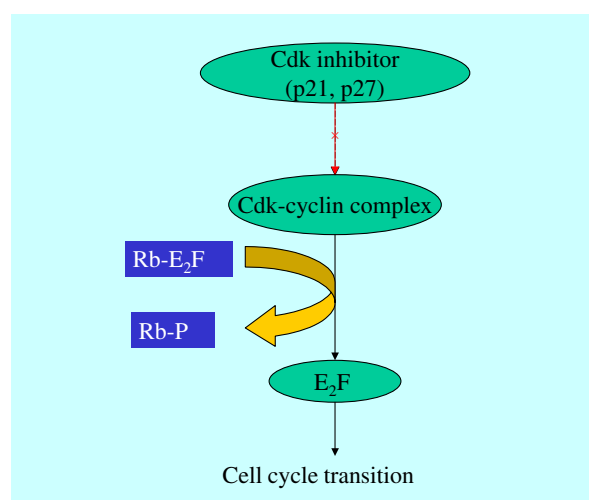


Figure 5 Regulation of cell cycle transition by Cdk inhibitors. Cdk, cyclin-dependent kinase; Rb, retinoblastoma protein; E₂F, transcription factor; -.-> inhibition; → stimulation.

Cdk-cyclin complexes, and thereby induce cell growth inhibition.¹²⁸

Lovastatin affects the control of G1/S transition by overexpression of a family of Cdk inhibitors that include p27^{Kip1} as well as p21^{Cip1/Waf1/Cap20} and p16^{Ink4}.^{130,131} Accordingly, several authors found a statin-induced increase in expression of p21^{14,16,24,34,100,132–135} and p27,^{16,121,132,135} indicating that statin-induced Cdk inhibitor expression may arrest cells in G1 phase.^{20,93,136} This was confirmed by Lee et al.,¹⁴ who showed that lovastatin-induced G1-arrest was accompanied by an increased association between p21 and Cdk2. Furthermore, Rb protein was hypophosphorylated and the association between Rb and the transcription factor E2F-1 was enhanced. Induction of growth arrest by double-thymidine block did not result in increased levels of p21 and p27, indicating that induction of p21 and p27 after lovastatin treatment is not a non-specific effect associated with cell cycle arrest, but is a specific effect of lovastatin treatment.¹³⁵

Katayose et al.¹³⁷ have shown that overexpression of the Cdk inhibitor p27 induces apoptosis in several cancer cell lines, indicating that p27 may mediate statin-induced apoptosis as well as growth arrest. Overexpression of cyclin E delayed lovastatin-induced growth arrest and apoptosis in NIH-3T3 cells,¹⁰² confirming that activity of cyclin-Cdk complexes affects induction of apoptosis in some cell lines. In contrast, statin-induced elevated p21 levels are not associated with induction of apoptosis.^{24,34} Cdk inhibitor p21 has been suggested to protect cells from apoptosis by induction of growth arrest.^{137,138} Normally differentiating neural progenitors become easily apoptotic, unless they are withdrawn from the cell cycle. Kim et al.²⁶ demonstrated that lovastatin induced differentiation and apoptosis of Ewing's sarcoma cell line CHP-100, without induction of p21 expression or growth arrest. Transfection of CHP-100 cells with p21 partially protected the cells from lovastatin-induced apoptosis. These results suggest that lovastatin induced differentiation in CHP-100 cells, but failed to elevate p21 levels and consequently, cells underwent apoptosis.

Statin-induced expression of the Cdk inhibitor p21 has been observed at both the transcriptional and translational level,^{14,24,34,133,135,139} suggesting that statins induce expression of Cdk inhibitor p21 by affecting transcription. However, Rao et al.¹³⁹ observed increased p21 mRNA levels in lovastatin-treated MDA-MB-157 breast cancer cells but concluded that induction of p21 protein expression is regulated posttranscriptionally because mRNA levels and protein levels are not regulated with the same kinetics.

Statin-induced expression of Cdk inhibitor p27 are regulated posttranscriptionally^{131,135,139,140} or by inhibition of p27 protein elimination.⁹³

Statin-induced upregulation of p21 and p27 protein levels were reported to be mediated mainly by RhoA.^{34,93,136} The association between RhoA and cell cycle regulatory proteins is complicated. RhoA negatively regulates expression of Cdk inhibitor p27^{136,140–142} by decreasing translational efficiency of p27 mRNA¹⁴⁰ and increasing p27 degradation.^{93,141,142} Recently, it was shown that RhoA induces cyclin E-Cdk2 activity.¹⁴² Sheaff et al.¹⁴³ have shown that p27 can be both an inhibitor and a substrate of the cyclin E-Cdk2 complex. Phosphorylation of p27 by cyclin E-Cdk2 is required for ubiquitination of p27 and consequent degradation.¹⁴⁴ These results indicate that RhoA can induce degradation of p27 by induction of cyclin E-Cdk2 activity and consequent phosphorylation of p27.¹⁴² Transition through the cell cycle requires reduction of Cdk inhibitor levels as well as induction of cyclin D expression. It was shown that cyclin D expression is regulated by the Ras/Erk pathway. Cell cycle transition depends on negative regulation of p27 expression by RhoA and upregulation of cyclin D expression by the Ras/Erk pathway.¹⁴² Inhibition of RhoA geranylgeranylation and consequent induction of p27 expression may contribute to cell cycle arrest in cell lines that are rescued from growth arrest by GGPP, whereas decreased Ras farnesylation and cyclin D expression may be more important in cells that are rescued by FPP.

Surprisingly, activation of the cell growth-stimulating Ras/Erk pathway has been reported to increase levels of Cdk inhibitor p21, resulting in inhibition of cell growth. Activated RhoA was shown to suppress Ras-induced elevation of p21 levels and consequently cells progress through the cell cycle when Ras and RhoA are both activated. These results demonstrate that regulation of p21 expression depends on Ras and RhoA activity.^{145,146}

Some experiments indicated that p27^{14,18,24,147} and p21^{18,136} are not essential for statin-induced effects. A possible explanation was provided by Rao et al.,¹⁴⁸ who demonstrated that lovastatin treatment resulted in G1 arrest in a wide variety of normal and tumour breast cells, but p21 and p27 protein levels were not increased in all cell lines. Nevertheless, binding of p21 and p27 to Cdk2 was significantly increased following lovastatin treatment. The increased binding of Cdk2 was shown to be achieved by redistribution of both p21 and p27 from Cdk4 to Cdk2 complexes, suggesting that p21- and p27-redistribution mediated lovastatin-induced G1-arrest independently of absolute levels of p21 and p27.

Alternatively, Park et al.¹⁸ suggested a p21- and p27-independent pathway for the effects of lovastatin. They observed that the proteasome inhibitors lactacystin and MG132 partially prevented lovastatin-induced E2F-1 degradation, suggesting that lovastatin modulates proteasomal degradation of E2F-1 which may be a critical regulatory mechanism of lovastatin-induced effects. However, lactacystin did not completely restore lovastatin-induced E2F-1 modulation, indicating that the ubiquitin-proteasome pathway cannot be the only critical regulatory mechanism.

Epidemiological studies with statins

Chemoprevention or carcinogenicity of statins in clinical and epidemiological studies

Lipid-lowering therapy has been associated with an increased risk of cancer. In 1996, Newman and Hulley¹⁴⁹ reviewed animal studies of lipid-lowering therapy and concluded that statins and fibrates (another class of lipid-lowering drugs) can cause cancer in rodents at doses comparable to those recommended for humans. However, Dalen and Dalton¹⁵⁰ argued that the statin doses used in these studies were substantially higher than recommended therapeutic doses. Additionally, extrapolation of data obtained in rodents to man has its limitations and must be done with greatest care. Two randomised trials showed an insignificant excess of cancer deaths among clofibrate-treated patients^{151–153} and patients treated with a cholesterol-lowering diet,^{153,154} respectively.

However, significant differences in cancer incidence or cancer deaths between treated and control groups were not found in most trials with statins (Table 1; Refs. 155–158). After completion

of one of these trials (LIPID trial), patients were offered open-label pravastatin therapy for two more years. The extended follow-up period did also not provide any evidence for an increased risk of cancer associated with the use of pravastatin.¹⁵⁹ Furthermore, a meta-analysis of these trials did not reveal a significant effect of statins on cancer.¹⁶⁰ Inclusion of smaller trials in the analysis did not substantially change the result.¹⁶¹ Additionally, when the experience of the three major trials (WOSCOPS, CARE, and LIPID) that compared pravastatin with placebo was combined in the prospective pravastatin pooling (PPP) project, differences between the incidence of fatal and non-fatal cancer between pravastatin treatment and placebo treatment were not detected.¹⁶²

Recently, results of the PROSPER trial were published. The study population of the PROSPER trial is relatively unique since the average age of study participants was 75 years at baseline. The incidence of newly diagnosed cancer was greater in pravastatin-treated patients as compared with placebo-treated patients (hazard ratio 1.25; 95% confidence interval: 1.04–1.51). The authors performed a meta-analysis of cancer rates in previous randomised placebo-controlled studies with statins. They did not find an association between the use of statins and the incidence of cancer, even when the PROSPER study was included. The authors suggest that the excess of newly diagnosed cases of cancer in the pravastatin-treated group is a chance finding, which may be partly explained by the recruitment of patients with pre-existing cancer at baseline.¹⁶³

When site-specific cancers were analysed, 12 cases of incident breast cancer were observed in the pravastatin-treated group, versus 1 case in the control group ($p = 0.002$) in the CARE trial (Table 1). Based on general cancer rates among women of the same age and race, 5 cases of breast cancer had to be expected in the control group.¹⁵⁷

Table 1 Overview of clinical trials with statins^{155–158}

| Study (statin) | Follow up (years) | Endpoint | N | | Cancer | | p value |
|---------------------------------|-------------------|-----------------|---------|---------|---------|---------|-------------------|
| | | | Placebo | Treated | Placebo | Treated | |
| 4S (simvastatin) | 7.4 | Cancer deaths | 2223 | 2221 | 68 | 52 | 0.087 |
| WOSCOPS (pravastatin) | 4.9 | Incident cancer | 3293 | 3302 | 106 | 116 | 0.55 |
| AFTEX ^a (lovastatin) | 5.2 | Incident cancer | 3301 | 3304 | 259 | 252 | 0.75 |
| LIPID (pravastatin) | 6.1 | Incident cancer | 4502 | 4512 | 403 | 417 | 0.43 |
| CARE (pravastatin) | 5.0 | Incident cancer | 2078 | 2081 | 161 | 172 | n.s. ^b |
| ALLHAT-LLT (pravastatin) | 4.8 | Cancer deaths | 5185 | 5170 | 369 | 378 | 0.66 |
| PROSPER (pravastatin) | 3.2 | Incident cancer | 2913 | 2891 | 199 | 245 | 0.02 |

^a AFTEX = AFCAPS/TexCAPS.

^b n.s., not significant.

In the LIPID study, a significant increase in incident breast cancer was not found after pravastatin treatment.¹⁵⁸ Similarly, the AFCAPS/TexCAPS study (Table 1) did not find an increased incidence of breast cancer in the lovastatin-treated group.¹⁵⁵ In addition, a case-control study that was designed to investigate the relation between the use of statins and the development of breast cancer among women did not reveal an increased risk for statin users.¹⁶⁴ Therefore, the high incidence of breast cancer as found in the CARE study was considered as an anomaly.¹⁵⁷

Significantly less cases of melanoma were found in the lovastatin-treated group as compared with the control group in the AFCAPS/TexCAPS trial.¹⁵⁵ In the 4S study, fewer patients treated with simvastatin died from cancer as compared with patients in the control group. The difference was just significant. In addition, the Lovastatin Study Groups reported 14 cases of cancer versus 21 expected on the basis of age- and gender-adjusted cancer rates.¹⁶⁵ In contrast, trials described in Table 1 did not show significant protective effects of statins against cancer suggesting that statins do not prevent cancer. It should be noted that these trials were not originally designed to investigate the association between use of statins and the occurrence of cancer. No attention was paid to possible pre-existing cancer in the treated or control groups. Furthermore, data are not available on the duration of statin use among patients that were diagnosed with cancer in the statin-treated groups. Since the development of malignancies may take many years, the duration of follow up was relatively short. Therefore, interpretation of these data is difficult.

Blais et al.¹⁶⁶ specifically investigated the relation between the use of statins and the occurrence of cancer in a nested case-control study. To avoid confounding by indication, they compared users of statins with users of bile-acid-binding resins. A protective effect of statins was found (odds ratio 0.72; 95% confidence interval: 0.57–0.92). However, the duration of follow up was again relatively short (2.7 years) and statin users were defined on the basis of a single prescription of statins, irrespective of the duration of therapy. Additionally, in a nested case-control study, we compared statin-treated cardiovascular patients with non-statin-treated cardiovascular patients. We found a risk reduction to develop cancer of 20% in users of statins (odds ratio 0.80; 95% confidence interval: 0.66–0.96^{166a}).

In vitro and in vivo studies have suggested a therapeutic effect of statins in cancer, whereas these epidemiological studies indicate a preventive

effect of statins in the development of cancer rather than a therapeutic effect. The mechanism of chemoprevention may well be different from mechanisms of statin-associated therapeutic effects. Recently, anti-oxidative properties of statins were reported. Angiotensin II-induced superoxide anion production was inhibited by simvastatin in cardiac myocytes and intracellular oxidative stress was blocked.¹⁶⁷ Atorvastatin reduced the amount of reactive oxygen species induced by angiotensin II and the angiotensin 1 receptor was downregulated in vascular smooth muscle cells. Experimental studies in vivo also showed a reduction in the amount of reactive oxygen species due to statin treatment.¹⁶⁸ Additionally, fluvastatin was shown to have anti-oxidative properties.¹⁶⁹ These anti-oxidative properties may contribute to chemopreventive properties of statins.

Statins as anticancer treatment in clinical studies

The first clinical study specifically investigating the effect of statins in the treatment of cancer was the phase I study of Thibault et al.³⁵ The authors found that lovastatin given p.o. at a dose of 25 mg/kg daily for 7 consecutive days is well tolerated. One minor response in a patient with recurrent high grade glioma was observed. Moreover, in a phase II study 35 mg/kg lovastatin was administered. The most severe side effects were myalgia and elevated serum creatine phosphokinase but both appeared to be transient. Antitumour effects were not observed, indicating that monotherapy may not be sufficient.¹⁷⁰ In a phase I–II trial of lovastatin in patients with anaplastic astrocytoma and glioblastoma multiforme, it was shown that high doses of lovastatin are well tolerated with concurrent radiation therapy. Response data were too premature to evaluate any antitumour activity.¹⁷¹

Only one randomised controlled trial has been performed so far. The effect of pravastatin on survival of patients with advanced hepatocellular carcinoma has been investigated. After standard treatment, patients were randomly assigned to either pravastatin treatment or no further treatment. Tumour regression was not observed, but patients receiving pravastatin survived significantly longer as compared with patients receiving no further treatment (18 versus 9 months; $p = 0.006$). Biochemical data suggest that liver function deteriorated faster in patients who were assigned to the control group. Although this trial was not blinded, these data suggest a role for statins in cancer therapy.¹⁷²

Studies that were specifically designed to investigate the relation between the use of statins and either development¹⁶⁶ or treatment¹⁷² of cancer indicate a protective effect of statins. Studies that report an increased incidence of cancer among users of lipid-lowering therapy were either performed with fibrates or diet or were only designed to investigate a cardiovascular endpoint.^{151–153,155–158,160,161} Therefore, statins may have anti-tumour effects despite evidence arguing for the opposite.

Farnesyltransferase inhibitors

Preclinical and clinical studies

Since the effects of statins were considered at first to be mediated by inhibition of Ras farnesylation, specific inhibitors of farnesyltransferase have been developed. Various FTIs are available: manumycin, R115777, SCH66336, L744,832, L739,749, L739,750, BMS186511, RPR115135, SCH56582, and BIM46228.^{174–178} R115777 and SCH66336 are currently referred to as Zarnestra and Sarasar, respectively.

FTIs induce reversion of morphological changes induced by Ras, block anchorage-independent growth and induce apoptosis *in vitro*.^{179–190} In addition, FTIs were shown to reduce expression of vascular endothelial growth factor (VEGF) in malignant cell lines^{180,191–193} and consequently, angiogenesis was decreased in mice bearing human tumours.¹⁹⁰ Over 70% of the cancer cell lines investigated are sensitive to FTIs.^{193,194} *In vivo* experimental studies revealed that FTIs block growth of both solid and non-solid tumours^{185,186,195–209} with little toxicity.^{199,202,205,206,209} These findings suggest that FTIs may be useful in the treatment of cancer and phase I clinical trials have been performed.

The results of eight phase I trials with SCH66336 and R115777 are reviewed in Table 2. Trials that were published only as abstracts are not included. Dose-limiting toxicities are gastrointestinal toxicities (nausea, diarrhoea, vomiting), renal insufficiency, dehydration, fatigue, neuropathy and myelosuppression (neutropenia and thrombocytopenia) (Table 2). Johnston,¹⁷³ Karp et al.,²¹⁰ and Eskens et al.²¹¹ summarised the results of phase I trials including trials that were published as abstracts. When evaluating the results of phase I trials with SCH66336, R115777, L778,123, and BMS-214662, it can be concluded that myelosuppression is a class effect of FTIs, whereas non-

hematological toxicities differ in essential aspects.²¹¹ Toxicity depends on dose and schedule of administration (Table 2; Ref. 173). The recommended dose is 500 mg twice a day, when R115777 was administered to patients with solid tumours for 5 days every 21 days. However, continuous therapy with R115777 resulted in a recommended dose of only 300 mg twice a day. Clinical activity has been observed in several studies (Table 2; Refs. 173,210). Although optimum dose and schedule remain to be determined, these early signs of clinical activity are promising.¹⁷³ Importantly, 29% of acute leukemic patients responded to R115777 therapy, indicating that R115777 should be particularly investigated in myeloid leukemic patients.²¹² Effectiveness of farnesyltransferase inhibition by SCH66336 was shown by the dose-dependent inhibition of farnesylation of prelamin A in buccal mucosa cells of SCH66336-treated patients.²¹³ Likewise, farnesyltransferase activity, lamin A farnesylation, and HDJ-2 farnesylation were shown to be decreased in bone marrow cells of R115777-treated patients.²¹⁴ Unfortunately, R115777 did not demonstrate clinical activity in phase II studies with non-small-cell lung cancer patients²¹⁵ and metastatic pancreatic cancer patients.²¹⁶ In conclusion, these clinical studies suggest that FTIs may have therapeutic value in the treatment of some cancer types.

Ras proteins

Initially, FTIs were designed to block Ras farnesylation. Indeed, several observations suggest that FTI-induced effects are mediated by the Ras protein. Cells expressing Ras-F are sensitive to FTI treatment but cells expressing either myr-Ras or Ras-GG were not affected by FTI treatment.^{180,185,217} Another finding that suggests that FTI-induced effects are mediated by Ras inhibition, is that cells activated downstream of Ras, such as Raf-transformed and MEK-transformed cells, are hardly susceptible to FTI treatment.^{180,185,202} Furthermore, the protection by insulin of Chinese hamster ovary (CHO) cells that express human insulin receptors against apoptosis induced by serum withdrawal was abrogated after treatment with manumycin or transfection with dominant-negative H-Ras.²¹⁸ This observation suggests that insulin protects against apoptosis by signalling via a Ras-dependent pathway that is inhibited by FTI treatment. K-Ras and N-Ras, but not H-Ras, are known to be geranylgeranylated in the absence of farnesylation.²¹⁹ Feldkamp et al.²²⁰ found that efficacy of FTI

Table 2 Overview of phase I trials with the FTIs SCH66336 and R115777

| FTI | Tumour | Scheme | No. of patients | Dose-limiting toxicities | Other toxicities | RD ^a | RS ^b |
|---------------------|---------------------------|----------------------------------------------|-----------------|------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|-----------------|--------------------------------------------|
| SCH66336 (Ref. 213) | Solid | 25–400 mg 2 dd 7 days | 20 | Gastrointestinal toxicities, fatigue, renal insufficiency, dehydration | Myelosuppression | 350 mg 2 dd | PR ^c : 1 SD ^d : 8 |
| SCH66336 (Ref. 211) | Solid | 25–400 mg 2 dd Continuous | 24 | Gastrointestinal toxicities, fatigue, renal insufficiency, dehydration, myelosuppression, neuropathy | Anemia, fever, mucositis | 200 mg 2 dd | SD: 2 |
| SCH66336 (Ref. 309) | Solid | 300–400 mg 1 dd 28 days and longer | 12 | Gastrointestinal toxicities, uremia, asthenia, myelosuppression | Upper gastrointestinal symptoms, abdominal symptoms | 300 mg 1 dd | PR: 0 CR: 0 |
| R115777 (Ref. 310) | Solid | 25–1300 mg 2 dd 5 days | 27 | Neuropathy | Fatigue, renal insufficiency, hematopoietic toxicity | 500 mg 2 dd | SD: 8 |
| R115777 (Ref. 214) | Acute leukaemia | 100–1200 mg 2 dd 21 days | 34 | Neuropathy | Myelosuppression, gastrointestinal toxicities, renal insufficiency, polydipsia, paresthesia | 600 mg 2 dd | CR ^e : 2 PR: 8 |
| R115777 (Ref. 311) | Solid | 50–500 mg 2 dd Continuous | 28 | Myelosuppression, neuropathy | Gastrointestinal toxicities, fatigue, exanthema | 300 mg 2 dd | PR: 1 SD: 3 |
| R115777 (Ref. 312) | Chronic myeloid leukaemia | 600 mg 2 dd 28 days 14 days rest | 16 | Fatigue, skin rash, peripheral neuropathy, myelosuppression | Gastrointestinal toxicities, pain, elevated transaminase/bilirubin levels, elevated creatinine levels | None | PR: 2 CR: 5 |
| R115777 (Ref. 313) | MDS ^f | 300–450 mg 2 dd 21 days 7 days rest | 21 | Myelosuppression, fatigue, myalgia, confusion | Rash, gastrointestinal toxicities, pain, elevated bilirubin levels, elevated creatinine levels, visual changes, headache | 400 mg 2 dd | PR: 5 CR: 1 |

^a RD, recommended dose.

^b RS, response.

^c PR, partial response.

^d SD, stable disease.

^e CR, complete response.

^f MDS = myelodysplastic syndrome.

treatment can be predicted on the basis of H-, K-, and N-isotype-specific Ras-GTP levels in astrocytoma cells. High levels of H-Ras-GTP and low levels of K-, and N-Ras-GTP were found to be predictive for FTI sensitivity. The finding that relative amounts of different isoforms of Ras protein can predict FTI-sensitivity suggests that FTI-induced effects are mediated by Ras.

Several observations argue against Ras farnesylation as a critical target of FTIs.^{221,222} In contrast to previous findings, cells transfected with myr-Ras appeared to be still susceptible to FTIs,²²³ whereas Ras mutations do not correlate with FTI sensitivity.^{184,185,193} Despite possible alternative geranylgeranylation of both K-Ras and N-Ras in the absence of farnesylation, malignant growth of these cells is inhibited by FTIs.^{193,199,202,219,224,225} Moreover, initiation of the FTI-induced effects do not correlate with inhibition of Ras processing.^{218,223,226} In addition, the reverted phenotype is maintained for several days after farnesyltransferase activity has returned to pretreatment levels.²²⁶

The latter observations suggest that Ras is not the critical target that mediates FTI-induced effects. However, Ras can still be a critical target of FTI-mediated effects despite these observations. Activated tyrosine kinases upstream of Ras may be responsible for Ras activation in FTI-sensitive cells with wild-type Ras.¹⁹³ Alternatively, human tumours with genetic mutations other than Ras may require normal Ras for malignant transformation.²²⁷ This may explain that FTI sensitivity is not correlated with the *ras* mutational status. Growth inhibition of cells expressing K-Ras and N-Ras that are geranylgeranylated when farnesylation is inhibited by FTI treatment can be explained when geranylgeranylated forms of these proteins are antagonists of tumour growth.²²² Finally, the observations that Ras suppresses the expression of long-lived growth inhibitory collagen $\alpha 2(I)$ ²²⁸ and tumorigenicity of Ras-transformed fibroblasts is decreased by transfection with collagen $\alpha 2(I)$ ²²⁹ might explain that FTI-induced reversion of the malignant phenotype is maintained for several days after farnesyltransferase activity has returned to pretreatment levels. Since the half-life of collagen $\alpha 2(I)$ is relatively long, this protein can continue to inhibit cell growth when Ras activity has returned to pretreatment levels.²³⁰

Rho proteins

RhoB has been suggested as an alternative for Ras as a critical mediator of FTIs. It is a low molecular weight GTPase that can be both farnesylated and geranylger-

anylated. FTI treatment results in decreased levels of RhoB-F whereas levels of RhoB-GG are increased.²³¹ In addition, the intracellular localisation pattern of RhoB is changed²²³ and growth stimulatory functions of RhoB are attenuated.²³¹

Several observations argue for RhoB as a critical target of FTI action. RhoB has a relatively short half-life (approximately 2 h), indicating that in contrast to Ras-F, RhoB-F could be depleted when FTI-induced morphological changes towards a less neoplastic phenotype are completed.^{223,232} Furthermore, transfection of FTI-sensitive cells with RhoB-GG resulted in reversion of the phenotype and growth inhibition in both Ras-transformed cells and human cancer cells with wild type Ras expression.^{233,234} In contrast, others did not find inhibition of cell proliferation or enhanced apoptosis after ectopic expression of RhoB-GG in leukemic cells.²⁰⁴

Another finding that argues for RhoB as a target of FTIs is that cells that ectopically express myr-RhoB are not sensitive to FTI treatment.^{187,223,231} In these cells, RhoB localisation is not dependent on isoprenylation. However, the biochemical properties of myr-RhoB are not exactly the same as those of wild type RhoB.²²² Finally, the presence of RhoB is crucial for Ras transformation,²³⁵ suggesting that inhibition of RhoB can revert malignant transformation. In contrast, it was shown that cell growth of murine fibroblasts defective in expression of RhoB was still partially inhibited by FTIs. Moreover, anchorage-independent growth was blocked by FTIs in these cells, indicating that RhoB is not required for FTI-induced inhibition of anchorage-independent growth.²³⁶

Arguments against RhoB as key mediator of FTI-induced reversion of malignant transformation are summarised by Sebt and Hamilton.²²² Both RhoB-F and RhoB-GG inhibit anchorage-dependent and anchorage-independent growth, induce apoptosis, and suppress tumour growth in nude mice, indicating that RhoB-F is not a critical target for FTIs.²³⁷ Moreover, RhoB has not been found to be mutated in human cancers²²² and the proportion of RhoB-GG in cells is larger than that of RhoB-F, and this raises the question whether changes in this ratio can explain the effect of FTIs on cells.^{98,231} Although transfection of *ras*-transformed fibroblasts with RhoB-GG resulted in inhibition of growth, apoptosis was not induced in cells which were not allowed to attach to a substratum.²³³

CENP-E and CENP-F

Conclusive evidence for either Ras or RhoB as a critical target of FTIs is not yet available. Cell cycle

analysis indicated that another protein may be a potential critical target of FTIs. In some cell lines, FTIs did not have an effect on cell cycle distribution, whereas other cell lines were arrested in G1 phase or accumulated in G2→M phase.^{133,200,201,204,238} In astrocytoma, accumulation in both G1 phase and G2→M phase was observed as well.¹⁷⁹ It was suggested that FTI-sensitive cell lines with H-Ras mutations are arrested in G1 phase, whereas other sensitive cell lines accumulate in G2→M phase.²³⁸ In H-Ras transformed cells, growth inhibitory effects of FTIs may be explained by effects on Ras processing. However, a farnesylated protein other than Ras has to be targeted by FTIs in cells lacking Ras mutations or with mutations other than H-Ras.²³⁹ Because cells that accumulate in G2→M phase are in prometaphase and bipolar spindle formation and chromosome alignment are inhibited,²⁴⁰ the farnesylated centromeric proteins CENP-E and CENP-F have been suggested as critical targets for FTIs. Indeed, CENP-E is an essential protein for the mitosis checkpoint²⁴¹ and blocking of CENP-E in farnesylation affects microtubular association, suggesting that CENP-E is a target of FTIs.²³⁹ However, cells that are deficient in CENP-E are able to establish a bipolar spindle and FTI-arrested cells are expected to have a bipolar spindle when CENP-E is a critical FTI target.²⁴⁰ Moreover, chromosome distribution patterns are different in CENP-E-deficient cells as compared with chromosome distribution patterns in FTI-arrested cells.²⁴⁰ In contrast, disruptions in Eg5 kinesin-related protein arrest cells in mitosis and show spindle poles that are not separated and are surrounded by a ring of chromosomes. H-Ras is known to activate cdc2, which in turn phosphorylates Eg5, offering a mechanism that may explain FTI-induced growth arrest.²⁴⁰ It is not known whether this mechanism is relevant in statin-induced effects as well.

Possible targets of FTIs are multiple but not unambiguous. Members of the Ras and the Rho family are candidates but other proteins, such as CENP-E may have a role as well. Whether a protein is a target of FTIs may depend on the exact genetic mutations and experimental conditions.

The MAPK and PI3K pathway

FTI treatment has been shown to inhibit signalling of the MAPK pathway *in vitro* and in experimental *in vivo* studies.^{181,201,202,218,243–245} In addition, activated Erk was undetectable in four out of eight FTI-treated patients in a phase I trial.²¹⁴ Furthermore, FTI-sensitivity correlated with the ability

to inhibit MAPK activation.¹⁹³ These data indicate a possible role of MAPK in FTI-induced effects. However, inhibition of the MAPK pathway by FTI treatment does not correlate with FTI-sensitivity in all systems. The effect of FTI treatment on MAPK was only modest in a FTI-sensitive astrocytoma cell line, whereas MAPK activity was markedly reduced in an astrocytoma cell line that was relatively resistant to inhibition of proliferation by FTI treatment.¹⁷⁹ Additionally, FTI treatment resulted in either weak MAPK inhibition or no MAPK inhibition in Balb-MK cells, whereas the same levels of inhibition of DNA synthesis were observed, whether MAPK inhibition was weak or absent.²⁴⁵ Suzuki et al. demonstrated that FTIs induce apoptosis by release of cytochrome c from the mitochondria and activation of caspase 3 in K-Ras-transformed normal rat kidney cells (KNRKs). Specific inhibition of either PI3K or MEK did not result in induction of apoptosis in serum-deprived KNRKs, suggesting that in K-Ras-transformed cells FTI-induced effects are not dependent on the MAPK or PI3K pathway.¹⁸⁸ These results suggest that the MAPK pathway is not the only signalling pathway that mediates FTI-induced effects.

The PI3K pathway was suggested to be involved as well in FTI-induced effects. Insulin-mediated Akt activation is blocked by manumycin treatment of CHO cells expressing the human insulin receptor.²¹⁸ Furthermore, Ras-transformed NIH-3T3 cells are less sensitive to FTI-induced apoptosis as compared with Akt2-transformed cells whereas cells expressing constitutively activated Akt2 are resistant to FTI-induced apoptosis.²⁴⁶ Moreover, FTI treatment results in inhibition of Akt1 activation in a tissue-specific manner, as H-Ras-transformed COS kidney cells and EGF-stimulated MCF-7 breast cancer cells are sensitive but transformed fibroblasts are not.²⁴⁷

Phosphorylation of PI3-kinase-dependent regulators of protein synthesis such as p70^{S6k} was inhibited by FTI-treatment in a mouse keratinocyte cell line and in mammary tumours in transgenic mice.^{200,245} The kinase p70^{S6k} was shown to be essential for G1 progression.²⁴⁸ Accordingly, inhibition of phosphorylation and activation of p70^{S6k} by FTI treatment was concomitant with reduced DNA synthesis,^{200,245,249} indicating that p70^{S6k} is involved in FTI-induced inhibition of cell proliferation. However, FTI-induced inhibition of p70^{S6k} is not mediated by PI3K in mouse keratinocytes.²⁴⁵

Phosphorylation of the proapoptotic protein BAD by the PI3K effector Akt was shown to be inhibited by FTI treatment in human ovarian epithelial cells and pancreatic cancer cells.²⁴⁶ Expression of the antiapoptotic protein Bcl-X_l results in suppression

of FTI-induced apoptosis in Ras-transformed fibroblasts.^{187,250} FTI treatment of human malignant astrocytoma cells resulted in increased expression of the proapoptotic proteins Bak and Bax and the antiapoptotic protein Bcl-2, whereas levels of Bcl-X_L were hardly affected.¹⁷⁹ Likewise, Bax and Bcl-X_S were upregulated by FTI treatment of human ovarian cancer cells.²⁵¹ These results demonstrate that disruption of the balance between proapoptotic and antiapoptotic proteins of the Bcl-2 family is involved in FTI-induced apoptosis. Jiang et al. have shown that inhibition of the PI3K pathway may contribute to disruption of this balance.²⁴⁶

Several studies indicate that serum withdrawal or denial of substratum attachment is required for FTI-induced apoptosis,^{187,188,204,250} suggesting that activity of another signal transduction pathway inhibits FTI-induced apoptosis.²⁵⁰ The combination of FTI treatment and specific inhibition of PI3K resulted in apoptosis, whereas either FTI treatment or PI3K inhibition alone did not induce apoptosis in Ras-transformed fibroblasts, indicating that the proapoptotic effects of FTIs can be masked by activation of the PI3K pathway.²⁵⁰ This finding was confirmed by Edamatsu et al.,²⁵² who showed that inhibition of PI3K enhanced FTI-induced apoptosis in human acute lymphoblastic leukaemia CEM cells and prostate cancer LNCaP cells. Overexpression or activation of cell survival pathways such as PI3K may explain why FTIs are not cytotoxic in all malignant cell lines.¹⁷³ It can be concluded that the MAPK and PI3K pathways may be targets of FTIs. The exact role of these signalling pathways is highly dependent on the tissue of origin of the cancer cell line and the exact experimental conditions.

Involvement of proteins that regulate the cell cycle

The tumour suppressor gene p53 is largely dispensable for FTI-induced apoptosis^{187,250} and growth inhibition²³⁴ in vitro. Ashar et al. observed that most human tumour cell lines with wild type p53 have an IC₅₀ < 100 nM for FTI SCH66336, whereas cell lines with mutant p53 have an IC₅₀ > 200 nM.²³⁸ Although FTI treatment resulted in a p53-independent decrease of tumour size in salivary tumours and mammary tumours in mice, the induction of apoptosis in p53^{-/-} tumours was somewhat attenuated as compared with that in p53^{+/+} tumours, suggesting that both p53-dependent and p53-independent apoptotic pathways may be activated by FTIs.¹⁹⁵ Recently, Moasser and Ro-

sen²⁵³ investigated a panel of breast cancer cell lines and reported that low FPTase expression and wild-type p53 may in part mediate FTI sensitivity. However, a single molecular marker could not be identified that accurately predicted FTI sensitivity.

Cdk inhibitor p21 is elevated by FTI treatment in several cell lines or in vivo tumour models.^{179,196,233,254} Induction of p21 without growth arrest has been observed in transformed fibroblasts, indicating that p21 may be necessary but not sufficient to mediate all biological responses to FTIs.²³³ In contrast, FTI treatment induced growth arrest in human malignant astrocytoma cell lines, but levels of p21 were not elevated in all cell lines.¹⁷⁹ Sepp-Lorenzino and Rosen²⁵⁴ investigated FTI-sensitive cancer cell lines and concluded that FTI treatment upregulates p21 in a p53-dependent manner and that the Cdk inhibitor p21 is required for growth arrest. In the absence of p53 and p21, FTI-induced growth arrest was annihilated and apoptosis was induced. These results demonstrate that p53 and p21 may have a role in FTI-mediated effects, but the effects of these proteins are highly cell line-specific.

FTIs have been shown to inhibit growth of a variety of human cancer cell lines in preclinical studies. In addition, results of phase I studies are promising. However, data published so far on the mechanism that mediates FTI-induced effects are rather conflicting. This can probably be explained by cell line specificity and variations in experimental conditions. Fibroblasts have been used in some in vitro studies, whereas most of the human tumours are of epithelial origin. The value of conclusions drawn in studies using fibroblasts needs to be established.²⁵⁴ Furthermore, rodent models have been used in in vitro and in vivo studies. Recently, striking differences in the mechanism of Ras-induced oncogenic transformation have been found between rodent and human cells.²⁵⁵ Consequently, findings on the mechanism of FTIs from studies using rodent models need re-evaluation in human cells.

Combinational therapies with statins or FTIs

Rationale for combining statins or FTIs with other agents

FTIs induce apoptosis only in cells deprived from growth factors or attachment to a substratum,¹⁸⁷ indicating that a second signal is required for FTI-

induced apoptosis.¹⁸⁸ Several signals have been suggested as a second death-promoting signal. FTI-induced apoptosis is inhibited by PI3K activity,²⁵⁰ suggesting that the second death-promoting signal required for FTI-induced apoptosis needs to inhibit PI3K. Accordingly, Edamatsu et al.²⁵² observed that inhibition of PI3K enhances FTI-induced apoptosis in a number of cell lines. However, roscovitine and olomoucine, both inhibitors of Cdk, were shown to be synergistic with FTI to induce apoptosis, independent of PI3K. Whether inhibition of PI3K or Cdk is more effective in synergy with FTI depends on the cell line. These results indicate that inhibition of Cdk activity is sufficient as second death-promoting signal in some cell lines. Treatment with an inhibitor of MEK enhanced FTI-induced apoptosis in Rat-transformed fibroblasts indicating that specific inhibition of the MAPK pathway may be sufficient as second death-promoting signal.²⁵⁶ These findings suggest that combinations of FTIs with other agents may have therapeutic value in the treatment of cancer. Furthermore, it has been shown that FTI-induced regression of salivary and mammary carcinomas is not maintained when FTI treatment is stopped in *ras* transgenic mice.¹⁹⁷ This observation indicates that combination with a cytotoxic agent is required for permanent tumour regression.¹⁸⁴

A phase I trial of lovastatin in patients with cancer has shown that plasma concentrations ranging between 0.1 and 3.9 μM are therapeutically achievable.³⁵ In vitro studies revealed that higher concentrations than 3.9 μM lovastatin are required to induce apoptosis or growth arrest in some cell lines. These results indicate that statin monotherapy is not sufficient for the treatment of all types of cancer. Combination of statin with another drug may overcome this problem.

Combinations with chemotherapeutics

An overview of in vitro experiments with combinations of chemotherapeutic agents and statins or FTIs is presented in Table 3. Several combinations were found to be more efficacious than monotherapy. These results have been confirmed in in vivo experimental models.^{65,90,258–265}

The effectiveness of combinations of either statins or FTIs and cytotoxic agents is highly dependent on the cell line, the cytotoxic agent, and the sequence of administration. The combination of cisplatin and the FTI SCH66336 was synergistic in non-small-cell lung cancer cells and glioblastoma cells, whereas antagonism was observed in breast, pancreatic, and colon cancer cell lines.²⁶⁵ Like-

wise, the combination of doxorubicin and lovastatin was synergistic in a colon cancer cell line and additive in lung and sarcoma cell lines.²⁵⁹ Combinational therapy was not similarly effective in different cell lines derived from the same tissue. Combination of cisplatin and manumycin A was synergistic in the anaplastic thyroid carcinoma cell lines DRO and KAT-18, whereas an additive effect was observed in other anaplastic thyroid carcinoma cell lines.²⁶⁶ The combination of doxorubicin and manumycin A was synergistic only in KAT-4, Hth-74, and C643 cell lines.²⁶⁶ When cisplatin was combined with lovastatin, enhanced apoptosis was observed but different colon cancer cell lines responded differently.⁸⁹ These data indicate that the effects of combinational therapy are not only dependent on the origin of the cancer cell lines, but also on the oncogenic mutations that are present.⁸⁹ This was confirmed by Russo et al.,²⁶⁷ who found that enhancement of 5-fluoruracil (5-FU)-induced antiproliferative effects by the FTI RPR-115135 was less distinct in HCT-116 cells transfected with dominant-negative p53 as compared with HCT-116 cells transfected with an empty vector. The sequence of treatment was found to be relevant as well, since antagonism was observed when cisplatin therapy preceded treatment with the FTI SCH66336 whereas the reverse order of treatment resulted in synergism in a non-small-cell lung cancer cell line.²⁶⁵

Little is known about mechanisms that cause synergism or antagonism in cancer cell lines. For some chemotherapeutic agents suggestions have been made concerning the mechanism. Taxanes (paclitaxel, docetaxel) and vinca-alkaloids (vincristin, vinblastin) are drugs interfering with the cellular microtubular system. During mitosis, microtubules are reorganised for chromosome segregation. Reorganisation of microtubules requires dynamic depolymerisation and polymerisation events. Interference of vincristin arrests cells in the G2/M phase of the cell cycle. G2/M arrest was enhanced synergistically by the combination of vincristin and FTI.¹⁸⁶ These results were confirmed by other authors,^{262,268} who found synergy between FTI and paclitaxel, which is another microtubule-affecting drug. Suzuki et al.¹⁸⁶ suggested that FTIs also interfere with microtubular dynamics, probably because farnesylated proteins modulate microtubular dynamics or FTIs affect a signalling pathway that affects microtubular dynamics. Alternatively, synergism between FTIs and paclitaxel may be explained by inhibitory effects of paclitaxel on protein isoprenylation.^{262,269} Furthermore, in vivo experiments suggest that the antineoplastic effects of the combination

Table 3 Effect of combinations of inhibitors of the mevalonate pathway and chemotherapeutic agents

| Combination | Effect | Cell line | Reference |
|---------------------------|-----------------|------------------------------------------------------------------------------------|-----------|
| <i>FTI SCH66336</i> | | | |
| 5-Fluoruracil | Antagonism | A-549, MCF-7, HCT-116, BxPC-3 | 265 |
| Melphalan | Additive | A-549 | 265 |
| Cisplatin | Synergism | A-549, T98G | 265 |
| Cisplatin | Antagonism | MCF-7, BxPC3, HCT-116 | 265 |
| Paclitaxel, docetaxel | Synergism | MDA-MB-468, DLD-1, NCI-H460, PA-1, DU-145, LNCaP, AsPC-1, BxPC-3, MIAPaCa2, PANC-1 | 262 |
| <i>FTI L-744832</i> | | | |
| Doxorubicin, cisplatin | Additive | MCF-7, MDA-MB-486 | 268 |
| Vinblastin, 5-fluoruracil | Additive | MCF-7, MDA-MB-486 | 268 |
| Paclitaxel | Synergism | MCF-7, MDA-MB-486 | 268 |
| <i>Lovastatin</i> | | | |
| Cytarabin | Synergism | K-562, ARAC8D | 87 |
| Cisplatin, 5-fluoruracil | Potentialiation | SW-480, HCT-116, LoVo, HT29 | 89 |
| Sodium phenylacetate | Synergism | A172 | 50 |
| Doxorubicin, radiation | Potentialiation | HOS-AD5 | 273 |
| Doxorubicin | Synergism | Colon-26 | 259 |
| Doxorubicin | Additive | LLC, Ras-NIH-3T3 | 259 |
| Sulindac | Potentialiation | HCT-116, SW-480, LoVo | 90 |
| <i>Manumycin A</i> | | | |
| Paclitaxel | Synergism | ARO, DRO, KAT-14, KAT-18, C643, Hth-74 | 266 |
| Cisplatin | Synergism | DRO, KAT-18 | 266 |
| Doxorubicin | Synergism | KAT-4, Hth-74, C643 | 266 |
| <i>Simvastatin</i> | | | |
| Cytarabin | Additive | HL-60, AML-2 | 314 |
| Carmustin | Synergism | Human glioma cells | 315 |
| <i>Cerivastatin</i> | | | |
| Doxorubicin, cisplatin | Synergism | T4-2 breast cancer cells | 316 |
| 5-Fluoruracil | Synergism | H630 and R10 colorectal cancer cells | 317 |
| <i>BIM-46228</i> | | | |
| Paclitaxel | Potentialiation | MIA-PaCa-2 | 175 |
| <i>FTI SCH56582</i> | | | |
| Vincristine | Synergism | Rat renal KNRK cells | 186 |

of FTI and paclitaxel are due to inhibition of angiogenesis.²⁶⁴

The combination of SCH66336 with cyclophosphamide, 5-FU, vincristin or taxanes results in enhanced antitumour activity in vivo.^{260–262} Since these chemotherapeutic agents are all substrates for the multidrug resistance protein P-glycoprotein (Pgp) and SCH66336 is a Pgp inhibitor, the synergism of SCH66336 and these chemotherapeutics may be explained by inhibition of Pgp by

SCH66336.²⁷⁰ Lovastatin, atorvastatin and simvastatin are inhibitors of Pgp as well,²⁷¹ suggesting that the interaction between statins and some chemotherapeutics may also rely on inhibition of Pgp.

Synergism between cytarabin and lovastatin in AML cell lines is based on inhibition of MAPK activation by lovastatin, which would otherwise be induced by cytarabin.⁸⁷ There is increasing evidence that exposure to some cytotoxic agents not only induces signalling pathways necessary for

the induction of apoptosis but that it also concurrently activates cytoprotective signalling pathways. It is suspected that the balance between the proapoptotic and the concurrent cytoprotective signals are decisive whether apoptosis occurs or not. Interference with cellular rescue mechanisms induced by cytotoxic treatment may therefore increase efficacy of anti-neoplastic treatment.²⁷²

Generation of activated oxygen species plays a role in cytotoxicity associated with radiotherapy and chemotherapy with compounds such as doxorubicin.²⁷³ Treatment with FTIs or lovastatin increases sensitivity to activated oxygen-associated cytotoxicity. Lovastatin lowers resistance to radiation, doxorubicin, and hydrogen peroxide of Ras-transformed human osteosarcoma cells.^{273,274} The combination of lovastatin and doxorubicin resulted in an additive effect on tumour growth of Lewis lung cancer cells and Ras-transformed sarcoma cells, whereas synergism was observed in tumour growth of colon cancer cells.²⁵⁹ The combination of FTI with doxorubicin has an additive effect on growth inhibition of breast cancer cells²⁶⁸ and a synergistic effect was observed in some but not all anaplastic thyroid carcinoma cells lines.²⁶⁶ The combination of lovastatin and doxorubicin resulted in significant reduction of tumour growth in experimental animals as compared with doxorubicin monotherapy, but improved survival was not observed. Lovastatin treatment significantly reduced troponin T release by cardiomyocytes in doxorubicin-treated mice, indicating attenuation of doxorubicin-induced cardiotoxicity by lovastatin.²⁵⁹

Pretreatment with FTI resulted in radiosensitisation of cancer cells *in vitro*^{176,276–278} and of tumours of H-Ras-expressing cancer cells *in vivo*.²⁵⁷ However, growth inhibition and radiosensitisation mediated by inhibitors of prenylation appeared to be independent processes, since radiosensitisation was observed only in Ras-mutated cell lines, whereas growth inhibition was independent of Ras mutations.²⁷⁵ Since hypoxic cells are more resistant to irradiation than well-oxygenated cells, the effects of FTI treatment on tumour oxygenation have been investigated. Tumour oxygenation was found to be improved after FTI treatment *in vivo*.²⁷⁸ Improved radiosensitivity of cancer cells themselves may contribute to the effectiveness of combinational therapy but these results suggest that improvement of tumour oxygenation contributes to increased radiosensitivity as well.²⁷⁸

The role of mutations in the interaction between FTIs and chemotherapeutics has been investigated by Adjei et al.²⁶⁵ and Russo et al.^{176,267} They concluded that interactions were cell line-specific

and not dependent on Ras mutations. Although Russo et al. observed that the presence of wild-type p53 was required to induce synergism between RPR-115135 and 5-FU, Adjei et al. reported that synergism between SCH66336 and cisplatin did not correlate with the status of p53. These findings imply that different FTIs may interact differently with cytotoxic drugs.¹⁷⁶

Interestingly, Prassana et al.⁵⁰ observed a synergistic effect of the combination of sodium phenylacetate and lovastatin in glioma cells. Sodium phenylacetate is an inhibitor of mevalonate pyrophosphate decarboxylase, which is an enzyme downstream of HMG-CoA reductase. Therefore, it would be interesting to study the effects of the combination of HMG-CoA reductase inhibitors and other inhibitors of the mevalonate pathway, e.g. FTIs.

Combinations with bisphosphonates

Bisphosphonates are drugs that suppress bone resorption and are currently used in skeletal disorders, such as osteoporosis. Although the exact mechanism remains to be elucidated, bisphosphonates are believed to reduce osteoclast activity. It was shown by Amin et al.²⁷⁹ that several nitrogen-containing bisphosphonates (N-BPs) inhibited rat liver squalene synthase. Pamidronate and alendronate, both N-BPs, are poor inhibitors of squalene synthase but these agents reduce sterol biosynthesis from mevalonate, indicating that they inhibit another enzyme of the mevalonate pathway (Fig. 2).²⁷⁹ Using crude enzyme preparations from bovine brain, N-BPs were shown to inhibit isopentenyl-PP isomerase, FPP synthase, or both.²⁸⁰ Bergstrom et al.²⁸¹ confirmed these results using rat liver cytosolic extracts and showed that recombinant human FPP synthase was inhibited by alendronate whereas purified rat liver IPP isomerase was unaffected. Other N-BPs were shown to inhibit FPP synthase as well and alendronate inhibited protein prenylation in osteoclasts and macrophages.²⁸² NE21650, a novel N-BP that inhibits both FPP synthase and IPP isomerase, is more effective in inhibition of protein prenylation as compared with alendronate.²⁸³

These findings suggest that N-BPs have antitumour potential, like other inhibitors of protein prenylation. Indeed, alendronate induced apoptosis in both J774 macrophages and osteoclasts^{282,284} and incadronate induced both cell cycle arrest and apoptosis in human myeloma cells.²⁸⁵ Furthermore, alendronate inhibited migration, adhesion, and invasion of PC-3 prostate cancer cells.²⁸⁶

Likewise, alendronate inhibited growth, adhesion and invasion of murine lung alveolar carcinoma cells.²⁸⁷ However, treatment with alendronate and R115777, or a combination of both drugs did not result in inhibition of tumour growth in mice inoculated with a murine lung alveolar carcinoma cell line. In contrast, alendronate enhanced the inhibitory effect of R115777 on the incidence of lung metastasis.²⁸⁸ Vincenzi et al.²⁸⁹ hypothesise that statins and bisphosphonates may inhibit cancer proliferation by induction of apoptosis and anti-angiogenic properties, respectively. Combination of these two classes of drugs could represent a new pharmacological approach in adjuvant cancer treatment.

Combinations with NSAIDs

Clinical, experimental, and epidemiological data suggest that NSAIDs have antitumour potential, particularly in cancers of the gastrointestinal tract (reviewed in Refs. 290–292). The exact molecular basis for NSAID-associated chemoprevention remains to be determined. Some findings indicate that inhibition of cyclo-oxygenase 2 (COX-2) is involved in the antitumour activity. However, COX-2-independent mechanisms such as downregulation of proto-oncogenes have been suggested as well.²⁹¹ Furthermore, salicylate has been reported to inhibit the mTOR/p70^{S6K} pathway.²⁹³ Consequently, combinations of NSAIDs with drugs blocking tumour growth signals may be effective in the treatment of cancer.²⁹¹ Agarwal et al.⁹⁰ investigated the combination of sulindac and lovastatin and reported that lovastatin enhanced sulindac-induced apoptosis in colon cancer cells. Moreover, in vivo experiments in rats indicated that combinational lovastatin and NSAID therapy enhances NSAID-associated chemoprevention. Additionally, cell growth was inhibited synergistically by lovastatin and the COX-2 inhibitor MF-tricyclic in two murine colorectal cancer cell lines²⁹⁴ and lovastatin-induced apoptosis was enhanced synergistically by the COX-2 inhibitor celecoxib in human HT-29 colon cancer cells.²⁹⁵ Findings on the effect of statins on COX-2 expression are somewhat conflicting. In human aortic smooth muscle cells, treatment with lovastatin and mevastatin resulted in increased COX-2 expression. This effect was mediated by inhibition of Rho protein.²⁹⁶ Statin treatment downregulated COX-2 expression in primary human umbilical vein endothelial cells.²⁹⁷ Agarwal et al.⁹⁰ observed decreased COX-2 expression in LoVo cells and COX-2 expression was undetectable in HCT-116 cells, both in the presence and absence of lovasta-

tin. Since lovastatin enhanced sulindac-induced apoptosis in both LoVo and HCT-116 cells, it is unlikely that lovastatin-induced elevated apoptosis is mediated through modulation of COX-2 expression. Celecoxib enhanced lovastatin-induced apoptosis and caspase-3 expression synergistically in HT-29 cells, indicating that agents with different modes of action may induce apoptosis synergistically by inducing caspase-3 activity.²⁹⁵

Combinations with GGTIs

Similar to statins, GGTIs have been shown to arrest human tumour cells in G₀/G₁ phase^{10,133} and reduce tumour growth in mice.^{263,298} Furthermore, the combination of GGTIs with chemotherapeutic agents was shown to be more efficacious than monotherapy.²⁶³ In order to acquire a more complete inhibition of protein prenylation, the effect of the combination of FTIs and GGTIs was investigated. In different cancer cell lines, combinational treatment with FTI and GGTI induced markedly higher levels of apoptosis than with either FTI or GGTI alone. However, doses of GGTIs that are sufficient to inhibit Ki-Ras prenylation are lethal to mice when continuously infused for more than 24 h.²⁹⁹ In contrast, Sun et al.^{263,298} did not report lethality associated with GGTI treatment. Because the degree of geranylgeranyltransferase inhibition of treated animals was not determined, Sun et al. may have used GGTI doses that cause only partial geranylgeranyltransferase inhibition. These observations indicate that GGTIs may have a narrow therapeutic window.²⁹⁹

Combinations with agents that target tyrosine kinase activity

Tyrosine kinase activity is important in signal-transduction cascades. The EGFR family consists of receptor tyrosine kinases that activate the Ras/MAPK and PI3K pathways (Fig. 4) resulting in cellular proliferation and survival. Four members of the EGFR family have been identified: HER-1 (EGFR-1; ErbB-1), HER-2 (ErbB-2), HER-3, and HER-4. It was shown that an EGFR autocrine loop contributes significantly to the Ras-transformed phenotype in rat intestinal epithelial cells. Accordingly, FTI-induced growth inhibition was enhanced by EGFR blockade³⁰⁰ and glioblastoma cells that overexpress EGFR are more sensitive to FTI treatment.²⁴² Furthermore, EGFR inhibitors potentiate lovastatin-induced apoptosis in head and neck squamous cell carcinoma cells that express EGFR.³⁰¹

Trastuzumab is a monoclonal antibody that blocks HER-2-induced survival and proliferation, particularly in breast cancer cells. Currently, an antibody that targets HER-1, and specific inhibitors of protein kinase activity are under investigation.³⁰² HMG-CoA reductase activity was shown to be affected by EGF and tyrosine kinase activity in SKBR-3 breast cancer cells that overexpress ErbB-2.^{303,304} The activity of Ras is dependent on HMG-CoA reductase activity and subsequent farnesylation. Furthermore, members of the EGFR family affect the activity of the Ras/MAPK pathway (Fig. 4). Therefore, the combination of agents that target tyrosine kinase activity (e.g. trastuzumab) with compounds that reduce protein prenylation (e.g. statins and FTIs) may be beneficial in anticancer therapy.

Recently, the non-receptor tyrosine kinase Bcr-Abl fusion protein was recognised to play a central role in the pathophysiology of chronic myeloid leukaemia (CML). Since this protein provides constitutive active tyrosine kinase, the tyrosine kinase inhibitor STI571 (currently referred to as imatinib mesylate) was investigated as a therapeutic agent in CML.³⁰⁵ Although, imatinib mesylate has been shown to have antitumour potential in leukaemias, treatment of the LAMA84 cell line resulted in the development of resistance to STI571-induced apoptosis.³⁰⁶ Combinational therapy may overcome mechanisms of resistance development.³⁰⁵ Indeed, it was shown that the combination of STI571 and FTI results in an additive inhibitory effect on leukaemic cell proliferation.³⁰⁷ Importantly, growth of chronic myeloid leukemic cells from patients with STI571 resistance was inhibited by the FTI SCH66336. It was shown that SCH66336 was able to restore STI571-induced apoptosis in otherwise resistant cells. Since SCH66336 inhibits downstream effector molecules of Bcr-Abl, such as Ras, combination of SCH66336 and STI571 may inhibit Bcr-Abl signalling below a critical threshold required for survival.³⁰⁸

Conclusion

The antitumour properties of statins and FTIs have been extensively investigated. Growth arrest and induction of apoptosis have been observed in various cell lines. Additionally, inhibition of primary tumour growth and metastasis was shown in experimental animals. Epidemiological and clinical trials that were designed to investigate the effect of statins on either the development or treatment of cancer indicated a beneficial effect of statins.

Unfortunately, evidence on the mechanisms that result in statin- or FTI-induced antitumour effects is rather conflicting. Possibly, mechanisms are not similar in different cell lines. Furthermore, chemoprevention and therapeutic effects may be mediated by different mechanisms. Prenylated proteins, such as Ras and Rho, have been shown to be involved in statin- and FTI-induced effects, but other proteins may be involved as well. Additionally, the contribution of downstream signalling cascades, such as MAPK and PI3K, requires further investigation. Finally, many *in vitro* studies and *in vivo* studies have been performed using rodent fibroblasts or other rodent cells. The value of findings in these studies needs to be established in human (epithelial) cells.

Several observations indicate that monotherapy with statins and FTIs in patients with cancer do not result in a permanent clinically relevant antitumour response. It was shown that a second signal, such as serum deprivation or PI3K inhibition, is required for FTI-induced apoptosis, indicating that FTI monotherapy is not sufficient. Furthermore, FTI-induced suppression of human tumour growth is not maintained when FTI treatment is halted, suggesting that either permanent FTI therapy or a combination with a second agent is required. In a phase I trial performed with lovastatin, doses as high as 25 mg/kg daily were administered but plasma lovastatin concentrations did not exceed 3.9 μM . Since higher concentrations are required for statin-induced antitumour effects in most cell lines, these findings indicate that combinational therapy is necessary when currently-used statins are applied for the treatment of cancer. Additionally, treatment of cancer with a single drug often results in development of resistance, as is the case for antitumour medication in general. Combinational therapy may overcome or delay this problem. In agreement with these findings, clinical responses have not been observed in a phase II trial that investigated lovastatin monotherapy in patients with gastric cancer.

In contrast, in a randomised placebo-controlled trial of pravastatin in patients with hepatocarcinoma monotherapy resulted in a clinically relevant response. However, combinations with other drugs may augment the statin-induced response, or may allow the use of lower doses. Several *in vitro* and *in vivo* studies confirmed that combinations of chemotherapeutic agents with either statins or FTIs result in additive or synergistic effects. Moreover, combination with other substances, such as bisphosphonates, NSAIDs, GGTIs, and tyrosine kinase inhibitors, was shown to be beneficial as well. Further exploration of the combination of statins

and FTIs with other compounds, such as chemotherapeutics, agents that interfere with the mevalonate pathway, and agents that block other signal transduction cascades is needed.

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