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Targeting protein prenylation for cancer therapy

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Abstract

Protein farnesylation and geranylgeranylation, together referred to as prenylation, are lipid post-translational modifications that are required for the transforming activity of many oncogenic proteins, including some RAS family members. This observation prompted the development of inhibitors of farnesyltransferase (FT) and geranylgeranyltransferase 1 (GGT1) as potential anticancer drugs. In this Review, we discuss the mechanisms by which FT and GGT1 inhibitors (FTIs and GGTIs, respectively) affect signal transduction pathways, cell cycle progression, proliferation and cell survival. In contrast to their preclinical efficacy, only a small subset of patients responds to FTIs. Identifying tumours that depend on farnesylation for survival remains a challenge, and strategies to overcome this are discussed. One GGTI has recently entered the clinic, and the safety and efficacy of GGTIs await results from clinical trials.

Interest in developing inhibitors of farnesylation as anticancer drugs was prompted by the realization more than 20 years ago that a sizable proportion of some, but not all, human cancers harbour activating oncogenic mutations in the *RAS* genes (between 8% and 93%, depending on the tumour type)[1], and that RAS GTPases require this lipid post-translational modification (PTM) for their malignant transforming activity[2]. Furthermore, many of the signal transduction pathways that are activated by RAS involve proteins that require

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Competing interests statement
The authors declare competing financial interests. See Web version for details.

DATABASES
ClinicalTrials.gov: http://clinicaltrials.gov/)
National Cancer Institute Drug Dictionary: http://www.cancer.gov/drugdictionary 5-fluorouracil | BMS-214662 | bryostatin 1 | carboplatin | cisplatin | cyclophosphamide | doxorubicin | gemcitabine | imatinib | lonafarnib | paclitaxel | tipifarnib | triciribine phosphate | vincristine
UniProt: http://www.uniprot.org/

FURTHER INFORMATION
Saïd M. Sebti's homepage:
Catalogue of Somatic Mutations in Cancer (COSMIC): http://www.sanger.ac.uk/cosmic
PrePS – Prenylation Prediction Suite: http://mendel.imp.ac.at/sat/PrePS/

SUPPLEMENTARY INFORMATION
See online article: S1 (table) | S2 (table) | S3 (table) | S4 (table) | S5 (table) | S6 (figure)

farnesylation or geranylgeranylation (together referred to as prenylation) for their ability to mediate tumour cell survival, growth, proliferation, migration and metastasis (FIG. 1). This, coupled with the fact that it has notoriously been difficult to design small GTPase inhibitors per se[3], prompted a global quest to develop farnesyltransferase (FT) inhibitors (FTIs) and geranylgeranyltransferase 1 (GGT1) inhibitors (GGTIs) (together referred to as prenyltransferase (PT) inhibitors (PTIs)) as potential anticancer drugs.

Preclinical studies in the 1990s demonstrated that FTIs are highly successful in killing cancer cells *in vitro* and in animals with very little toxicity, thus generating much excitement and raising the hope that, finally, a RAS inhibitor may be developed as a novel anticancer drug. Contrary to expectations, however, responses to FTIs, whether in cells, animals or human patients, do not seem to depend on *RAS* mutations; and the inhibition of KRAS farnesylation leads to its geranylgeranylation (discussed below). Furthermore, in most clinical trials FTIs have not been as successful as expected, with no survival advantages, for example, to patients with advanced solid cancers[4–6] or with acute myeloid leukaemia (AML)[7]. However, monotherapy with FTIs demonstrates antitumour activity in a subset of cancer patients, particularly those with haematological malignancies, whereas combinations of FTIs with cytotoxic agents improve the responses of patients with locally advanced breast cancer or other advanced solid tumours[8–11]. At present, we do not understand why some tumours are resistant while others are sensitive to FTIs. Clearly, the identification of the farnesylated proteins the inhibition of which is responsible for the antitumour effects of FTIs will lead to a better understanding of their mechanism of action and to the selection of patients whose tumours are sensitive to FTI treatment.

GGTIs as potential anticancer agents were developed for several reasons. First, KRAS (predominantly the alternatively spliced KRAS4B variant), which is the most frequently mutated isoform of RAS[1], and NRAS become geranylgeranylated and remain fully functional when cells are treated with FTIs[12–15]. Second, in some human malignancies, such as pancreatic cancer with KRAS mutated in 90% of patients[1], pathways that are mediated by geranylgeranylated proteins downstream of RAS, such as RALA and RALB, may be more relevant to oncogenesis than those mediated by MEK or AKT[16,17]. Third, the exclusively geranylgeranylated RHOC has an essential role in metastasis[18,19]. Fourth, the small GTPases cell division cycle 42 (CDC42) and RAC, which are exclusively geranylgeranylated, are crucial downstream targets for RAS-dependent transformation in rodent fibroblasts[20,21]. Furthermore, RAC1 is required to induce KRAS-driven lung cancer in mice[22]. Thus, GGTIs should be more efficient in cancer cells that are addicted to geranylgeranylated proteins, whereas FTI–GGTI combinations or dual prenylation inhibitors might be required to combat KRAS-dependent human tumours[23]. Similar to FTIs, GGTIs have shown promising results *in vitro* and in animal models, and one GGTI (GGTI-2418) has recently entered Phase I clinical trials[24].

A better understanding of the aberrant signalling pathways that a given tumour is addicted to and the effects of PTIs on these pathways will lead to strategies that exploit the vulnerabilities of individual tumours and ultimately to predicting which patient populations are most likely to respond to PTIs, either alone or in combination. Work has begun in pursuing this goal with the identification of a two-gene expression ratio that potentially

predicts the response of patients with AML to the FTI tipifarnib (also known as R115777)[25,26] (discussed below).

General aspects of protein prenylation

The importance of FT and GGT1 for normal physiology and tumorigenesis: lessons from knockout mice

The biochemistry of prenylation and pioneering findings in the field of prenylation research are summarized in BOX 1 and the TIMELINE, respectively. Protein prenylation is required for the membrane localization of otherwise cytosolic proteins. Studies in yeast[27] and mammalian cells[28] suggest that protein prenylation is also required for the normal function of at least some proteins. Additionally, defective prenylation has been attributed to the pathogenesis of several diseases other than cancer (BOX 2) Contemporary reviews have estimated that several hundred proteins are subject to prenylation[29,30]. Therefore, the inhibition of FT activity probably prevents many proteins from functioning properly, and it is perhaps not surprising that genetic disruption of the catalytic FT β-subunit (*Fntb*) in mice causes embryos to die very early in development[31] (TABLE 1). Whether the constitutive ablation of GGT1 activity in mammals is also embryonically lethal has not yet been determined. However, studies conducted in *Saccharomyces cerevisiae*[32], *Drosophila melanogaster*[33] and mouse embryonic fibroblasts[34], as well as the fact that more proteins are geranylgeranylated than are farnesylated[35], suggest that GGT1 function is essential for survival and development, and that the functions of FT and GGT1 are not redundant.

More interesting in this context are the results obtained with conditional PT deletions. The first such study by Barbacid and colleagues[31] suggested that FT is not required for tumour initiation in mice, either in mice developing KRAS-G12V-induced lung adenocarcinoma or in mice subjected to carcinogen-induced skin carcinoma, but that it is required for tumour progression and maintenance[31]. The study also suggested that adult mice lacking *Fntb* show normal tissue homeostasis except for slight defects in wound healing or liver regeneration. Finally, in these conditional FT-knockout mice, the authors suggested that wild-type HRAS still associates with cellular membranes[31]. Recently, these unexpected results have been called into question by Yang *et al.*[36] who re-analysed the conditional *Fntb*-null allele generated by the Barbacid laboratory: it produced a transcript that encoded a protein with a short in-frame deletion rather than, as expected, a transcript with a frameshift mutation that resulted in a true null allele. Thus, the results described above may have been due to a 'leaky' null allele that permitted the expression of partially active FT.

Two other recent studies by Bergo and colleagues have shown that conditional *Fntb* deficiency[37] or conditional *Pggt1b* (which encodes the catalytic β-subunit of GGT1) deficiency[34] reduces the formation of KRAS-G12D-induced lung cancer in mice. Furthermore, simultaneous knockout of both *Fntb* and *Pggt1b* has a far greater effect on KRAS-G12D-induced lung tumour onset and progression than either deletion alone[37]. Also, loss of both *Fntb* and *Pggt1b* significantly extends the lifespan of mice that express activated KRAS-G12D in their lungs, which validates FT and GGT1 as important targets for cancer therapy. In contrast to the earlier study by Barbacid *et al.*[31], Bergo and colleagues

demonstrated that *Fntb* transcripts were not detectable, and HRAS does not associate with cell membranes in the absence of FT[37], confirming earlier cell-based studies.

Bergo and colleagues have also recently described the effect of GGT1 deficiency in the haematopoietic system[38]. Mice harbouring an inducible $Kras^{G12D}$ oncogene in haematopoietic cells develop a lethal myeloproliferative disease (MPD), and up to one-third also develop acute lymphoblastic leukaemia (ALL). Whereas the absence of GGT1 markedly reduced the severity of MPD, it had no effect on ALL[38]. As KRAS can be farnesylated in the absence of GGT1, the antitumour effects of GGT1 depletion with regards to MPD can be attributed to defective prenylation of other GGT1 targets[38] that are downstream of KRAS, such as RALA and RALB.

Regulation of PTs

Given the importance of PTs, it is surprising that their regulation in response to external signals has not been investigated in great detail. The α-subunit of FT and GGT1, FNTα, has been shown to be phosphorylated in a transforming growth factor-β (TGFβ)-dependent manner, which either does not affect[39] or decreases FT activity[40]. FNTβ was also shown to be phosphorylated[41], raising the possibility that both of these events are necessary to modulate FT activity. Furthermore, insulin stimulates activating phosphorylation of FNTα by a member of the RAF1–MEK–MAPK pathway[42].

FNTα is cleaved by caspase 3 during apoptosis[43], suggesting that some signals may induce apoptosis by indirectly inhibiting the prenylation and the function of proteins that are involved in cell survival. Interestingly, dietary fish oil, which contains high levels of Ω3 polyunsaturated fatty acids, inhibits the expression of FT and colon tumorigenesis in rats[44], which is consistent with the observation that FT activity is increased in human colon cancer[45]. These findings also raise the possibility that FTIs may function as chemopreventive agents, an idea that has received experimental support in mouse models of lung cancer[46,47].

Mechanism of action of FTIs

The observation that FT and GGT1 may be dispensable for adult tissue homeostasis, but may be required for KRAS-driven tumorigenesis[34,37], further validated the concept of developing PTIs as novel anticancer drugs. Several strategies, including structure-based drug design and high-throughput screens, were used to identify a variety of PTIs (see Supplementary information S1 (table) for structures and potencies of representative compounds).

Depending on the context, the treatment of cancer cells with FTIs results in the induction of apoptosis, cell cycle arrest and the inhibition of anchorage-dependent and anchorage-independent cell proliferation, cell migration and angiogenesis (see Supplementary information S2 (table)). The exact mechanisms by which FTIs induce the antitumour effects described above are unknown mainly because the identity of the crucial farnesylated proteins, the inhibition of which mediates these FTI effects, is unknown (discussed further

below). However, several studies have shown that FTIs affect oncogenic and survival signal transduction pathways, which can explain some of their antitumour effects.

Cell proliferation

FTIs inhibit signalling pathways that are involved in anchorage-dependent and anchorage-independent proliferation. In well-defined systems in which mutant HRAS drives the transformation of NIH3T3 cells, FTIs inhibit the farnesylation of HRAS, prevent its association with the plasma membrane, inhibit downstream signal transduction pathways such as RAF–MEK–MAPK[48] and inhibit tumour growth[15]. In human tumour cell lines with multiple genetic alterations, FTIs inhibit PI3K–AKT signalling, particularly in ovarian and pancreatic cancer cells that overexpress AKT2 (REF. 49), although this seems to be context-specific, as, in lung cancer cells with low or undetectable levels of phospho-AKT, lonafarnib (also known as SCH66336)-induced apoptosis does not rely on AKT inhibition[50]. In nude mice, many FTIs inhibit the growth of human tumours harbouring a variety of genetic alterations, including *KRAS* mutations, *TP53* deletions and silenced cyclin-dependent kinase inhibitor 2A (*CDKN2A*^{INK4A})[51]. Similarly, in transgenic mice that express mutant KRAS or that overexpress wild-type NRAS, FTIs only inhibit tumour growth and do not induce tumour regression[52,53]. However, in mutant *Hras*-transgenic mice, FTIs cause tumour regression (see below) (see Supplementary information S3 (table)).

Cell cycle progression

FTIs primarily accumulate cells at prometaphase by preventing bipolar spindle formation and chromosome alignment[54,55], which may rely on the inhibition of the farnesylation of the centromere-associated protein E (CENPE) and CENPF[56,57], as well as phosphatase of regenerating liver (PRL) protein tyrosine phosphatases (PTPs)[58]. However, in some human cancer cell lines FTIs can induce G1 phase arrest. For example, L-744,832 induces p21 accumulation and inhibition of RB phosphorylation[59]. Similarly, in Rat1 fibroblasts transformed with HRAS^{G12V}, the FTI HR-12 causes the accumulation of p27, which results in the inhibition of cyclin-dependent kinase 2 (CDK2) and subsequent G1 arrest[60]. This compound inhibits both anchorage-dependent and anchorage-independent cell growth and blocks cell motility in wound healing assays. Rat1 cells transformed with myristoylated HRAS^{G12V} are resistant to HR-12 (REF. 60), indicating that the inhibition of HRAS farnesylation is responsible for HR-12 effects.

Apoptosis

FTIs cause breast tumour regression very effectively in mutant *Hras*-transgenic mice[61] that express *Myc* or that lack *Trp53*, but not in *Erbb2*-transgenic mice[62], suggesting that these drugs may induce apoptosis, which was confirmed by studies in cultured cells. For example, FTIs inhibit integrin-mediated and growth factor-mediated activation of the PI3K–AKT pathway, which results in the dephosphorylation of AKT substrates, including the pro-apoptotic BCL-2 family member BCL-2 antagonist of cell death (BAD), which leads to its activation. In this setting, overexpression of constitutively active AKT2 rescues FTI-induced apoptosis[49]. However, in other cells, FTIs induce apoptosis only when deprived of growth factors or of substratum attachment, suggesting that growth factors and integrins can rescue

FTI-induced apoptosis[63–65]. FTIs can also induce apoptosis by enhancing death receptor signals[66] or by inhibiting nuclear factor-κB (NF-κB)-dependent induction of cyclin D1, survivin, inhibitor of apoptosis proteins (IAPs) and BCL-2 (REF. 67). Another FTI that has been explored in preclinical and clinical studies, BMS-214662, has pro-apoptotic activity and induces tumour regression in nude mouse xenografts, but this seems to be related to the inhibition of GGT2 and not FT or GGT1 (REF. 68).

Angiogenesis

Lonafarnib inhibits angiogenesis in lung and head and neck tumour cells by decreasing hypoxia and insulin-like growth factor 1 (IGF1)-stimulated hypoxia inducible factor 1α (HIF1α) expression[69]. This study also showed that lonafarnib inhibits vascular endothelial growth factor A (VEGFA) production by inhibiting HIF1α binding to heat shock protein 90 (HSP90), which results in the degradation of HIF1α. Consistent with this, other FTIs, such as L-744,832 (REF. 70) and tipifarnib[71], affect angiogenesis, possibly by inhibiting HIF1α expression and hypoxia. Finally, another FTI, LB42708, inhibits angiogenesis, possibly by inhibiting pathways that are mediated by MAPK and AKT[72].

Combinations

In cell culture, structurally unrelated FTIs can enhance the growth inhibitory and apoptotic effects of radiation[73], taxanes[74,75], cisplatin[76,77], 5-fluorouracil[78], MEK inhibitors[79], CDK inhibitors[80] and the breakpoint cluster region (BCR)–ABL inhibitor imatinib (also known as STI-571)[81]. In nude mouse xenografts, beneficial combinations have been reported for lonafarnib and cytotoxic agents such as cyclophosphamide, 5-fluorouracil, vincristine[82] and paclitaxel[83], or for FTI-2148 and paclitaxel, cisplatin and gemcitabine[84]. We have recently found that a combination of tipifarnib and the inhibitor of AKT activation triciribine phosphate (TCNP)[85] (but not the single agents alone) causes breast tumour regression in *Erbb2*-transgenic mice[86].

Several studies by Giannakakou and colleagues have recently provided a mechanistic explanation for the commonly observed synergy between FTIs and taxanes. First, they showed that lonafarnib in combination with paclitaxel enhances tubulin acetylation more than the effect of either drug alone, and that this is due to the inhibition of histone deacetylase 6 (HDAC6)[87], an enzyme that functions as a tubulin deacetylase and that is involved in stress response, microtubule stability and cell migration. FTIs increase the amount of microtubule-bound paclitaxel, even in cells that are resistant to paclitaxel alone, and this is dependent on functional HDAC6 (REF. 88). Finally, FT and HDAC6 physically associate with each other at microtubules, and FTIs induce the dissociation of FT from microtubules, resulting in the inhibition of HDAC6 activity, an effect that was duplicated by stable knockdown of FNTα using short hairpin RNA (shRNA)[89]. Most interestingly, HDAC6 does not contain a carboxy-terminal CaaX motif and is thus a very unlikely FT substrate. It is possible that other FT targets that are present in the microtubule–protein complex mediate FT regulation of HDAC6.

GGTI effects in cultured cells and *in vivo*

Similar to FTIs, GGTIs induce apoptosis and inhibit tumour cell growth, both in cultured cells and in animal models (see Supplementary information S3 (table)). In contrast to FTIs, GGTIs result in G1 arrest and not mitotic arrest[90]. Their ability to induce G1 arrest may be due to inducing the expression of the CDK inhibitors p21 and p27, inhibition of CDK2 and CDK4, and hypophosphorylation of RB[91]. GGTIs induce the accumulation of p27 in the nucleus through the inhibition of CDK2-mediated phosphorylation of Thr187 in p27, and this is important for their ability to induce tumour cell death[92]. Tamanoi and colleagues recently described P61-A6, a GGTI that causes G1 arrest, probably (at least in part) by inhibiting RHOA geranylgeranylation and inducing p21 expression (REFS 93,94). Furthermore, GGTI-induced apoptosis may also depend on their ability to reduce the levels of phosphorylated and thus activated AKT and survivin[95]. GGTIs can also induce apoptosis by increasing death receptor 5 (DR5; also known as TNFRSF10B) expression, decreasing cellular FLICE-like inhibitory protein (cFLIP; also known as CFLAR) expression and enhancing TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis in human non-small-cell lung cancer (NSCLC) cells[96]. In addition, GGTIs inhibit platelet-derived growth factor (PDGF)-stimulation of PDGF receptor (PDGFR)-mediated tyrosine phosphorylation and MAPK signalling, suggesting that PDGFR phosphorylation is mediated by a GGT1 substrate[97].

The ability of our lead GGTI, GGTI-2418 (currently in Phase I clinical trials), to inhibit anchorage-dependent and anchorage-independent growth may also depend on its ability to inhibit the geranylgeranylation of RALB or RALA, respectively[98]. In addition, GGTI-2418 effectively prevents xenograft tumour growth in nude mice and results in breast tumour regression in *Erbb2*-transgenic mice[92]. Casey and colleagues described GGTI-DU40 (REF. 99), which is a highly potent and selective GGT1 inhibitor that inhibits the prenylation of several cellular proteins, including RHO GTPases. In MDA-MB-231 breast cancer cells, GGTI-DU40, but not the FTI L-744,832, inhibits thrombin-induced cell rounding. GGTIs with novel scaffolds were recently identified by a virtual screen of 9.5 million compounds in conjunction with quantitative structure–activity relationship modelling[100].

FTIs in the clinic

Starting in 2000 (REF. 101), four FTIs have been evaluated in at least 75 clinical trials: tipifarnib, lonafarnib, BMS-214662 and L-778123. In 64 of these studies (with 35 being Phase I trials), the clinical response has been determined (TABLES 2,3; see Supplementary information S4 and S5 (tables)). Sixteen years after curing transgenic mice that developed mouse mammary tumour virus (*MMTV*)–*Hras*-driven breast tumours with FTIs[61], and after accumulating other very impressive preclinical data, it has become clear that in human clinical trials, monotherapy with FTIs shows limited antitumour activity in haematopoietic cancers, and generally no or very little activity in solid tumours. Thirty-eight of the 64 clinical trials (59%) concerned tipifarnib, either alone or in combination with other agents. Eighteen of the 64 trials (28%) — all but one of which was conducted in patients with solid tumours — reported no objective responses. Seventeen of the 18 trials (94%) with no objective responses were conducted with FTI monotherapy. Twenty-three of the 64 trials

(36%) conducted in both solid and haematological malignancies showed very little antitumour activity (the rate of objective responses was <15%). The median objective response was 2.3% for monotherapy with FTIs and 11.4% for combinations with FTIs. Furthermore, in 28 of the 64 trials (44%), FT activity or the prenylation status of marker proteins (most commonly HDJ2 (also known as DNAJA1**)** or prelamin A) was assessed to determine whether the FTI treatment had affected its intended targets. There was no correlation between FT inhibition and clinical responses. Most importantly, two FTIs that have so far advanced to Phase III clinical trials are lonafarnib[6] and tipifarnib[4,5,7], and, unfortunately, these drugs were unable to improve the outcome for advanced pancreatic cancer[4], advanced colon cancer[5], advanced NSCLC[6] or AML[7], whether alone[7,5] or in combination with carboplatin and paclitaxel[6] or with gemcitabine[4].

What accounts for this discrepancy between laboratory findings and clinical data? First, in humans, KRAS, and not HRAS, is most frequently mutated[1]. Second, unlike HRAS, which is exclusively farnesylated, KRAS (and possibly NRAS) can escape FTI-mediated inhibition as it can be alternatively prenylated by GGT1 and thus is fully functional[12–15] (BOX 1). Third, the lack of antitumour activity may be due to the fact that most of the clinical trials enrolled patients with advanced and/or metastatic disease. It is also important to note that even though it was known preclinically that KRAS function is resistant to FTIs, Phase III clinical trials were carried out in patients whose tumours harboured mutant KRAS (that is, patients with pancreatic cancer). Perhaps this is because preclinical studies had shown that some cancer cells that harbour mutant KRAS are sensitive to FTIs, possibly owing to the inhibition of exclusively farnesylated proteins downstream of KRAS. In another clinical trial, attempts were made to directly inhibit KRAS function by using L-778123, which inhibits both FT (half-maximal inhibitory concentration (IC_{50}) = 2 nM) and GGT1 (IC_{50} = 98 nM). Unfortunately, in peripheral blood mononuclear cells from patients treated with this drug, KRAS prenylation was not inhibited[102].

When used in combination with other agents, FTIs have fared better. For example, Phase I studies based on a combination of tipifarnib with gemcitabine and cisplatin have shown some promise in advanced solid tumours (33.3% complete response rate or 26% partial response rate)[8,9]. Similarly, in Phase II neoadjuvant settings, tipifarnib increases the rate of pathological complete responses from the historical 10% to 25% when combined with chemotherapy (doxorubicin and cyclophosphamide) in patients with locally advanced breast cancer[10,11]. The fact that some patients respond to FTIs suggests that some human tumours depend on farnesylated proteins for survival. The identification of these proteins remains a challenge that must be overcome in order to select patients whose tumours are most likely to respond to FTIs. To this end, a strategy to predict clinical response to FTIs was recently developed by Raponi *et al.* Following the analysis of gene expression profiles from patients with untreated AML, these authors found that a high ratio of expression of two genes, RAS guanyl releasing protein 1 (*RASGRP1*), which encodes a RAS guanine nucleotide exchange factor (GEF) that activates RAS, and aprataxin (*APTX*), which encodes a protein involved in DNA excision repair, predicts a tipifarnib-positive response of patients with AML[25,26]. Moreover, in patients with advanced solid cancers, low mRNA levels of *FNTB*, but not

FNTA, are associated with improved response to lonafarnib plus taxane and significantly better survival[103]. Similar studies are needed for other types of cancer.

Targets crucial for the antitumour activity of FTIs

As the data described above have illustrated, and as can be expected of drugs that affect a large number of PT substrates, PTIs trigger a plethora of molecular and cellular effects, whether in cell culture, animal models or in human cancer patients. A key question is, what are the crucial PT substrates that mediate these effects? The answer to this question may uncover why FTIs and GGTIs are only effective in a subset of cells and tumours.

RAS proteins as crucial targets for FTIs

FTIs were originally developed to inhibit RAS function. However, the ability of FTIs to inhibit tumour growth is not correlated with mutations in RAS, whether in cells[104,105], animals[105] or human patients[106] (discussed above). KRAS, the most frequently mutated human oncoprotein, becomes geranylgeranylated and is fully functional in tumour cells treated with FTIs[12–15]. However, FTIs are effective at inhibiting the growth of mutant KRAS-harbouring tumours in nude mice[107] and transgenic mouse models[53], suggesting that the inhibition of KRAS farnesylation is not required for FTI antitumour activity, and that, in these models, tumours are addicted to farnesylated proteins other than KRAS. Similar considerations apply to NRAS, because it can also escape FTI-mediated inhibition. HRAS, conversely, is not alternatively geranylgeranylated in cells treated with FTIs. Therefore, the inhibition of HRAS farnesylation can still contribute to FTI antitumour activity in tumours that are addicted to mutant or wild-type HRAS for survival. Thus, it may be worthwhile to design clinical trials that involve FTIs for patients with *HRAS*-mutant bladder cancers, a tumour that has clearly been understudied so far (TABLES 2,3).

RHEB as a crucial target for FTIs

Like HRAS, RAS homologue enriched in brain (RHEB) is exclusively farnesylated[75]. The GTPase-activating protein (GAP) for RHEB is the tumour suppressor tuberous sclerosis complex TSC1–TSC2 (REFS 108–110). AKT phosphorylates and inactivates TSC1–TSC2, causing activation of RHEB[111–113]. RHEB stimulates the protein kinase mTOR, which results in activating phosphorylation of the mTOR substrates S6 kinase (S6K) and eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1)[114,115], which is consistent with RHEB being essential for cell growth and cell cycle progression in *D. melanogaster*[116]. RHEB may activate mTOR either by directly binding to it[117] or by binding to the mTOR antagonist FKBP38 (also known as FKBP8)[118]. Most importantly, the ability of RHEB to stimulate mTOR depends on its farnesylation[75,114]. Therefore, cancer cells that are addicted to RHEB may be sensitive to FTIs. For example, this could include tumours that overexpress RHEB or that harbour persistently activated pathways that lead to constitutive RHEB activation (including, PTEN deficiency, PI3K and AKT mutations, AKT overexpression or TSC1–TSC2 deficiency). Consistent with this idea, RHEB is frequently upregulated in transformed cells and human cancer cells[75,119], and, in several NSCLC cell lines, the ability of FTIs to inhibit proliferation or induce apoptosis depends on RHEB expression levels, which in turn are correlated to the degree of S6K phosphorylation by

mTOR[77]. Similarly, RHEB is overexpressed in some lymphomas, which have increased mTOR activity and enhanced sensitivity to FTIs[120]. The fact that an exclusively geranylgeranylated mutant of RHEB (RHEB-GG) renders PTEN-deficient lymphoma cells resistant to FTIs further supports the idea that the inhibition of RHEB farnesylation contributes to the antitumour activity of FTIs[120]. Similar results were obtained when RHEB-GG was shown to rescue the ability of FTIs to synergize with paclitaxel[75] and cisplatin[77]. As mTOR is an inhibitor of autophagy[121], and RHEB activates mTOR, it may not be surprising that FTIs can induce autophagy, possibly by blocking RHEB function[122]. The induction of autophagy may be particularly important in cancer cells that are resistant to apoptosis. Collectively, these findings suggest that the inhibition of RHEB farnesylation contributes to the antitumour activity of FTIs, which should perhaps be evaluated in clinical trials for patients with tumours that express high levels of RHEB, as well as for patients with tuberous sclerosis, which is a syndrome caused by the loss of TSC1 or TSC2 function.

Inhibition of RHOB farnesylation is unlikely to contribute to the antitumour activity of FTIs

Under physiological conditions, RHOB is found both geranylgeranylated (RHOB-GG), which accounts for 70% of all RHOB, and farnesylated (RHOB-F), which accounts for the remaining 30%, in cells. It has been suggested that the inhibition of RHOB farnesylation accumulates RHOB-GG and contributes to FTI-induced apoptosis[123,124]. Consistent with this, in murine fibroblasts, RHOB-GG but not RHOB-F, suppresses *RAS*-induced transformation[125]. However, the fact that a large proportion of RHOB is already in the geranylgeranylated form in the absence of FTI treatment argues against a major contribution of inhibition of RHOB farnesylation to FTI antitumour activity. More importantly, in human cancer cells of epithelial origin, both RHOB-F and RHOB-GG have tumour suppressive activity, further arguing against the inhibition of RHOB farnesylation playing a part in FTI antitumour activity[126]. Consistent with RHOB functioning as a tumour suppressor, RHOB is downregulated in several human cancers[127–129]. Although RHOB was shown to be farnesylated *in vitro* by FT, and the treatment of cultured cells with the FTI L-739749 increases the levels of RHOB-GG[130], the laboratory of Goldstein and Brown has shown using purified components that RHOB is farnesylated by GGT1 but not by FT[131]. This, of course, would disqualify RHOB as a target.

However, FTI treatment activates the *RHOB* promoter and accumulates large amounts of the RHOB protein[132]. Furthermore, mutant HRAS-transformed $RHOB^{-/-}$ cells are less sensitive to FTI-induced apoptosis and FTI inhibition of anchorage-dependent but not anchorage-independent tumour growth. Taken together, these results suggest that increased RHOB protein levels, not inhibition of RHOB farnesylation, may contribute to some of the effects of FTIs[133].

Other possible targets

PTIs are bound to have effects not only on oncogenic RAS family members, but also on other prenylated proteins some of which are not yet known. Some candidate targets for prenylation include tumour suppressors, ARHI (also known as NOEY2 and DIRAS3)[134], RAS-related and oestrogen-regulated growth inhibitor (RERG)[135], deleted in breast cancer 2 (DBC2; also known as RHOBTB2)[136], RAS-related inhibitor of cell growth (RIG)[137], RAS-

related protein on chromosome 22 (RRP22; also known as RASL10A)[138] and maybe others. The non-discriminatory action of PTIs may abrogate crucially important tumour suppressor functions and may at least partially compromise their effects on oncogenic pathways. Therefore, it needs to be established whether the proteins mentioned above are exclusively farnesylated or geranylgeranylated, and whether their prenylation is crucial to their growth inhibitory function. For proteins that pass these tests it is recommended that their functional status is assessed in future clinical trials.

The inhibition of the farnesylation of several other proteins could contribute to the antitumour activity of FTIs. These include CENPE, CENPF, the PRL phosphatases PRL1 (also known as PTP4A1), PRL2 (also known as PTP4A2) and PRL3 (also known as PTP4A3), lamins A and B, HDJ2, RND3, peroxisomal biogenesis factor 19 (PEX19), RHOD, RHO6, RHO7 (also known as RHON), TC10 (also known as RHOQ) and prostacyclin receptor (PTGIR). Although some of these may be associated with known effects of FTIs such as the possible role that CENPE and CENPF and PRLs may have in FTI-induced mitotic arrest, the contributions of others require further investigations.

Alternative approaches

RCE1 and ICMT inhibitors

Interestingly, prenylation seems to be constitutive, but prenylated proteins can undergo up to three additional PTMs, the last two of which are reversible (BOX 1). First, the last three C-terminal amino acids, aaX, are proteolytically removed by RAS-converting enzyme 1 (RCE1)[139]. Second, the carboxyl group in the now C-terminal prenylated cysteine is methylated by isoprenylcysteine carboxyl methyltransferase (ICMT)[140]. Third, many prenylated proteins become palmitoylated on upstream cysteines by membrane-bound palmitoyl transferases[141]. The carboxymethylation, which neutralizes the carboxyl negative charge, coupled with the palmitoylation, further stabilizes membrane association and anchoring.

RCE1 and ICMT, which function downstream of PTs, have also attracted attention as potential targets for cancer therapy. Conditional lack of *Rce1* expression in skin carcinoma cells that express activated HRAS has much less severe effects on cell proliferation than the effect of lonafarnib[142]. By contrast, conditional deletion of *Icmt* efficiently blocks transformation by either human KRAS-G12V or human BRAF-V599E oncogenic mutants[140]. Considering these results, ICMT inhibitors are more likely to be successful than RCE1 inhibitors. Indeed, pharmacological inhibition of ICMT inhibits the growth of HepG2 tumour xenografts in nude mice[143]. As postprenylation inhibitors will affect both farnesylated and geranylgeranylated proteins, these drugs may be more toxic than FTIs or GGTIs.

Prenylated proteins that are phosphorylated

Several small GTPases are subject to reversible phosphorylation. For example, protein kinase C (PKC)- or PKA-mediated phosphorylation of CDC42, KRAS, RAP1A and RHOA induces their removal from the cell membrane[144–147]. The question is whether this relocation translates into a loss or change of function. With regard to KRAS, this has

recently been investigated. Interestingly, PKC-mediated phosphorylation of Ser181 in KRAS promotes its relocation to mitochondrial membranes where it associates with BCL-X_L (also known as BCL2L1) and promotes apoptosis. Overexpression of KRAS-S181E is sufficient to induce apoptosis, an effect that is rescued by co-transfection with BCL-2 (REF. 145). This raises the intriguing possibility that PKC agonists such as bryostatin 1[145] may be more efficient for killing and/or less toxic to KRAS-dependent tumours than PTIs. Phosphorylation of RAB6, RHOE and RALA activates their function[148–151]. Most interestingly, aurora kinase A enhances, and protein phosphatase 2A (PP2A) inhibits, the transforming activity of RALA[150,151]. Provided that activating phosphorylation can occur independently of prenylation, these findings may indicate that GGTI-mediated inhibition of RALA may not be sufficient to completely block its transforming ability. However, these findings also suggest that GGTIs that inhibit RALA might synergize with aurora kinase A inhibitors or with PP2A agonists. Phosphorylation of CDC42, RHOB and RHEB inhibits their function[152–154], cautioning against combinations of PTIs and protein kinase inhibitors to target CDC42- or RHEB-dependent tumours.

Synthetic lethality

As is well documented, many human cancers depend on oncogenic KRAS for survival, and this dependency confers a vulnerability that is unique to these cancer cells. Indeed, recent unbiased RNA interference-based screens[155–159] and other approaches[160] identified six genes the knockdown of which kills only human tumours that depend on mutant KRAS. Such a synthetically lethal strategy would be of benefit if some of the identified targets are more druggable than KRAS (see Supplementary information S6 (figure)). Of the six studies cited above, the one identifying the lethal interaction between oncogenic KRAS and CDK4 is particularly interesting[160]. It has been previously reported that RAS-mediated transformation requires the expression of functional RB in mouse fibroblasts[161]. This is consistent with the fact that activated RAS induces cyclin D1 (REF. 162), which activates CDK4 and other kinases and ultimately leads to inhibitory phosphorylation of RB, thus permitting the G1/S transition[163]. These considerations might explain why human tumours very rarely display loss-of-function mutations in RB together with activating RAS mutations[164]. Furthermore, they provide a rationale for investigating whether PTIs and CDK inhibitors act in a synergistic fashion, both in animals and in clinical trials.

Considering the enormous number of proteins affected by the RAS signalling network, we predict that further synthetic lethal interactions will be identified. For example, small interfering RNA (siRNA) screens silencing protein kinases or protein phosphatases may reveal molecular targets the inhibition of which sensitizes cancer cells to PTIs. Conversely, siRNA screens silencing the prenylome may reveal crucial prenylated proteins the inhibition of which sensitizes cancer cells to drugs that target other signal transduction pathways; for example, PI3K–AKT, RAF–MEK–MAPK and CDKs.

Future directions and challenges

Despite the conceptual advances that have been made over the past decade, in our opinion, the major challenge in this field is the following question: which PT substrates are crucial for the proliferation or survival of different cancer types? Or, in other words, does the

antitumour activity of PTIs in a given tumour depend on the inhibition of certain prenylated proteins? At present, the exact size of the prenylome is unknown[165]. Although more than 100 proteins have been experimentally confirmed to undergo prenylation[166], a recent search of the UniProt database (release 20 April 2010; see Further information) returned 587 human genes that encode proteins bearing a C-terminal CXXX motif. Although not all of these proteins will qualify as PT substrates, this suggests that many prenylated proteins have not yet been identified.

Current standard methods to characterize protein prenylation are, for the most part, designed to follow individual proteins[167] and are thus not practical to address the above questions. Therefore, this field should develop and streamline techniques that are capable of analysing prenylation on a global scale, in a manner that is reasonably rapid, feasible and convenient for many laboratories. As a step towards characterizing the entire prenylome, Maurer-Stroh *et al.*[165] have recently developed a sequence-based software suite that is designed to predict whether proteins hitherto unknown to be prenylated are likely to be modified by FT, GGT1 and/or GGT2. Combining this with approaches that uncover actual prenylation patterns in various cancer cells, as well as changes in prenylation patterns in response to PTIs, will eventually reveal which prenylated proteins the inhibition of which is responsible for the antitumour effects of PTIs and which patients are most likely to respond to treatment with these inhibitors. Several techniques pursuing this goal have recently been described[168–173]. For example, labelling cells with modified tractable prenyl donors in lieu of the natural farnesyl diphosphate (FPP) or geranylgeranyl diphosphate (GGPP) is a step in that direction. This can involve the labelling of cells with azido-farnesyl, followed by the affinity purification of farnesylated proteins with a biotinylated phosphine capture reagent[168]. Similarly, labelling cells with azido-GG analogues, followed by the selective labelling of the resulting azido-GG proteins with a modified rhodamine, can be used to detect geranylgeranylated proteins by fluorescent imaging[172].

It is our belief that proteome-wide or prenylome-wide approaches, such as those discussed above, are urgently needed to identify the subsets of prenylated proteins that are affected by FTIs and/or GGTIs, which in turn should help to link the physiological effects of various PTIs to their molecular targets, and thus will help to design improved clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Farnesylation	One of two types of prenylation. This involves the transfer of a farnesyl moiety to the cysteine of the C-terminal CaaX box of the target protein. Catalysed by farnesyltransferase

Geranylgeranylation	This prenylation is catalysed by geranylgeranyltransferase 1 (GGT1) or GGT2. GGT1 transfers a geranylgeranyl moiety to the cysteine of the C-terminal CaaX box, and GGT2 acts on the cysteines of C-terminal CXC or CC motifs
Prenylation	Also known as isoprenylation. An irreversible post-translational modification of proteins consisting of the covalent attachment of an isoprenyl lipid to a cysteine within four residues of the C terminus
Myristoylated	A universal and irreversible co-translational modification of proteins involving the covalent attachment of a myristoyl group to an N-terminal amino acid of a nascent polypeptide. It is important for membrane targeting of the modified protein
CaaX motif	This refers to the last four C-terminal amino acids that serve as a recognition motif for farnesyltransferase or geranylgeranyltransferase 1. C (cysteine) is the amino acid being modified, a is an aliphatic residue and X is any residue
Intimal hyperplasia	The thickening of the innermost layer of a blood vessel as a complication of a reconstruction procedure or endarterectomy. It is the universal response of a vessel to injury and is an important reason for late bypass graft failure, particularly in vein and synthetic vascular grafts
Neointima	A new or thickened layer of arterial intima (innermost layer of an artery or a vein) formed especially on a prosthesis or in atherosclerosis by migration and proliferation of cells from the media
Palmitoylated	A post-translational modification, consisting of the covalent attachment of fatty acids to cysteine residues of membrane proteins, thought to further enhance membrane anchoring of previously prenylated proteins. In contrast to prenylation and myristoylation, it is reversible
Prenylome	The subset of proteins in a cell or organism that is modified by prenylation

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At a glance

- Post-translational modifications with the lipids farnesyl or geranylgeranyl (together referred to as prenyl) are catalysed by farnesyltransferase (FT) or geranylgeranyltransferase 1 (GGT1) and are required for the cellular localization, function and cancer-causing activities of some proteins. Among the hundreds of proteins that are estimated to be prenylated most are either exclusively farnesylated (for example, HRAS and RAS homologue enriched in brain (RHEB)) or geranylgeranylated (for example, RHOA, RHOC, RALA and RALB); some are both farnesylated and geranylgeranylated (RHOB), and others are naturally farnesylated but become geranylgeranylated when FT is inhibited (for example, KRAS and NRAS).
- These and other important observations prompted the design and development of inhibitors of FT (FTIs) and GGT1 (GGTIs) as potential anticancer drugs. Several FTIs have been tested clinically but only one GGTI has recently entered clinical trials.
- Further validation of FT and GGT1 as anticancer drug targets was recently provided by genetic mouse models: conditional loss of FT and/or GGT1 hampers mutant KRAS-induced tumorigenesis and extends the lifespan of mice.
- FTI treatment results in the reversal of several hallmarks of cancer, including mitotic arrest at prometaphase, induction of apoptosis, inhibition of anchorage-dependent and anchorage-independent growth, invasion, angiogenesis and tumour growth, as well as induction of tumour regression in animal models. These effects seem to be mediated by interference with aberrant signal transduction pathways such as RAF–MEK–ERK, PI3K–AKT, and other oncogenic and survival pathways.
- GGTI treatment also results in the reversal of the cancer hallmarks mentioned above except that they block cells in the G1 phase of the cell cycle, and this seems to be owing to their ability to induce the accumulation of the cyclin-dependent kinase (CDK) inhibitors p21 and p27 and to inhibit CDKs and induce hypophosphorylation of RB. GGTI treatment also decreases the levels of phospho-AKT and survivin, and this seems to mediate their ability to induce apoptosis.
- Although in preclinical models FTIs are highly effective as antitumour agents, in clinical trials limited efficacy was observed. This is primarily due to poor patient selection. This in turn is due to our lack of understanding of the mechanism of action of FTIs. In the future, a major effort must be dedicated to identifying the prenylated proteins the inhibition of which is responsible for the antitumour effects of PTIs. This will be of great value not only for enhancing our understanding of the mechanism of action of FTIs and GGTIs, but also for selecting patients whose tumours are addicted to specific prenylated proteins and who are more likely to respond to these agents. Recent advances in

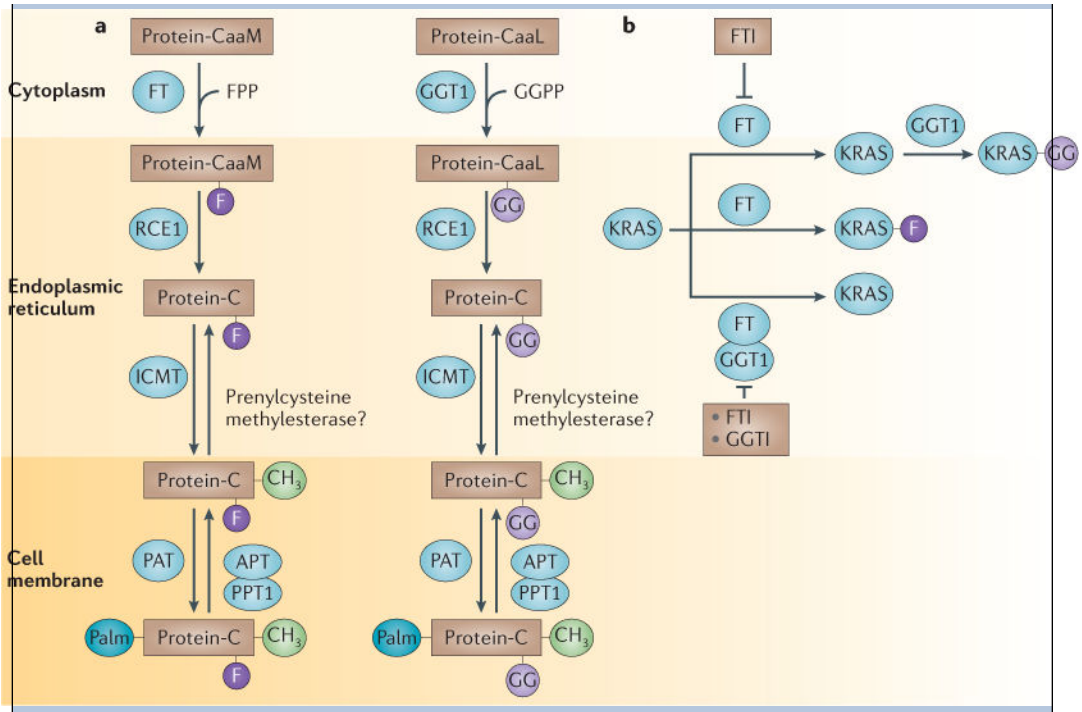
techniques to characterize the human prenylome are likely to accelerate achieving these crucial goals in the prenylation field.

Box 1

Biochemistry of protein prenylation

Prenylation is a universal lipid post-translational modification (PTM) of cysteine residues near the carboxyl terminus that facilitates membrane association[174,175]. Using farnesyl diphosphate (FPP) or geranylgeranyl diphosphate (GGPP) as the lipid donor, this enzymatically catalysed PTM transfers either a C_{15} farnesyl (F) or a C_{20} geranylgeranyl (GG) group to the sulphydryl group of the cysteine residue, thus forming covalent thioether bonds. In eukaryotic cells, prenylation is catalysed by three 'housekeeping' enzymes, farnesyltransferase (FT), gernaylgeranyltransferase 1 (GGT1) and GGT2. We focus on FT and GGT1. FT and GGT1 are cytosolic heterodimeric proteins that share a common α-subunit[176], but that have homologous but distinct β-subunits[177]. The crystal structure of FT shows a crescent-shaped helical hairpin domain and an α–α barrel domain[178]. Both FT and GGT1 are metalloenzymes that require zinc for catalysing the covalent binding of the prenyl group carbon to the CaaX cysteine thiol[179].

Enzyme kinetics and other biochemical studies indicated that FPP first binds to FT, which is followed by the binding of the protein substrate, with prenylation of the substrate occurring much faster than the release of the farnesylated protein product. Proteins modified by FT or GGT1 seem to share a conserved C-terminal CaaX recognition motif (in which C is cysteine, a is an aliphatic amino acid and X is variable). The nature of the C-terminal residue X specifies whether a protein is a substrate for FT or for GGT1: whereas FT prefers X to be methionine, serine, glutamine or cysteine, GGT1 prefers X to be leucine or isoleucine[180]. However, these rules are not absolute: for example, a CaaX protein with a C-terminal phenylalanine can be farnesylated or geranylgeranylated[181]. And although GGT1 clearly prefers X to be leucine[182], some CaaL motifs can also be farnesylated by FT[183]. Furthermore, at least one protein, RHOB (CaaX box sequence: CKVL), is naturally both farnesylated and geranylgeranylated[35]. Proteins with a C-terminal CaaX box can undergo up to three additional PTMs (see part **a** of the figure). Some proteins such as KRAS-4B (CaaX box sequence: CVIM) and NRAS (CaaX box sequence: CVVM) are naturally only farnesylated, but can be geranylgeranylated and remain fully functional in the presence of an FTI (see part **b** of the figure). To block KRAS function would thus require the inhibition of both FT and GGT1 (REFS 12–15). It is not known how many other farnesylated proteins can be cross-prenylated by GGT1, but the presence of a C-terminal methionine seems to be important for the ability of proteins to undergo cross-prenylation.



APT, acylprotein thioesterase; ICMT, isoprenylcysteine methyltransferase; PAT, protein acyltransferase; PPT1, palmitoylthioesterase 1; RCE1, RAS-converting enzyme 1.

Box 2

The use of PTIs in other diseases

An unexpected benefit of farnesyltransferase (FT) inhibitors (FTIs) may be their potential to treat diseases other than cancer. Hutchinson–Gilford progeria syndrome (HGPS) is a genetic disease that is associated with premature ageing and death, normally from heart failure, at about 13 years of age. HGPS is linked to a mutation in *LMNA* that prevents prelamin A (a substrate for FT) from proper maturation into lamin A. Normal processing of prelamin A involves, among other steps, the removal of a carboxy-terminal peptide containing farnesylcysteine. Children with HGPS are unable to perform this step, which results in a mutant form of lamin A, termed progerin[184], which is persistently farnesylated and non-functional[185]. In fibroblasts derived from patients with HGPS and mice, as well as HeLa cells, FTIs can prevent the aberrant nuclear morphology of cells that express progerin[186–188]. In mouse models of HGPS, FTIs greatly improve the phenotype of HGPS with respect to lifespan, body weight and bone integrity[189]. Furthermore, the crucial target for FTI treatment was confirmed to be progerin[190]. These results suggest that FTIs may be beneficial for children with HGPS, and lonafarnib is currently being tested in clinical trials.

A common problem in the treatment of cardiovascular diseases is the hyperproliferation of smooth muscle cells, as seen in restenosis (intimal hyperplasia) of coronary arteries following balloon angioplasty or bypass surgery. Local administration of FTIs or geranylgeranyl transferase inhibitors (GGTIs) can prevent restenosis by blocking neointima formation[191]. GGTIs may also assist in the therapy of cardiovascular diseases by increasing nitric oxide synthase expression[192].

Parasitic diseases such as malaria, Chagas disease, African sleeping sickness, Toxoplasmosis and Leishmaniasis cause millions of deaths in tropical and subtropical regions, and the therapeutic potential of FTIs for these diseases has also been explored[193]. FTIs specifically designed to inhibit parasitic FT and not mammalian FT are significantly more toxic to parasitic protozoa[194,195]. Recently, a GGT1 from *Trypanosoma cruzi*, the parasite responsible for Chagas disease, was cloned[196], and GGTIs may also be effective against these diseases.

FTIs also show strong antiviral activity in mice infected with the hepatitis δ virus[197]. Other diseases that may also be amenable to therapy with prenyltransferase (PT) inhibitors (PTIs) are multiple sclerosis[198] and metabolic bone disorders[199], as well as a wide variety of undesirable fibrotic reactions[200].

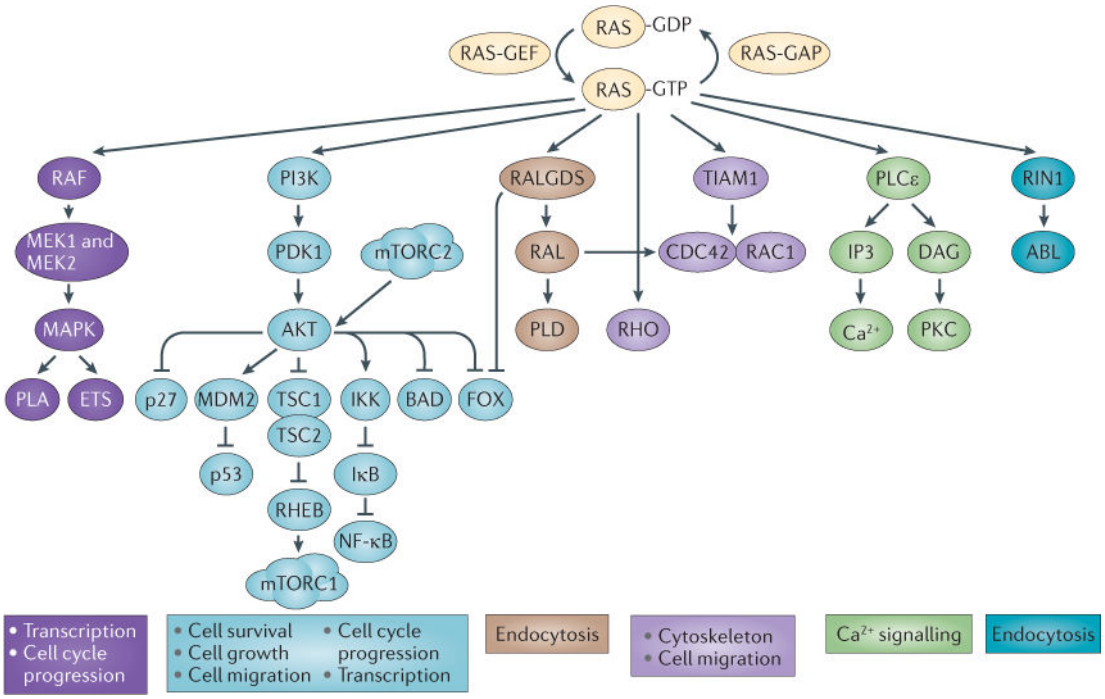
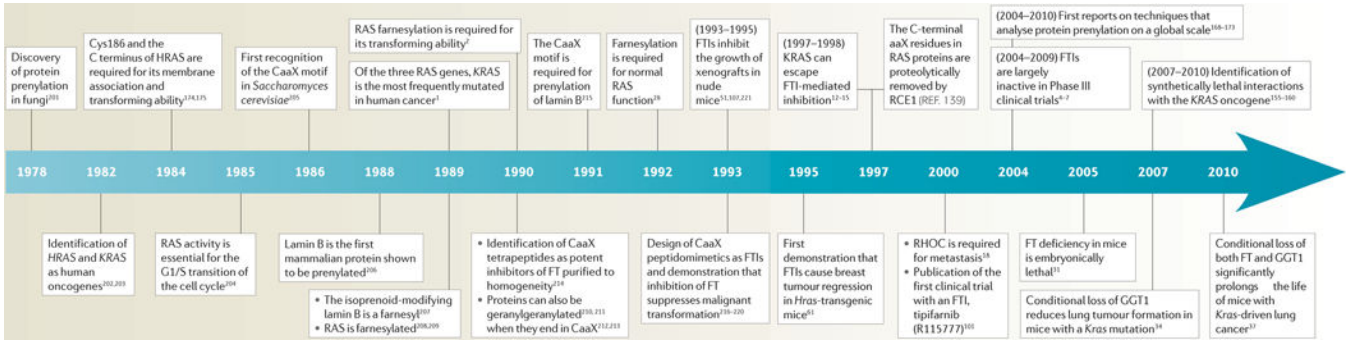


Figure 1. RAS signalling pathways in mammalian cells

Active (farnesylated, membrane-bound and GTP-bound) RAS modulates a number of signalling pathways. Oncogenic RAS mutations tend to lock RAS in its GTP-bound state, resulting in constitutive RAS signalling. The major RAS effector pathways are shown. The two best-studied pathways that are activated by RAS are the RAF–MEK–MAPK signalling cascade and the PI3K–AKT pathway. The RAF–MEK–MAPK pathway ultimately activates the ETS family of transcription factors, which induce multiple genes that promote cell cycle progression and cell migration. Likewise, AKT phosphorylates multiple cellular proteins, leading to the inhibition of several tumour suppressors (such as p27, p53, tuberous sclerosis 1 (TSC1), TSC2 and BCL-2 antagonist of cell death (BAD)) or leading to the activation of several oncogene products. RAS also activates other small GTPases such as RALA and RALB, which have recently been shown to mediate RAS transformation in human pancreatic tumours, for example. Farnesyltransferase inhibitors (FTIs) were originally developed to block the function of RAS. However, as numerous studies *in vitro* and *in vivo* have shown, their antitumour activity is not correlated to the mutation status of KRAS isoforms. This suggests that the antitumour activity of FTIs relies on blocking the activity of other prenylated proteins. However, the inhibition of RAS protein function may still be important, particularly for tumours with mutant HRAS and tumours addicted to wild-type RAS. CDC42, cell division cycle 42; DAG, diacylglycerol; FOX, forkhead transcription factor; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; IKK, IκB kinase; IP3, inositol-1,4,5-trisphosphate; mTORC, mTOR complex; NF-κB, nuclear factor-κB; PDK1, phosphoinositide-dependent kinase 1; PKC, protein kinase C; PLA, phospholipase A; PLCε, phospholipase Cε; PLD, phospholipase D; RALGDS, RAL guanine nucleotide dissociation stimulator; RHEB, RAS homologue enriched in brain; RIN1, RAS and RAB interactor 1; TIAM1, T cell lymphoma invasion and metastasis 1.



Timeline.

Protein prenylation and human cancer

FT, farnesyltransferase; FTI, FT inhibitor; GGT1, geranylgeranyl transferase 1; RCE1, RAS-converting enzyme 1.

Table 1

Effects of PT-targeted gene deletions

Affected gene product	Animal model	Type of gene deletion	Tissue	Phenotypes	Refs
GGT1 β-subunit	*Saccharomyces cerevisiae*	Constitutive	NA	Lethal	32
GGT1 β-subunit	*Drosophila melanogaster*	Constitutive	NA	Lethal	33
FT β-subunit	Mouse	Constitutive	NA	Embryonically lethal	31
		Conditional	Lung	• Delayed wound healing* • No effect on tumour initiation* • Lack of tumour progression*	31
		Conditional	Lung	Lack of HRAS association with cell membranes Delay of KRAS-induced lung tumours	37
		Conditional	Haematopoietic cells	Reduced severity of MPD	38
GGT1 β-subunit	Mouse	Conditional	Lung	**1** Disruption of actin cytoskeleton **2** Reduced cell migration **3** Cell cycle arrest of embryonic fibroblasts **4** Inhibition of KRAS-induced lung tumour formation	34
FT β-subunit and GGT1 β-subunit	Mouse	Conditional	Lung	**1** Significant delay of KRAS-G12D-induced lung tumour onset **2** Increased lifespan	37

FT, farnesyltransferase; GGT1, geranylgeranyltransferase 1; MPD, myeloproliferative disease; NA, not applicable; PT, prenyltransferase.

*Owing to a ‘leaky’ null allele these results have been called into question[36].

Table 2

Clinical trials with FTIs*

Disease	Phase	Patients (median age)	Clinical response and number of patients‡	FT activity or prenylation	Response rate (%)	Other comments
Tipifarnib						
Acute leukaemia	I	34 (63)	2 CR and 8 PR	FT ↓§ and HDJ2 ↓	29.4	No NRAS mutations in patient tumours
Advanced bladder cancer	II	34 (64)	2 PR and 13 SD	ND	5.9	Response rate does not warrant further investigation
Advanced breast cancer	II	76 (54)	9 PR and 9 SD	ND	11.8	All responders had wild-type RAS genes
Advanced colon cancer	II	55 (69)	1 PR, 11 SD, and 31 PD and MD	ND	1.8	Tipifarnib is ineffective
Advanced NSCLC	II	44 (71)	7 SD	HDJ2 ↓ and prelamin A ↓. FT ↓ in 83% of patients	0	No objective response. Future studies should be done with combinations
Advanced solid tumours	I	25 (58)	0 CR, 0 PR, 8 SD, and 17 PD and MD	Data not shown	0	No objective response
Advanced solid tumours	I	9 (53)	1 SD, and 8 PD and MD	ND	0	No objective response
Advanced solid tumours	I	28 (56)	2 PR and 3 SD	ND	7.1	5 of 15 patients had KRAS mutations
Advanced solid tumours	I	21	6 SD	ND	0	No objective response. Phase II trial recommended
AML	II	252 (62)	11 CR and 8 PR	ND	7.5	MS for patients with CR: 369 days
AML	II	145 (74)	22 CR, 3 PR, 50 SD and 58 PD and MD	HDJ2 ↓	17.2	Median duration of CR: 7.3 months
AML	III	228 (76)	18 CR, 20 PR, 105 SD, and 36 PD and MD	ND	16.7	MS: 107 days. 8% of patients had a CR with an MS of 666 days
		229//	0 CR, 3 PR, 130 SD, and 46 PD and MD		1.3	MS: 109 days
Brain tumours	II	81 (11)	2 PR	ND	2.5	Very little activity
CML, myelofibrosis and multiple myeloma		40 (57)	7 CR and 7 PR	ND	17.5	Clinical activity in CML and myelofibrosis
Metastatic pancreatic cancer	II	20 (61)	1 SD	FT ↓ by 50% and HDJ2 ↓ by 33%	0	No objective response. MS: 19.7 weeks
Multiple myeloma	II	36 (62)	0 CR, 0 PR, 23 SD, and 13 PD and MD	FT ↓ and HDJ2 ↓	0	No objective response. No correlation between FT ↓ and disease stabilization

Nat Rev Cancer. Author manuscript; available in PMC 2014 May 28.

Disease	Phase	Patients (median age)	Clinical response and number of patients‡	FT activity or prenylation	Response rate (%)	Other comments
MDS	I	20 (66)	1 CR, 5 PR, and 1 PD and MD	FT ↓ and HDJ2 ↓	30	No correlation between FT ↓ and response. No correlation between RAS mutation status and response
MDS	II	27 (66)	2 CR and 1 PR	ND	11.1	Modest antitumour activity
MDS	II	82 (67)	26 PR and 37 SD	ND	31.7	Median duration of CR: 11.5 months
MDS	I	61 (68)	3 CR and 13 PR	FT ↓ by 75%	26.2	Only one responder with a KRAS mutation. No correlation between FT ↓ and dose
Neuro-fibromatosis and neurofibromas	I	40 (≤15)		FT ↓ by 43% and HDJ2 ↓	0	No objective response
Pancreatic cancer	II	53 (65)		ND	0	No objective response. MS: 2.6 months
Small-cell lung cancer	II	20 (62)	1 SD	ND	0	No objective response. MS: 6.8 months. Progression-free MS: 1.4 months
Advanced colon cancer	III	235 (61)	0 CR, 1 PR, 57 SD, and 155 PD and MD	ND	0.4	MS: 174 days. SD >3 months: 24%
		133¶ (62)	0 CR, 0 PR, 17 SD, and 107 PD and MD		0	MS: 185 days. SD >3 months: 13%
Lonafarnib						
Advanced solid tumours	I	24 (57)	0 CR, 0 PR and 2 SD	ND	0	No objective response
Advanced solid tumours	I	12 (61)	0 CR and 0 PR	Prelamin A ↓	0	No objective response
Advanced solid tumours	I	22 (54)	1 CR and 1 PR	FT ↓	12.5#	Sponsor terminated study early owing to negative interim efficacy. FT ↓ not correlated with response
Advanced solid tumours	II	15 (57)	0 CR, 0 PR and 7 SD	ND	0	No objective response
CML	Pilot	13 (62)	0 CR and 2 PR	ND	15.5	
CNS tumours	I	48 (12)	0 CR, 1 PR and 9 SD	ND	2.1	
Metastatic colon cancer	II	21 (64)	0 CR, 0 PR and 3 SD	ND	0	No objective response
MDS or sAML	II	16 (70)	1 PR	ND	6.7	
NSCLC	II	29 (58)	3 PR, 11 SD, and 15 PD and MD	ND	10.3	MS: 39 months. Well-tolerated. Further clinical trials recommended
Refractory urothelial cancer (transitional cell carcinoma)	II	10 (65)	0 CR, 0 PR, 2 SD, and 8 PD and MD	Small HDJ2 ↓	0	No objective response
Solid tumours	I	20 (59)	1 PR and 8 SD	Prelamin A ↓	5.0	
BMS-214662						

Disease	Phase	Patients (median age)	Clinical response and number of patients‡	FT activity or prenylation	Response rate (%)	Other comments
Acute leukaemia	I	30 (53)	4 CR and 1 PR	Short-lived FT ↓	16.7	
Advanced solid tumours	I	44 (54)	0 CR, 0 PR and 1 SD	Transient FT ↓ by 89.5%	0	No objective response. One patient with pancreatic cancer survived for >3.5 years
Advanced solid tumours	I	68 (60)	0 CR, 0 PR and 5 SD	Short-lived FT ↓	0	No objective response
Advanced solid tumours	I	19 (55)	1 SD, and 18 PD and MD	ND	0	No objective response
Solid tumours	I	25 (57)	1 PR, 16 SR, and 8 PD and MD	Short-lived FT ↓	4.0	Response was minor

AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; CNS, central nervous system; CR, complete response; FNTB, farnesyltransferase β-subunit; FT, farnesyltransferase; FTIs, FT inhibitors; GGTI, geranylgeranyltransferase 1 inhibitor; HI, haematological improvement; MD, metastatic disease; MDS, myelodysplastic syndrome; MS, median survival; ND, not determined; NSCLC, non-small-cell lung cancer; OS, overall survival; PD, progressive disease; PR, partial response; sAML, secondary acute myeloid leukaemia; SD, stable disease.

*See Supplementary information S4 (table) for a table with references. This table only considers FTIs because, to our knowledge, only one GGTI, GGTI-2418, is currently in clinical trials. Studies that did not evaluate tumour response are not included. Median ages are rounded to the closest integer. The number of evaluable patients is stated whenever possible. The response rate was calculated by dividing the sum of complete and partial responses by the number of evaluable patients.

‡PR includes haematological improvement.

§Downward arrows indicate a reduction of enzyme activity (in the case of FT) or a reduction in farnesylation (in the case of HDJ2 or prelamin A).

//This patient cohort received best supportive care.

¶In this study all patients received best supportive care, with 235 receiving tipifarnib and 133 receiving a placebo.

#Number differs from the authors' calculation as they included SD, resulting in a response rate of 37.5%.

Table 3

Clinical trials with combinations including FTIs*

Drugs	Disease	Phase	Patients (median age)	Clinical response and number of patients‡	FT activity or prenylation	Response rate (%)	Other comments
Tipifarnib + capecitabine	Advanced solid tumours	I	41 (57)	5 PR and 11 SD	FT ↓§ and HDJ2 ↓	12.2	No correlation between FT ↓ and response
Tipifarnib + doxorubicin + cyclophosphamide	Advanced breast cancer	I and II	32 (51)	7 CR	FT ↓ by 55–100%	21.9	
	Stage IIB–IIIC breast cancer	II	44 (51)	11 CR	Median FT ↓ by 91%	25	
Tipifarnib + etoposide	AML	I	84 (77)	20 CR	p-S6 ↓	23.4	
Tipifarnib + gemcitabine	Advanced solid tumours	I	19 (59)	2 PR	HDJ2 ↓	10.5	
Tipifarnib + gemcitabine	Advanced pancreatic cancer	III	341 (61)	6 CR, 6 PR, 53 SD, and 28 PD and MD	ND	1.8	MS: 193 days. 6-month survival: 53%. 1-year survival: 27%
			347// (62)	8 CR, 8 PR, 52 SD, and 30 PD and MD	ND	2.3	MS: 182 days. 6-month survival: 49%. 1-year survival: 24%
Tipifarnib + gemcitabine + cisplatin	Advanced solid tumours	I	27 (58)	1 CR and 8 PR	Prelamin A ↓	33.3	
	Advanced solid tumours	I	31 (58)	8 PR and 12 SD	ND	25.8	Phase II trial recommended
Tipifarnib + idarubicin + cytarabine	AML and MDS	I/II	95 (50)	61# CR and 9 PR	ND	74	MS: 17 months
			108¶ (52)	65#		70	MS: 13 months
Tipifarnib + imatinib	CML	I	25 (62)	17 PR	ND	68	11 patients withdrew (lack of response)
Tipifarnib + irinotecan	Solid tumours	I	35 (52)	3 PR, 14 SD, and 13 PD and MD	ND	8.6	
Tipifarnib + letrozole	Advanced breast cancer	II	74 (60)	3 CR, 19 PR, 29 SD, and 23 PD and MD	ND	29.7	No improvement by tipifarnib
			39// (61)	1 CR, 14 PR, 15 SD, and 9 PD and MD	ND	38.5	
Tipifarnib + sorafenib	Advanced solid tumours	I	43 (56)	3 PR, 15 SD, and 20 PD and MD	25% of patients with >50% FT ↓	7.0	
Tipifarnib + tamoxifen	Metastatic breast cancer	I	12 (50)	2 PR and 1 SD	FT ↓ 42–54%	16.7	

Drugs	Disease	Phase	Patients (median age)	Clinical response and number of patients‡	FT activity or prenylation	Response rate (%)	Other comments
Lonafarnib + carboplatin + paclitaxel	Advanced NSCLC	III	308 (unknown)		NA	NA	OS: 144 days. TTP: 137 days
			308// (unknown)			NA	OS: 168 days. TTP: 152 days
Lonafarnib + docetaxel	Advanced solid tumours	I	29	1 CR and 6 SD		3.4	Response (CR + SD) correlates with low FNTB mRNA levels
Lonafarnib + gemcitabine	Advanced bladder cancer	II	31 (64)	1 CR and 9 PR	ND	32.3	MS: 11.5 months. TTP: 7 months
Lonafarnib + imatinib	CML	I	23 (55)	6 CR and 2 PR	ND	34.7	
Lonafarnib + paclitaxel	Solid tumours	I	21 (60)	6 PR	ND	28.6	6 patients previously treated
BMS-214662 + cisplatin	Advanced solid tumours	I	23 (57)	15 SD	Short-lived FT ↓	0	No objective response
BMS-214662 + paclitaxel	Advanced solid tumours	I	26 (60)	2? PR	Short-lived FT ↓	≤7.7	
BMS-214662 + paclitaxel + carboplatin	Advanced solid tumours	I	30 (58)	3 PR and 8 SD	Short-lived FT ↓ and HDJ2 ↓	10.0	No correlation between dose and HDJ2 ↓
L-778,123 + radiotherapy	Advanced solid tumours	I	7 (59)	5 CR, 1 PR, and 6 PD and MD	ND	85.7	No RAS mutations
	Advanced pancreatic cancer	I	10 (59)	1 PR, 5 SD, and 4 PD and MD	HDJ2 ↓	10.0	3 out of 4 patients examined have KRAS mutations

AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; CR, complete response; FNTB, farnesyltransferase β-subunit; FT, farnesyltransferase; FTIs, FT inhibitors; GGTI, geranylgeranyltransferase 1 inhibitor; HI, haematological improvement; MD, metastatic disease; MDS, myelodysplastic syndrome; MS, median survival; NA, not applicable; ND, not determined; NSCLC, non-small-cell lung cancer; OS, overall survival; p, phosphorylated; PD, progressive disease; PR, partial response; S6, ribosomal protein S6; SD, stable disease; TTP, median time to progression.

*See Supplementary information S5 (table) for a table with references. This table only considers FTIs because, to our knowledge, only one GGTI, GGTI-2418, is currently in clinical trials. Studies that did not evaluate tumour response are not included. Median ages are rounded to the closest integer. The number of evaluable patients is stated whenever possible. The response rate was calculated by dividing the sum of complete and partial responses by the number of evaluable patients.

‡PR includes haematological improvement.

§Downward arrows indicate a reduction of enzyme activity (in the case of FT) or a reduction in farnesylation (in the case of HDJ2 or prelamin A).

//This patient cohort received a placebo instead of the FTI.

¶This patient cohort received idarubicin plus cytarabine (referred to by the authors as a historical control).

#As the reference only provides percentage response rates, the patient numbers were calculated.