thematic review

Thematic review series: Lipid Posttranslational Modifications

Farnesyl transferase inhibitors

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Abstract Some proteins undergo posttranslational modification by the addition of an isoprenyl lipid (farnesyl- or geranylgeranyl-isoprenoid) to a cysteine residue proximal to the C terminus. Protein isoprenylation promotes membrane association and contributes to protein-protein interactions. Farnesylated proteins include small GTPases, tyrosine phosphatases, nuclear lamina, cochaperones, and centromere-associated proteins. Prenylation is required for the transforming activity of Ras. Because of the high frequency of Ras mutations in cancer, farnesyl transferase inhibitors (FTIs) were investigated as a means to antagonize Ras function. Evaluation of FTIs led to the finding that both K- and N-Ras are alternatively modified by geranylgeranyl prenyltransferase-1 in FTI-treated cells. Geranylgeranylated forms of Ras retain the ability to associate with the plasma membrane and activate substrates. Despite this, FTIs are effective at inhibiting the growth of human tumor cells in vitro, suggesting that activity is dependent on blocking the farnesylation of other proteins. FTIs also inhibit the in vivo growth of human tumor xenografts and sensitize these models to chemotherapeutics, most notably taxanes. Several FTIs have entered clinical trials for various cancer indications. In some clinical settings, primarily hematologic malignancies, FTIs have displayed evidence of single-agent activity. Clinical studies in progress are exploring the antitumor activity of FTIs as single agents and in combination. This review will summarize the basic biology of FTIs, their antitumor activity in preclinical models, and the current status of clinical studies with these agents.—Basso, A. D., P. Kirschmeier, and W. R. Bishop. **Farnesyl transferase inhibitors.** ***J. Lipid Res.*** **2006.** 47: **15–31.**

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Mammalian cells express three protein prenyltransferases. Farnesyl protein transferase (FTase) catalyzes the transfer of a 15 carbon isoprenyl lipid from farnesyl diphosphate (FPP) onto a cysteine residue in the C-terminal CaaX box (C = cysteine, a = aliphatic amino acid, and X = C-terminal amino acid) of various proteins. CaaX-containing proteins terminating in serine, methionine, glutamine, and alanine are recognized by FTase and include H-Ras, N-Ras, K-Ras, prelamin A, HDJ2, PTP-CAAX/PRL tyrosine phosphatases, RhoB and other Rho family GTPases, Rheb, CENP-E, and CENP-F (1–9). Proteins with a C-terminal leucine residue (CaaL) are modified with a 20 carbon isoprene by the related enzyme geranylgeranyl prenyltransferase-1 (GGTase-1) (2). A third protein prenyltransferase, GGTase-2, catalyzes the addition of the geranylgeranyl isoprene onto the C terminus of the Rab family of small GTPases. Rab proteins regulate intracellular vesicular traffic and typically contain two cysteine residues adjacent to their C termini (XXCC, XCXC, or CCXX) (10).

Both FTase and GGTase-1 are composed of α and β subunits. The α subunit is common to both enzymes, whereas distinct β subunits impart isoprene and protein substrate selectivity (11). Enzyme activity requires zinc, which activates the thiolate of the cysteine in the peptide substrate for nucleophilic attack of the farnesyl group (12). Substrate binding to FTase is ordered binding to FPP occurs first, followed by binding of the CaaX peptide (13). The prenylation reaction step is relatively fast, followed by rate-limiting release of the farnesylated protein product (13). High-resolution X-ray structures of various catalytic states of FTase have been solved (14–18).

Because of the important role ascribed to Ras in human malignancies (19), Ras proteins are considered important points for potential intervention in anticancer drug discovery (see below). With the elucidation of the Ras posttranslational modification pathway in the late 1980s, FTase became a logical pharmacologic target to affect Ras function, and the search for farnesyl transferase inhibitors (FTIs) was initiated. Initial approaches focused on the identification of CaaX-peptide inhibitors of FTase, which were competitive with the protein substrate (20). Some CaaX tetrapeptides acted as alternative substrates some of these tetrapeptides (e.g., Cys-Val-Phe-Met) were true inhibitors in that they were not substrates for farnesylation (1, 20–22). These peptides were not efficiently taken up into cells and were subject to rapid intracellular degra-

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dation. CaaX-peptidomimetic inhibitors, in which the aliphatic aa portion of the CaaX tetrapeptide was replaced by benzodiazepine (C-BZA-M) or aminomethylbenzoic acid (C-AMBA-M), were effective inhibitors of FTase and were more stable than the tetrapeptides (23–26). Additional FTIs were designed to be competitive with FPP (27–29). Small molecule inhibitors of FTase were identified through high-throughput screening of chemical libraries followed by structure-directed medicinal chemistry optimization. These include two of the compounds that have advanced into clinical evaluation, lonafarnib and tipifarnib.

Lonafarnib (SCH 66336) is a nonpeptidic CaaX-competitive selective inhibitor of FTase (FTase IC_{50} = 1.9 nM, GGTase-1 IC_{50} > 50,000 nM) (30, 31). R115777 (tipifarnib) inhibits the FTase prenylation of K-Ras in vitro with an IC_{50} of 7.9 nM, also with high selectivity versus GGTase-1 (40% inhibition at 50,000 nM) (32). Similarly, BMS-214662 is a selective inhibitor of FTase (FTase IC_{50} = 1.35 nM, GGTase-1 IC_{50} > 1,000 nM) (33). A recent study showed that analogs of BMS-214662 also inhibited Rab GGTase-2, although the activity of BMS-214662 against this enzyme was not reported (34). L-778,123 is less selective for FTase (FTase IC_{50} = 2 nM, GGTase-1 IC_{50} = 98 nM) (35) (**Fig. 1**).

Ras AS A TARGET OF FTase

There are three *ras* genes encoding four Ras proteins (H-Ras, K-Ras4A, K-Ras4B, and N-Ras). Ras is a small GTPase that binds and hydrolyzes GTP and can exist in an active (Ras-GTP) or inactive (Ras-GDP) state. Ras-GTP positively regulates cell growth. Ras is required for serum-stimulated cell growth and entry into the S phase of the cell cycle (36). The critical role of Ras in oncogenic transformation was demonstrated using various methods, including expression of dominant-negative forms of Ras and homologous recombination to disrupt activated *ras* genes in human tumor cell lines (37, 38). Upon tyrosine kinase receptor activation, Grb2 binds the phosphorylated receptor and recruits SOS, a Ras guanine nucleotide exchange factor that stimulates Ras GTP binding (39, 40).

Ras activates several signal transduction pathways, including the Raf-MEK-ERK kinase cascade. Activated Ras recruits Raf to the plasma membrane, an essential event for Raf activation (41). Forms of Raf engineered to be directly targeted to the plasma membrane by addition of a CaaX motif are activated in a Ras-independent manner (42). After Raf activation, there is sequential activation of the downstream kinases MEK1,2 and ERK1,2 (MAPK1,2). ERK phosphorylates several substrate proteins, including the Elk-1 transcription factors (43–45). Ras also activates other signaling pathways, including phosphatidylinositol 3-kinase (PI3K) and Ral-guanine nucleotide exchange factor (46, 47). Activation of these signal transduction pathways by Ras is critical for cell growth and survival.

There is a high frequency of activating mutations in the *ras* genes (codons 12, 13, and 61) in human cancer. These activating mutations encode Ras proteins with suppressed GTPase activity, allowing Ras to remain in the active GTP-bound state independent of upstream activation (19). This results in constitutive signal transduction by GTP-Ras to downstream effectors. Activating mutations in K-Ras are prevalent in some epithelial cancers, including pancreatic cancer (>90%), colorectal cancer (~50%), and lung cancer (~30%). Activating mutations in N-Ras occur in melanoma (10–20%) and some hematologic malignancies. Mutations in H-Ras are rare in human cancer but have been reported in 15–20% of bladder cancers (19). Ras

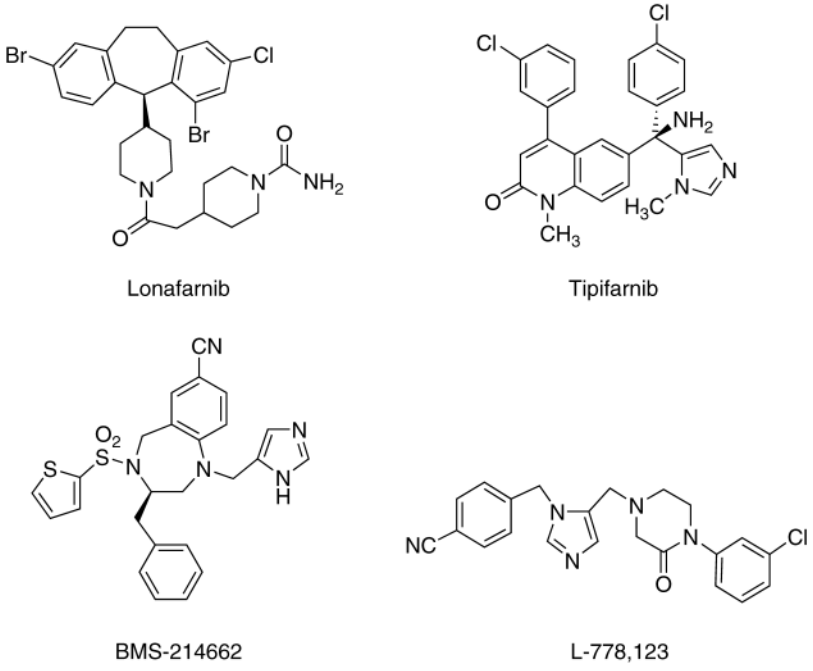

Fig. 1. Farnesyl transferase inhibitors (FTIs).

mutations are rare in some cancers (e.g., breast cancer and glioma) however, Ras signaling is frequently upregulated in these tumors as a result of the activation of upstream growth factor signaling pathways.

Ras is posttranslationally modified by several sequential enzymatic steps. After farnesylation of the CaaX-box cysteine, many farnesylated proteins are cleaved by Ras-converting enzyme (Rce1), a protease that removes the three C-terminal amino acids (aaX). Carboxymethylation of the farnesylated cysteine is catalyzed by isoprenylcysteine methyltransferase (ICMT) in a reaction using *S*-adenosylmethionine as the methyl group donor (48). Both Rce1 and ICMT are associated with the endoplasmic reticulum. These reactions are important in imparting additional hydrophobic character onto the C terminus. After these reactions, both H-Ras and N-Ras undergo a further lipid modification by the addition of palmitoyl residues adjacent to the farnesyl group in a reaction catalyzed by protein palmitoyl transferase (49). Palmitoylation serves to further increase the membrane affinity of fully modified Ras. K-Ras4B does not undergo palmitoylation but contains a highly basic stretch of amino acids adjacent to the farnesylated cysteine, which serves to strengthen membrane affinity by electrostatic interactions with the membrane surface. Mature forms of Ras are associated with the plasma membrane. H- and N-Ras are transported from the endoplasmic reticulum to the plasma membrane along the secretory pathway, whereas K-Ras may be transported by a distinct route.

The role of each posttranslational modification was evaluated using in vitro translated proteins containing various modifications and using lipid-modified peptides (50). Farnesylated, noncleaved, nonmethylated Ras associates inefficiently with membranes. Cleavage of the C-terminal three amino acids enhances membrane association by 2-fold. Cleavage of the aaX residues followed by carboxyl methylation enhances membrane association by 4-fold (51). Conversely, Cre-mediated loss of Rce1 in fibroblasts eliminates the endoproteolytic processing of Ras and results in the partial mislocalization of Ras to the cytoplasmic fraction (52). Similarly, ICMT null embryonic stem cells lack the ability to methylate farnesylated Ras and subsequently have mislocalized Ras (53). The presence of an upstream polybasic domain or palmitoylation is required for full membrane association (51). The effect of lipid modification on Ras localization in various intracellular membrane compartments and subdomains was reviewed recently (54).

All Ras isoforms are farnesylated in vitro and in cells (3), with K-Ras being a higher affinity substrate for FTase than H-Ras (55, 56). In in vitro biochemical assays, both N-Ras and K-Ras, but not H-Ras, are weak substrates for GGTase-1 (56). In cell culture, FTIs prevent H-Ras farnesylation, membrane localization, and reverse H-Ras-induced cellular transformation (30, 57). In addition, FTIs induce tumor regressions in MMTV-vH-Ras (58) and WAP-H-Ras transgenic mice without causing any systemic toxicity.

In contrast to the dramatic effects of FTIs on H-Ras function, K- and N-Ras are alternatively prenylated by

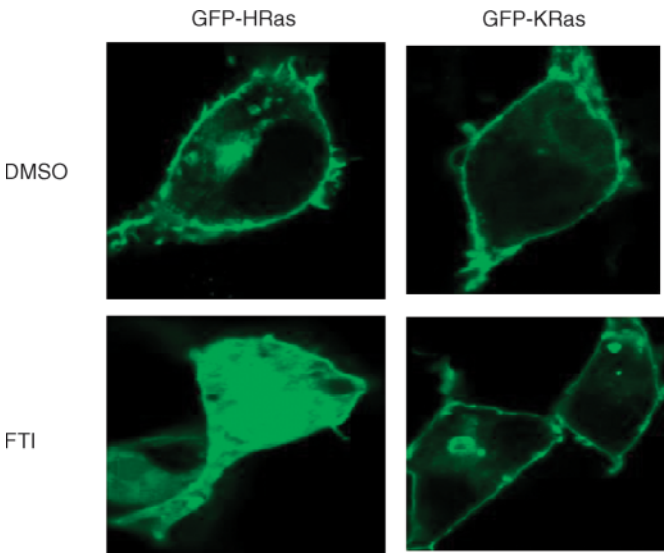

Fig. 2. FTIs disrupt the membrane localization of H-Ras but not K-Ras. Cos cells transfected with green fluorescent protein (GFP)-Ras were treated with DMSO or 1 μM lonafarnib (SCH 66336) for 48 h immediately after transfection. FTI treatment results in a relocalization of GFP-H-Ras from the plasma membrane to the cytosol. However, GFP-K-Ras remains associated with the plasma membrane in FTI-treated cells. Under these conditions, the isoprene content of K-Ras is entirely shifted to geranylgeranyl (59).

GGTase-1 in FTI-treated cells (55, 59). This alternative prenylation results in persistent membrane association for K-Ras and N-Ras in FTI-treated cells (**Fig. 2**), although the alternative prenylation of N-Ras appears to be less efficient than that of K-Ras, resulting in some observable accumulation of soluble N-Ras in some cell types (unpublished data). K-Ras-mediated activation of ERK/MAPK is also not inhibited by FTIs in cells (60, 61). Despite the failure to abrogate the prenylation of K- and N-Ras, FTIs block the growth of numerous tumors in preclinical models, including K- and N-Ras transgenic onco-mouse models (62–64). Unlike the large tumor regressions observed in H-Ras transgenic models, FTIs induce modest regressions in N-Ras transgenic models and complete tumor growth inhibition, but not regressions, in K-Ras transgenic models. Activity in these models in the face of alternative prenylation suggests that the antitumor activity of FTIs is dependent upon blocking the farnesylation of proteins other than K-Ras and N-Ras.

There are a number of candidate farnesylated proteins whose inhibition may contribute to this activity. We and others have surveyed many farnesylated proteins to determine whether they are subject to alternative prenylation (**Table 1**). Proteins that are not geranylgeranylated in FTI-treated cells are more likely to play a critical role in the biological response to FTIs (although it cannot be ruled out that switching of the isoprene content of some proteins results in subtle changes in function). Among these candidate proteins is the H-Ras protein. H-Ras function is completely inhibited by FTIs, and this inhibition may play

TABLE 1. Farnesylated proteins

Protein	CAAX	Alternatively Prenylated? (Reference)	Function
H-Ras	CSVL	No (56)	GTPase/signal transduction
Rheb	CSVM	No (77)	GTPase/signal transduction
Rheb2	CHLM	No (77)	GTPase/signal transduction
HDJ2	CQTS	No (5)	Cochaperone
Prelamin A	CSIM	No (120)	Nuclear envelope protein
CENP E	CKTQ	No (9)	Kinesin motor protein
CENP F	CKVQ	No (9)	Chromosome passenger protein
K-Ras	CVIM	Yes (56)	GTPase/signal transduction
N-Ras	CVVM	Yes (56)	GTPase/signal transduction
RhoB	CKVL	Yes (65)	GTPase/signal transduction
PRL1	CCIQ	Yes (inefficient) (108)	Tyrosine phosphatase
PRL2	CCVQ	Yes (inefficient) (108)	Tyrosine phosphatase
PRL3	CCVM	Yes (inefficient) (108)	Tyrosine phosphatase
RhoD	CCVT	Yes (unpublished data)	GTPase
Rho6	CSIM	Yes (unpublished data)	GTPase
Rho7	CNLM	Yes (unpublished data)	GTPase
Tc10	CLIT	Yes (unpublished data)	GTPase

a role in the antitumor activity of FTIs in some tumors in which wild-type H-Ras functions in signal transmission.

ROLE OF FTase TARGETS OTHER THAN Ras

RhoB

RhoB is an unusual protein in that it exists in both farnesylated and geranylgeranylated forms in non-FTI-treated cells. The geranylgeranylated form of RhoB (RhoB-GG) is more abundant, accounting for up to 70% of the total RhoB protein in untreated cells (7). In the presence of FTIs, all of the cellular RhoB is geranylgeranylated (65). Several lines of evidence suggest that inhibition of RhoB farnesylation may contribute to the antitumor activity of FTIs. RhoB expression in Rat1 cells stimulates proliferation, and this can be inhibited by FTIs (66). Moreover, ectopic expression of a myristoylated form of RhoB in Ras-transformed Rat1 cells leads to FTI resistance (66). Unlike Ras, which has a half-life of ~24 h (67), the half-life of RhoB is ~2 h (66). It has been suggested that this short half-life corresponds to the rapid morphological reversion observed in some FTI-treated cells (66).

Some of the responses to FTI treatment may be a result of a loss of farnesylated RhoB (RhoB-F), a gain of RhoB-GG, or both. Some studies have suggested functional differences between RhoB-F and RhoB-GG and that FTI treatment results in a gain of growth-inhibitory function for RhoB-GG (65). Supporting a growth-inhibitory role of RhoB-GG, overexpression of a form of RhoB engineered to be only geranylgeranylated reverses the transformed phenotype of Ras-transformed Rat1 fibroblasts (68). Furthermore, in Ras-transformed NIH-3T3 cells, RhoB-F enhanced and RhoB-GG suppressed cell growth. In these cells, RhoB-GG, but not RhoB-F, inhibited the Ras-induced activation of Akt and NFκB (69). Recently, it was found that RhoB-GG inhibits expression from the cyclin B promoter and that under low serum conditions FTI treatment reduced cyclin B levels, leading to cell cycle arrest and apoptosis (70).

Despite these intriguing observations, other studies have suggested that RhoB is not a critical target of FTIs. Most studies with RhoB have been performed in fibroblasts and may not reflect RhoB function in epithelial cells. Expression of wild-type RhoB, RhoB-F, or RhoB-GG was reported to inhibit cell focus formation and growth in soft agar of the human pancreatic tumor cell line Panc-1 (71, 72). In addition, FTIs were found to inhibit the anchorage-independent growth of RhoB null cells (73, 74). The contribution of RhoB to the biological response to FTIs remains controversial. Final resolution of this controversy may require precise definition of the cellular functions of the two prenylated forms of RhoB.

Rheb

Rheb (Ras homolog enriched in brain) is a GTPase identified as a protein upregulated in rat brain by synaptic activity and growth factors (75). Two highly related human Rheb proteins exist, Rheb1 and Rheb2. Both forms of Rheb are ubiquitously expressed in human tissue and are upregulated in transformed cells and human tumor cell lines (76, 77). Tuberous sclerosis complex TSC1/TSC2 (hamartin/tuberin) serves as a GTPase-activating protein (GAP) for Rheb. GAPs accelerate the rate of GTP hydrolysis by their target proteins and promote the formation of the inactive GDP-bound form therefore, TSC1/TSC2 regulates Rheb function and reduces the level of activated Rheb-GTP (78–81). Mutations in TSC1/TSC2 result in a loss of its tumor-suppressor function. These mutations cause tuberous sclerosis, an autosomal dominant genetic disorder that occurs in 1 in 6,000 people. Tuberous sclerosis is characterized by benign tumors in the brain, heart, kidney, skin, and eyes, and common clinical manifestations of tuberous sclerosis include seizures, mental retardation, autism, and organ failure (82, 83).

The TSC2/TSC1 complex is negatively regulated by Akt-mediated phosphorylation of tuberin as a result, Rheb activity is increased (84–88). The TSC2/TSC1 complex is positively regulated by the tumor-suppressor protein LKB kinase (89, 90). Mutations in LKB lead to enhanced Rheb activity and are associated with Peutz-Jeghers syndrome, an autosomal dominant inherited disorder that leads to gastrointestinal polyps and predisposes people to various tumors (91, 92).

Rheb positively regulates mTOR signaling, and overexpression of Rheb induces phosphorylation of mTOR substrates, S6 kinase, and 4EBP-1 (93, 94). Recently, Rheb was demonstrated to directly bind mTOR and to enhance

its activity (95, 96). As a consequence, Rheb overexpression in *Drosophila* causes overgrowth of multiple tissues as a result of increased cell size (97–99). Both Rheb1 and Rheb2 are farnesylated proteins, and upon treatment with FTI-277, Rheb-induced phosphorylation of S6 kinase is inhibited (8, 93). Human Rheb1 and Rheb2 are in vitro substrates for FTase, but not GGTase-1, and treatment of cells with FTIs, including lonafarnib, completely inhibits prenylation of Rheb (77). Ectopic expression of a Rheb construct engineered to be geranylgeranylated (CSVM → CSVL) abrogates lonafarnib enhancement of tamoxifen- and taxane-induced apoptosis. Additionally, expression of dominant negative Rheb or Rheb short interfering RNA (siRNA) in human tumor cells mimics lonafarnib treatment in sensitizing tumor cells to tamoxifen- and taxane-induced apoptosis (77). These studies suggest that inhibition of Rheb farnesylation may play a role in FTI enhancement of the antitumor response to other chemotherapeutics.

These observations also suggest that FTIs may have utility in treating benign tumors in patients with tuberous sclerosis. Recently, it was demonstrated that treatment of TSC-null mouse embryonic fibroblasts with the FTI BMS-214662 inhibits their growth in serum-free medium. Furthermore, TSC-null cells display an altered, rounded morphology and contain actin filaments predominantly at the cell periphery. FTI treatment also reverses this abnormal morphology and leads to the reappearance of intracellular actin filaments (100). Interestingly, the mTOR inhibitor rapamycin also blocked the serum-free growth of these cells but did not result in normalization of the actin cytoskeleton. This suggests that FTIs lead to a more complete inhibition of the Rheb signaling pathway than do mTOR/Raptor inhibitors such as rapamycin. FTIs inhibit signaling downstream of Rheb, including both mTOR/Raptor, which regulates growth, and mTOR/Rictor, which regulates the actin cytoskeleton.

CENP-E and CENP-F

CENP-E is a centromere-associated kinesin motor protein that functions in microtubule attachment to kinetochores, an event needed for the segregation of sister chromatids during mitosis (101, 102). CENP-E is also essential for positioning chromosomes at the metaphase plate. CENP-F is a cell cycle-regulated chromosome passenger protein that functions in mitosis (103). Both CENP-E and CENP-F are farnesylated proteins not subject to alternative prenylation. Peptides derived from the C termini of these proteins are in vitro substrates for FTase, but not GGTase-1, and lonafarnib treatment of cells inhibits the prenylation of both proteins (9). Interestingly, FTI-treated cells have defects in both the alignment of chromosomes at the metaphase plate and the formation of bipolar spindles (104). In cells treated with FTIs, CENP-E and CENP-F still localize to kinetochores during prometaphase (9, 104). However, it has been reported that FTIs reduce the levels of CENP-F at the kinetochores (105). FTIs also block CENP-E association with microtubules (9). Inhibition of the farnesylation of CENP-E and CENP-F may alter their function and may help explain the failure of chromosomes to properly align in metaphase and the subsequent accumulation of some human tumor cells in the G2/M phase of the cell cycle in response to FTI treatment. Recent experiments showed that ectopic expression of the kinetochore binding domain of CENP-F (C terminus) delayed progression through G2/M (105). This phenomenon was dependent upon farnesylation of the ectopically expressed peptide, suggesting that a fully functional farnesylated CENP-F is required for efficient G2/M progression. Because of the role of CENP-E and CENP-F in mitotic spindle function, inhibition of their farnesylation may contribute to the synergistic interaction observed between taxanes and FTIs. Further studies are needed to define the role of CENP proteins versus the role of Rheb in the interaction between taxanes and FTIs.

PTP-CAAX/PRL

The PTP-CAAX or PRL family of protein tyrosine phosphatases plays a role in regulating cell growth and mitosis. The PRL family includes three members, all of which are farnesylated proteins (106). PRL1 mRNA is increased in numerous human tumor cell lines, and a role for PRL in tumorigenesis has been demonstrated (6). D27 pancreatic ductal cells can be transformed by ectopic expression of PRL1 or PRL2, and these transformed cells are tumorigenic in nude mice (106). Additionally, CHO cells overexpressing PRL1 or PRL3 exhibit enhanced motility, as seen in transwell migration and wound-healing assays. In contrast to the control cells, PRL1-overexpressing CHO cells exhibit a metastatic phenotype in mice (107).

In vitro, incubation of HeLa cell lysates with [^{3}H]FPP results in labeling of PRL1, PRL2, and PRL3. PRL1 and PRL2 can also be labeled with [^{3}H]geranylgeranyl diphosphate in this system, suggesting that these proteins may be susceptible to alternative prenylation (108). When PRL1 is transiently overexpressed in Cos monkey kidney cells, its labeling with [^{3}H]mevalonic acid is greatly reduced by FTI treatment but, in contrast to H-Ras, PRL1 labeling is not completely abolished (P. Kirschmeier, unpublished data). This is consistent with the inefficient alternative prenylation of PRL1 in cells.

In transfected CHO cells, all three forms of PRL are localized to the plasma membrane. Upon FTI treatment, these proteins are reported to relocalize to the nucleus and intracellular punctate structures (107). These experiments were performed with cells overexpressing PRL. When endogenous PRL1 was studied by microscopy using PRL antibodies, a different story was revealed. PRL1 was found to be localized to the endoplasmic reticulum and to relocalize to the mitotic spindle and centrosomes during mitosis. Localization to the endoplasmic reticulum, but not to the spindle, was disrupted by FTI treatment or by mutation of the CaaX box to a sequence that cannot be prenylated (CCIQ → SCIQ). Cells ectopically expressing PRL1-SCIQ display defects in mitosis and cytokinesis characterized by chromosome bridges and lagging chromosomes. Cells expressing mutant PRL1 properly arrest in

mitosis after nocodazole treatment, suggesting that farnesylated PRL1 is required for proper spindle dynamics rather than the spindle checkpoint (6). Thus, inhibition of PRL farnesylation may in part account for FTI-induced accumulation of cells in the G2/M phase of the cell cycle.

HDJ

Human DnaJ homologs include HDJ2, HSJ1, and HDJ1/Hsp40. Similar to DnaJ, these proteins serve as cochaperones and stimulate the ATPase activity of Hsp70 (109, 110). In so doing, they increase the levels of Hsp70 in the ADP-bound form, a form that has greater affinity for unfolded polypeptide substrates. This facilitates the folding of newly synthesized proteins and the folding of proteins as they translocate into organelles (111, 112). These cochaperones also facilitate Hsp70-mediated nuclear trafficking and steroid receptor signaling (113, 114).

HDJ2 is a farnesylated protein whose prenylation status has been used as a marker for FTase inhibition in clinical trials. The functional significance of HDJ2 farnesylation remains unclear, although studies in yeast support a functional role. Expression of an unprenylated form of YDJ1 (the *Saccharomyces cerevisiae* homolog of HDJ2), in which the CaaX cysteine is replaced with a serine residue, fails to rescue the temperature-sensitive phenotype of yeast cells lacking YDJ1 (5). These yeast cells also display defects in the transport of polypeptides across intracellular membranes (111). Together, these studies suggest that farnesylation may regulate the activity, localization, or complex formation that is required for YDJ1 function. There is little work reported to date on the functional consequences of blocking HDJ2 farnesylation in mammalian cells.

Nuclear lamins

The nuclear lamina is a protein meshwork lining the nucleoplasmic surface of the nuclear envelope, providing a structural framework for the nuclear envelope (115). The nuclear lamina consists of lamin proteins that are required for nuclear envelope assembly (116). Lamin B was one of the first proteins shown to be modified by prenylation (4). Lamin A is also farnesylated, but it is unique among farnesylated human proteins. After modification by FTase, Rce1, and ICMT, prelamin A undergoes a farnesylation-dependent proteolytic cleavage in which the C-terminal 15 amino acids, including the farnesylated cysteine, are removed (117, 118). As a consequence, mature lamin A lacks a farnesyl modification. This cleavage reaction is catalyzed by the ZMPSTE24 protease (119). In the absence of farnesylation, cleavage by ZMPSTE24 does not occur. An antibody that recognizes the portion of prelamin A that is removed by ZMPSTE24 cleavage selectively detects prelamin A but not the mature protein. This antibody can be used to detect the accumulation of unfarnesylated prelamin A and provides another marker for FTase inhibition (120). This marker has also been used to demonstrate FTase inhibition in clinical studies (121, 122).

Similar to HDJ2, the functional role of lamin farnesylation remains unclear however, it is thought to play a role in targeting prelamin A to the nuclear membrane, where mature lamin A is released by the action of ZMPSTE24. It has been shown that in cells treated with the HMG-CoA reductase inhibitor lovastatin (which blocks all isoprene synthesis), unprocessed prelamin A accumulates in nucleoplasmic particles (123). However, others have demonstrated that despite the inhibition of prenylation, prelamin A is still competent for assembly into the nuclear lamina (124, 125). It was also reported that FTI treatment or deletion of the prelamin A CaaX box prevents binding to nuclear prelamin A recognition factor (126).

A mutation in prelamin A occurs in children with Hutchinson-Gilford progeria syndrome (HGPS), an extremely rare syndrome characterized by premature aging. HGPS is characterized by retarded growth, osteoporosis, thin skin, and premature atherosclerosis, resulting in death by myocardial infarction or cerebrovascular accident at an average age of 12 years. The mutation in the lamin A gene in these children results in the use of a cryptic splice site and the production of a form of prelamin A protein termed progerin that lacks 50 amino acids near the C-terminal end of the protein. As a result, the ZMPSTE24 proteolytic cleavage site is lost and cells accumulate farnesylated progerin (127, 128). Expression of this mutant lamin A results in misshapen nuclei, a characteristic of progeria cells (129). A similar nuclear phenotype is observed in cells derived from *Zmpste24*-deficient mice, a strain of mice that mirrors many of the traits seen in patients with HGPS (130). In addition, a deficiency in ZMPSTE24 results in misshaped, blebbed nuclei and is responsible the human disease restrictive dermopathy, a lethal perinatal progeroid disorder (131). It has been hypothesized that accumulation of the farnesylated form of prelamin A at the nuclear envelope may be responsible for the cellular pathology seen in these settings. Recent studies with FTIs support this hypothesis. FTIs, including lonafarnib, were shown to reverse the nuclear phenotype in cells derived from mice and from patients with HGPS or restrictive dermopathy (132 133 134). Studies are ongoing to evaluate the effects of FTIs in mouse models of HGPS. Depending upon the outcome of these studies, the potential benefits of using FTIs to treat patients with HGPS (and other laminopathies and/or progeroid syndromes in which alterations in lamin A play a role) will likely be explored.

Additional farnesylated proteins

A number of other farnesylated proteins have been identified that may contribute to the biological activity (both antitumor efficacy and toxicity) of FTIs. These include other small GTPases: RhoD, Rho6, Rho7, and TC10, all of which terminate in methionine and have been demonstrated to be substrates for alternative prenylation by GGTase-1 (P. Kirschmeier, unpublished data). Similarly, the peroxisomal protein HK22 (PxF) is a farnesylated protein subject to alternative prenylation (P. Kirschmeier, unpublished data).

Additional farnesylated proteins include the GTPase RRP22 and the prostacyclin receptor. RRP22 and the small GTPases Rig and Noey2 can inhibit cell growth and have a potential tumor-suppressor function (135). Although these proteins all possess a C-terminal methionine (suggesting the potential for alternative prenylation), RRP22 was shown to incorporate radiolabeled farnesyl but not geranylgeranyl in a rabbit reticulocyte system. Preventing the prenylation of RRP22 with FTIs may inactivate its tumor-suppressor properties.

The prostacyclin receptor is a G protein-coupled receptor that contains a CSLC CaaX motif and was shown to be farnesylated. This receptor plays a negative role in platelet aggregation and vasodilation. FTI treatment has been shown to inhibit prostacyclin receptor-mediated cAMP generation and calcium flux (136). This suggests that FTIs may affect physiologic processes in which prostacyclin and its receptor are implicated.

PRECLINICAL ACTIVITY OF FTIs

FTIs block the growth of a variety of tumor cell lines both in vitro and when grown as xenografts in vivo (137). FTIs induce G2/M accumulation in a number of human tumor cell lines and G1 arrest in cells harboring a mutant H-Ras (138). When combined with a second signal, FTIs can induce apoptosis. For example, FTIs induce apoptosis when cells are deprived of serum or substratum attachment (139, 140) or when used in combination with other signal transduction inhibitors (141, 142).

The ability of FTIs to inhibit tumor cell growth does not correlate with Ras mutational status or Ras isoform expression (61, 143). FTIs are also effective at inhibiting the growth of cells in which Ras activity is upregulated as a result of upstream signaling events, including mutational inactivation of the neurofibromin GAP protein, overexpression of TGFα, and mutationally activated upstream growth factor receptors (144–146). It has been suggested that the sensitivity of astrocytoma cell lines to FTIs correlates with the amount of H-Ras-GTP and that resistance correlates with the amount of K- and N-Ras-GTP, irrespective of total Ras-GTP levels (147). Additionally, tumor cells with wild-type p53 are highly sensitive to FTI-induced growth inhibition, and FTI treatment induces the expression of p53 and its downstream target p21 (138, 148). Although wild-type p53 tumors are especially sensitive, FTIs also inhibit the growth of tumor xenografts that lack functional p53 (149).

FTIs display antitumor activity in a wide range of preclinical tumor xenograft models. For example, tipifarnib inhibits the growth of CAPAN-2 pancreatic, LoVo colon, and C32 melanoma xenograft models. Tumor growth is inhibited 11–90% in these models over a dose range of 25–100 mg/kg twice daily (b.i.d.) (32). Likewise, lonafarnib shows antitumor efficacy in a variety of human tumor xenograft models, including DU-145 human prostate, NCI-H460 lung, A549 lung, MiaPaCa pancreatic, and HCT116 colon xenograft models. In these models, tumor growth inhibition ranged from 67% to 86% at a dose of 40 mg/kg four times per day (137). Additionally, when dosed b.i.d. (60 mg/kg), lonafarnib inhibited tumor growth by 47–64% in MDA-435 breast, ES2 ovarian, IGROV1 ovarian, and TOV-112D ovarian xenograft models (B. Long, unpublished data). Regressions were observed in several xenograft models, including EJ bladder, MCF-7 breast, and LOX melanoma tumor xenografts, when treated with lonafarnib (M. Liu and B. Long, unpublished data).

In contrast to lonafarnib and tipifarnib, which result in tumor growth inhibition in most models, BMS-214662 (300–800 mg/kg once daily) induces tumor regression in a broad spectrum of tumor xenograft models, including EJ-1 bladder, MiaPaCa-2 pancreatic, Calu-1 lung, and HT-29 and HCT-116 colon tumors (150). Compared with other FTIs, BMS-214662 is also a more potent inducer of tumor cell apoptosis under standard in vitro growth conditions (i.e., 10% serum). The reason for the distinct behavior of this FTI is unclear, although it is likely related to the inhibition of a second target in addition to FTase (150). Recent work suggests that this second target may be GGTase-2, an enzyme responsible for posttranslational prenylation of Rab family GTPases (34).

Lonafarnib has displayed significant activity in preclinical models of leukemia driven by the Bcr-Abl fusion oncoprotein. In vitro, lonafarnib inhibits the growth of Bcr-Abl-transformed cells and of primary tumor cells derived from patients with chronic myelogenous leukemia (151). Accumulation of cells in the G2/M phase of the cell cycle and limited induction of apoptosis were observed. In a syngeneic model, mice injected with BaF3 cells expressing the 210 kDa form (p210) of the Bcr-Abl fusion protein develop acute leukemia, resulting in the death of all control mice within 4 weeks of inoculation. Treatment with lonafarnib (40 mg/kg) for 32 days prolonged survival for >1 year in 13 of 15 treated mice (151). Similarly, transgenic mice that express the p190 form of Bcr-Abl all die of leukemia/lymphoma within 100 days after detection of Bcr-Abl expression in peripheral blood. In contrast, 80% of mice treated with lonafarnib survive without signs of leukemia until the termination of the study at 200 days (152). Other studies have confirmed that lonafarnib prolongs the survival of Bcr-Abl transgenic animals (153). Importantly, two groups have reported that lonafarnib retains activity against chronic myeloid leukemia cells that are resistant to the Bcr-Abl kinase inhibitor STI-571 (imatinib/Gleevec™) (154, 155).

The antitumor activity observed with FTIs has been attributed to various downstream responses. Several studies suggested that the mechanism of action of FTIs may be attributable, in part, to inhibition of PI3K/Akt signaling. Sensitivity to FTIs was reported to correlate with the expression of Akt2 in ovarian and pancreatic cancer cells, and FTIs were reported to block Akt activity in these cells (156). Additionally, it was proposed that FTI-induced loss of Akt protein expression may play a role in FTI action in head and neck cancer cell lines (157). However, other studies have shown that Akt expression and activity remain unaffected by FTIs (60, 158). In a panel of human breast

and ovarian cancer cell lines, we failed to detect an effect of lonafarnib treatment on the phosphorylation of AKT (A. D. Basso, unpublished data).

Several studies have reported that FTI treatment affects the apoptotic pathway. Increases in proapoptotic Bax and Bak protein expression and proapoptotic conformational changes of these proteins were reported in response to FTIs (159 160 161). However, in some cases, the expression of antiapoptotic BCL2 protein was also increased (160). Additionally, some FTIs were reported to increase levels of reactive oxygen species and cause double-stranded DNA breaks (162). This effect is likely unrelated to FTase inhibition and was seen using relatively high concentrations of these agents.

PRECLINICAL FTI COMBINATION WITH CHEMOTHERAPIES

FTIs enhance both the growth inhibition and apoptotic response of tumor cells to various other cancer therapeutic agents. Enhanced activity was shown in combination with taxanes (163), cisplatin (164), MEK kinase inhibitor (141), tamoxifen (165), cyclin-dependent kinase inhibitors (166), 5-fluorouracil (167), and the Bcr-Abl kinase inhibitor STI-571 (imatinib/Gleevec™) (155). In xenograft models, lonafarnib shows enhanced antitumor activity when combined with paclitaxel (168), cyclophosphamide, 5-fluorouracil, and vincristine (137). FTIs also sensitize tumor cells to radiation (169). The radiation combination studies were reviewed recently (170).

Of all the FTI combinations investigated, the most striking results were achieved in combination with STI-571 and taxanes. The combination of the FTI lonafarnib with the Bcr-Abl kinase inhibitor imatinib (STI-571) resulted in enhanced growth inhibition and induction of apoptosis in Bcr-Abl-transformed cells, including cells that are resistant to STI-571 alone (154). Similar results were reported by Nakajima et al. (155). Recently, Jorgensen et al. (171) reported that the combination with lonafarnib reduced the resistance of primitive quiescent chronic myeloid leukemia (CML) cells to imatinib treatment. These quiescent Bcr-Abl-positive cells persist in patients after imatinib monotherapy.

A synergistic interaction with taxanes was reported for several structurally distinct FTIs and appears to be an on-target class effect. The initial report by Rosen and colleagues (163) was conducted with the peptidomimetic FTI L-744,832. They found enhanced taxane-induced mitotic arrest and taxane-induced apoptosis in breast tumor cells in culture and suggested that a farnesylated protein may regulate the mitotic checkpoint. Similar in vitro observations were made with tipifarnib in multiple myeloma cells (172). Similarly, lonafarnib synergistically inhibits in vitro growth when combined with either paclitaxel or docetaxel in a panel of human tumor cell lines and enhances the in vivo antitumor response to paclitaxel in several xenograft models (168). Recently, we expanded this evaluation to a panel of breast, prostate, and ovarian tumor xenograft models. Although treatment with single-agent lonafarnib (20–60 mg/kg b.i.d.) or taxane (paclitaxel or docetaxel) administered at the maximal tolerated dose caused partial tumor growth inhibition, the combination of these two agents resulted in marked tumor regressions in multiple models (A. D. Basso, unpublished data B. Long, unpublished data).

The mechanism of this positive interaction is not fully defined. Several farnesylated proteins (e.g., CENP-F) play a role during progression through mitosis. The accumulation of some tumor cells in G2/M, perhaps as a result of the inhibition of CENP-F farnesylation, may contribute to the sensitization to taxane treatment. Recent data also suggest that the inhibition of Rheb farnesylation may contribute to the positive interaction between FTIs and taxanes (77). In support of this notion, inhibition of signaling downstream of Rheb by the mTOR inhibitor rapamycin also sensitizes cells to taxanes (77). A role for tubulin acetylation in this interaction has also been suggested (173). Regardless of the mechanism, the robustness of the preclinical data with this combination suggests that it should be fully evaluated in the clinical setting.

CLINICAL ACTIVITY OF FTIs

Several FTIs have advanced into clinical testing in oncology. Below is a summary of the results published to date with these agents. Two of these compounds (lonafarnib and tipifarnib) are administered orally, and two (BMS-214662 and L-778,123) are intravenous agents.

Clinical activity of lonafarnib (SCH 66336)

Based on several phase I studies in cancer patients, the maximum tolerated dose (MTD) for lonafarnib as a single agent was identified as 200 mg b.i.d. (121, 174, 175). Toxicities observed were primarily gastrointestinal (diarrhea, vomiting, and nausea). Lonafarnib inhibits FTase in treated patients, as demonstrated by the accumulation of prelamin A in buccal mucosa cells (121, 174) and the accumulation of unfarnesylated HDJ2 in tumor biopsies and peripheral blood mononuclear cells (PBMCs unpublished data). Some single-agent clinical activity was reported in phase I studies in patients with solid tumors, including one partial response in a patient with previously treated metastatic non-small-cell lung cancer (NSCLC) (121) and two NSCLC patients with stable disease for >7 months (175).

Most of the development of lonafarnib in solid tumors has focused on combination therapy. A phase I study of 24 patients with solid tumors was carried out to determine the tolerability of the combination of lonafarnib and paclitaxel. Patients were treated with 100, 125, or 150 mg of lonafarnib (b.i.d. throughout the 21 day cycle) in combination with paclitaxel (135 or 175 mg/m^2 on day 8 of a 21 day cycle). Six of 15 previously treated patients had durable partial responses, including 3 of 7 patients previously treated with taxanes. The MTD determined by this study was 100 mg of lonafarnib b.i.d. and 175 mg/m^2

paclitaxel the most common toxicity was diarrhea (176). These doses were used in a phase II study in stage IIIB/IV NSCLC patients who had progressed on or within 3 months of previous taxane therapy. Of 29 patients, 3 had partial responses and 11 had disease stabilization (177). The combination was generally well tolerated. Based on these positive phase II data in a taxane-refractory/resistant NSCLC population, a phase III study was initiated in first-line NSCLC in combination with paclitaxel (175 mg/m^2 every 3 weeks) and carboplatin (area under the curve = 6). This study was terminated upon second interim analysis because of insufficient activity (unpublished data). The reason for these contrasting results is unclear.

The combination of lonafarnib (125 mg b.i.d.) with paclitaxel (80 mg/m^2 weekly) was also evaluated in a phase I/II study of 19 patients with metastatic breast cancer (178). One complete response, five partial responses, and two minor responses were reported. The remaining patients in this study had stable disease. Further studies of this combination in breast cancer were recommended.

Other solid tumor studies evaluating lonafarnib have been completed or are in progress. In a phase II study of patients with metastatic colon cancer refractory to 5-fluorouracil and irinotecan who received lonafarnib monotherapy, no objective responses were observed, although stable disease for several months was reported in several patients (179). Similarly, no objective responses were seen with single-agent lonafarnib in a phase II study in refractory urothelial cancer (180). A phase I study in recurrent/progressive pediatric brain tumors was reported in 2004 (181). The MTD was determined to be 150 mg/m^2 b.i.d. Evidence for pharmacodynamic activity (presence of unfarnesylated HDJ2 in PBMCs) and antitumor activity (1 partial response and 11 stable diseases in 30 patients) was presented.

Lonafarnib has also been evaluated in hematologic malignancies, including chronic myeloid leukemia in blast crisis (CML-BC), advanced myelodysplastic syndrome (MDS), chronic myelomonocytic leukemia (CMML), and acute lymphoblastic leukemia (182, 183). The MTD of lonafarnib as a single agent in these patients was 200 mg b.i.d. In a phase I study, pharmacodynamic and clinical activity was reported in patients with MDS/CMML and CML-BC. Responses were also seen in a phase II study in MDS/CMML, including two complete responses. In several patients with proliferative CMML, a leukemia differentiation syndrome was observed, requiring lonafarnib withdrawal and administration of steroids. This syndrome requires further monitoring in ongoing studies (184). Responses were also reported when lonafarnib was administered in combination with imatinib in a phase I study in patients with CML after failure of imatinib alone (185). The MTD of lonafarnib in combination with a standard dose of imatinib was 100 mg b.i.d. The potential role of lonafarnib and other FTIs in the treatment of hematologic malignancies has been reviewed (186).

Overall, few objective responses have been seen when lonafarnib was used as a single agent to treat solid tumors. This is not surprising, given the finding that FTIs are static agents in most of the preclinical models in which they have been evaluated. In contrast, single-agent activity was observed in hematologic malignancies, most notably CMML. Studies are ongoing to further explore this activity. Studies are also ongoing in solid tumor indications to further define the activity of lonafarnib when used in combination with standard therapies.

Clinical activity of tipifarnib (R115777)

Several single-agent phase I studies with tipifarnib have been reported. In an initial study, tipifarnib was dosed orally b.i.d. for 5 days every 2 weeks. The MTD with this schedule was identified as 500 mg b.i.d. Grade 2 or 3 adverse events included nausea, vomiting, and fatigue myelosuppression was mild and infrequent (187). A second study explored a more prolonged schedule of 28 days of treatment followed by 1–2 weeks of rest in patients with advanced solid tumors. The MTD on this schedule was 300 mg b.i.d. Myelosuppression was dose-limiting in this study (188). In a third study, using continuous treatment, 300 mg b.i.d. was identified as the MTD. Myelosuppression and neurotoxicity were dose-limiting, and one partial response was seen in a NSCLC patient (189).

Similar to the clinical observations with lonafarnib, the most promising clinical results with tipifarnib have been seen in hematologic malignancies, including acute myeloid leukemia (AML), CML, MDS, and multiple myeloma. In a phase I study of 35 adults with refractory and relapsed acute leukemia, 10 patients responded, including 2 with complete responses. Patients were treated for 21 days, and dose limiting toxicities were seen at the 1,200 mg b.i.d. dose. Toxicities included central neurotoxicity, nausea, and myelosuppression (190). A follow-up study in patients with relapsed/refractory hematologic malignancies demonstrated optimal inhibition of protein farnesylation when tipifarnib was dosed at 300 mg b.i.d. for 21 days of a 28 day cycle (191).

Separate studies have confirmed clinical activity in patients with MDS, CML, multiple myeloma, and AML. In 21 patients with MDS, tipifarnib (300 mg b.i.d., 3 weeks on/1 week off) resulted in one complete and two partial responses and three hematologic improvements. FTase activity was inhibited by >75% in 12 of 17 evaluated patients (192). Responses were also seen with tipifarnib in MDS using a 4 week on/2 week off schedule (193). In patients with CML, tipifarnib (600 mg b.i.d. for 4 weeks out of every 6 weeks) showed clinical activity of 22 total patients, 7 had complete or partial responses (194). In patients with advanced multiple myeloma, tipifarnib (300 mg b.i.d. for 3 of 4 weeks) resulted in disease stabilization in 64% of patients. In these patients, FTase activity and HDJ-2 farnesylation were inhibited in PBMCs (195). A recent study suggests that intermittent dosing (b.i.d. on alternative weeks) may be more effective, in that higher doses (600 mg) can be given without toxicities (196). Activity has also been reported with single-agent tipifarnib in AML patients. These data were recently reviewed (197). In 2005, the Oncologic Drugs Advisory Committee rejected the approval of tipifarnib for the treatment of

elderly patients with newly diagnosed poor-risk AML, citing an insufficient complete response rate. It was noted that completion of the ongoing phase III trial comparing tipifarnib with best supportive care may provide the necessary confirmatory data for approval.

Tipifarnib single-agent treatment has shown limited clinical activity in solid tumors. Phase II studies in relapsed small-cell lung cancer (198), advanced NSCLC (199), and metastatic pancreatic cancer (200) failed to demonstrate single-agent activity in these advanced disease settings. Similarly, a phase III study of tipifarnib monotherapy in patients with advanced refractory colorectal cancer showed no improvement in overall survival (201). Limited single-agent activity was reported in patients with advanced urothelial tract transitional cell carcinoma (2 partial responses and 13 stable diseases among 34 patients) (202). More significant single-agent activity has been reported in breast cancer. A phase II study of patients with advanced breast cancer determined that 300 mg b.i.d. given for 3 of 4 weeks was better tolerated than continuous 300 mg b.i.d. dosing. Objective responses were observed with both schedules, but hematologic toxicity was significantly lower with the intermittent dosing regimen. Of the 41 patients on the continuous schedule, 4 had partial responses and 6 had stable disease. Of the 35 patients on the intermittent dosing regimen, 5 had partial responses and 3 had stable disease (203).

Many of the solid tumor studies with tipifarnib have investigated the benefit of adding it to standard chemotherapy. Phase I combinations with a number of agents have been reported, including the combination with irinotecan (204) and gemcitabine (205) and the triple combination with gemcitabine and cisplatin (206). These studies were designed to define the MTD of the combination, although some responses were noted. Building on these studies, a phase III trial in 688 patients with advanced pancreatic cancer was conducted using tipifarnib (200 mg b.i.d. continuously) in combination with gemcitabine compared with placebo plus gemcitabine. The addition of tipifarnib failed to prolong overall survival in that study (207).

It was demonstrated recently in a phase I study in patients with metastatic, hormone receptor-positive breast cancer that tipifarnib (200 mg b.i.d. given for 21 days of a 28 day cycle) can be safely administered with tamoxifen. Of 12 patients treated, two partial responses and one stable disease for >6 months were reported. Further studies are needed to define the clinical efficacy of this combination (208). Importantly, in preclinical studies, the combination of hormonal agents (both antiestrogens and aromatase inhibitors) with FTIs has demonstrated enhanced activity, resulting in regressions in some models (209) (B. Long, unpublished data).

Clinical activity of BMS-214662

Recently, several phase I studies were reported for BMS-214662. A study in patients with advanced solid tumors determined the MTD to be 200 mg/m^2 (1 h intravenous infusion) given once every 3 weeks. Toxicities included increased hepatic transaminases, nausea, vomiting, and diarrhea. FTase activity in patient PBMC samples declined gradually during the 5 h after infusion but returned to baseline within 24 h (210). Similar FTase inhibition in PBMC samples was seen in a second study in which patients refractory to standard chemotherapy were treated with BMS-214662 followed by cisplatin once every 3 weeks. Of these 29 patients, 15 had reported disease stabilization (211). A third phase I study demonstrated that this agent could be safely administered at 160 mg/m^2 in combination with paclitaxel (225 mg/m^2) and carboplatin (area under the curve = 6). Among 30 patients, a partial response was observed in 1 patient with taxane-resistant esophageal cancer, and partial responses were observed in 2 additional patients (endometrial and ovarian), along with stable disease in 8 patients (212). An additional phase I study exploring weekly dosing demonstrated activity in 5 patients with acute leukemia or high-risk MDS (among 30 patients), including two complete responses (213). Phase I studies exploring weekly 1 h infusions and weekly 24 h infusions in solid tumor patients have also been reported (214, 215). The toxicity profiles for the two regimens differed significantly.

Clinical activity of L-778,123

Few clinical studies have been reported for L-778,123. A phase I study in patients with solid tumors determined the MTD to be 1,120 mg/m^2/day given as a continuous intravenous infusion for 7 consecutive days of a 21 day cycle. Toxicities included prolongation of QTc interval, fatigue, confusion, thrombocytopenia, and neutropenia. This study demonstrated that L-778,123 could be given safely and at doses that inhibited HDJ2 prenylation in PBMC samples (216). Two phase I studies were performed in combination with radiotherapy. A study in pancreatic cancer determined that 280 mg/m^2/day (continuous intravenous) could be given safely concomitant with radiation (217). Using the same schedule, an additional study of nine patients (NSCLC and head and neck carcinoma) reported one partial response and five complete responses (218). In one study with L-778,123, inhibition of prenylation of both the FTase substrate HDJ2 and the GGTase-1 substrate Rap1A was reported (219). This finding is consistent with this molecule being a more potent GGTase inhibitor than tipifarnib or lonafarnib. Surprisingly, no inhibition of K-Ras prenylation was detected in patient samples.

CONCLUDING REMARKS

The clinical development of FTIs has proven to be challenging. To date, convincing single-agent activity has been demonstrated in hematologic malignancies, in particular CML, MDS, and AML. In contrast, single-agent activity in solid tumors has been limited, although the reports of single-agent responses to tipifarnib in breast cancer are intriguing (203). If FTIs are to find utility in solid tumor therapy, it will most likely be in combination with standard cytotoxic or hormonal agents. The most promising combinations, based on preclinical data, are those with taxanes

and antiestrogens. Although the initial clinical studies of the combination of paclitaxel and lonafarnib in refractory NSCLC were encouraging, this was not borne out in a randomized phase III trial in the first-line setting. The reason for this discrepancy is unclear, but based on the compelling preclinical data, the combination of FTIs with taxanes deserves further clinical evaluation, perhaps in a more chemosensitive tumor type.

This class of agents presents some unique challenges to clinical development.

1) In most preclinical models, FTIs induce tumor growth inhibition, rather than tumor regressions, when used as monotherapy. This suggests that standard response criteria, which have proven valuable in the clinical development of cytotoxic agents, cannot be applied with these compounds. This is likely to be an issue with other new classes of targeted signal transduction inhibitors. It also suggests that relatively chemoresistant, aggressive cancers such as lung and pancreatic cancer may not be appropriate settings to explore activity with this class of agents. Greater activity is anticipated in the maintenance or adjuvant setting.

2) Although the biochemical target of these compounds is well defined, the downstream biology resulting from blocking protein farnesylation is complex. Clearly, some proteins, most notably the Ras family, can escape FTase inhibition by alternative prenylation. It remains to be defined which protein (or more likely set of proteins) is critical for the antitumor activity observed.

3) A related challenge is the inability to identify a sensitive tumor type based on a specific genetic profile. Clearly, the activity of FTIs is independent of K- and N-Ras mutational status. Are there H-Ras-driven tumors (in which H-Ras is activated either by mutation or by upstream signaling) that are appropriate for testing FTIs? Are there other markers that can be identified that may prove useful to predict response? To date, such markers have proven elusive. A number of biochemical markers have been used successfully in the development of FTIs to demonstrate target inhibition in the clinic. These include the accumulation of unfarnesylated proteins (prelamin A and HDJ2) and direct ex vivo measurement of FTase inhibition. Recently, Raponi et al. (220) reported the identification of a common set of genes that were regulated by tipifarnib in three AML cell lines and in leukemic blast cells isolated from two AML patients who had been treated with tipifarnib. Expression of these candidate genes might also be used as surrogate biomarkers of drug activity.

It is striking that many of the preclinical observations with FTIs (e.g., efficacy in combination with taxanes and impressive single-agent activity in models of CML) are highly consistent across structurally diverse molecules, suggesting that this biology is mechanism-driven. If this preclinical biology can be translated into the clinic, it is likely that FTIs will find utility in hematologic cancers and in solid tumors in combination with chemotherapy. For this to be realized, it is critical to learn from the negative trials in chemoresistant tumor types and build on the observations of clinical activity in various phase II settings.

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