

## Ras Protein Farnesyltransferase: A Strategic Target for Anticancer Therapeutic Development

By Eric K. Rowinsky, Jolene J. Windle, and Daniel D. Von Hoff

**Abstract:** Ras proteins are guanine nucleotide-binding proteins that play pivotal roles in the control of normal and transformed cell growth and are among the most intensively studied proteins of the past decade. After stimulation by various growth factors and cytokines, Ras activates several downstream effectors, including the Raf-1/mitogen-activated protein kinase pathway and the Rac/Rho pathway. In approximately 30% of human cancers, including a substantial proportion of pancreatic and colon adenocarcinomas, mutated ras genes produce mutated proteins that remain locked in an active state, thereby relaying uncontrolled proliferative signals. Ras undergoes several posttranslational modifications that facilitate its attachment to the inner surface of the plasma membrane. The first—and most critical—modification is the addition of a farnesyl isoprenoid moiety in a reaction catalyzed by the enzyme protein farnesyltransferase (FTase). It follows that inhibiting FTase would prevent Ras from maturing into its biologically active form, and FTase is of considerable interest as a potential therapeutic target. Different

classes of FTase inhibitors have been identified that block farnesylation of Ras, reverse Ras-mediated cell transformation in human cell lines, and inhibit the growth of human tumor cells in nude mice. In transgenic mice with established tumors, FTase inhibitors cause regression in some tumors, which appears to be mediated through both apoptosis and cell cycle regulation. FTase inhibitors have been well tolerated in animal studies and do not produce the generalized cytotoxic effects in normal tissues that are a major limitation of most conventional anticancer agents. There are ongoing clinical evaluations of FTase inhibitors to determine the feasibility of administering them on dose schedules like those that portend optimal therapeutic indices in preclinical studies. Because of the unique biologic aspects of FTase, designing disease-directed phase II and III evaluations of their effectiveness presents formidable challenges.

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A DETAILED UNDERSTANDING of the mechanism by which mutated genes confer a neoplastic phenotype on cells is anticipated to result in mechanism-based cancer therapeutics that specifically target the underlying defects in cellular growth regulation. By virtue of their specificity, these therapeutics may prove more effective and much less toxic than the chemotherapeutic agents now available, thereby resulting in superior therapeutic outcomes. One potential target is the Ras family of proteins, which are mutationally activated in a wide range of human tumor types and are important contributors to the neoplastic phenotype.<sup>1-3</sup>

In addition to the role of *ras* in neoplasia, normal *ras* genes, which are present in all eukaryotes, are critical regulators of numerous physiologic processes.<sup>1-3</sup> Experimental studies of Ras protein structure, function, and regulation indicate that Ras is a key intermediate in signal transduction pathways that mediate proliferative and other types of signals largely from upstream of receptor tyrosine kinases to a downstream cascade of protein kinases, which control a wide variety of cellular processes, including growth, differentiation, apoptosis, cytoskeletal organization, and membrane trafficking.<sup>4,5</sup> Because of its central role in regulating these processes, Ras, along with several of the Ras effector pathways, provides opportunities to develop novel therapeu-

tics that specifically target the aberrant signaling pathways operative in tumor cells.

This review is an overview of current knowledge of the role of Ras in signal transduction. Its focus is the principal posttranslational process involved in Ras activation, farnesylation, which is required for Ras to transform cells and is a novel target for development of therapeutics against cancer. The current state of efforts targeting Ras protein farnesylation is examined, including preliminary results of the first generation of therapeutics to enter clinical trials.

### THE *ras* PROTO-ONCOGENE AND Ras PROTEIN

Three *ras* proto-oncogenes have been identified: the *H-ras* gene (homologous to the oncogene of the Harvey murine sarcoma virus), the *K-ras* gene (homologous to the

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*From the Institute for Drug Development, Cancer Therapy and Research Center; and the University of Texas Health Science Center, San Antonio, TX.*

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*Address reprint requests to Eric K. Rowinsky, MD, Institute for Drug Development, Cancer Therapy and Research Center, 8122 Datapoint Dr, Suite 700, San Antonio, TX 78229-3272; email erowinsk@saci.org.*

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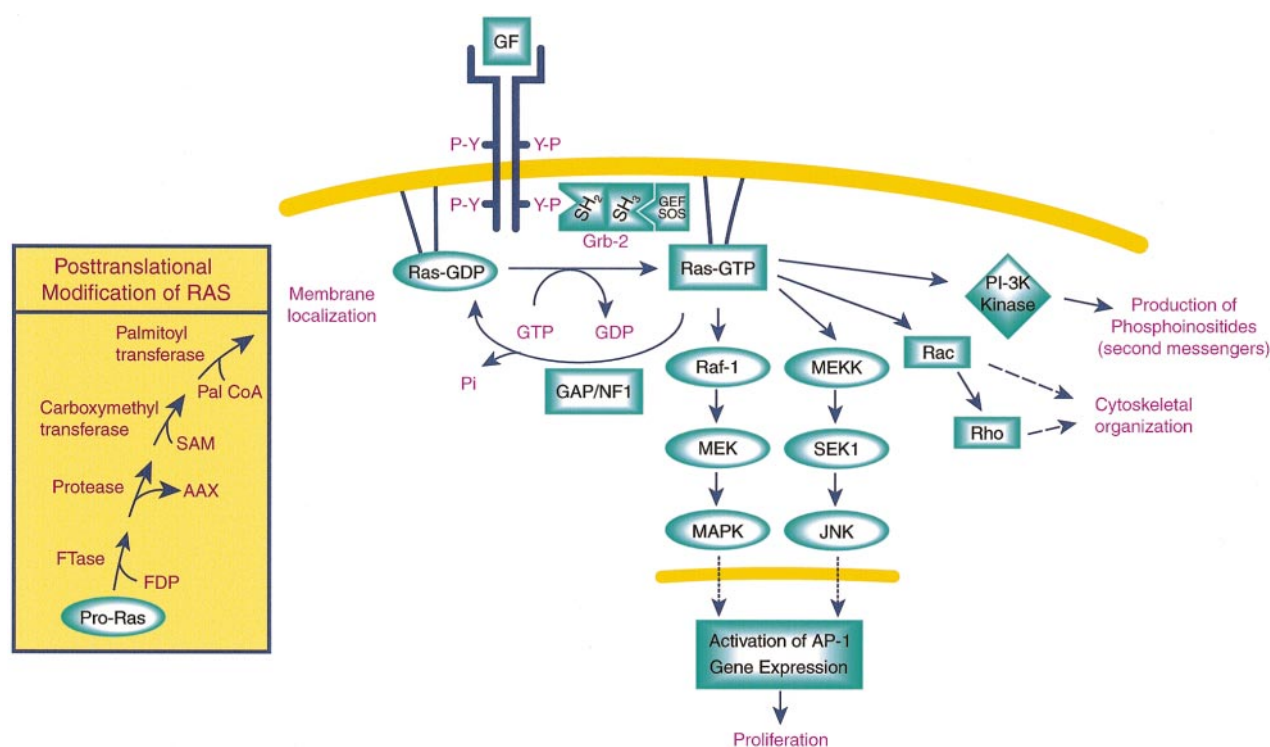
oncogene of the Kirsten murine sarcoma virus), and the *N-ras* gene (which does not have a retroviral homolog and was first isolated from a neuroblastoma cell line).<sup>4-9</sup> The *ras* oncogenes encode four 21-kd proteins, called p21<sup>ras</sup> or Ras (H-Ras, N-Ras, and K-Ras4A and K-Ras4B, resulting from two alternatively spliced K-Ras gene products), that are localized to the inner surface of the plasma membrane in mammalian cells.

Ras proteins contain 188 or 189 amino acids and exhibit high sequence homology, with the first 86 amino acids being identical, the next 78 having 79% homology, and the following 25 amino acids being highly variable.<sup>5,6</sup> The highly conserved nature of the variable region across mammalian species indicates that Ras proteins serve specific functions. The final four amino acids play an important role in specifying subcellular localization of the Ras protein. All Ras proteins have a specific amino acid sequence motif at the carboxyl (C) terminus, commonly referred to as the

CA<sub>1</sub>A<sub>2</sub>X box, in which C represents a cysteine residue; A<sub>1</sub> and A<sub>2</sub> represent aliphatic amino acids, usually valine, leucine, or isoleucine; and X is either methionine or serine.

#### Regulation of Ras Activity

Ras proteins are members of an extended family of GTPases, which include proteins involved in protein synthesis and signal transduction.<sup>4</sup> Ras functions as a molecular switch that cycles between an inactive guanosine 5'-diphosphate (GDP)-bound form and an active guanosine 5'-triphosphate (GTP)-bound state. The processes by which Ras is activated and functions in intracellular signaling are depicted in Fig 1. Ras is synthesized as a biologically inactive cytosolic propeptide (Pro-Ras) and is localized to the inner surface of the plasma membranes only after it has undergone a series of closely linked posttranslational modifications at the C-terminus, thereby increasing its hydrophobicity and facilitating its association with the plasma mem-



**Fig 1.** Ras pathways. Ras is synthesized as a propeptide (Pro-Ras) and undergoes a series of posttranslational lipid modifications that enable it to associate with the inner surface of the plasma membrane. The first modification is catalyzed by FTase to cause covalent addition of the farnesyl group from farnesyl diphosphate (FDP) onto the cysteine residue of the CAAX sequence. Next the AAX residues are removed by CAAX protease, followed by carboxymethylation of the farnesyl-cysteine residue from a *s*-adenosyl-L-methionine (SAM) donor. In some Ras proteins, palmitoyl transferase catalyzes an additional modification of upstream cysteine residue(s) by the fatty acid palmitate. These modifications enhance protein hydrophobicity and plasma membrane association, in which Ras cycles from an inactive GDP-bound state to an active GTP-bound state. In response to growth factors (GF), GEF mediates exchange of GTP for GDP. Ras-GTP then activates several effectors, including Raf-1 and the MAPK pathway, the Rac/Rho pathway, kinase kinase kinase MEKK, and PI3K. GTPase activator proteins (GTPase activator protein [GAP] and neurofibrin [NFI]) then enhance hydrolysis of Ras-GTP and return it to an inactive state. GEF, guanine-nucleotide-exchange factors; Grb-2, growth factor receptor-binding protein; JNK, Jun amino-terminal kinase; SOS, son-of-sevenless (*Drosophila* homolog of *ras*).

brane.<sup>10-14</sup> The first and most critical step, farnesylation, adds a 15-carbon farnesyl isoprenoid group to H-, K-, and N-Ras and is catalyzed by protein farnesyltransferase (FTase).

Ras proteins transmit a wide array of extracellular signals from cell surface receptors to the cytoplasm, initiating a cascade of protein kinases that ultimately regulates both nuclear and cytoplasmic processes. Considerable progress has been made in elucidating the details of the signal pathway upstream of Ras (ie, from the binding of a growth factor to its receptor to the activation of Ras). In its normal wild-type state, Ras-GDP is rapidly and transiently converted to Ras-GTP in response to diverse extracellular stimuli. These stimuli include growth factors that stimulate proliferation of fibroblasts and other types of cells (eg, epidermal growth factor, c-erb2, and platelet-derived growth factor), growth factors that activate lymphocytes and stimulate the proliferation of hematopoietic cells (eg, interleukin 2, interleukin 3, and granulocyte-monocyte colony-stimulating factor), hormones (eg, insulin), and neurotransmitters (eg, carbachol).<sup>15,16</sup> Typically, the cell-surface receptors for these growth factors are receptor tyrosine kinases; the binding of growth factors and other signals to the receptor promotes receptor dimerization that leads to autophosphorylation.<sup>15,17,18</sup> In a similar manner, cytokines and other transmitters may bind to receptors that activate nonreceptor tyrosine kinases such as the Src family (eg, Lck, Lyn, and Fyn).<sup>15,17,18</sup> The tyrosine-phosphorylated growth factor receptor provides a binding site for an "adapter protein" such as growth factor receptor-binding protein (Grb2), which "connects" other signaling proteins through its *src*-homology 2 and *src*-homology 3 binding domains (Fig 1). In essence, Grb2 binds to one of the tyrosine residues on the activated tyrosine kinase receptor through its *src*-homology 2 domain, and Grb2 then recruits Ras activator proteins to the plasma membrane. Ras activator proteins such as SOS (an acronym for the *Drosophila* homolog of this gene, son-of-sevenless) function as Ras guanine-nucleotide-exchange factors (GEF) that bind to the *src*-homology 3 domain of Grb2, thereby forming a stable complex. GEF then mediates the exchange of GTP for GDP on Ras by facilitating the dissociation of GDP from Ras-GDP; subsequent GTP binding promotes the release of GEF and leaves Ras in its activated form.<sup>4</sup> When stimulated by receptor activation to bind GTP, Ras promotes cellular proliferation and other effects.

During normal cell growth, continuous stimulation by extracellular growth factors is required to maintain wild-type Ras in an activated state; otherwise, it reverts rapidly to the inactive form. Although wild-type Ras has low intrinsic GTPase activity, GTPase activator proteins (ie, GTPase activator or accelerator protein [GAP] or neurofibromin

[NFI]) enhance the hydrolysis of bound GTP to GDP, converting Ras to an inactive form. Biochemical studies suggest that although mutant Ras exhibits slightly less intrinsic GTPase activity than does wild-type Ras, the principal functional effect conferred by mutant Ras is a marked decrease in the ability of Ras to interact with GAP.<sup>19-21</sup> Instead of reverting to its inactive GDP-bound state, mutant Ras remains in an active GTP-bound state and continues to activate downstream effectors despite the absence of growth factor stimulation.

#### *Activation of Effector Proteins*

In its GTP-bound state, Ras can activate several downstream effector pathways, of which the pathway involving the serine-threonine kinase Raf-1 has been most thoroughly elucidated. There are multiple branch points in this pathway, and Raf is only one of many effectors of Ras signaling.<sup>22</sup> Other effectors include the small GTP-binding proteins (called G proteins) Rac and Rho, phosphatidylinositol-3'-kinase (PI3K), and mitogen-activated protein (MAP) kinase kinase kinase (MEKK). Two mechanisms have been proposed to explain how Ras-GTP activates its downstream effectors.<sup>23</sup> In the recruitment model, Ras is anchored to the plasma membrane, where it binds to the cytoplasmic effector and allows other membrane proteins to induce activation. Alternatively, in the allosteric model, Ras binding induces a conformational change in the effector molecule, resulting in activation. Both mechanisms may be involved, depending on which effector protein is activated.

*Activation of Raf-1.* The activation of the effector Raf-1 occurs after it is recruited to the cell membrane; however, the precise mechanism by which Ras activates Raf-1 is unknown.<sup>24-31</sup> Once activated, Raf-1 phosphorylates two MAP kinase kinases, MEK<sub>1</sub> and MEK<sub>2</sub>, which in turn phosphorylate the mitogen-activated protein kinases (MAPK), p44<sup>MAPK</sup> and p42<sup>MAPK</sup> (also known as extracellular signal-regulated kinases 1 and 2 or ERK1 and ERK2).<sup>32</sup> On activation, MAPKs translocate to the nucleus, where they phosphorylate and activate a variety of substrates, including the Elk1 nuclear transcription factor, ultimately leading to the activation of other kinases, transcription factors, and *c-fos* and other downstream target genes associated with proliferation.<sup>22</sup>

Several lines of evidence indicate that Raf is a critical effector of Ras function. First, dominant-negative mutants of Raf can impair Ras-transforming activity.<sup>33,34</sup> Constitutively activated forms of Raf also exhibit transforming activity comparable to that of Ras<sup>30,35</sup> and are themselves sufficient to transform some murine cells.<sup>36-38</sup> However, Raf is certainly not the sole effector of Ras. Although activated Raf

generally has potent transforming activity in rodent fibroblasts, it is less efficient in transforming other cell types.<sup>39,40</sup> Other proteins that associate with the "effector" domain of Ras-GTP have been identified, including several members of the Rho family, MEKK, and PI3K, each of which exhibits transformation activity or Ras-induced signaling activity, in many cases independent of Raf.<sup>22</sup>

*Activation of Rac and Rho.* Ras-GTP also activates the G proteins Rac and Rho through an activation pathway often referred to as the cell morphology pathway.<sup>41-43</sup> Like Ras, these proteins cycle between GDP- and GTP-bound states and are regulated by factors similar to GEF and GAP.<sup>15</sup> One of the principal functions of the Rho proteins appears to be the regulation of the actin cytoskeleton, affecting such processes as membrane ruffling and formation of stress fibers, focal adhesions, and filopodia.<sup>25</sup> The activation of Rac and Rho by oncogenic Ras may lead to morphologic changes that increase the invasive properties of transformed cells. Cells with constitutively activated Rac exhibit a dramatic increase in membrane ruffling, with an increase in actin polymerization, whereas cells with constitutively activated Rho are associated with significant cytoskeletal reorganization and increased numbers of focal adhesions.<sup>44,45</sup>

*Activation of MEKK.* MEKK, like Raf, is a serine-threonine protein kinase, one that is activated by GTP-Ras and in turn phosphorylates and activates MEK-family proteins, independently of Raf.<sup>46,47</sup> Although MEKK can activate MEK when overexpressed, its primary target appears to be a related kinase, SEK1, which in turn phosphorylates another MAPK family member, Jun amino-terminal kinase (JNK).<sup>48-51</sup> JNK in turn activates the c-Jun transcription factor. Even though c-Jun appears to be required for Ras transformation, the MEKK pathway has not been implicated in tumorigenesis.<sup>52,53</sup> JNK activation may promote different cellular consequences, such as apoptosis and proliferation, depending on the coordinate activation of other pathways.<sup>22</sup>

*Activation of PI3K.* In addition to the Raf-1 and Rac/Rho pathways, Ras activates the downstream effector PI3K, which is a member of a family of lipid kinases that phosphorylate phosphoinositides.<sup>54</sup> PI3K forms a high-affinity complex with Ras-GTP, resulting in an increase in PI3K activity.<sup>55-57</sup> PI3K signaling has been linked to a number of cellular processes that may be significant in oncogenic transformation, including control of the actin cytoskeleton, motility, invasiveness, prevention of cellular senescence, and suppression of apoptosis.<sup>58-63</sup> Of particular interest is the finding that PI3K mediates the inappropriate survival of Ras-transformed epithelial cells in the absence of attachment to the extracellular matrix and the suppression of c-Myc-induced apoptosis by Ras.<sup>64,65</sup> Activation of PI3K results in the production of a number of phosphoinositides,

which are assumed to function as second messengers. However, only a single downstream target for PI3K activation, protein kinase B (PKB; also Akt), has been clearly identified.<sup>66</sup> Another protein, pp70<sup>S6k</sup>, is also activated by PI3K, although the mechanism of activation is unknown.<sup>67</sup> It is known that pp70<sup>S6k</sup> mediates phosphorylation and activation of the 40S ribosomal protein S6, which is necessary for cell cycle progression from G<sub>1</sub> into S phase.<sup>68-70</sup>

*Potential Effectors of Ras.* Other effectors of Ras function have been identified, including the zeta isoform of protein kinase C and Ral guanine nucleotide dissociation stimulator.<sup>27-29</sup> However, the functional significance of Ras interactions with these signaling proteins is not known. In a recent study, Cripto-1, a member of the epidermal growth factor family that does not activate known growth factor receptors, was shown to stimulate growth of mouse mammary epithelial cells and block lactogenic hormone-mediated expression of beta-casein via Ras signaling, with the former effect dependent on a MAPK-mediated pathway but the latter dependent on a PI3K pathway.<sup>61</sup> Thus it is likely that in any given cell type, multiple Ras effector pathways may cooperate to produce the full effect of Ras activation.

#### *Mutations of ras in Cancers*

Mutated *ras* oncogenes were first identified by their ability to transform NIH 3T3 cells after DNA transcription.<sup>1,71-73</sup> Subsequent analysis of a variety of tumor samples revealed that in part of the human tumors, one of the three *ras* genes harbored a point mutation; as a result, the protein product has an altered amino acid, most commonly at one of the critical positions 12, 13, or 61. In human tumors, the mutation at residue 12, in which the glycine residue is mutated to serine, cysteine, arginine, asparagine, alanine, or valine, is the most commonly found.<sup>1,71-73</sup> Mutations of *ras* occur in approximately 30% of all human cancers, including a significant proportion of pancreatic and colorectal carcinomas.<sup>1,15,19</sup> Most mutationally activated forms of *ras* genes identified in tumors result in disrupted guanine nucleotide regulation and constitutive activation of Ras.<sup>5</sup> With regard to the three *ras* genes, mutation of *K-ras* is most commonly found in human tumors, whereas *N-ras* mutations are encountered less often and *H-ras* mutations rarely.<sup>1-5</sup> The clinical significance of these different mutations is not completely understood, although there is evidence that each Ras isoform leads to distinct biochemical consequences because of the quantitative differences in activation of the many downstream effector pathways.<sup>74</sup> In addition, the type of *ras* mutation seems to correlate with tumor type.<sup>1-5</sup> Although activating *ras* mutations are particularly associated with myeloid malignancies and carcinomas of the



**Table 1. *ras* Mutations and Human Tumors**

Type of Tumor/Cancer	No. of Tumors Assessed	<i>ras</i> Mutation Frequency (% Positive)	Predominant <i>ras</i> Mutation
Acute myelogenous leukemia	302	23	N
Bladder	67	11	H
Breast	80	2	H, K
Myelodysplastic syndrome	138	28	N
Cervix	106	6	---
Cholangiocarcinoma	35	56	N
Colon			
Adenocarcinoma	751	36	K
Adenoma	349	24	K
Embryonal rhabdomyosarcoma	36	14	---
Endometrial carcinoma	174	21	K
Liver	31	10	N
Lung			
Large-cell carcinoma	61	21	K
Adenocarcinoma	626	22	K
Kidney	30	10	H
Melanoma	50	16	N
Multiple myeloma	144	46	---
Oral squamous cell carcinoma	109	23	---
Ovarian carcinoma	148	23	K
Pancreatic carcinoma	247	78	K
Seminoma	54	43	K, N
Skin: keratoacanthoma	66	26	H
Thyroid			
Follicular adenoma	71	28	H, K, N
Follicular carcinoma	30	53	H, K, N
Papillary carcinoma	69	25	---
Undifferentiated carcinoma	12	58	H, K, N

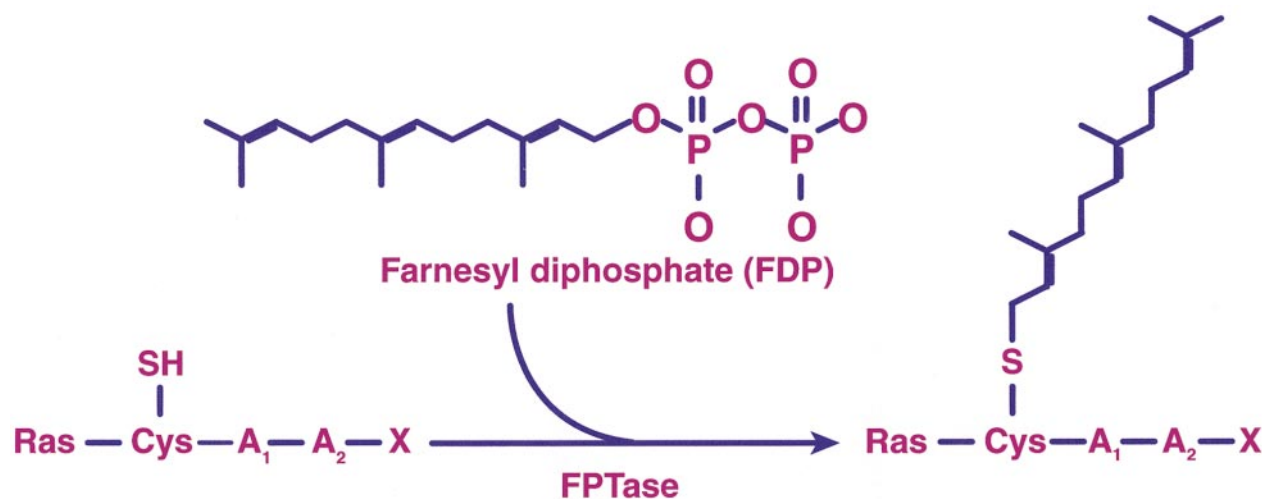
NOTE. Data adapted.<sup>1,15,19</sup>

colon, pancreas, lung, and thyroid, they have also been detected in many other types of cancer (Table 1).<sup>1,3,15,19,75</sup> There do not appear to be major functional differences among the three Ras proteins when mutated, and in most tumor types there does not appear to be absolute specificity for any particular type of *ras* mutation.

#### POSTTRANSLATIONAL MODIFICATION OF Ras

For Ras to transduce the extracellular signals provided by growth factors and cytokines, it must be associated with the inner surface of the plasma membrane. This association is facilitated by a series of posttranslational chemical modifications. After its synthesis as cytoplasmic Pro-Ras, Ras is sequentially modified by farnesylation of the cysteine residue, proteolytic cleavage of the AAX peptide by proteases, and carboxymethylation of the new C-terminal carboxylate by carboxymethyl transferase. As the first step in this sequence, farnesylation is the most critical part of the process.<sup>10,18,74,76-81</sup>

FTase catalyzes the farnesylation step by recognizing the CAAX motif of the Ras C-terminus and transferring a 15-carbon farnesyl isoprenoid from farnesyl diphosphate (FDP) to form a thioether bond with the Ras cysteine (Fig 2).<sup>78</sup> In another principal prenylation reaction relevant to cell signaling, geranylgeranylation, protein geranylgeranyl transferases (GGTases) transfer either one or two 20-carbon geranylgeranyl isoprenoids from geranylgeranyl diphosphate to proteins.<sup>10,76</sup> Both farnesylation and geranylgeranylation result in more hydrophobic proteins. The proteins modified by geranylgeranylation are more hydrophobic than are those modified by farnesylation, and geranylgeranylation may also serve as part of a recognition sequence for



**Fig 2.** The first step in Ras posttranslational modification is mediated by Ftase, which transfers a farnesyl moiety from FDP to the cysteine moiety in the CAAX motif at the carboxyl terminus of Ras. Ftase inhibitors block this enzymatic step.

protein-protein interactions.<sup>76</sup> Prenylated proteins share characteristic C-terminal amino acid sequences, including CAAX, XXCC, or XCXC.

All Ras proteins except K-Ras4B undergo an additional modification, in which the enzyme palmitoyltransferase catalyzes attachment of a fatty acid palmitate on cysteine residue(s) near the farnesylated cysteine.<sup>82,83</sup> K-Ras4B has not a palmitoylation site but rather a cluster of lysine-rich sequences that may be responsible for increased affinity with the cell membrane, by electrostatically interacting with acidic phospholipids and other negatively charged membrane groups on the inner membrane surface.<sup>11,83-85</sup> With regard to the respective roles of farnesylation and palmitoylation, it has been proposed that farnesylation brings a finite amount of Ras to all cellular membranes and that palmitoylation is then required to trap the protein, at least reversibly, in the membrane. Although each of these posttranslational modifications increases the hydrophobicity of Ras and contributes to its association with the plasma membrane, the initial farnesylation step alone is sufficient to promote substantial membrane association and confer transforming potential.<sup>13,14</sup> Studies in *Xenopus* oocytes that had physiologic amounts of H-ras indicated that Ras activates oocytes very poorly, unless the protein is palmitoylated.<sup>83</sup> Strategies that are capable of blocking FTase and preventing farnesylation may be expected to inhibit the maturation of Ras into a biologically active molecule, thus turning off signal transduction. An in-depth understanding of other posttranslational processes related to Ras, including palmitoylation, methylation, and proteolysis, is emerging, and studies to determine whether they have a role as strategic targets for anticancer therapeutic development seem warranted.

#### *FTase and Other Prenyltransferases*

Most prenylation reactions are catalyzed by three prenyl protein transferases that differ in their isoprenoid substrates and protein targets. These enzymes have  $\alpha$ - and  $\beta$ -subunits. The  $\alpha$ -subunits have all evolved from a common ancestral gene and contain a repetitive sequence motif with conserved amino acids that are important for function.<sup>6,79,85</sup>  $\beta$ -Subunits are also ancestrally related and contain internal repeats that facilitate zinc ( $Zn^{2+}$ ) binding. FTase consists of a 48-kd  $\alpha$ -subunit and a 46-kd  $\beta$ -subunit.<sup>6,79,85</sup> Zinc ( $Zn^{2+}$ ) appears to be required for substrate CAAX binding, whereas magnesium ( $Mg^{2+}$ ) is required for catalysis. Although whole proteins are substrates for cellular FTase, CAAX tetrapeptides act as *in vitro* substrates that behave kinetically, like the corresponding complete protein. In addition to influencing the affinity of substrates for FTase or GGTase, the terminal residue of the CAAX box also confers enzyme specificity.

For example, although mammalian FTase prenylates CAAX substrates in which X is either methionine, serine, or glycine, the affinity for the enzyme is 10- to 30-fold higher for substrates in which X is methionine (eg, K-Ras4B and lamin B) than for substrates in which X is either serine or glycine (H-Ras).<sup>86,87</sup> This implies that intracellular proteins have different sensitivities to FTase inhibitors.

Recent studies of the crystalline structure of FTase indicate that the enzyme contains two clefts, which may represent the FDP and CAAX binding sites.<sup>88</sup> At the junction of these clefts lies a  $Zn^{2+}$  atom, which coordinates the thiol group of the cysteine into a ternary complex. The results of cross-linking studies suggest that both FDP and the CAAX region may bind to the  $\beta$ -subunit, whereas the  $\alpha$ -subunit may stabilize the  $\beta$ -subunit and catalyze the transfer of the farnesyl isoprenoid moiety.<sup>89</sup> Further, the  $\alpha$ -subunit undergoes phosphorylation, which controls the activity of the enzyme.<sup>90</sup> A second FTase isoform, with a molecular weight of 250 kd, was recently isolated from human Burkitt lymphoma Daudi cells; it has a  $\beta$ -subunit that is identical to that of the principal FTase but has a distinct  $\alpha$ -subunit.<sup>91</sup> The functional significance of this new isoform in the posttranslational modification of Ras has not yet been established.

Two other structurally related protein prenyltransferases, GGTase-I and GGTase-II, prenylate critical proteins by attaching either one or two 20-carbon geranylgeranyl isoprenoid lipid moieties to the C-terminal end of the proteins. GGTase-I and FTase share an identical  $\alpha$ -subunit and have similar, but distinct,  $\beta$ -subunits. Like FTase, GGTase-I is a  $Zn^{2+}$  metalloenzyme that requires  $Mg^{2+}$  for catalysis and recognizes proteins with a CAAX motif or CAAX tetrapeptides as substrates.<sup>6,79,85</sup> In contrast to FTase, which binds FDP only 30 times as tightly as it does GGPD, GGTase-I binds GGPD 300 times tighter than it does FDP.<sup>92,93</sup> GGTase-I preferentially prenylates proteins in which the X residue is leucine. The enzyme consists of three subunits, of which the catalytic unit is an  $\alpha\beta$ -heterodimer with homology to the subunits of FTase and GGTase-I. However, GGTase-II requires an additional protein, known as the Rab escort protein, to facilitate interaction of its substrate proteins with the enzyme.<sup>86</sup> Unlike FTase, it is inhibited by  $Zn^{2+}$  and recognizes other domains of the target protein in addition to its C-terminal substrate.<sup>74,85,94</sup>

#### *Selectivity of Proteins for Prenylation Reactions*

Although FTase and GGTase-I have distinct protein substrate preferences, their substrate specificities are not absolute. It has been shown *in vitro* that GGTase-I can prenylate proteins in which the X amino acid is methionine (usually a substrate for FTase) and FTase can prenylate

proteins in which the X is leucine (usually a substrate for GGTase-I).<sup>94</sup> Furthermore, GGTase-I can geranylgeranilate K-Ras4B and other proteins that are typically farnesylated,<sup>95</sup> and it can both farnesylate and geranylgeranilate a single substrate, Rho B.<sup>96</sup> Although it is apparent that geranylgeranilated Ras proteins produced by mutant *ras* genes are capable of transforming cells, geranylgeranilated proteins produced by normal *ras* are inhibitory. The cumulative results of studies indicate that although membrane localization is critical for Ras function modification with a specific isoprenoid lipid moiety (eg, FDP or GDPD), it is not essential.<sup>97</sup> The cross-prenylation of Ras by GGTase-I has also been observed in yeast strains in which the *RAM1* gene for the FTase  $\beta$ -subunit has been deleted.<sup>98</sup> The potential for cross-prenylation of FTase and GGTase-I implies that GGTase-I might be able to restore the function of Ras and other proteins after FTase inhibition, which may have implications for the development of resistance to such targeted therapeutics.<sup>95,96,98</sup>

Many mammalian proteins besides the four forms of Ras have a CAAX motif and are substrates for either FTase or GGTase (Table 2).<sup>80,86,94,100</sup> Why some proteins are modified by farnesylation, others by geranylgeranylation, and still others by double geranylgeranylation remains unknown. Many of these substrates are oncogenic and/or have roles in mitogenic signaling. Protein farnesylation is essential for many physiologic processes, including skeletal muscle function (phosphorylase kinase) and vision (transducin  $\gamma$ -subunit, cyclic guanosine 3',5'-monophosphate [cGMP] phosphodiesterase  $\alpha$ -subunit of cGMP, and rhodopsin kinase).<sup>79,80</sup> GGTase-II catalyzes proteins of the Rab family that are involved in protein secretion. Thus proteins involved in Ras signal transduction are not the only proteins that undergo prenylation, suggesting that therapeutic efforts directed at disrupting these processes may in fact inhibit multiple pathways.

Prenylated proteins have vastly different affinities for FTase and GGTases, however, which largely depend on the specific amino acids that comprise the C-terminus CAAX tetrapeptide and the binding constant ( $K_m$ ) of the enzyme. Although most data suggest that the minimal recognition sequence of proteins that are farnesylated by FTase is the CAAX tetrapeptide, there is evidence that additional sequences outside the CAAX region influence the binding affinity of substrates to the enzymes and the kinetics of prenylation.<sup>96,101</sup> The differences in protein affinity imply that various intracellular proteins exhibit a range of sensitivities to FTase inhibitors. Because protein farnesylation is involved in many physiologic processes, a major concern

**Table 2. Mammalian CAAX Proteins That Are Known or Likely to Be Prenylated**

CAAX Protein(s)	Function(s)
<b>Farnesylated</b>	
H-Ras, K4B-Ras, and N-Ras	Signaling for growth, differentiation, apoptosis
Lamins A and B	Nuclear membrane structure
Rap2	Platelet function
Rho-B and Rho-E	Cytoskeletal organization; gene expression; cell cycle control
Pxf	Peroxisomal location
Phosphorylase kinase $\alpha$ and $\beta$	Skeletal muscle function
PRL-1/PTP CAAX 1 and 2	Protein tyrosine phosphatase
Transducin $\gamma$	Visual signal transduction
cGMP phosphodiesterase $\alpha$	Visual signal transduction
Rhodopsin kinase	Visual signal transduction
YDJ1 homolog	Chaperone protein
Inositol-1,4,5-triphosphate 5-phosphatase type I	Lipid phosphatase; calcium signaling
<b>Geranylgeranilated</b>	
G-proteins $\gamma$ -subunits	Signaling for growth, differentiation, apoptosis
Rap1	Competes with Ras for various effectors
Rho A, B, C, and G	Cytoskeletal organization; gene expression; cell cycle control
Cdc42	Rho family; cytoskeletal organization; cell polarity in <i>Saccharomyces cerevisiae</i>
Rac 1 and 2	Membrane ruffling; actin reorganization
R-Ras 1 and R-Ras 2/TC21	Binds to bcl-2, which regulates apoptosis
Ra1 A and B	Unknown
cGMP phosphodiesterase $\beta$	Visual signal transduction
2'-5' oligo (A) synthetase	Role in protein synthesis
2'-3'-Cyclic nucleotide 3'-phosphodiesterase	Composed of myelin; MAP
Inositol-1,4,5-triphosphate 5-phosphatase type I	Lipid phosphatase, calcium signaling

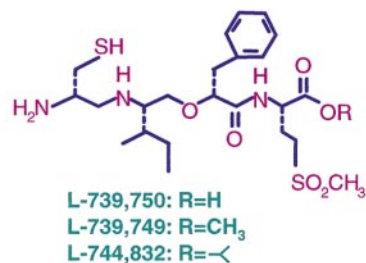
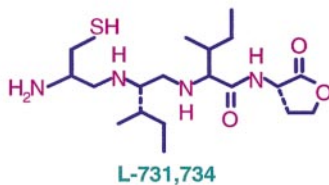
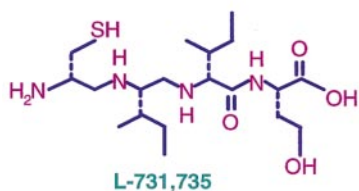
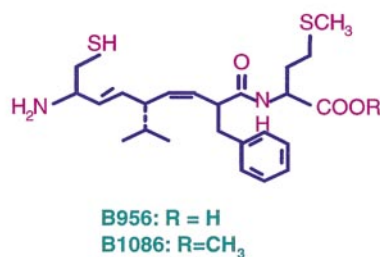
NOTE. Data adapted.<sup>77,78,99</sup>

regarding the development of therapeutics targeting FTase is whether sufficiently high therapeutic indices can ever be achieved.

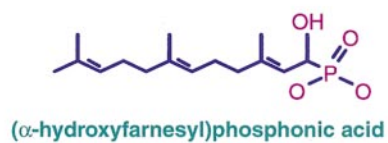
#### TYPES OF FTase INHIBITORS

The acquisition of detailed kinetic information about the FTase reaction and the physicochemical nature of FTase substrates has led to the rational design of FTase inhibitors.<sup>6,77-80,102,103</sup> Three general approaches have been used: design and synthesis of FDP analogs that compete with the substrate FDP for FTase; design and synthesis of peptidomimetics or CAAX mimetics that compete with the CAAX portion of Ras for FTase; and design and synthesis of bisubstrate analogs that combine the features of both FDP analogs and peptidomimetics (Fig 3). Still other approaches have resulted in the development of several types of structurally and functionally unrelated compounds that are

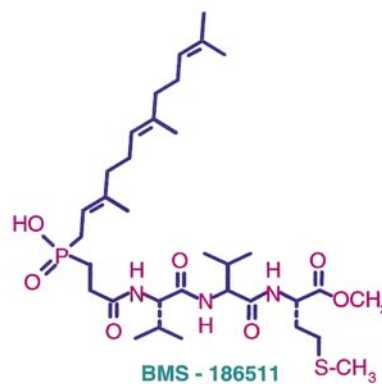
### I. Peptidomimetics (CAAX Mimetics)



### II. Farnesyl diphosphate analogs



### III. Bisubstrate Inhibitors



### IV. Inhibitors identified from compound libraries

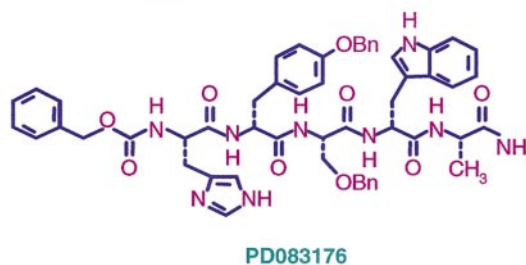
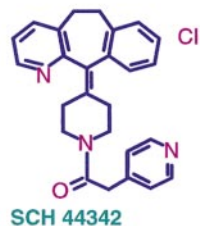


Fig 3. Structures of representative agents from different classes of F1ase inhibitors.



nonpeptidomimetic inhibitors of FTase. The recent elucidation of the crystalline structure of FTase most likely will further our understanding of the binding of specific classes of inhibitors and provide insight into the optimal design of FTase inhibitors.<sup>88</sup>

#### *FDP Analogs*

Inhibitors of FTase have been designed based on the farnesyl moiety of the FDP substrate. Among the first FTase inhibitors to demonstrate activity in cell culture systems was the nonhydrolyzable FDP  $\alpha$ -hydroxyfarnesyl-phosphonic acid, which inhibits FTase with an inhibitory constant ( $K_i$ ) of 5 nmol/L.<sup>6,79,95</sup> The agent inhibited Ras processing in H-*ras*-transformed NIH 3T3 fibroblasts at concentrations as low as 1  $\mu$ mol/L.<sup>40</sup> Other, more highly selective FDP analogs that inhibit FTase at submicromolar concentrations in vitro have also been synthesized and have been shown to inhibit H-Ras processing in whole cells at concentrations of approximately 1  $\mu$ mol/L.<sup>6,95</sup> These FDP analogs have also been demonstrated to block H-Ras-mediated transformation of NIH 3T3 fibroblasts at concentrations of 100  $\mu$ mol/L, and none were toxic to untransformed cells at concentrations of up to 250  $\mu$ mol/L.<sup>6</sup> However, these agents have not yet demonstrated relevant antitumor activity in animal models.

Although FDP binds to FTase at low nanomolar affinity, intracellular FDP concentrations are approximately 1  $\mu$ mol/L, which means that most FDP binding sites on FTase in the cell are occupied. Thus FDP analogs likely need to possess higher affinity than does FDP for FTase. Further, other enzymes use FDP in many cellular processes, which implies that FDP analogs may produce appreciable toxicity and therefore clinically useful compounds need to be much more selective for FTase than do other FDP-using enzymes in the cell.

#### *Peptidomimetics*

The finding that CAAX tetrapeptides contain the primary determinants for enzyme recognition led to the synthesis of a number of peptides as FTase inhibitors, using the principles of rational drug design. The demonstration that tetrapeptides with aromatic amino acid substitutions at the second aliphatic amino acid position two residues away from the cysteine group were nonsubstrate FTase inhibitors aroused interest in developing low-molecular-weight CAAX peptidomimetics as a principal strategy for FTase inhibition.<sup>100,104</sup>

Although CAAX peptides are potent FTase inhibitors in acellular systems, several physicochemical aspects limit their usefulness against tumor cells growing in tissue culture and in animals, and these compounds generally lose two or three logs of potency in whole cells. First, the free C-terminal carboxylate residue of CAAX mimetics is nega-

tively charged, which makes the plasma membrane relatively impermeable to such compounds. To mask the negative charge, a prodrug strategy has been used to synthesize ester or lactone derivatives, with the assumption that the ester or lactone would be hydrolyzed to the more active acid in the cell. Nevertheless, these prodrugs are susceptible to cleavage by esterases and other hydrolytic enzymes in plasma, and thus the challenge has been to develop prodrugs that are resistant to hydrolysis in plasma but still sensitive to the intracellular hydrolysis required to generate the active FTase inhibitor. Second, the labile peptidic bonds of these compounds are rapidly degraded by intracellular proteases, and additional chemical modifications to enhance compound stability are required. A pseudopeptide strategy, whereby peptide bonds in CAAX are reduced to their methyleneamino forms, has been used to create several potent and stable peptidomimetics. For example, reduction of the first and second amide linkages and substitution of homoserine for methionine has been used to synthesize L-731,735, which is relatively stable in the cell.<sup>105</sup> L-731,735 is a potent inhibitor of FTase (concentration that inhibits function or growth by 50% [ $IC_{50}$ ], 18 nmol/L); the  $IC_{50}$  of its prodrug, L-731,734, is much greater ( $IC_{50}$ , 282 nmol/L). A further application of this approach involves the synthesis of the methyleneoxy-isostere L-738,750, a potent FTase inhibitor ( $IC_{50}$ , 1.8 nmol/L) that is prepared by replacing the amide linkages between the two central amino acids in CAAX with an oxyether bridge.<sup>106</sup> Both L-738,750 and its prodrug methyl ester derivative, L-739,749, inhibit H-Ras processing at concentrations of 0.1 to 1.0  $\mu$ mol/L and suppress the growth of mutated H-Ras-transfected tumors in nude mice.<sup>107</sup> A similar prodrug, L-744,732, has been demonstrated to inhibit the growth of more than 70% of tumor cell lines in vitro at concentrations of 2 to 20  $\mu$ mol/L.<sup>107</sup>

A more recent approach to developing peptidomimetic FTase inhibitors is to eliminate the prodrug strategy. One permutation of this approach involves deletion of the X residue in the CAAX box, followed by further modifications of the resultant C-terminal elements.<sup>108</sup> This strategy has produced cell-permeable compounds that are pure competitive inhibitors of the protein substrate but are not themselves substrates of FTase. These agents also possess in vitro potencies for FTase in the range of 25 to 500 nmol/L. In addition, despite deletion of the X residue, which determines prenylation specificity, these pseudopeptides retain more than 100-fold selectivity for FTase versus GGTase-I. The development of these agents has been limited by non-mechanism-based cytotoxicity.

Another related approach involves replacing the peptidic features of the two central amino acids of the CAAX tetrapeptide with stable hydrophobic spacers. This approach,

using 4-aminobenzoic acid and its derivatives to replace the amino acids, has been used to synthesize FTI-276, which is one of the most potent compounds in its class, and its prodrug, FTI-277.<sup>109</sup> In vitro, FTI-276 inhibits FTase (IC<sub>50</sub>, 0.5 nmol/L), and in vivo FTI-277 inhibits H-Ras processing (IC<sub>50</sub>, 100 nmol/L). Still another pseudopeptidomimetic approach, in which alkaline spacers are used to replace the central two amino acids in the CAAX tetrapeptide, led to the synthesis of B956 and its prodrug, B1086.<sup>110</sup> B956 inhibits both H-Ras and K-Ras processing (IC<sub>50</sub>, 0.5 and 25 μmol/L, respectively). These agents have been shown to inhibit the growth of transformed cell lines without Ras mutations at concentrations ranging from 16 to 80 μmol/L and to inhibit tumor growth in nude mice.<sup>110</sup>

Random, high-volume screening of histamine-receptor antagonists from compound libraries led to the identification of a class of novel nonpeptidic, nonsulfhydryl tricyclic inhibitors of FTase that do not depend on a prodrug strategy.<sup>6,102</sup> The prototypical tricyclic FTase inhibitor SCH44342 actively competes with the CAAX substrate. This agent inhibits human FTase (IC<sub>50</sub>, approximately 250 nmol/L) and Ras processing in Cos-7 monkey kidney cells that transiently expressed H-Ras (IC<sub>50</sub>, 3 μmol/L).<sup>111-114</sup> The pentapeptide PD083176 was also identified by high-volume screening of a compound library, and further structure-activity studies led to a series of potent derivatives.<sup>115</sup> PD083176 lacks the cysteine residue common to most potent FTase inhibitors and was shown to be competitive with FDP. Although this agent inhibits human FTase (IC<sub>50</sub>, 10 nmol/L), it does not penetrate cells. However, when 5 pmol was microinjected into *Xenopus* oocytes, PD083176 inhibited insulin-induced cell maturation, a Ras-mediated process, but not progesterone-induced maturation, a process not dependent on Ras.

#### Bisubstrate Analogs

Structural and kinetic analyses of FTase revealed a sequential mechanism whereby an enzyme-FDP-CAAX ternary complex is formed before catalysis and raised the possibility that bisubstrate analogs that mimic the transition state of the enzyme might be both potent and specific inhibitors of the enzyme. Instead, bisubstrate analogs that incorporate the structural motifs of both FDP and the CAAX tetrapeptide are highly potent in vitro.<sup>116</sup> The bisubstrate analog BMS-186511 is 2,000-fold more specific for FTase than for GGTase and has a minimal effect on normal cells.<sup>117</sup> The compound also inhibits Ras signaling and growth in H-*ras*-transformed and K-*ras*-transformed NIH 3T3 cells at concentrations as low as 0.1 μmol/L, with farnesylation of Ras almost completely inhibited at 100 μmol/L. Furthermore, the agent has been shown to inhibit the anchorage-

independent growth of ST88-14, a malignant schwannoma cell line that is deficient in the expression of neurofibromin.<sup>117</sup> Because neurofibromin has intrinsic Ras GTPase-activating activity and cells deficient in it have increased levels of Ras-GTP, it is conceivable that inhibitors of FTase will be useful in treating patients with type I neurofibromatosis.<sup>118,119</sup>

#### Natural Products

A number of other FTase inhibitors have been identified by high-throughput screening of natural products or libraries of compounds that inhibit the ability of FTase to catalyze the addition of FDP to recombinant H-Ras in vitro. Random screening of microbial and natural products, using a yeast genetic screen for cell-permeable Ras inhibitors, has led to identification of the microbial product manumycin and related compounds as inhibitors of FTase.<sup>120</sup> Some natural products, including the chaetomelic acids, actinoplanic acid A, and manumycin analogs, compete with FDP, whereas other inhibitors, such as the pepticinnamins, compete with the Ras CAAX tetrapeptide.<sup>6</sup> Most of these agents inhibit human FTase at IC<sub>50</sub> values of approximately 100 nmol/L. Manumycin has been shown to inhibit the growth of several human pancreatic cancer cell lines (IC<sub>50</sub>, 3.5 to 7.5 μmol/L).<sup>121</sup> Interestingly, inhibitory activity in cell lines containing a mutated K-*ras* gene was comparable to that in cell lines with the wild-type *ras* gene. The number of nude mice that developed tumors after inoculation with these cells was reduced by 80% when the cells were pretreated with manumycin 30 μmol/L for 2 hours before inoculation. Further, the number of liver metastases was reduced significantly. Other natural products, such as fusidienol, preusomerin, gliotoxin, 10'-desmethoxystreptonigrin, and cylindrol A, inhibit FTase noncompetitively at IC<sub>50</sub> values of 1 to 2 μmol/L.<sup>6</sup>

#### Nonpeptidomimetic FTase Inhibitors

The first FTase inhibitor to be studied in human clinical trials, R115777 (Fig 4), is a nonpeptidomimetic FTase inhibitor that is an oral quinolone analog of imidazole-containing heterocyclic compounds initially developed as antifungals. Screening led to identification of the compound 4-phenyl-6-(phenyl-1H-imidazole-1-ylmethyl)-2(1H)-quinolinone as a lead, and subsequent directed synthesis led to the discovery of an analog, R115777, with oral antitumor activity.<sup>122</sup> In vitro tests of human tumor cell lines showed 80% overall sensitivity to R115777, and 100% growth inhibition at ≤ 120 nmol. K-*ras* mutations were identified as a marker for resistance to the agent (the remaining 20% of tumors had < 50% growth inhibition at 500 nmol).<sup>123</sup> R115777 inhibits the farnesylation of lamin B (IC<sub>50</sub>, 0.08

## Nonpeptidomimetic FTase inhibitor



Fig 4. Structure of the nonpeptidomimetic FTase inhibitor R115,777.

nmol) and K-rasB ( $IC_{50}$ , 7.9 nmol) and proliferation of H-Ras–transformed fibroblasts ( $IC_{50}$ , 1.7 nmol). Proliferation of CAPAN-2, HCT-116, and LoVo tumor cells was inhibited at  $IC_{50}$  measurements of 16 to 22 nmol.<sup>124</sup>

### GGTase Inhibitors

Most of the FTases described thus far were developed as selective inhibitors of FTase, and these compounds are more than 1,000-fold more potent at inhibiting FTase than are either GGTase-I or GGTase-II. It can be argued that selectivity is desirable to avoid toxicities that might result from GGTase inhibition, given that a far greater number of physiologic proteins are known to be substrates for GGTase-I. Selective inhibitors of GGTase, including GGTI-297 and GGTI-298, have been synthesized, and these compounds might be useful in determining the role of GGTase in cells.<sup>125-127</sup> In addition, it is possible that the coordinate use of GGTase inhibitors and FTase inhibitors will be more effective against cells harboring mutated K-ras, which is a substrate for GGTase-I.

### Antisense Oligonucleotides

Although the focus of most efforts at blocking the activation of mutant Ras has been on inhibiting FTase, antisense oligonucleotides that block Ras function have also been designed. The expression of mutant H-Ras can be inhibited by antisense nucleotides that interact with H-Ras mRNA codon 12, where mutation most frequently occurs.<sup>128</sup> When absorbed to polymeric nanoparticles, these oligonucleotides were shown to inhibit the growth of tumors with mutant Ras implanted in nude mice. Antisense oligonucleotides can also be used against other proteins in the signaling

cascade activated by Ras proteins, such as the Raf protein.<sup>128</sup> Although an advantage of this approach is specificity of the oligonucleotide to the target gene, it is likely that blocking the expression of only one of the downstream proteins will not be sufficient to reverse the full effect of activated Ras expression.

## ANTITUMOR ACTIVITY OF FTase INHIBITORS

### *In Vitro* Studies

FTase inhibitors block the farnesylation of Ras in a dose-dependent manner in cancer cells growing in tissue culture, although most studies have been performed in tumor cells in which the substrate is a mutated form of H-Ras.<sup>123-131</sup> Although these agents are much more effective at inhibiting protein farnesylation than geranylgeranylation, FTase inhibitors also inhibit farnesylation of many other identified and probably unidentified protein substrates of FTase. However, much higher concentrations of FTase inhibitors are required to block the farnesylation of many of these other substrates, particularly lamin B.<sup>129,131</sup> One caveat is that inhibitors of FTase are generally much less effective at modulating the processing of K-Ras, whose gene is the most frequently mutated *ras* in human cancer. One possible explanation is that the affinity of K-Ras for FTase is 10- to 30-fold higher than for other forms of Ras.<sup>86,87,132</sup> Alternatively, this phenomenon may reflect the ability of GGTase-I–catalyzed geranylgeranylation to restore K-Ras processing in cells treated with FTase inhibitors, because K-Ras possesses the specific CAAX motif and the upstream polylysine region, which are requirements for GGTase-I substrates.<sup>109,133,134</sup>

At concentrations that inhibit the farnesylation of H-Ras in tumor cells in vitro, FTase inhibitors prevent many changes associated with neoplastic transformation in rodent fibroblasts, including rapid and anchorage-independent growth, morphologic transformation, and cytoskeletal alterations.<sup>79</sup> The concentration of FTase inhibitors required to elicit these effects is similar to that required to inhibit intracellular farnesylation, suggesting that these actions are mechanism based. However, many of the cellular effects induced by FTase inhibitors may be considered “cytostatic,” as suggested by the return of H-ras–transformed fibroblasts growing in Petri dishes to the flattened appearance of a transformed phenotype once the FTase inhibitor is removed from the culture medium.<sup>130,131</sup> In addition, FTase inhibitors downregulate signaling pathways activated by Ras in cells growing in tissue culture. For example, treatment of H-ras–transformed fibroblasts with FTase inhibitors inactivates the Raf/MEKK/MAP kinase cascade by preventing Raf from binding to membrane-bound Ras-GTP.<sup>135,136</sup> After treatment, Raf is involved in an inactive complex associated with

nonprenylated, soluble Ras.<sup>136</sup> Some FTase inhibitors have been shown to inhibit MAPK activation in cells transformed by H-*ras*, but not in cells transformed by the geranylgeranylated form of K-*ras*.<sup>109,133,135,136</sup> The specificity of these agents is further illustrated by studies showing that cells transformed by activated *raf* are resistant to the actions of FTase inhibitors at doses that clearly inhibit cells transformed by H-*ras*.<sup>105,116,131</sup>

In human tumor cell lines, FTase inhibitors block anchorage-independent cell growth. In a study involving 42 human tumor cell lines, L-744,832 inhibited anchorage-independent growth in more than 70% of the cell lines (IC<sub>50</sub>, < 20 μmol/L).<sup>107</sup> Many of these cell lines had multiple genetic alterations, including mutant K-*ras*, mutant *p53*, and dysregulated *myc*, as well as overexpression of growth factor receptors. Of interest, 11 of 17 cell lines with wild-type Ras were also sensitive to L-744,832.

Similarly, R115777 completely inhibited the growth of 80% of a series of human tumor cell lines at concentrations of less than 120 nmol/L.<sup>123</sup> Although several of the cell lines harboring K-*ras* mutations were drug sensitive, much higher drug concentrations were generally required, and the cell lines were more likely to be resistant to the inhibitor. In addition, FTI-276 inhibits growth of human tumor cells bearing many relevant types of mutations, including oncogenic K-*ras* and a *p53* deletion, but it was not active against a lung cancer xenograft that lacked these mutations.<sup>137</sup> In another study, in which 19 human tumor cell lines were evaluated for FTase-mediated inhibition of anchorage-independent growth after treatment with B956 and its methyl ester B1086, the drug sensitivity in the 14 cell lines with Ras mutations was greatest in cells with mutant H-Ras, followed by cell lines expressing mutant N-Ras.<sup>110</sup> Tumor cell lines expressing mutant K-Ras and those without Ras mutations were more resistant. Drug sensitivity in the cell lines with mutant K-*ras* spanned two orders of magnitude.

Taken together, these findings indicate that FTase inhibitors may be effective against a broader range of cancer cells than originally anticipated, including tumors that are not solely dependent on *ras* mutations; however, their optimal activity is likely to be against tumors expressing H-*ras*. This finding may reflect the ability of K-Ras and N-Ras to alternatively be prenylated by GGTase-I when farnesylation is blocked.<sup>133,138,139</sup> Alternatively, the incomplete correlation between Ras mutational status and sensitivity to FTase inhibitors suggests that not all cells with *ras* mutations depend on Ras for transformed growth. Indeed, these cells may have other mutations that make mutant Ras redundant. Another possible explanation that may have far-reaching ramifications is that farnesylation of other proteins, in

addition to Ras, is important for cancer cell growth. Supporting this hypothesis is evidence that many other critical proteins are targets for FTase inhibitors in transformed cells and may play a role in conferring tumor cell sensitivity to FTase inhibitors.<sup>99,140</sup> One putative target is the protein RhoB, which is both farnesylated and geranylgeranylated *in vivo*; however, RhoB appears to be farnesylated primarily by GGTase-I *in vitro*.<sup>97</sup>

Investigations of alternative or complementary mechanisms by which FTase inhibitors cause tumor regression are warranted. Because these agents do not exhibit significant toxicity *in vitro* and *in vivo*, they obviously differ from available chemotherapeutic agents. In *ras*-transformed cells that are not allowed to attach to a substratum, L-739,749 has been demonstrated to induce massive DNA degeneration and cell death that is independent of *p53* but inhibited by the apoptosis suppressor Bcl-xl.<sup>141</sup> Another FTase inhibitor, FPT inhibitor III, has been demonstrated to augment the expression of the apoptosis-promoting proteins Bax and Bcl-xs and to induce apoptosis in human ovarian cancer cells.<sup>142</sup> Additionally, substantial growth suppression of a C32 human melanoma xenograft harboring wild-type Ras was shown to emanate predominately from an apoptotic response.<sup>143</sup> The proapoptotic effects of the FTI L-744,832 have also been demonstrated to be masked by activation of P13K, which is modulated by cytokines and integrins.<sup>144</sup> Furthermore, the results suggested that efforts to inhibit the P13K pathway may unmask the proapoptotic effects of FTIs in malignantly transformed but not normal cells.<sup>144</sup> These collective findings suggest that under certain conditions, FTase inhibitors may inhibit tumor growth by promoting apoptosis, which may have important implications for the clinical development of FTase inhibitors. With regard to the promotion of apoptosis by FTase inhibitors, it also must be determined whether the principal cellular target is Ras itself or another signaling protein that undergoes farnesylation. Again, a possible target is Rho B, which is intimately involved in adhesion and undergoes farnesylation. It has been suggested that FTase inhibitors block Rho B signaling, which causes transformed cells to revert to a state in which cell attachment is necessary for continued viability.<sup>141</sup>

There is even evidence that if they carry oncogenic *ras* mutations, malignant cells with multiple genetic abnormalities (a scenario that more closely resembles the typical clinical one) may be sensitive to FTase inhibitors. However, because FTase inhibitors may also block normal Ras function, they may be active against tumor cells transformed by mutations upstream of Ras. Although redundancies in cell signal transduction pathways that bypass Ras to activate the MAP kinase pathway represent potential mechanisms of cellular resistance to FTase inhibitors, these agents have



additional actions downstream of Ras that enable them to exert activity despite parallel upstream signaling. For example, unfarnesylated oncogenic H-Ras acts as a dominant negative inhibitor of Ras activity.<sup>145</sup> In the unfarnesylated state, it forms a stable complex with Raf, preventing its translocation from the cytoplasm to the plasma membrane. However, a nononcogenic Ras, modified so that it cannot be farnesylated, does not interact with Raf, which implies that cells with oncogenic Ras may be more sensitive to FTase inhibitors than are normal cells.<sup>146</sup> Nevertheless, even incomplete inhibition of FTase might provide a pool of oncogenic Ras to inhibit Raf activity in tumor cells with *ras* mutations, whereas Raf activity in normal cells with wild-type *ras* would not be affected. Because *K-ras* mutations are much more common in human malignancies than are *H-ras* mutations, these implications would be more profound if unfarnesylated oncogenic K-Ras behaves in a similar manner.

The frequency with which resistance develops, as well as its mechanism, will need to be explored if FTase inhibitors prove to be clinically effective anticancer drugs. In an *in vitro* study, *ras*-transformed 749<sup>F</sup>-1 cells, which are derived from Rat1 cells, were unaffected by treatment with the peptidomimetic FTase inhibitor L-739,749 at concentrations up to 30-fold higher than those sufficient to revert *ras*-transformed cells.<sup>147</sup> Resistance correlated with a reduced ability of L-739,749 to inhibit farnesylation of Ras and lamin B and to regulate growth and cytoskeletal activation. In addition, endogenous FTase was less susceptible to drug inhibition. Further studies indicated that the resistance was not related to mutations of the FTase subunits, changes in intracellular drug accumulation, nor amplification of the multidrug-resistance gene. Given these results and related findings in transgenic mouse models, it may be important to formulate dosing strategies to ensure that tumor cells are not exposed to FTase inhibitors under conditions in which resistant cells can be selected.

#### *Antitumor Activity of FTase Inhibitors in Xenograft Models*

One of the first demonstrations of the *in vivo* antitumor activity of FTase inhibitors was against transformed rodent NIH 3T3 cells transplanted into nude mice. R115777, administered orally twice daily for 15 days at doses of 6.25, 12.5, and 25 mg/kg, inhibited the growth of *H-ras*-transformed cells by 56%, 84%, and 86%, respectively.<sup>147</sup> In nude mice with LoVo human colon tumors, R115777 given for 32 days at doses of 25, 50, and 100 mg/kg inhibited tumor growth by 11%, 68%, and 81%, respectively. In neither of these studies was any overt toxicity observed.<sup>148</sup> Other FTase inhibitors have also been effective at inhibiting tumor growth in nude mice. Daily subcutaneous administra-

tion of L-739,749 suppressed the growth of *H-ras*-, *K4B-ras*-, or *N-ras*-transformed fibroblasts in a murine xenograft model in a dose-dependent manner, the extent of which suppression correlated with the inhibition of H-Ras processing in the tumor.<sup>105</sup> In contrast, the growth of *raf*- or *mos*-transformed tumors was not suppressed. The agent was also active against a PSN-1 pancreatic cancer cell line carrying mutations in *K-ras*, *p53*, and *myc*.<sup>105</sup> In addition, daily intraperitoneal administration of the CAAX peptidomimetic FTI-276 inhibited the growth of *H-ras*-transformed NIH 3T3 cells in nude mice, which correlated with the ability of the agent to inhibit Ras processing in the cells.<sup>137</sup>

Many types of FTase inhibitors inhibit growth of human tumor xenografts in nude mice. For example, the tricyclic FTase inhibitor SCH66336, which inhibits the growth of tumor cells with and without activated *ras* oncogenes *in vitro*, demonstrated impressive activity against a wide array of human tumor xenografts, including tumors of colon, lung, pancreas, prostate, and urinary bladder origin.<sup>111</sup> R115777 was also effective against human tumor xenografts expressing *K-ras* mutations, including LoVo human colon and CAPAN-2 pancreatic xenografts.<sup>149</sup> In the LoVo tumors, R115777 predominantly inhibited malignant angiogenesis, whereas the principal effect in the CAPAN-2 tumors was growth arrest. These results indicate that FTase inhibitors inhibit tumor growth by several mechanisms.

#### *Toxicity in Preclinical Studies*

An unexpected but desirable aspect of FTase inhibitors is their apparent lack of growth inhibitory activity against nonmalignant cells *in vitro* and their tolerability in animal and human studies.<sup>105,116,129,130,136,149</sup> For example, histologic examination of the tissues of animals treated with L-744,832 or L-739,749 for protracted periods has revealed no abnormalities in rapidly dividing tissues (eg, bone marrow and gastrointestinal tissue) or in tissues in which farnesylated proteins play critical physiologic roles (eg, eyes and skeletal muscle).<sup>105,150</sup> Although one possible explanation for the absence of toxicity in normal tissues at FTase-inhibitor doses that inhibit tumor growth is cross-prenylation of unmutated N- and K-Ras by GGTase in normal cells, this raises the question of why cells with mutated N-Ras and K-Ras are sensitive to FTase inhibitors. A second possible explanation, somewhat related to the first, is that other geranylgeranylated proteins related to Ras can functionally overlap with Ras proteins, thereby compensating for the loss of Ras function.<sup>77</sup> A third possibility is that endogenous Ras proteins are more likely to be K-Ras4B or N-Ras, whose prenylation and function are not as effectively blocked by FTase inhibitors as are those of H-Ras.<sup>133</sup> It is also possible that a greater redundancy of pathways in normal cells may

allow them to downregulate Ras function. Finally, less toxicity to normal tissues may result if FTase inhibitors can reduce the function of oncogenic Ras to below the critical threshold required for transformation yet not so low that essential Ras functions are disrupted.<sup>77</sup> The implication of these observations is that FTase inhibitors may produce relatively high therapeutic indices in patients with malignant diseases. The preliminary results of phase I studies of both peptidomimetic (L-778,123) and nonpeptidomimetic (R115777, SCH66336) FTase inhibitors support these pre-clinical findings.<sup>151-157</sup>

#### *FTase Inhibitors Against Tumors Arising in Transgenic Mice*

One of the advantages of using cell culture models is the ease of genetic manipulation, because various mutants of a gene of choice can be readily introduced by transfection, and the effect on a given cellular property can be assessed. However, tumors arising in transgenic mice more closely resemble human tumors with regard to cellular environment and natural history of tumor development than do xenograft models. One such transgenic model that may be ideal for the evaluation of FTase inhibitors is the mouse mammary tumor virus (MMTV)-*H-ras* transgenic mouse. These mice express the activated *H-ras* oncogene under control of the MMTV promoter, which directs expression to the mammary and salivary glands. As a result, the mice develop both mammary and salivary adenocarcinomas at an average age of approximately 8 months.<sup>158,159</sup>

MMTV-*H-ras* transgenic mice with palpable mammary and salivary adenocarcinomas were treated with subcutaneous L-744,832 at daily doses of 10 to 40 mg/kg.<sup>149</sup> At the highest dose, these established tumors regressed in all mice and were no longer measurable after 2 weeks of treatment. At the dose of 20 mg/kg, all animals exhibited at least a partial reduction in tumor size, but at the dose of 10 mg/kg, several animals did not respond. In these later animals, subsequent treatment with doses of 40 mg/kg proved ineffective, which raises the possibility that exposure to suboptimal doses of FTase inhibitor will select for resistant tumor cells. Further, tumors regrew in most animals after treatment was discontinued. Nevertheless, L-744,832 was more effective than doxorubicin administered at its maximally tolerated dosage. Similarly, prophylactic oral treatment of *H-ras* transgenic mice with SCH66336 delayed tumor onset, reduced the number of tumors per mouse, reduced the average tumor weight per animal, and produced significant regression of established tumors in a dose-dependent fashion when administered in a therapeutic mode.<sup>111</sup>

The MMTV-*ras* transgenic model has been used to investigate the mechanism of response to FTase inhibitors and to determine whether it is dependent on the *p53* tumor suppressor gene that plays a critical role in the G<sub>1</sub> cell cycle checkpoint, inducing either growth arrest or apoptosis in response to DNA damage and other cellular perturbations.<sup>160</sup> Interbreeding MMTV-*ras* transgenic mice with *p53* “knock-out” mice produces animals that develop *ras*-expressing tumors that either possess or lack *p53* function.<sup>161,162</sup> The loss of *p53A* results in greatly accelerated tumorigenesis, and tumors arising in *p53*-deficient mice have higher histologic grades, increased growth rates, and greater genomic instability than do tumors arising in *p53* wild-type mice.<sup>159</sup> Of interest, the level of spontaneous apoptosis in MMTV-*ras/p53*<sup>+/+</sup> tumors was found to be very low, probably because of the apoptosis-inhibitory properties of activated *ras*. In contrast to doxorubicin or paclitaxel treatment, treatment of transgenic mice with L-744,832 resulted in marked tumor regression that was associated with marked apoptosis and reduced numbers of S-phase cells in MMTV-*ras/p53*<sup>+/+</sup> tumors. Thus, tumors expressing activated *ras* were resistant to apoptosis, even in response to agents that readily induce apoptosis in other settings. However, the administration of L-744,832 renders the tumors sensitive to apoptosis, resulting in a dramatic tumor response. Tumors from MMTV-*ras/p53*<sup>-/-</sup> mice responded similarly to the FTase inhibitor as did *p53* wild-type tumors, indicating that apoptosis due to Ras inhibition is largely *p53* independent. In mice with *neu* and *c-myc* transgenes, L-744,832 produced modest tumor regression by reducing the fraction of S-phase cells, but the agent did not induce apoptosis. Thus, depending on the genetic alterations present, FTase inhibitors may promote tumor regression by multiple mechanisms, including apoptosis and cell cycle regulation. Finally, tumors arising in other transgenic models in which *ras* is not activated (eg, MMTV-*c-myc*, or MMTV-*neu*) are relatively unresponsive to inhibitors of FTase, again suggesting that this class of agents may be most active in tumors bearing *H-ras* mutations.

#### EVALUATING FTase INHIBITORS IN THE CLINIC

##### *Phase I and Feasibility Studies*

Several FTase inhibitors are currently being evaluated in phase I clinical investigations. In contrast to the development of cytotoxic agents, in which toxicities in rapidly growing tissues correlate, albeit loosely, with antitumor activity and can be used as general measures of drug effect, selecting an optimal dose of FTase inhibitors in phase I studies for subsequent disease-directed studies is a great challenge. Toxic effects may not be evident at doses that

inhibit Ras farnesylation, or may not be quantifiable or even related to FTase inhibition. Pharmacologic studies may be used to gauge whether plasma concentrations associated with maximal inhibition of Ras farnesylation and antitumor activity in preclinical studies are being achieved in patients. However, interspecies differences in tissue distribution of drug, protein binding, pharmacokinetics, and metabolic processes may preclude extrapolating from animals to humans, thereby limiting the usefulness of pharmacologic studies in this regard. The development and validation of assays of protein prenylation in accessible tissues that reflect farnesylation of Ras in tumors will undoubtedly facilitate efforts to determine the optimal doses of FTase inhibitors in phase I evaluations. Protein prenylation can be assessed using a diverse series of assays. For example, prenylation of a specific protein (eg, nuclear lamins) or global protein prenylation can be measured by labeling cellular proteins with [<sup>3</sup>H]mevalonic acid, the precursor of the isoprenoids, or metabolically labeling in vitro with [<sup>3</sup>H]mevalonic acid, [<sup>3</sup>H]FDP, or [<sup>3</sup>H]geranylgeranyl diphosphate.<sup>163</sup> Alternatively, inhibition of prenylation of marker proteins can be quantified using gel mobility shift assays. These assays may be helpful in selecting doses of FTase inhibitors that achieve maximal inhibition of prenylation of marker proteins validated to correlate with a desirable target effect.

An important clinical issue is how best to administer FTase inhibitors. There is experimental evidence indicating that continuous drug exposure, perhaps optimally achieved with continuous treatment, is required to achieve maximal efficacy. However, protracted dosing raises concerns about both acquired drug resistance and toxicity. Acquired drug resistance has been noted with the FTase inhibitors in both tumor cells growing in cell culture and animals.<sup>9,110,147,150</sup> In addition, the most likely long-term toxic effects of protracted continuous treatment may not be fully appreciated on the basis of the standard procedures used in preclinical toxicology studies of new anticancer agents in animals. In both preclinical and early clinical investigations, it will be important rigorously to monitor organs, such as the eyes and skeletal muscle, that require essential farnesylated proteins. Because many other farnesylated proteins have yet to be identified, it will also be prudent to monitor patients carefully for unexpected toxicity, particularly long-term effects.

The preliminary results of a phase I study of the first FTase inhibitor to enter clinical evaluations, R115777, which was administered orally on a twice-daily schedule for 5 consecutive days every 2 weeks in patients with solid malignancies, have indicated rapid gastrointestinal absorption, a plasma half-life of approximately 5 hours, and

achievement of biologically relevant steady-state plasma concentrations within 2 to 3 days of initiating twice-daily dosing.<sup>151</sup> At doses of less than 1,300 mg twice daily, R115777 was well tolerated, although an unacceptably high rate of dose-limiting toxicity, consisting of neuropathy (one patient), fatigue, and gastrointestinal complaints, was observed at the dose level of 1,300 mg twice daily.<sup>151</sup> Other adverse events included nausea, vomiting, diarrhea, fatigue, headache, and reversible renal toxicity.<sup>151</sup> The recommended dose for phase II evaluations was 500 mg twice daily, which results in biologically relevant plasma concentrations.<sup>151</sup> A study of the feasibility of administering R115777 on a twice-daily, 21-day continuous-dosing schedule is in progress.<sup>152</sup> To date, neutropenia and thrombocytopenia are projected to preclude treatment with doses exceeding 240 mg twice daily, and plasma steady-state concentrations at this dose inhibit tumor growth in vitro. Investigators have also begun to determine the safety, tolerability, and pharmacokinetic behavior of the peptidomimetic L-774,123, administered in a continuous 7-day intravenous infusion before commencement of a protracted administration schedule.<sup>153,154</sup> This peptidomimetic FTase inhibitor has a benzylimidazole core and low nanomolar activity against FTase and inhibits prenylation of Ras proteins and anchorage-independent growth of *ras*-transformed cells in vitro at low micromolar concentrations. In the phase I study, the feasibility of achieving steady-state plasma concentrations associated with the maximal FTase inhibition in preclinical studies is being assessed, and the inhibition of farnesylation of a marker chaperone protein in peripheral-blood mononuclear cells is being studied. Similarly, the tolerability and pharmacokinetic profiles of BMS214662 and SCH66336 are also being evaluated in phase I studies.<sup>111,155-157</sup> In phase I studies of SCH66336 administered on a twice-daily oral continuous-dosing schedule, vomiting, diarrhea, myelosuppression, and fatigue were the principal toxicities, and the recommended phase II dose is 240 mg twice daily.<sup>155-157</sup> In several of these trials, the inhibition of farnesylation of the marker protein prelamin A, which is converted to lamin A, is being assessed in both buccal mucosal cells and peripheral-blood mononuclear cells.<sup>155,156</sup> A partial response in a pretreated patient with non-small-cell lung cancer was a reported preliminary result.<sup>155</sup>

#### *Use With Other Agents and Therapeutic Modalities*

Because multiple pathways are important for the proliferation, invasion, and metastases of malignant cells, and because combination therapies are often far more effective than are single-agent regimens, the FTase inhibitors may complement other anticancer agents that may or may not

affect Ras-mediated pathways. Additionally, although FTase inhibitors demonstrated the capacity to rapidly reduce and nearly ablate large tumors in preclinical studies (rather than simply prevent tumor growth), residual tumors proliferated after withdrawal of the agents. Therefore, combinations of FTase inhibitors and classic cytotoxic chemotherapeutic agents may result in greater cytoreduction and may reduce the need for protracted therapy. The overlapping antitumor spectra and nonoverlapping toxicity profiles of FTase inhibitors and cytotoxic agents provide a rationale for assessing the efficacy and feasibility of combination regimens. Although the choice of chemotherapeutic agents to be evaluated in combination with inhibitors of FTase will ultimately be dependent on the logistics and appropriateness of the agents for the particular clinical setting, the selection may also be based on a unique mechanistic rationale. For example, the FTase inhibitor L-744,832 and antimicrotubule agents that prevent tubulin depolymerization, such as the taxanes and epothilones, have been shown to inhibit the growth of several breast cancer cell lines *in vitro* in a synergistic manner, whereas interactions between the FTase inhibitor and antimicrotubule agents that induce tubulin depolymerization are much less pronounced, albeit still additive.<sup>111,164</sup> Further, the results of mechanistic studies have indicated that L-744,832 enhances the mitotic block induced by antimicrotubule agents that prevent tubulin polymerization. The combination of paclitaxel or cisplatin with minimally effective concentrations of R115777 was demonstrated to produce additive antiproliferative activity against human MCF-7 breast, CAPAN-2 pancreatic, and C32 melanoma cells growing in tissue culture and as well-established tumor xenografts.<sup>165</sup> The interaction between R115777 and paclitaxel was additive irrespective of the order of drug administration, and the duration of the response to R115777 was not enhanced by paclitaxel. In another study, the combination of the FTase inhibitor SCH66336 and paclitaxel demonstrated either synergistic or additive activity against a broad panel of human tumor cell lines, except for one breast cancer cell line against which the combination demonstrated antagonism.<sup>166</sup> The results were independent of p53 mutational status, *ras* mutational status, or tissue of origin. Additive interactions have also been noted between FTase inhibitors and cisplatin, cyclophosphamide, doxorubicin, and fluorouracil.<sup>111,164</sup>

FTase inhibitors may also augment the responsiveness of tumors to other therapeutic modalities, such as the inhibition of malignant angiogenesis or ionizing irradiation. Oncogenic Ras is known to drive pathways involved in angiogenesis, and FTase inhibitors are capable of inhibiting angiogenesis.<sup>148,153,167</sup> In one study, L-739,749 was shown to block

the expression of vascular endothelium-derived growth factor in H-*ras*-transformed cells,<sup>168</sup> and it is conceivable that FTase inhibitors will be used with therapeutics that principally target malignant angiogenesis. H-*ras* and other oncogenes have also been demonstrated to confer resistance to the cytotoxic effects of ionizing radiation, and the inhibitors of FTase have demonstrated radiation-sensitizing properties in tumors growing in tissue culture and animals.<sup>146,147</sup> The augmentation of radiation may be attributed to the enhancement of irradiation-induced apoptosis of transformed cells by FTase inhibitors.<sup>169</sup> Furthermore, the radiosensitivity of normal cells is not enhanced, indicating a selective radiosensitizing effect, which provides a rationale for clinical evaluations of FTase inhibitors and ionizing radiation.<sup>169,170</sup>

#### *Disease-Directed Clinical Evaluations*

The design of disease-directed (phase II and III) clinical evaluations to determine whether FTase inhibitors may play a role in the treatment of specific malignancies presents several formidable challenges. FTase inhibitors have induced regressions of established tumors in animal models, but in contrast to the appreciable cytoreductive response that is the traditional end point in phase II “screening” evaluations of conventional chemotherapy agents, tumor growth inhibition or “cytostasis” may be the principal therapeutic effect of FTase inhibitors. Therefore, a developmental plan that provides for a clinical situation that is sufficiently sensitive for detection and measurement of tumor growth inhibition will need to be implemented in disease-directed evaluations. Although experimental evidence exists indicating that FTase inhibitors may inhibit the growth of tumors with or without *ras* mutations, phase III and earlier, exploratory (phase II) evaluations may have the greatest likelihood of detecting meaningful clinical activity if the studies are performed in tumor types that are highly likely to have *ras* mutations. Further, such detection is more likely if only patients who have malignancies with well-documented *ras* mutations are enrolled. After rigorous “proof of principle” trials, the scope of disease-directed evaluations can be broadened, and patient eligibility requirements can be less restrictive. For example, in advanced pancreatic cancer, a phase III study can be designed so that patients are randomized to treatment with either gemcitabine (conventional arm) or gemcitabine plus an FTase inhibitor (experimental arm). Alternatively, similar patients—or those who are at high risk for recurrence (ie, postpancreatectomy patients with metastases to lymph nodes and disease at the margins of resection)—may be randomized to treatment with either conventional therapy alone or an FTase inhibitor.



The principal end points for such trials would be median survival, percentages of patients who are alive at relevant intervals (eg, 1-year survival rate for patients with advanced pancreatic cancer), time to progression, clinical benefit (eg, performance status, weight loss, and pain control), and improvement in quality of life.

Realistically, some type of "lead" or indication that the FTase inhibitors possess relevant clinical activity and may be capable of modifying the natural history of disease progression will ultimately need to be observed before resource-intensive phase III studies are begun. One possible way to obtain such early leads before launching large randomized studies is to measure the relative time to tumor progression in patients receiving single-agent treatment with the FTase inhibitor (period B) against that produced by treatment with a relevant standard therapy or supportive care, measured just before administration of the experimental agent (period A). On the basis of experience with agents that were later shown to have relevant clinical activity in randomized trials (ie, 30% of patients had a longer time to progression when treated with the new agent in earlier, single-arm studies than when treated with the agent that they received before receiving the new agent), a 30% prolongation in the time to progression may be a reasonable threshold to use before proceeding to phase III studies. Alternatively, "exploratory" single-arm or randomized phase II studies that are designed with sufficient power to detect and quantify the relevant indices of tumor growth inhibition may provide meaningful leads about activity before randomized evaluations.<sup>171</sup> For example, in advanced pancreatic cancer, the percentage of patients surviving for at least 1 year in exploratory nonrandomized studies may be considered a reasonable end point to use in gauging whether to proceed with randomized phase III evaluations.<sup>171,172</sup> Given the results of phase II and III studies of gemcitabine in patients with advanced pancreatic cancer, an FTase inhibitor demonstrating a 1-year survival rate with a lower limit of a 95% confidence interval of at least 20% might be viewed as a candidate for phase III development.

On a similar note, the proportion of patients who have progressive disease as their best response appears to relate inversely to the ultimate utility of any particular agent in a specific clinical setting, and a maximum acceptable threshold of patients with progressive disease as their best response may be used to forecast the potential usefulness of the agent. A retrospective analysis of National Cancer Institute of Canada Clinical Trial Group phase II studies of new agents indicated that the rates of disease progression of agents thought to be most promising in breast and lung carcinomas and glioma appear to be less than 20%, 30%, and

40%, respectively.<sup>173</sup> The use of such thresholds, particularly those that have been validated, may be useful in screening FTase inhibitors before undertaking large randomized trials.

#### SUMMARY

The results of preclinical studies of FTase inhibitors suggest that therapeutics specifically designed on the basis of an understanding of the primary molecular defects governing malignant cell proliferation will be more efficacious and less toxic than will traditional, nonspecific cytotoxins. However, in spite of the encouraging experimental results to date, it remains to be determined whether FTase inhibitors can inhibit tumor growth in patients with advanced disease, and many obstacles will have to be overcome before we can fully evaluate these agents in the clinic. In addition to the usual need to optimize the pharmacologic characteristics of any new class of agents, there are several preclinical and clinical developmental concerns that are uniquely applicable to the FTase inhibitors.

Perhaps one of the most important concerns is that the animal tumor models used to evaluate FTase inhibitors do not fully recapitulate the complexity of genetic alterations in human tumors. The fact that mutated *ras* alone is but one genetic lesion essential for the complete conversion of a normal cell to the fully malignant phenotype raises the question of whether approaches to correct the *ras* defect alone will have any significant antitumor activity. However, limited experimental evidence suggests that correction of just a single defect, such as the *ras* mutation, can significantly impair the aberrant growth of tumor cells.<sup>174,175</sup>

The selectivity and therapeutic indices of FTase inhibitors are important related issues. Selectivity is a multifaceted phenomenon that relates not only to the prenylation of the various Ras proteins but also to the selectivity of protein substrates for FTase versus GGTase and the prenylation of many other proteins that are important from both physiologic and pathophysiologic standpoints. Further understanding of the relative roles of FTase and GGTase in cross-prenylating Ras, particularly K-Ras, and of the relative contribution of FTase-induced inhibition of the prenylation of Ras and other proteins will ultimately determine the requirements for the selectivity of inhibitors of FTase. Nevertheless, the accumulated biologic data obtained thus far indicate that these agents possess remarkable potential as components of our therapeutic armamentarium against malignant diseases and, possibly, nonmalignant disorders involving aberrant cellular proliferation.

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