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Perspective

Inhibitors of Farnesyltransferase: A Rational Approach to Cancer Chemotherapy?

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Introduction

Despite major breakthroughs in many areas of medicine over the past 100 years, the successful treatment of cancer remains a significant challenge at the start of the 21st century. The elucidation of molecular mechanisms that promote malignancy is yielding new therapeutic targets for drug discovery efforts. The goal is to produce novel agents that selectively kill tumor cells or inhibit their proliferation without the general toxicity that limits traditional cancer chemotherapy. One well-studied target is the oncogene *ras*. Ras proteins are small GTPases that function as on−off switches, regulating cellular functions such as proliferation.[1] When the protein binds GTP, it becomes transiently activated but normally reverts to the inactivated GDP-bound state. Ras is mutated in 30% of all human cancers,[2,3] and the mutant proteins persistently bind GTP and are constitutively active. It is generally believed that this aberrant function contributes to the development of cancer.[1] The discovery that Ras required prenylation for activity led to the targeting of the enzyme responsible, farnesyltransferase (FTase).[4,5] Thus, farnesyltransferase inhibitors (FTIs) were conceived as a rational way to treat cancer by inhibiting the function of the oncogene *ras*. Since they were last reviewed here,[6] FTIs have shown efficacy as anticancer agents in clinical trials. At the same time, significant doubt has been cast on a Ras-mediated mechanism of action.

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Farnesyltransferase Structure and Function

Farnesyltransferase catalyzes the reaction between farnesyl diphosphate (FPP) and the cysteine residue of a polypeptide's C-terminal CaaX motif (C is Cys; a is usually an aliphatic amino acid; X is the C-terminal residue, typically Met) to give a farnesyl thioether (Figure 1).[7] The enzyme is a heterodimer consisting of 48 kDa α and 46 kDa β subunits. The former subunit is also a component of the closely related enzyme geranylgeranyltransferase type I (GGTase-I), which utilizes geranylgeranyl diphosphate (GGPP) as the prenyl donor and has a different CaaX specificity (X is typically Leu).[7] Farnesylation is usually followed by additional modification of the prenylated protein, including proteolytic removal of the last three residues of the "CaaX box" and carboxymethylation of the new C-terminal farnesylated cysteine.[6] The initial prenylation step is required for the correct membrane localization and function of Ras.

A combination of enzymology and crystallography has illuminated the mechanistic details of catalysis by FTase.[8] Recently, the publication of a series of structures that represent the major steps on the reaction pathway afforded an excellent overview of this enzyme-catalyzed process.[9] The reaction proceeds via an ordered mechanism, with FPP binding first, followed by the CaaX substrate. The structures of ternary complexes (using nonreactive FPP or peptide analogues) provide an explanation for this ordering: the isoprenoid forms a substantial part of the binding surface for the CaaX peptide. The peptide binds in an extended conformation with the cysteine sulfur coordinated to the active site zinc ion, which apparently lowers the pK_a of the thiol, significantly increasing the local concentration of the

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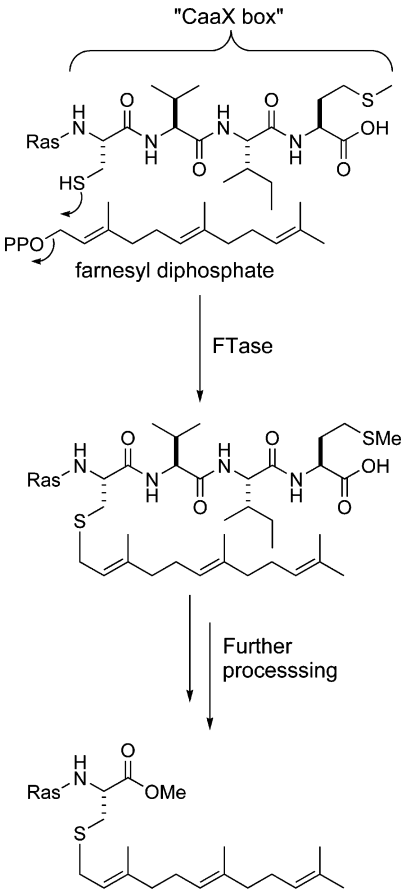


Figure 1. Posttranslational modification of Ras (K-Ras C-terminal CVIM shown).

reactive thiolate.[10,11] It is proposed[9] that a rotation of the FPP backbone brings its C_1 carbon into proximity with the thiolate, leading to a transition state that is consistent with previous mechanistic studies. Release of the farnesylated peptide product is slow and requires the addition of FPP.[12] The FPP displaces the product isoprenoid group to a new binding site, the "exit groove", with concomitant change in the CaaX backbone from an extended to a β-turn conformation. Product release regenerates the FTase/FPP complex.

Interestingly, published crystal structures have revealed that FTIs may inhibit the enzyme by blocking the peptide substrate site or by occupying part of the peptide site and the exit groove in a manner similar to the farnesylated peptide product.[9] Representative examples are illustrated in Figure 2, showing approximately the same view of three FTase/FPP/FTI ternary complexes. The peptidomimetic inhibitor **1** (Figure 2A and Chart 1) occupies the same site as a CaaX peptide but binds in a nonproductive conformation that prevents reaction with FPP.[13] The 3-aminopyrrolidinone **14** (Figure 2B and Chart 2) is also bound in the peptide site, with the imidazole group ligating the active site zinc ion.[14] Tricyclic FTIs such as **20** (Figure 2C and Chart 4) do not bind to the zinc ion but block both the peptide site and the exit groove.[15]

Development of Farnesyltransferase Inhibitors

The development of various classes of FTIs has been reviewed thoroughly elsewhere.[6,16−20] The following is a brief summary of some of these efforts, with an emphasis on the development of the various clinical candidates.

Early attempts to discover inhibitors of FTase focused on modifications of the isoprenoid and CaaX polypeptide substrates of the enzyme. While potent FPP-derived inhibitors have been discovered, most attention has been paid to analogues of the CaaX peptide.[6,16] Two of the more notable CaaX-derived inhibitors are **1** (L-739,750; Merck; FTase $IC_{50} = 1.8$ nM)[21] and **3** (FTI-276; Hamilton and Sebti groups; FTase $IC_{50} = 0.6$ nM),[22] often employed as their ester prodrugs **2** (L-744,832) and **4** (FTI-277), respectively (see Chart 1). These peptidomimetic FTIs have been used widely to probe the biochemical and biological consequences of FTase inhibition. For example, **2** was found to exhibit impressive efficacy in a transgenic mouse model of cancer.[21] MMTV-v-Ha-*ras* mice express a mutant H-*ras* gene and spontaneously grow tumors that in many ways more closely resemble human cancers than tumors grown in xenograft mouse models. In this transgenic model, **2** induced complete tumor regression in the absence of any obvious toxicity (see Figure 3). In contrast, the cytotoxic

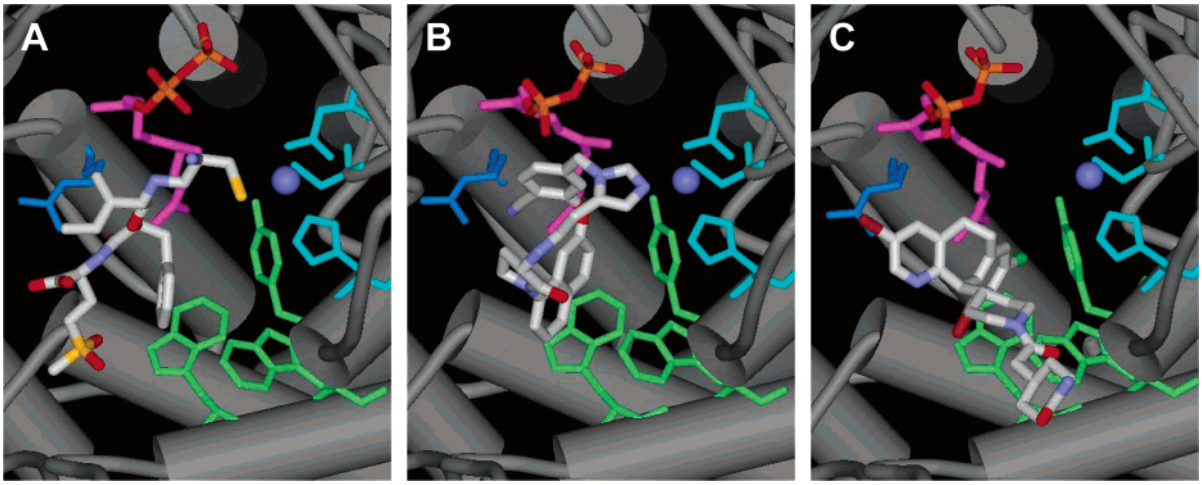
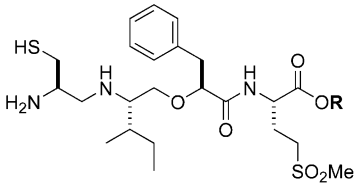
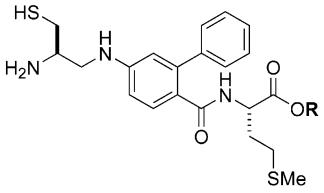


Figure 2. X-ray crystal structures of the FTase/FPP/FTI ternary complexes with compound **1** (A),[13] compound **14** (B),[14] and compound **20** (C).[15] The farnesyl group of FPP is colored magenta. The three zinc-ligating residues (Asp297β, Cys299β, His362β) are shown in cyan, Arg202β is shown in blue, and the three aromatic side chains that define a hydrophobic pocket (Trp102β, Trp106β, Tyr361β) are shown in green.

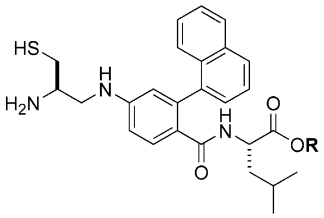
Chart 1. Thiol-Based FTIs



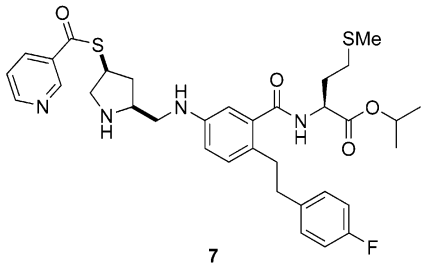
1 (**R** = H) FTase IC_{50} = 1.8 nM
2 (**R** = *i*-Pr)



3 (**R** = H) FTase IC_{50} = 0.6 nM
4 (**R** = Me)



5 (**R** = H) GGTase-I IC_{50} = 54 nM
6 (**R** = Me)



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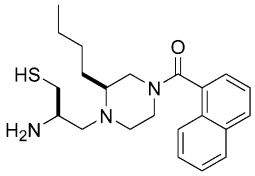
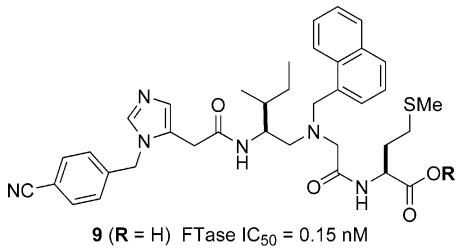
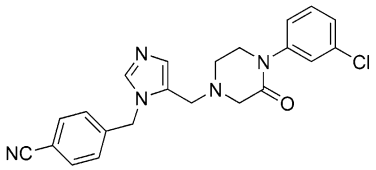


8 FTase IC_{50} = 1 nM

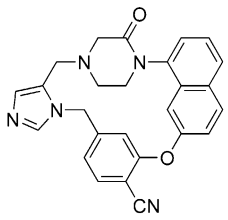
Chart 2. Imidazole-Based FTIs



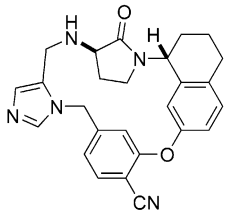
9 (**R** = H) FTase IC_{50} = 0.15 nM
10 (**R** = Me)



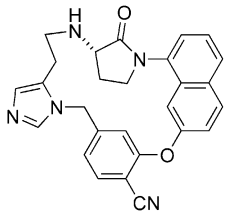
11 FTase IC_{50} = 2 nM



12 FTase IC_{50} = 0.2 nM



13 FTase IC_{50} < 1 nM

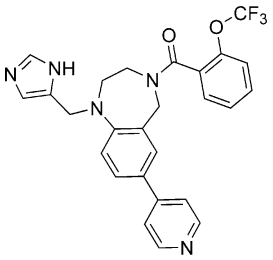


14 FTase IC_{50} = 1.1 nM

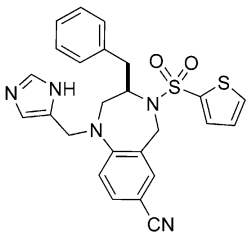
agent doxorubicin failed to shrink tumors in this model at its maximum tolerated dose (MTD).[21] Such observations suggested that FTIs might indeed fulfill the promise of effective cancer chemotherapy without toxicity. Despite the encouraging in vivo data, there were reservations about the clinical utility of peptidomimetics such as **2** primarily because of the potential for thiol-based toxicity and the requirement of a prodrug strategy. Nonetheless, the AstraZeneca compound **7** (AZD3409; Chart 1), which has both the key thiol and carboxylate groups blocked in a double prodrug strategy, has recently advanced to the clinic.[23] The parent drug is a potent inhibitor of FTase (K_i < 1 nM) but also has significant activity against GGTase-I (K_i = 8 nM). The prodrug **7** inhibited the growth of H-*ras*-transformed 3T3 fibroblasts (IC_{50} = 49 nM) and demonstrated in vivo activity in nude mouse tumor models.

At Merck, an extensive effort to address the concerns about the peptidomimetic mercaptan and carboxylate groups was undertaken. One way to remove the need for an ester prodrug was truncation of the CaaX tetrapeptide and subsequent reengineering to give the piperazine-based non-peptide thiol **8** (Chart 1; FTase IC_{50} = 1 nM).[24] Another early success was the identification of a general thiol replacement in (4-cyanobenzyl)imidazole. This moiety was designed to simultaneously ligate the active site zinc ion and occupy a nearby hydrophobic binding site, and it afforded highly potent peptidomimetic FTIs such as **9** (FTase IC_{50} = 0.15 nM), shown in Chart 2.[25] The corresponding methyl ester prodrug **10** was found to inhibit the growth of H-*ras*-transformed Rat1 cells with an IC_{50} value of 630 nM. The discrepancy between the intrinsic potency of **9** and the activity of its methyl ester in cells is

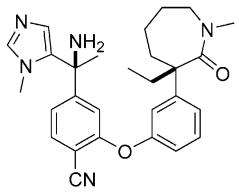
Chart 3. Imidazole-Based FTIs



15 FTase IC_{50} = 24 nM



16 FTase IC_{50} = 1.4 nM

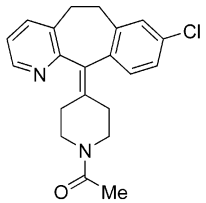


17 FTase IC_{50} = 0.06 nM

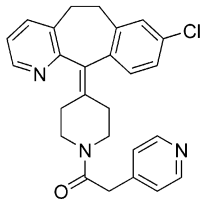
characteristic of such peptide-like prodrug FTIs and is probably due to poor cell permeability. Fusion of the non-peptide **8** with the thiol replacement in **9** and optimization of the piperazine template led to piperazinone FTIs, which displayed significantly improved cell-based activity compared with earlier peptidomimetic FTIs.[26] For example, the Merck clinical candidate **11** (L-778,123; Chart 2; FTase IC_{50} = 2 nM) inhibited the growth of H-*ras*-transformed cells with an IC_{50} value of 15 nM.[27] It was discovered that in the presence of anions such as ATP, **11** inhibited GGTase-I (GGTase-I IC_{50} = 100 nM) and, interestingly, was competitive with GGPP rather than the CaaX substrate.[28] This dual activity differentiated **11** from other clinically investigated FTIs, which were highly selective inhibitors of FTase. In PSN-1 human tumor cells, **11** inhibited the prenylation of the FTase substrate HDJ2 (EC_{50} = 92 nM) and the GGTase-I substrate Rap1A (EC_{50} = 6800 nM).[29] Studies directed at optimization of such non-peptide cyanobenzylimidazoles utilized transferred nuclear Overhauser effect (NOE) data to design macrocyclic FTIs such as compound **12** (FTase IC_{50} = 0.2 nM) in Chart 2.[30] Members of this macrocyclic class of FTIs exhibited the highest cell potency yet described for inhibitors of FTase: compound **13**, for example, inhibited the processing of HDJ2 in PSN-1 cells with an EC_{50} value of 180 pM.[14]

Chemists at Bristol-Myers Squibb sought analogues of thiol **8**, in which the mercaptan was replaced by imidazole,[31] and evolved their initial micromolar leads into a tetrahydrobenzodiazepine-based series of compounds represented by **15** (Chart 3; FTase IC_{50} = 24 nM).[32] It was discovered that the potency of analogues such as **15** was enhanced by addition of a phenylmethyl moiety at the 3-position and replacement of the 4-position amide with a sulfonamide. Incorporation of a 7-cyano substituent simultaneously improved potency and aqueous solubility, and these modifications led to the clinical candidate **16** (BMS-214662; Chart 3; FTase IC_{50} = 1.4 nM).[33] It is notable that these researchers independently discovered the potency-enhancing effects of a cyanophenyl group in the context of imidazole-based FTIs. It seems likely that this moiety is taking advantage of similar binding interactions to the cyanobenzyl in many Merck compounds (vide supra), a possible example of "convergent evolution" in medicinal chemistry. Compound **16** is a potent and selective FTI that is reported to promote apoptosis to a greater extent than other FTIs of comparable potency, suggesting that the apoptosis might result from a mechanism unrelated to FTase.[34] Additionally, **16** reverted H-*ras*-transformed Rat1 cells to a normal phenotype with an IC_{50} value of

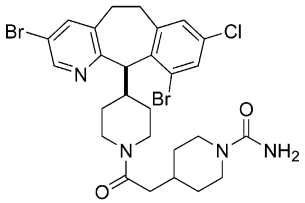
Chart 4. FTIs Derived from Screening of Compound Libraries



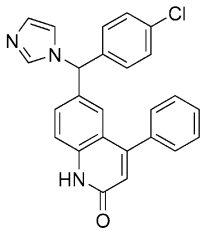
18 FTase IC_{50} = 27,000 nM



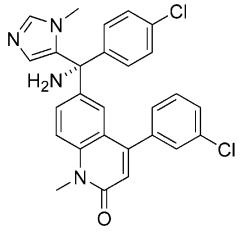
19 FTase IC_{50} = 250 nM



20 FTase IC_{50} = 1.9 nM



21 FTase IC_{50} = 180 nM



22 FTase IC_{50} = 0.86 nM

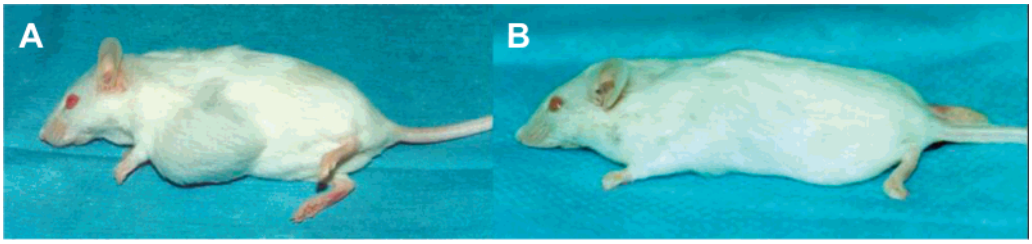


Figure 3. Regression of a doxorubicin-insensitive mammary adenocarcinoma by compound **2**. The MMTV-v-Ha-*ras* mouse was treated with dioxorubicin (2 mpk/day) for 9 days. After 12 days, the volume of the primary tumor was approximately 3000 mm^3 (A). Treatment with **2** (40 mpk/day) was initiated the next day and continued for 27 days, at which time complete tumor regression had been achieved (B).[21] Reprinted with permission from *Nature Medicine* (http://www.nature.com/).[21] Copyright 1995 Nature Publishing Group.

100 nM. The soft agar growth of several human tumor cell lines (HTLs) was inhibited by **16** with reported IC_{50} values as low as 25 nM. The compound exhibited broad cytotoxic activity against a panel of HTLs as well as impressive activity in vivo. Oral dosing of **16** (600 mpk/day) to mice bearing human tumor xenografts resulted in complete and lasting regression of established tumors in some cases.[34]

In contrast to the gradual evolution from CaaX peptides to small molecules such as **11** and **16**, other researchers obtained attractive leads for the development of FTIs from screening of compound libraries. For example, screening at Schering-Plough identified micromolar FTIs such as **18** (SCH-37370; FTase IC_{50} = 27 000 nM) in Chart 4, a close analogue of the H_1 receptor antagonist loratadine.[20] Structure−activity exploration led to **19** (SCH-44342; Chart 4), which exhibited significantly enhanced potency (FTase IC_{50} = 250 nM). Addition of bromo substituents and saturation of the vinyl bond were found to further improve potency, and alternatives to the pyridine side chain were explored to improve pharmacokinetic properties, ultimately leading to the clinical candidate **20** (SCH-66336, lonafarnib, Sarasar; FTase IC_{50} = 1.9 nM).[35] This tricyclic inhibitor is distinguished from the other FTIs tested in humans by its lack of a ligand for the active site zinc ion in FTase. Compound **20** blocked anchorage-independent growth of H-*ras*-transformed fibroblasts in soft agar with an IC_{50} value of 75 nM and was active ($IC_{50} \leq 500$ nM) against approximately 60% of a panel of HTLs, with soft agar IC_{50} values as low as 40 nM.[36] In nude mouse xenograft experiments, orally administered **20** demonstrated good activity and achieved complete growth inhibition for some tumor types.[36]

At the Janssen Research Foundation, screening for FTIs afforded lead quinolinone compounds such as **21** (FTase IC_{50} = 180 nM) in Chart 4. It was found that attachment of the imidazole moiety via the 5-position, combined with N-methylation of the imidazole, increased potency against FTase and selectivity against cytochrome P450-dependent enzymes. Incorporation of a benzylic amino group was shown to improve cell potency, and additional optimization produced **22** (R115777, tipifarnib, ZARNESTRA; FTase IC_{50} = 0.86 nM), the first FTI to advance to human clinical trials (see Chart 4).[37] Compound **22** inhibited the growth of H-*ras*-transformed NIH 3T3 cells with an impressive IC_{50} value of 1.7 nM. The compound also inhibited proliferation of approximately 75% of a panel of 53 HTLs, and it was found to have IC_{50} values below 10 nM against many of them,[38] indicating that it may be the most potent FTI to be evaluated in the clinic. Orally administered **22** inhibited the growth of human tumor xenografts in nude mice, although histological analysis revealed heterogeneity in the responses. The FTI produced different levels of antiangiogenic, antiproliferative, and apoptotic responses in different tumor lines, suggesting that the complexity of tumor responses to FTIs may extend to tumor−host interactions.[38] One in vitro study with potential clinical significance was the generation of a cell line with resistance to FTIs by continuous exposure of the human colon cancer cell line KM12 to **22**.[39] The stably resistant line demonstrated more than 10-fold resistance to **22** and the structurally unrelated FTI **4** but no resistance to the GGTase-I inhibitor **6** (GGTI-298; Chart 1) or a variety of other antitumor agents. The mechanism of resistance was not fully defined, but it appeared to be related to reduced levels of FTase activity in the resistant cells.

While FTIs have been developed for anticancer therapy, there is active interest in exploring their potential as antiparasitic agents.[40] Initial studies suggest that it will be possible to prepare analogues that are highly selective for the parasite vs mammalian FTase enzymes. FTIs such as **4** (Chart 1) have shown in vitro antiparasitic activity, and in vivo studies are ongoing.[40]

What's Ras Got To Do with It?

The development of FTIs was conceived as an indirect way to block the function of *ras* oncogenes because Ras proteins are farnesylated as the first step of a series of posttranslational modifications that are critical for their activity. Indeed, many early observations were consistent with this hypothesis. FTIs were shown to reverse the morphological transformation, and block the growth, of H-*ras*-transformed rodent fibroblasts.[41] Moreover, they exhibited significant efficacy in vivo, for example regressing tumors in H-*ras* transgenic mouse models with little apparent toxicity.[21,36]

However, a number of observations have questioned whether the biology of FTIs is due to inhibition of Ras function. First, the time course of FTI-induced morphological reversion of H-*ras*-transformed fibroblasts does not correlate well with the disappearance of farnesylated H-Ras.[41] Second, although FTIs have been found to inhibit the growth of a wide range of HTLs, the sensitivity of the cell lines does not relate to their *ras* mutation status.[42] Third, of the three human Ras proteins (H-Ras, N-Ras, and K-Ras), FTIs inhibit only

the cellular processing of H-Ras. In the absence of FTase activity, N-Ras and K-Ras act as substrates for GGTase-I, leading to geranylgeranylated proteins that appear to be functionally equivalent to the farnesylated forms.[43] In human cancers, it is the K-*ras* gene that is by far the most commonly mutated while H-*ras* mutations are rare,[2,3] so it is unlikely that FTIs will have widespread success as inhibitors of mutant Ras.

The most direct way to address the alternative prenylation of K-Ras is to inhibit both FTase and GGTase-I simultaneously. Compound **11** is an inhibitor of both enzymes (vide supra) and has been shown to inhibit the processing of K-Ras in PSN-1 cells in vitro (EC_{50} = 6300 nM).[29] In human clinical trials, the pharmacodynamics of **11** were assessed by monitoring the prenylation of HDJ2 and Rap1A in peripheral blood mononuclear cells. Dose-dependent inhibition of HDJ2 farnesylation was seen, as well as inhibition of Rap1A prenylation, the first demonstration of inhibition of GGTase-I in humans.[29] However, no inhibition of K-Ras processing was detected in this study, even in patients who exhibited significant levels of unprocessed Rap1A. Studies using **4** (Chart 1) and the related GGTase-I inhibitor **6** (the ester prodrug of **5**, GGTI-297; GGTase-I IC_{50} = 54 nM) showed that treatment of K-*ras*-transformed cells with both inhibitors did result in inhibition of K-Ras prenylation, but the combination afforded no more inhibition of cell growth than treatment with the FTI alone.[44] Researchers at Merck evaluated the consequences of inhibiting K-Ras prenylation in xenograft and transgenic mouse models of cancer, using both combinations of FTIs and GGTIs as well as novel dual FTI-GGTIs such as **17** (see Chart 3; FTase IC_{50} = 0.06 nM; GGTase-I IC_{50} = 3.6 nM),[45] which inhibited the prenylation of K-Ras in PSN-1 cells with an EC_{50} value of 340 nM. While high doses of FTI + GGTI or FTI-GGTI produced unprocessed K-Ras in vivo, the levels of GGTase-I inhibition required were found to be lethal to the mice, so this approach is not clinically viable.[46]

The disconnection between FTase inhibition and oncogenic *ras* could have proved fatal to the development of FTIs for the treatment of cancer, but preclinical data indicated that the compounds possessed antitumor properties that were not related to the oncogene. It should be noted that H-*ras*-transformed cells are among the most sensitive to the inhibitory effects of FTIs, and it seems likely that inhibition of H-Ras function plays a role in these cases. It is also possible that in cancers with up-regulated cell surface receptor activity, wild-type H-Ras could be persistently activated.[47] One study indicated that the sensitivity of astrocytoma cells to **20** correlated with high levels of activated H-Ras (H-Ras·GTP) combined with low levels of N-Ras·GTP and K-Ras·GTP, regardless of *ras* mutational status.[48] However, the observed activity against a wide variety of other cell lines is most likely due to interference with the function of other FTase substrates. Since the first suggestions that Ras may not be the sole target of FTase inhibition, there has been considerable interest in discovering the other protein(s) responsible for the biological activity of FTIs. This knowledge should facilitate the design of clinical studies and perhaps suggest other novel approaches to chemotherapy.

The observation that morphological changes in H-*ras*-transformed fibroblasts occurred on a faster time scale than depletion of farnesylated H-Ras led to the identification of RhoB, which is turned over more rapidly, as a candidate FTI target.[41] RhoB is a small GTPase that is a substrate for both FTase and GGTase-I, and Prendergast and colleagues have made compelling arguments that FTI treatment increases the cellular concentration of geranylgeranylated RhoB and that this is growth inhibitory.[49] Consistent with this hypothesis, transformed fibroblasts derived from RhoB −/− knockout mice exhibited reduced sensitivity to FTIs but FTI-induced growth inhibition was still observed, suggesting that other proteins are involved.[50] Additional evidence questioning the relevance of RhoB came from experiments in which HTLs were transfected with engineered versions of RhoB that were designed to be exclusively farnesylated (RhoB-F) or geranylgeranylated (RhoB-GG).[51] It was found that RhoB-F and RhoB-GG, as well as wild-type RhoB, were growth inhibitory under these conditions. Thus, at least some of the effects of FTIs must be due to proteins other than Ras and RhoB.

It has been observed that while H-*ras*-transformed cells accumulate in the G_1 phase of the cell cycle when treated with FTIs, most HTLs that are sensitive to FTIs accumulate in $G_2 \rightarrow M$ upon FTase inhibition. This has led to the suggestion that the centromere-binding proteins CENP-E and CENP-F may be biologically relevant targets of FTase inhibition.[52] CENP-E and CENP-F are substrates for FTase but not GGTase-I and they have been implicated as mediators of the $G_2 \rightarrow M$ checkpoint.[53] In the presence of **20**, the association of CENP-E with microtubules was disrupted in A549 lung carcinoma cells, suggesting a mechanistic link between FTIs and disruption of mitosis.[52] Studies that utilized expression of CENP-F mutants have indicated that the proper localization and function of CENP-F requires an intact CaaX box and FTase activity.[54] However, others have argued that the effects of FTIs on chromosome morphology and alignment are not consistent with CENP-E being the key target of FTase inhibition.[55]

The emerging picture of the effects of FTIs on cells is complex. It appears that cells with mutant H-*ras* respond differently, and tend to be more sensitive, to FTIs than other transformed cells. Genotypic analyses have indicated that cells with wild-type p53 are also especially sensitive to FTIs.[56,57] Other evidence has implicated inhibition of the PI3-kinase/Akt-2 survival pathway in FTI-induced apoptosis.[58] It appears that the various effects of FTIs on cells, such as growth inhibition, apoptosis, and morphological alteration, depend on multiple proteins and pathways. Moreover, the mechanistic details of these responses may depend on the cell type and the environmental context of the cells. Continued work is needed to clarify the relevant target(s) of FTase inhibition.

Clinical Trials with FTIs

In preclinical in vitro and in vivo studies, FTIs exhibited both cytostatic and cytotoxic properties, and this ambiguity has complicated the selection of relevant endpoints in clinical trials.[18] The traditional strategy for evaluation of cytotoxics in terms of their ability to shrink tumors may be suboptimal if the primary effect

of FTIs is growth inhibition (cytostasis) rather than cytotoxicity. Since the *ras* hypothesis has fallen out of favor, the clinical studies have targeted a variety of cancers, regardless of their historical frequency of *ras* mutations. As anticipated, there appears to be no general relationship between clinical response and *ras* status.[59,60] Pharmacodynamic effects of FTIs in patients have been monitored by quantitation of unprocessed FTase substrates such as HDJ2 and prelamin A[29,61] and by measuring residual FTase activity in bone marrow samples.[62] In general, it appears that significant levels of inhibition of the enzyme have been achieved. One indication that FTase inhibition has been achieved clinically is that similar patterns of toxicity have been noted for these structurally diverse compounds, with myelosuppression, gastrointestinal toxicity, and fatigue apparently being mechanism-based. A summary of some of the more significant clinical findings follows.

Clinical Data: Monotherapy

In a phase I clinical trial, **22** was administered orally b.i.d. to 28 patients with advanced solid tumors in a dose-escalating protocol.[59] The dose-limiting toxicities (DLTs) were myelosuppression and neurotoxicity, and the MTD was determined to be 300 mg b.i.d. One patient with non-small-cell lung cancer (NSCLC) showed a partial response (PR) (75% decrease in tumor size). In a phase II breast cancer trial examining continuous and intermittent dosing schedules of **22** (300 mg b.i.d.), 8 patients out of 76 (11%) showed a PR (≥50% tumor reduction).[63] However, **22** did not improve survival time of patients with advanced colorectal cancer in a phase III trial.[64] Perhaps the most impressive clinical efficacy reported for **22** as monotherapy has been in hematologic malignancies. A phase I dose-escalating trial in patients with refractory and relapsed acute leukemias encountered DLT of central neurotoxicity at 1200 mg b.i.d. Out of 34 evaluable patients, clinical responses occurred in 10 (29%), including 2 complete remissions (CRs).[62] Other clinical trials with **22** have shown objective responses in chronic myeloid leukemia (CML)[65] and other hematological malignancies, and the clinical evaluation of this FTI continues.

In a phase I trial, **20** was dosed orally twice a day in a dose escalating fashion to 20 patients with solid tumors.[66] The DLTs were fatigue and gastrointestinal toxicity and the MTD was 350 mg b.i.d. One PR was observed in a patient with NSCLC. In a phase II study of patients with pancreatic cancer, **20** was compared with standard gemcitabine therapy and two PRs were seen with **20** (200 mg b.i.d.).[67] Compound **20** has also achieved clinical responses in patients with hematological malignancies, with gastrointestinal toxicity and myelosuppression as the major adverse events. In one study of advanced hematological malignancies, **20** (200 mg b.i.d.) produced a hematologic response in 19% of patients.[20] A number of phase II and phase III trials are ongoing.

A phase I study examined the administration of **11** as a continuous infusion to patients with advanced solid malignancies.[68] The observed DLTs were myelosuppression, prolongation of the QT_c interval, and fatigue, and the MTD was 560 (mg/m^2)/day. No objective responses were seen, and the clinical development of this dual inhibitor has been discontinued.

Compound **16** has been investigated clinically using both oral and intravenous delivery routes with later trials favoring infusion, in part to minimize gastrointestinal toxicity.[69] There have been several reports of minor clinical responses with **16** in solid tumors.[69] In a phase I study, treatment with **16** led to objective responses in 24% of patients with advanced hematologic malignancies,[60] but the current development status of **16** is unclear.

Clinical Data: Combination Therapy

Preclinical studies have revealed that FTIs can exhibit additivity or synergy when employed with other anticancer therapies.[70,71] FTIs have exhibited synergy with taxanes in particular and have been found to act as radiosensitizers.[47,69] Recently, **20** has been shown to enhance the activity of imatinib (Gleevec) against BCR-ABL-expressing cell lines,[72,73] and evaluation of this combination in clinical trials was initiated. Other clinical studies have evaluated combinations of FTIs with a variety of current therapies, including taxanes, gemcitabine, cisplatin, the antibody trastuzumab (Herceptin), and radiotherapy. Objective responses have been reported for **22**, **20**, and **16** in combination with paclitaxel or docetaxel.[47,69] Compound **11** was evaluated in conjunction with standard radiotherapy in patients with either head and neck cancer or NSCLC. In the six patients with evaluable disease, there were two CRs in head and neck cancer and three CRs and one PR in NSCLC, suggesting that FTIs may be clinically useful as radiosensitizers.[74] A phase I clinical trial examined the combination of **22** with gemcitabine and cisplatin in patients with advanced solid tumors, and objective responses were seen in 33% of patients.[75] However, in a phase III study of advanced pancreatic cancer, **22** in combination with gemcitabine was found to offer no benefit compared with gemcitabine and placebo.[76]

Conclusion

The development of farnesyltransferase inhibitors was initiated with the goal of targeting mutant Ras, which is constitutively activated in many cancers. Early efforts to develop FTIs focused on modification of CaaX tetrapeptides, and a number of important peptidomimetic FTIs have been synthesized, including the recent clinical candidate **7**. Peptidomimetic analogues helped to define the biological effects of FTIs, and early results, which often involved inhibition of H-Ras farnesylation, were encouraging.

While the first-generation peptide-like FTIs were being evolved into non-thiol non-peptides, the *ras*-driven rationale began to unravel. It was discovered that the responses of cells to FTI treatment could not be fully explained by inhibition of Ras function, which is not surprising because there are many other known farnesylated proteins.[69] There was also a lack of correlation between the mutational status of *ras* in HTLs and their sensitivity to FTIs. Most disturbingly, it was found that K-Ras, the most clinically relevant form of Ras, was rescued by GGTase-I when FTase was inhibited.

Nonetheless, encouraging preclinical results helped FTIs advance to the clinic. Five structurally diverse clinical candidates have been described, three were evolved from the CaaxX peptide and two were derived

from screening leads. The ease of discovery of these FTIs was strongly influenced by the quality of the original lead structure (peptide vs druglike), highlighting the critical importance of screening technology and diverse compound collections.

The difference between the preclinical reports of profound antitumor effects in the absence of toxicity and the clinical experience with FTIs is at once striking and disappointing. While FTIs are generally well tolerated, at least compared with many cytotoxics, they do exhibit similar DLTs to standard chemotherapy, such as myelosuppression, gastrointestinal toxicity, and neurotoxicity. As monotherapy, FTIs have generally performed modestly, although there appears to be significant efficacy in myeloid leukemias and breast cancer. Like other cancer therapeutics, FTIs will probably be more effective when used in combination. Clinical studies on FTIs combined with other therapies have revealed activity against a variety of cancers, although it is difficult to attribute efficacy to the FTI in small phase I combination trials with any certainty. Both preclinical and clinical data suggest that FTIs may be useful additions to therapy with taxanes and radiation.

In the years since FTase was implicated as a target for inhibition of *ras* function, our understanding of the complexity of this system has increased. It is clear that FTIs are not highly selective inhibitors of mutant Ras function. Rather, they are antiproliferative agents that have shown clinical efficacy for the treatment of some cancers. The actual downstream target(s) of FTase inhibition remain controversial. There are probably multiple farnesylated proteins involved in the varied biology of FTIs, and the relative importance of each may vary between cell types. Despite the relative rarity of H-*ras* mutations in cancer, tumors that depend on upregulated H-Ras function may be uniquely responsive to inhibition of FTase. In such cases, FTIs would be rationally designed antitumor agents, but it is to be hoped that, even as non-Ras inhibitors, they will be a useful addition to the arsenal of anticancer therapies.

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Biography

Ian M. Bell was educated at Gonville and Caius College, Cambridge University, and received an M.A. in Natural Sciences. He continued his studies at Cambridge University with Dr. Chris Abell and graduated with a Ph.D. in Organic Chemistry in 1992. He was awarded a SERC−NATO postdoctoral fellowship to work at the Scripps Research Institute with Dr. Donald Hilvert. In 1994, he joined the Department of Medicinal Chemistry at Merck Research Laboratories, West Point, PA, where he is currently a Senior Research Fellow.

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