Farnesyltransferase Inhibitors: An Overview of the Results of Preclinical and Clinical Investigations

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Abstract

This article presents an overview of preclinical studies and clinical trials of a number of independently derived farnesyltransferase inhibitors (FTIs). Potential targets and biological modes of action of FTIs are discussed, and the results of clinical trials are summarized. The significant efficacy of FTIs as single or combined agents in preclinical studies stands in contrast with only moderate effects in clinical Phase II-III trials. These results reveal a substantial gap in the understanding of the complex activity of FTIs and their interactions with cytotoxic agents. We conclude that the rational combination of FTIs with other therapies, taking into account the biological activities of the individual agents, may improve the clinical results obtained with FTIs.

Introduction

FTIs were developed to specifically inhibit the activity of oncogenic ras in tumor cells. A large number of preclinical studies have been undertaken with the various FTIs to test their spectrum of efficacy and to better understand the biological mechanism of their antitumor activity. In part, these studies have been prompted by the unexpected efficacy of FTIs in tumor cells without mutated ras. At present, the mechanisms of action of FTIs appear complex and are not completely understood. The role of Ras inhibition in the antitumor activity of these inhibitors is a topic of debate, and other farnesylated targets that could play a major role in FTI effects have been identified. Thus, although clinical trials with these agents are under way, further investigation into their biological activity is warranted. For the purposes of this review, Ras proteins will serve as the prototypical target. This is both for historical reasons and because, although it is clear that ras mutations are not required for tumor cell susceptibility to FTIs, inhibition of activated wt Ras signaling by these inhibitors in tumor cells lacking ras mutations remains a likely contributor to their activity.

Prenyltransferases and Their Inhibition

Prenyltransferases

Prenylation of proteins is carried out by a family of transferases that catalyze the addition of C\textsubscript{15}-farnesyl or C\textsubscript{20}-geranylgeranyl isoprenoids to proteins (reviewed in Ref. 1). Two of the three prenyltransferases, FTase and GGTase-1, can act on Ras proteins and have been targeted for inhibition in the context of cancer therapy. These two members of the prenyltransferase family are heterodimeric proteins that share an identical α-subunit but have different β-subunits (2). Physiologically, only FTase is involved in the posttranslational processing of Ras proteins in mammalian cells. However, under FTI treatment, there is alternative prenylation of certain proteins, including RhoB, K-Ras, and N-Ras, by GGTase-1 (3–5).

The recognition site for prenylation by FTase or GGTase-1 is called the CAAX box, a COOH-terminal tetrapeptide sequence composed of cysteine, two aliphatic amino acids, and a terminal amino acid that serves as the primary determinant of farnesylation versus geranylgeranylation. FTase is preferentially active on protein substrates with CAAX motifs ending with serine, methionine, or glycine. The enzymatic affinity in vitro is 10–30-fold higher for substrates with X being methionine (K-Ras A and B have the sequences CVIM and CIIM, respectively) compared with X being serine or glycine (the H-Ras recognition sequence is CVLHS). GGTase-1 is preferentially active on proteins terminating with CAAX motifs that end with leucine (6). As mentioned above, all three Ras isoforms are normally farnesylated, but K-Ras and N-Ras are also substrates for prenylation by GGTase-1 in the presence of a FTI. K-Ras is more resistant to inhibition by FTIs both because of its higher affinity for FTase and due to alternate prenylation by GGTase-1. Complete inhibition of the prenylation of K-Ras therefore requires a combination of FTIs and GGTIs (7).

Classification of Prenyltransferase Inhibitors

Within the last decade, a variety of structurally different molecules have been developed that inhibit prenyltransferases with high potency and selectively. The development and characterization of these inhibitors has been the subject of many recent reviews, including Refs. 8–13. Peptidomimetic inhibitor development was based on the findings that CAAX tetrapeptides contain the primary signals for the recognition of prenyltransferase enzymes (14). These inhibitors act as competitors for prenyltransferase binding to the CAAX box of full-length proteins. The first pseudosubstrate FTIs were tetrapeptides with aromatic amino acid substitutions at the second position from the COOH-terminal end (at a distance of two amino acids from cysteine). Initially these compounds had poor membrane permeability and were relatively unstable (reviewed in Ref. 10). Subsequent refinement has overcome these problems, and the current generation of these compounds shows excellent bioavailability and potency (reviewed in Ref. 13).
The structure of a second class of FTIs is based on the farnesyl group. α-Hydroxyfarnesylphosphate is the prototype of this class. Improved derivatives are able to inhibit FTase selectively in vitro but revealed no relevant antineoplastic activity in animal models (15).

Bisubstrate analogues are based on the enzyme FDP-CAAX complex that forms before the process of catalysis itself is initiated. Inhibitors in this class contain both the structural motifs of FDP and CAAX. They are highly potent and specific for FTase and exhibit antineoplastic activity in vitro and in vivo (16).

Another approach to developing FTIs has been the screening of drug libraries. FTIs of varied structure have been identified by this approach. Examples of these inhibitors include SCH66336, a tricyclic inhibitor, and R115777, a nonpeptidomimetic inhibitor. Natural compounds, too, have been identified as potent inhibitors of FTase. Manumycin is a naturally occurring compound isolated from Streptomyces sp. that competes with FDP (15).

**Preclinical Studies and Possible Mechanisms of FTI Biological Activity**

**FTI Targets: The Ras Protein as Prototype**

**Structure and Isoforms of Ras.** The three mammalian ras genes yield four Ras proteins: H-Ras; N-Ras; K-Ras4A; and K-Ras4B. The two K-Ras proteins result from alternative splicing of the fourth exon of a single gene. K-Ras4B, H-Ras, and N-Ras are ubiquitously expressed. K-Ras4A is the only isoform demonstrating tissue-specific expression (17). Only K-Ras expression is essential in development because K-ras knockout mice die at embryonic day 11 (18, 19). H- and N-ras knockout mice are viable (20, 21), as are H- and N-ras double-knockout mice (21).

The first 165 amino acids of the different Ras isoforms are largely homologous. The effector, the exchange factor, and the nucleotide-binding sites are all found in the NH2-terminal conserved domains. The COOH-terminal region after amino acid 165 is called the HVR. The HVR can be divided into two domains: the linker domain, and the membrane-targeting domain. The latter consists of the COOH-terminal CAAX box common to all Ras proteins and a secondary membrane-targeting domain, which contains cysteine palmitoylation sites in H-Ras, N-Ras, and K-Ras4A or a polylysine domain in K-Ras4B (Fig. 1; Refs. 14 and 22–24). Palmitoylation or the presence of a polybasic domain (K-Ras4B) is essential for efficient transportation to the plasma membrane. Studies suggest that the second targeting region in the HVR determines the route taken by the different Ras proteins out of the endoplasmic reticulum to the plasma membrane. Whereas K-Ras takes an uncharacterized Golgi-independent route to the disordered plasma membrane, H-Ras traffics via the classical secretory pathway through the Golgi to caveolae and lipid rafts (25). Studies on the role of the linker domain of the H-Ras HVR (26) showed that incorrectly localized H-Ras interferes with the communication between Raf, MEK, and MAPK.

In addition to membrane binding, microlocalization within the membrane differs between Ras isoforms and may influence both activation and signaling to downstream effectors. It was also recently shown that H- or N-Ras signaling, but not K-Ras signaling, can occur not only at the plasma membrane but also from the endoplasmic reticulum and the Golgi (27).

**Ras Posttranslational Modification and Transformation.** Ras must be bound to the cell membrane to have transforming activity (22). Oncogenic Ras proteins lose their transforming activity when attachment to the plasma membrane is blocked by mutations in the CAAX sequence (22, 28–30). The cysteine residue to which the isoprenyl group is attached is critical in this respect. These findings were the basis for developing pharmacological inhibitors of prenylation as discussed above. After prenylation, the next steps in post-translational modification are AAX proteolysis by Rce1 and then α-carboxymethylation of farnesylated cysteine residues by isoprenylcysteine carboxyl methyltransferase (reviewed in Ref. 25). H-Ras and N-Ras are subsequently palmitoylated by palmitoyltransferase.

**The Functional Specificity of Ras Isoforms.** Several pieces of evidence indicate that the different isoforms of Ras serve distinct functions. Table 1 gives an overview of the different functions of Ras isoforms described to date. Because cell type differences are also known to exist in Ras signaling (reviewed in Ref. 31), the cell types in which these observations have been made are indicated in the table.

Another hint for the different functions of Ras isoforms is that cancers arising in different organs are most often associated exclusively with mutation in only one ras gene (Table 1). A study using Ras chimeras between H-Ras and K-Ras of the HVR showed that the secondary membrane-targeting domain of the HVR is a major contributor to the specificity of H-Ras in rat mammary carcinogenesis (32). This effect mapped to the Ras CAAX box, suggesting that
posttranslational modification can be a determinant of oncogenic potential.

**Ras Functions and Pathways.** Ras proteins are activated by the binding of GTP. Under normal conditions, this is triggered by a signal initiated by growth factor binding to plasma membrane receptors with a tyrosine kinase activity. Oncogenic mutations in Ras reduce GTP hydrolysis, resulting in constitutive Ras signaling. Increased signaling can also result from growth factor receptor mutation or amplification. Deregulated Ras signaling can result in loss of contact inhibition, anchorage-independent growth, ruffling of the plasma membrane, and stimulation of DNA synthesis.

Among the many pathways affected by Ras signaling, two well-characterized pathways include the Raf-MAPK pathway and the PI3K pathway (reviewed in Ref. 33). The Raf-MAPK pathway results in activation of the ETS family of transcription factors. Through these transcription factors, Ras signaling can induce a variety of genes involved in the process of tumor growth, invasion, and stroma formation.

Ras proteins also stimulate PI3K, which itself has multiple targets such as phosphoinositide-dependent kinases, p70S6K, Rac, and guanine exchange factors (34). Phosphoinositide-dependent kinases activate Akt (protein kinase B). Akt is activated in cells exposed to diverse stimuli such as hormones, growth factors, and extracellular matrix components. Protein kinase B/Akt phosphorylates and regulates the function of many cellular proteins, including those involved in metabolism, apoptosis, and proliferation, through regulation of cell cycle progression (35). The effects of Ras on the cytoskeleton are also mediated through the PI3K pathway (rearrangement of cortical filaments, formation of lamellipodia, and membrane ruffling).

Other downstream pathways of Ras lead to Raf guanine exchange factors. Raf-GDS, Rgl, and Rif are activated by Ras at the plasma membrane. This in turn leads to the formation of the GTP-bound state of the Raf family GTPases (36, 37). Evidence for an important role of Raf downstream of Ras comes from the observation that dominant negative Raf blocks Ras transformation (38, 39). The activated Ras effector mutant Ras (12V,37G), which stimulates endogenous Raf guanine exchange factors without activating Raf or PI3K, promotes growth in low serum and transformation but not metastasis (40–42).

**FTI Efficacy and the Influence of Ras Status**

Original reports using ras-transfected mouse cell lines and one report with human tumor lines appeared to support the specificity of FTI treatment for cells with ras mutations. Initial studies using L-731,734 showed that soft agar colony formation by v-ras-transformed cells was blocked, whereas cells transformed by v-raf or v-mos oncogenes were resistant to this inhibition (43). Nagasui et al. (44) tested colony formation in 14 human cancer cell lines expressing different ras oncogenes with the FTI B956/B1086. Sensitivity was highest for cell lines with H-ras mutations, followed by cell lines harboring N-ras mutations and K-ras mutations. Inhibition of colony formation in soft agar of wt ras cell lines required the highest doses of the compound. In this study, comparison of FTI inhibition of tumor xenografts of three cell lines [EJ-1 (bladder carcinoma), HT1080 (fibrosarcoma), and HCT116 (colon carcinoma); which express mutant H-, N-, or K- isoforms of Ras, respectively] showed that FTI treatment inhibited the growth of tumors best in the H-ras-mutated cell line and less in the N-ras- and K-ras-mutated cell lines.

Other studies have shown that these inhibitors have a broader spectrum of activity that includes tumor cells without ras mutation. Sepp-Lorenzino et al. (45) tested the effect of the FTI L-744,832 on the anchorage-dependent and -independent growth of 42 human tumor cell lines. The FTI R115777 was tested in a panel of 53 human tumor cell lines (46). The tumors tested in these studies included those originating from brain, bladder, breast, cervix, colon, lung, skin, ovaries, pancreas, prostate, eye, and connective tissues. More than 70% of the cell lines were sensitive to the drug as determined by inhibition of tumor cell proliferation in both of these studies. The sensitive cell lines harbored mutations in H-, N-, and K-Ras, but interestingly, among them there were also cell lines with wt ras and activated tyrosine kinases. Inhibition of proliferation was not correlated with the tumor type or with the presence or absence of ras mutation. In the first study, it was established that in resistant cells, the mechanism of cellular drug resistance was not a function of altered drug accumulation or FTase insensitivity. Moreover, when sensitive cells were compared with resistant cells, there was no difference in either the specific inhibition of lamin B processing (which is only prenylated by FTase and not by GGTase) or the functional activation of Ras. However, resistance seemed to be associated with the presence of Ras mutation-independent pathways for MAPK activation by tyrosine kinases because the epidermal growth factor-induced activation of MAPK was only inhibited in sensitive cells. The study of R115777 (46) demonstrated that cell lines with K-ras mutations required higher dose concentrations to achieve growth inhibition. Accordingly, inhibition of K-Ras4B protein processing could not be detected at doses where inhibitions of the other isoforms of Ras and of lamin B were seen. Another group tested the FTI BIM-46228 on several tumor cell lines in vitro (47). Again, growth was inhibited in most cell lines independently of the ras mutational status.

Studies of effects of FTI on mouse tumors and in xenograft models have also shown a wide spectrum of antitumor effects. Histological studies of human tumor xenografts revealed three different patterns of responses to R115777 (46). The first pattern was found in C32 melanomas with wt ras, where induction of apoptosis in the tumor cells was the predominant observation. Interestingly, no increase in

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**Table 1 Differentiation of the respective isoforms of Ras**

<table>
<thead>
<tr>
<th>Described effect (Ref. no.)</th>
<th>Cell type used</th>
<th>Ki-Ras4A</th>
<th>Ki-Ras4B</th>
<th>N-Ras</th>
<th>Ha-Ras</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAF-1 activation (142, 143)</td>
<td>COS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>RAF-1 activation (144)</td>
<td>Fibroblasts</td>
<td>+ + +</td>
<td>+</td>
<td>n.d.</td>
<td>+</td>
</tr>
<tr>
<td>PI3K activation (26)</td>
<td>COS</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+ + +</td>
</tr>
<tr>
<td>Focus formation (142)</td>
<td>BHK COS</td>
<td>+ +</td>
<td>+</td>
<td>+</td>
<td>+ + +</td>
</tr>
<tr>
<td>Anchorage-independent growth (142)</td>
<td>RIE-1</td>
<td>+ + +</td>
<td>+</td>
<td>n.d.</td>
<td>+ + +</td>
</tr>
<tr>
<td>Migration (142)</td>
<td>COS</td>
<td>No</td>
<td>+ + +</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Apoptosis suppression (145, 146)</td>
<td>Fibroblasts</td>
<td>No</td>
<td>No</td>
<td>+</td>
<td>No</td>
</tr>
<tr>
<td>Human tumor greatest prevalence (147)</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Pancreatic, colon, NSCLC</td>
<td>+</td>
<td>No</td>
</tr>
</tbody>
</table>

<sup>a</sup> COS, fibroblastoid monkey kidney cells; BHK, hamster kidney cells; RIE-1, rat intestinal epithelial cells; n.a., not applicable; n.d., not determined.
apoptosis was observed in these cells grown as monolayers in vitro. This result is in accord with another report using the FTI L-739,749, where increased apoptosis was observed in cultures in which attachment was blocked, but not in cultures growing as an attached monolayer (48). The second pattern of response was seen in LoVo colon tumors (expressing mutant K-Ras) that exhibited inhibition of angiogenesis measured as a significant reduction of Factor VIII. The third pattern was seen in Capan-2 pancreatic tumors that also bear a K-ras mutation. Tumors derived from these cells showed an antiproliferative effect upon treatment with R115777 accompanied by an increase in apoptosis in the host endothelial cells of the tumor vasculature; however, there were no significant changes in angiogenesis-related markers (46). The FTI BIM-46228 (47) was tested on xenografts derived from MIA-PaCa-2 and HCT-116 cells, both with mutant K-ras. BIM-46228 reduced the tumor growth rate in both cell lines but did not lead to tumor regression. Other reports showed that the growth of tumors with wt ras can be successfully inhibited in vivo (49–51).

Growth inhibition in vitro and in vivo was also reported in cell lines with K-ras mutations after treatment with either a FTI (FTI-276) or a GGTI (GGTI-297), despite the fact that both inhibitors together are required for inhibition of K-ras processing (7). Thus, inhibition of growth in many K-ras mutant cell lines did not require inhibition of oncopgenic K-Ras prenylation. This conclusion is supported by the observation that the growth of mammary adenocarcinomas in MMTV K-ras4B transgenic mice was inhibited by the FTI L-744,832, despite continued prenylation of K-Ras (52). However, the isoform of Ras that is activated can still influence the response to FTIs because these investigators observed a rapid and nearly complete tumor regression in MMTV-v-H-ras mice treated with L-744,832.

The observation that large established tumors regressed after treatment with FTIs (53) and the association of ras mutations with strong VEGF expression (54) led to the hypothesis that FTI activity in vivo was mediated in part by antiangiogenesis. Rak et al. (54) demonstrated a significant reduction of both VEGF mRNA and VEGF functional protein in H-ras-transformed rat intestinal epithelial cells. However, Ras also acts in an inverse manner: H-ras-transformed cells strongly suppress the expression of thrombospondin-1, an endogenous angiogenesis inhibitor (55). Furthermore, Cohen-Jonathan et al. (56) showed that FTI treatment enhanced tumor oxygenation. Feldkamp et al. (57) proposed that FTI-mediated astrocytoma tumor growth inhibition was due to a combination of factors that affect both the tumor cells and host vasculature. Based on the results from the other studies cited here, it appears that this conclusion may apply to most tumor types. Thus, although several FTI compounds with different chemical structures have been shown to have antiangiogenic properties (58) (57), the net effect of FTIs on tumor vasculature, angiogenesis, and oxygenation is still being investigated.

Of importance to the clinical application of these inhibitors were the findings that a subset of oncogenic ras transgenic mice developed resistance to FTI-mediated tumor regression after prolonged treatment as well as rapid return of the tumor after the cessation of FTI administration (59). These results are in accord with the lack of durable responses in the recently reported Phase II studies in which FTIs were used as single agents.

The combination of FTIs and GGTIs has been tested preclinically in K-ras mutant PSN-1 pancreatic cancer cells and was found to elicit a greater apoptotic response than either agent alone in vitro. However, in vivo studies revealed pronounced toxicity of prolonged infusion of either combined FTIs and GGTIs or dual prenylation inhibitors (60). The toxicity observed in mice contrasts with acceptable toxicity of a continuous i.v. infusion of the dual prenylation inhibitor L-778,123 at 280 mg/mg2/day in combination with radiotherapy [see below (61)]. Good tolerance of L-778,123 was confirmed in another Phase I study at 560 mg/mg2/day, a dose where plasma concentrations at steady-state exceeded IC50 values required for growth inhibition and cytotoxicity in preclinical studies (62). It should be noted that inhibition of K-Ras prenylation was not detected in patients treated with L-778,123 (63). Further development of L-778,123 was abandoned because of compound-specific electrocardiographic QTc prolongation at doses at or above 560 mg/m2/day. Thus, whereas trials of dual-specificity inhibitors are not currently under way, the possibility remains that other dual-specificity inhibitors or inhibitors with activity against K-Ras may be developed successfully in the future.

The preclinical studies of FTI activities against a wide range of tumor cell types have demonstrated that the action of FTIs is not dependent upon the presence of ras mutations, although the presence of H-ras mutations has been shown to confer a more sensitive phenotype in several of these studies. Because H-Ras will be the primary target of FTIs, what remain to be defined are the role of H-Ras signaling inhibition in tumors without H-ras mutation and its contribution to the antitumor activity of these agents.

Alternative Targets for FTI Antitumor Activity

The results described in the previous section suggest that farnesylated proteins that are pivotal to transformation in addition to oncogenic Ras, may also be important targets for FTI antitumor effects. More than 20 mammalian proteins are farnesylated (reviewed in Ref. 10). Possible candidate targets in the context of cancer treatment include RhoB (64); the phosphatases PRL-1, 2, and 3 (65); and CENP-E and CENP-F centromeric proteins (reviewed in Ref. 66).

RhoB is a small GTPase involved in regulation of endosomal traffic (67) that normally exists in either farnesylated or geranylgeranylated forms (68). RhoB is very rapidly induced by DNA damage (69) and has been implicated in the FTI-induced apoptosis of Ras-transformed cells (70). FTI treatment leads to a shift toward geranylgeranylation of RhoB and altered cellular localization and function (reviewed in Ref. 71). Elevations of RhoB-GG were implicated in the antineoplastic responses after FTI treatment, specifically by inducing p21(Waf-1) expression and cell cycle arrest, as well as by increasing apoptosis in certain cells (72, 73). The contribution of the altered balance of RhoB prenylation toward RhoB-GG is still controversial because another group reported that both RhoB-F and RhoB-GG are potent suppressors of transformation and human tumor growth (74).

Other possible targets for the antiproliferative effects of FTIs are the centromeric proteins CENP-E and CENP-F. These proteins are preferentially expressed during mitosis and appear to be critical to transit through that portion of the cell cycle (reviewed in Ref. 66). These proteins have a CAAX motif and are farnesylated (75). Delay of progression through G2-M was achieved by ectopic expression of the kinetochore-targeting domain of CENP-F. The delay required the presence of a CAAX box and FTase activity (76). Localization of CENP-F to the nuclear envelope at G2-M and kinetochores, as well as its degradation after mitosis, also appears to require farnesylation (76). The association of CENP-E with microtubules is dependent upon farnesylation (75). However, it was also reported that CENP-E and CENP-F localization to the kinetocore was not affected by FTI treatment resulting in abnormal spindle formation and prophase arrest (77). Furthermore, the FTI-mediated arrest at prophase appears to be independent of transformation state (78). Thus, the antitumor effects of inhibiting farnesylation of these CENP proteins have not yet been defined.

Still other potential targets for FTI action in tumors include Rheb, another farnesylated small G-protein protein. A mutant of FTase resulted in G2-M enrichment of yeast that was reversed by introduction of a geranylgeranylated Rheb (79). The effects of human Rheb...
inhibition have not yet been studied. Members of the PRL family of tyrosine phosphatases are candidates by virtue of their farnesylation (65) and the finding that overexpression can lead to transformation (80, 81).

**FTI Effects on Cell Cycle Progression**

To better understand how the FTIs might be used in the treatment of human tumors, Barrington et al. (82) further explored the mechanisms by which L-744,832 induces tumor regression in a variety of transgenic mouse tumor models. ras/myc tumors were found to respond nearly as well to L-744,832 treatment as MMTV-v-H-ras tumors, although no induction of apoptosis was observed. Rather, tumor regression in the ras/myc mice appeared to be mediated by a large reduction in the S-phase fraction. These findings suggested that the effect of Ras inhibition on tumor response is affected by the presence of genetic alterations that abrogate growth control at the G1-S-phase boundary. In tumors with an intact G1 checkpoint, inhibition of Ras relieved the suppression of apoptosis. However, in the presence of genetic alterations that impaired the G1 checkpoint and therefore enhanced Ras proliferative signals, such as p53 or c-myc overexpression, Ras inhibition by L-744,832 resulted in suppression of cell cycle transit. Interestingly, the mechanism of tumor response in the ras/p53−/− tumors was mediated by both an increase in apoptosis and a decrease in the tumor cell S-phase fraction. L-744,832 has been shown to induce G1 arrest in a subset of sensitive lines (50). p53-dependent induction of p21 was found to be responsible for the arrest. However, neither p53 nor p21 was required for inhibition of cell proliferation. In the absence of p21, cells became polyploid and underwent apoptosis. These data suggest that farnesylated protein(s) may be involved in regulating activity of p53. Another group reported accumulation of A549 and Calu-1 in M phase due to the inability to progress from prophase to metaphase after treatment with FTI-2153 (77). Some cells also arrested in the G1 phase. This group also reported that the formation of bipolar spindles and proper chromosome alignment in M-phase were inhibited. Similar findings were described with the FTI L-744,832 when combined with Taxol as described below (83). These effects may reflect inhibition of centromeric protein farnesylation by FTIs because both CENP-E and CENP-F are farnesylated as discussed above.

**FTI-induced Apoptosis**

There are many reports that describe apoptosis in tumor cells and tumor vasculature after treatment with FTIs (46, 48, 70, 84–87). However, the mechanism of FTI-induced apoptosis is still a topic of controversy. Suzuki et al. (84) reported that several FTI compounds (SCH56582, BMS191563, B1088, and FTI-277) induced apoptosis in low serum conditions only in v-K-ras-transformed but not in transformed rat kidney cells. The group also observed a release of cytochrome c into the cytosol and a subsequent activation of caspase 3 but not caspase 1. In this study, neither the MEK1/2 inhibitor PD98059 nor the PI3K inhibitor wortmannin induced apoptosis in these cells. Another group, using the FTI-277 [which was used by Suzuki et al. (84)], reported that FTI induced apoptosis in mouse 3T3 cells that overexpress Akt2 but not in mouse 3T3 cells expressing oncogenic H-Ras (88). The same report showed that constitutively active Akt2 blocked FTI-induced apoptosis in human cells. One possible mechanism for FTI-induced apoptosis was uncovered by a recent report showing that treatment of ras-transformed fibroblasts with the FTI LB42722 reversed Ras-mediated inhibition of fas gene expression (85), which would increase susceptibility of these cells to apoptosis induced by Fas ligands.

Apoptosis induction by FTIs might partially explain the tumor regression, but the mechanism of such induction remains unclear because some FTIs do not induce apoptosis in all sensitive human tumor cells. Whereas some cells react with apoptosis upon treatment with FTIs, others do not, even if they show tumor regression (46, 50, 71, 88–90). Even if apoptosis upon treatment with FTIs has been reported in many reports, it should not be forgotten that the dominant effect of FTIs used as single agents appears to be antiproliferative.

**Normal Tissue Toxicity**

A very important feature of FTIs in terms of their use in the clinic is that they are known to have very low toxicity for normal cells at concentrations that block growth of transformed cells. This is surprising because the farnesylation of Ras and other farnesylated proteins in both normal and transformed cells is inhibited. Although the reasons for this are not known, several points deserve consideration. Higher concentrations of CAAX-based FTIs are required to inhibit farnesylation of the nuclear lamins than are required to inhibit Ras processing (91, 92). Another possible explanation for the low toxicity of FTIs in normal cells is the observation that R-Ras2/TC21 signals through some (93) but not all (94) Ras signal transduction pathways. Carboni et al. (95) reported that BMS18651 completely blocked the function of oncogenic Ras without affecting the function of R-Ras2/TC21, which is expressed ubiquitously in many cells. This protein could potentially provide sufficient signaling to maintain normal cell viability under conditions where H-Ras was inhibited.

The studies discussed above show that FTIs affect cells in a complex manner that may be influenced by the complement of mutations present in the tumor cells. Moreover, these studies have also shown that tumors are often quite sensitive to FTIs at doses that show little toxicity in animals (53). In keeping with the broad activity of FTIs, several mechanisms of FTI action including antiproliferative, proapoptotic, and antiangiogenic effects have been identified. These effects have yet to be fully defined, but each may contribute individually or in combination to the antitumor activity of these agents. The varied effects of FTI treatment may further enhance the interaction of FTIs with other forms of therapy.

**Preclinical Studies Combining FTIs with Other Treatment Modalities**

**Combining FTIs and Chemotherapeutic Agents**

Combining FTIs with other chemotherapeutic agents is an attractive approach for several reasons: FTIs have distinct spectra of activity compared with classical chemotherapeutic agents (90). Furthermore, the growth inhibition by FTIs can supplement cytotoxic effects of other drugs in an additive or even supra-additive way under certain conditions. However, the cell cycle blocks induced by FTIs could inhibit the activity of certain cytotoxic agents. For this reason, careful preclinical study of FTI/drug interactions is essential. Several groups have reported on the combination of FTIs with other drugs (47, 83, 96–98). Reports where FTIs are tested with several chemotherapeutic agents under similar conditions and in the same mode are of special interest because they allow for comparisons between the interactions of FTIs with different agents.

Several human cancer cell lines with K-ras mutations or wt ras status were tested for sensitivity to FTI-2148 combined with 5-FU, melphalan, and cisplatin in vitro (89). Combining FTI with 5-FU was less than additive, whereas combinations with melphalan as well as gemcitabine were strictly additive. In A549 lung adenocarcinoma and T98G glioblastoma cells, SCH66336 combined with cisplatin was more than additive; in the other three cell lines, the combination was
less than additive (96). ras status did not predict for the efficacy of combined treatment, but timing of drug administration was critical. A supra-additive effect could only be demonstrated when SCH663636 administration preceded cisplatin treatment. This effect was not related to the intracellular retention of platinum-DNA adducts, but cisplatin-induced apoptosis was shown to be enhanced. It was speculated that SCH663636 might be enhancing the effects of cisplatin by inhibiting the farnesylation of a polypeptide that ordinarily participates in cisplatin resistance, but the exact mechanism remains to be determined.

Doxorubicin, cisplatin, vinblastine, 5-FU, Taxol, and an epothilone drug were tested in combination with FTI L-744,832 in an in vitro model using the wt ras breast cancer cell lines MCF-7 and MDA-MB-468 (83). In contrast to the additive effects seen with most chemotherapeutic compounds, the combination with Taxol and desoxyepothilone was synergistic. The latter two drugs share a common pharmacological mechanism insofar as they both stabilize microtubule polymerization. This effect was independent of the sequence of drug exposure. Similar synergistic effects were reported for a breast cancer and a prostate cancer cell line (T47D and DU-145). The fact that epothilones do not share structural similarity to Taxol but also stabilize microtubules and exhibit synergistic effects in combination with the FTI was interpreted as a mechanistic relationship between FTIs and microtubule-stabilizing agents. The combination was shown to block a majority of the cells in G2-M, and further analysis described an abnormal chromosome alignment and disordered spindle apparatus. It was proposed that a farnesylated protein involved in the regulation of mitosis is responsible for the effects observed. The effects of the FTI BIM-46228 on anchorage-independent growth of MIA-PaCa-2 cells in combination with Taxol were supra-additive as determined using the Pearson correlation coefficient (47). Another FTI, SCH663636, also was able to synergize with paclitaxel and to a somewhat lesser degree with docetaxel when 11 cell lines of different tumor entities were tested in vitro (97). Interestingly, in a mouse model, a wap-ras/F strain that develops mammary tumors with resistance to paclitaxel was shown to be re-sensitized to paclitaxel when combined with SCH663636.

Additional in vivo studies of drug interaction have also been reported. Human lung adenocarcinoma A549 xenografts were tested for combination therapy with FTI L-2148 (98). In this experiment, the FTI was given over 2 weeks with micro-osmotic pumps, and the chemotherapeutic agents were administered three times with intervals of 4 days. Under these conditions, growth inhibition was significantly better for all three combinations. Importantly, regrowth of tumors to the initial volume (100 mm^3) was delayed substantially. This study did not discern a clear advantage of one or another combination of chemotherapeutic agents with the FTI. In another study combining SCH663363 with cyclophosphamide, 5-FU, and vincristine in a HTB177 human lung carcinoma xenograft (K-ras mutant), additive effects of combining FTI with these agents were reported (99).

Supra-additive effects have primarily been observed when FTIs were combined with cisplatin or the microtubule-stabilizing agents Taxol/epothilone. Theoretically, chemotherapeutic agents that do not require cell cycle progression for their activity should be more effective in combination with FTIs because FTIs can induce cell cycle blocks. This prediction is in accord with the observation that cisplatin, which is not cell cycle specific, has a supra-additive interaction with FTIs. The interaction between FTIs and Taxol or epothilone may, on the other hand, reflect the influence of FTIs on the function of centromeric proteins mentioned previously, an effect that could be amplified in combination with microtubule-stabilizing drugs.

**Combining FTIs and Radiation**

**Ras Activation and Radiation Resistance.** Radiation survival of transformed cells has been shown to be influenced by Ras activation in many studies. The link between ras oncoprotein expression and increased radiation survival was first described in N-ras-transfected NIH-3T3 cells (100) and then expanded to other forms of ras in NIH-3T3 (101). These findings were confirmed in primary rat embryo fibroblasts transformed at early passage with H-ras (102, 103). Activating mutations in the respective genes coding for the different isoforms of Ras (H-, K-, and N-Ras) have also been shown to contribute to the intrinsic radiation resistance of several human tumor cell lines (104–106).

**FTIs and Radiosensitivity.** We have demonstrated that inhibiting Ras by treating cells with prenyltransferase inhibitors before radiation has a synergistic effect on radiation-induced cell killing in both rodent and human tumor cells with ras mutations (105, 107). Because H-Ras only undergoes farnesylation, blocking FTase alone can sensitize cells with activated H-ras. Combined blocking of FTase and GGTase is required to sensitize cells harboring activated K-ras to radiation because K-Ras can undergo alternate prenylation by GGTase when FTase activity is blocked. The tumor cell lines tested were derived from a broad variety of tissues of origins including bladder (T24), breast (HS578T), colon (SW480), lung (A549), and fibroblasts (HT1080). These results could be achieved both in vitro and in vivo in the case of H-Ras-expressing tumors using various FTIs including FTI-276, FTI-277 (13), L-744,832 (53), L-778,123 (62), and R115777 (46). Radiosensitization in vivo of tumors expressing K-ras mutations has been more difficult to demonstrate because the correct balance of FTase and GGTase inhibition for blocking K-Ras prenylation in vivo has not yet been established. GGTIs also show a higher level of toxicity in vivo than FTIs (60).

Because many of the human tumor models we have studied as xenografts contain extensive regions of hypoxia, and hypoxic cells are markedly more resistant to killing by radiation, we investigated the effects of FTase inhibition on tumor xenograft hypoxia (56). To detect hypoxia, we used 5-fluoro-substituted 2-nitroimidazole (EF5; Ref. 108). This compound is metabolized to a protein-thiol reactive species in the absence of oxygen. A Cy-3-conjugated monoclonal antibody specific for EF5 served as a detector of EF5 binding and thus for hypoxia (109). Treatment with FTI L-744,832 resulted in a reduction in hypoxia in tumors that expressed activated H-Ras (cell lines T24 and 141-1), but not in tumors with normal ras (cell lines HT-29 and RT-4). These results suggest that FTase inhibition might contribute to radiosensitization in vivo by increasing tumor oxygenation. This finding could be of significance to combined treatment with FTIs and chemotherapeutics as well because hypoxic tumors show resistance to these agents (110). More recent studies have demonstrated that H-Ras activation through EGFR signaling is also susceptible to FTase inhibition, as shown by radiosensitization of SQ20B tumor cells. SQ20B tumor xenografts also showed enhanced tumor oxygenation after treatment with the FTI L-778,123 accompanied by enhanced tumor apoptosis.4

**The Ras Pathway and Radiation Resistance.** Other components of the signaling pathways leading from Ras have been studied as targets for radiosensitization. The Raf-MEK-MAPK pathway was implicated in radiation sensitivity because antisense c-raf-1 was able to sensitize human squamous cell carcinoma cells to irradiation (111). Moreover, DU-145 prostate carcinoma cells expressing wt ras were more radiosensitive after MEK inhibition (112). The role of the MAPK pathway in radiation resistance may be tissue specific, how-

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4 Unpublished observations.
ever, because the MEK inhibitor PD98059 failed to radiosensitize two bladder cancer cell lines \([H\text{-ras} \text{ activated } T24\text{ and } ras \text{ wt RT-4 (113)}]\). In a subsequent study, Gupta et al. (114) further examined the pathways downstream of Ras for their influence on Ras-mediated radiosistance using specific pharmacological inhibitors. Again, the Raf-MEK-MAPK pathway was inhibited with PD98059 but did not affect radiosensitivity in T24 cells. Likewise, the p38 inhibitors SB 203580 and PD169316 as well as the p70S6K inhibitor rapamycin did not radiosensitize this cell line. In contrast, inhibition of the PI3K pathway using LY294002 increased radiation sensitivity in T24 cells as well as in 3.7 H-ras-transformed rat embryo fibroblasts and DLD-1 colon cancer cells expressing mutant K-Ras. Cells expressing wt Ras, MR4 rat embryo fibroblasts immortalized with \(v\text{-myc}\), and the RT-4 bladder cancer line were not radiosensitized. These observations were confirmed by transfection of MR4 and RT-4 with a constitutively active PI3K. Induction of this PI3K made the cells more resistant to radiation, whereas concurrent treatment of the transfected cells with LY294002 sensitized them to radiation. Further confirmation of the significance of the PI3K pathway in radiation resistance has been obtained by transfecting HT1080 cells expressing activated N-Ras with a plasmid encoding PTEN phosphatase. Overexpression of this phosphatase, which antagonizes PI3K activity by dephosphorylating phosphatidylinositol 3,4,5 trisphosphate (115), rendered HT1080 cells more sensitive to radiation compared with the parental cells with normal PTEN expression.\(^5\) In another study, activation of PI3K by EGFR signaling in SQ20B cells was blocked using the EGFR inhibitor Iressa, the FTI L744,832, and the PI3K inhibitor LY294002. Radiosensitization was achieved with all three agents (116). The mediation of Ras-dependent radiosensitivity through PI3K and its reversal with LY294002 were recently confirmed in a study in which RIE-1 rat intestinal epithelial cells were transfected with Ras (117). In addition to PI3K, this study reported that MAPK-independent Raf signaling also contributed to mediation of Ras-induced radiation resistance. Taken together, these data show that Ras activation by mutation or upstream signaling can increase intrinsic radiation resistance of tumor cells through activation of PI3K. Inhibitors of the Ras signaling pathway are able to reduce tumor radiation resistance when Ras is activated indirectly by receptor signaling or directly by mutation. Ras inhibition can enhance both apoptosis and oxygenation in tumors. Either effect or both effects could contribute to tumor radiosensitization and might improve the effectiveness of chemotherapeutic drugs as well. In this regard, a recent report demonstrated in mice that prenyltransferase inhibitors, in combination with either cisplatin, gemcitabine, or Taxol, resulted in greater antitumor activity than either the prenyltransferase inhibitors or the cytotoxic drugs alone (98). Abrogation of Ras activity in the setting of chemotherapy might thus intensify the effects of anticancer chemotherapeutics in vivo in much the same way as it does radiation. It is possible that differences in the signal transduction specificity of the different isoforms of Ras could be significant in this regard. Because K-ras is the most frequently mutated form of ras in human malignancies, a perceived problem in the development of FTIs has been their poor activity against K-Ras. However, if sensitivity to drugs and radiation is primarily influenced by PI3K, which in turn is preferentially activated by H-Ras, then FTI-mediated inhibition of H-ras may in many cases suffice to sensitize cells to cytotoxic therapies.

\(^5\) Unpublished observations.

Clinical Trials with FTIs

\textbf{Phase II Trials of FTIs as Single Agents}

A number of Phase I trials evaluating the toxicity and maximally tolerated dose of the FTIs and \(ras\) antisense agents have been performed. In general, the results of Phase I studies demonstrate that these agents are well tolerated and that the routes of administration (oral, i.v., or s.c.) are suitable for prolonged administration to patients. Many of these drugs have entered Phase II and III evaluation, and the first results of some Phase III studies have recently been reported. In addition, Phase I studies of these inhibitors in combination with radiation and other chemotherapeutic agents have also been completed. Studies designed to assess the efficacy of these compounds should be forthcoming.

Ten Phase II studies have been reported to date (Table 2). The compound R115777 has been tested in eight Phase II studies. The best results have been achieved in patients with hematological malignancies; 7 of 42 patients with relapsed or refractory acute myelogenous leukemia had a reduction of leukemic blasts below 5% (118). Two CRs and two PRs were described in a total of seven patients with myeloproliferative disorders (119). Two additional patients with myeloproliferative disorders had SD. In solid malignancies, PRs were observed in patients with metastatic breast cancer [4 of 41 with continuous treatment and 4 of 35 in the intermittent dosage schedule (120)]. PRs were also reported in 3 of 42 patients with glioma (121). SD has also been reported for patients with metastatic breast cancer [6 of 41 continuous schedule and 4 of 35 intermittent schedule (120)], stage IIIB/IV NSCLC [6 of 44 patients with SD over more than 6 months (122)], and recurrent glioma [2 of 42 patients over more than 6 months (121)]. Eight of 22 patients with small cell lung cancer maintained a good performance status during treatment (123). No major clinical responses were achieved for 23 patients with stage IIIB/IV NSCLC treated with L-778,123 (124). Further details of these studies’ results including toxicities are presented in Table 2. In general, the FTIs have been tolerable, with hematological toxicity observed in nearly all trials. Interestingly, compound-specific toxicities have also been described (125).

\textbf{Phase III Trials of FTIs}

The results of two Phase III trials have recently been reported in abstract form (126, 127). The first study was a randomized double-blind placebo-controlled trial of R115777 in metastatic colorectal cancer (126). In this study, 235 patients receiving R115777 were compared with a placebo group of 133 patients. There was no statistical difference between the two groups for median overall survival. A second Phase III trial of R115777 was also recently completed in patients with advanced pancreatic carcinoma. This study enrolled 688 patients in 133 centers (127). Gemcitabine and R115777 were compared with gemcitabine and placebo. No statistically significant differences were observed in median overall survival (193 days versus 182 days), in the 6-month and 1-year survival rates, or in progression-free survival between the treatment groups. Ten drug-related deaths were reported for gemcitabine and R115777, and seven drug-related deaths were reported for gemcitabine and control. Neutropenia and thrombocytopenia were observed somewhat more frequently in the combination group [40% and 15% (combination) versus 30% and 12% (gemcitabine alone), respectively]. Nonhematological adverse effects were similar in the two groups, except for diarrhea and hypokalemia [38% and 15% (combination) versus 25% and 8% (gemcitabine alone), respectively].
Trials of FTIs with Other Treatment Modalities

The FTIs R115777, BMS214662, SCH66336, and L-778,123 have been tested in combination with other agents as shown in Table 3. R115777 was tested together with 5-FU and leucovorin, gemcitabine, gemcitabine and cisplatin, topotecan, CPT-11, docetaxel, and capecitabine in patients with diverse malignancies (128–134). Tolerable schedules were identified for most of the combinations. The maximally tolerated dose was most often defined by myelotoxicity. Clinical responses have been described for the combinations of R115777 with gemcitabine and cisplatin, topotecan, and CPT-11. One CR was reported for the combination of R115777 with gemcitabine and cisplatin, and one CR was reported for the combination with docetaxel (130, 133). SCH66336 was tested in combination with gemcitabine, gemcitabine plus cisplatin, paclitaxel, and paclitaxel plus carboplatin (135–139).

Again, major responses have been described for the combinations of SCH66336 with gemcitabine and SCH66336 with paclitaxel. BMS214662 and L-778,123 were each tested together with paclitaxel (140, 141). An objective response (that was not further specified) has been described in a patient with recurrent laryngeal cancer after treatment with BMS214662. A biochemical response has been observed in patients treated with BMS214662. Only disease stabilization has been observed in patients treated with L-778,123 combined with paclitaxel.

The results of a multicenter Phase I clinical trial of L-778,123 in combination with radiotherapy was also reported recently (125). Three patients with HNC and 6 patients with NSCLC received 70 or 65 Gy, respectively, in addition to the FTI. Eligibility was independ-
<table>
<thead>
<tr>
<th>No. of Pts.</th>
<th>No.</th>
<th>Escalated drug schedule</th>
<th>Schedule other drug(s)</th>
<th>RPTD</th>
<th>DLT (gr 3/4 toxicities)</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU Leucovorin</td>
<td>128</td>
<td>FTI: 200, 300, 400, 500 mg CD or 200 mg d1–21 q29 ID</td>
<td>d1–2 q15 5-FU: 400 mg/m² bolus, then 600 mg/m², 22 h. LV: 200 mg/m² 2 h</td>
<td>n.a.</td>
<td>9 ANC</td>
<td>2 fatigue, 1 paresthesia</td>
</tr>
<tr>
<td>18 colorectal, 12 pancreatic &amp; 30 pts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No DLT in pts @ ID schedule</td>
</tr>
<tr>
<td>Gem</td>
<td>129</td>
<td>FTI: DL1 200 mg, DL2 300 mg Gem: 1 g/m² d1, d8, d15 q29</td>
<td>Gem 1 g/m² d1, d8, d15 q29 200 mg bid</td>
<td>n.a.</td>
<td>9 ANC</td>
<td>2 fatigue, 1 paresthesia</td>
</tr>
<tr>
<td>Gemcitabine &amp; CDDP</td>
<td>130</td>
<td>FTI: 100, 200, 300 mg q22Gem: (750, 1000 mg/m²d1, d8 cDDP: 75 mg/m² d1</td>
<td>lowest DL is too toxic: R115777 100 mg bid Gem: 75 mg/m², cDDP 75 mg/m²</td>
<td>n.a.</td>
<td>10 ANC, 6 PLT</td>
<td>2 rash, 5 nausea</td>
</tr>
<tr>
<td>Diverse advanced</td>
<td>131</td>
<td>FTI: d1–21 q29 DL1 1.0</td>
<td>R115777 200 mg bid p.o. DL1–21 q29 Gem: 75 mg/m², cDDP 75 mg/m²</td>
<td>n.a.</td>
<td>10 ANC, 6 PLT</td>
<td>2 rash, 5 nausea</td>
</tr>
<tr>
<td>Topotecan</td>
<td>132</td>
<td>FTI: 26 pts</td>
<td>Topotecan 1.0 mg/m²d1–21 q29 × 5d1–21 q29</td>
<td>5: 100 (3 pts) 3: 100 (3 pts) 2: 100 (4 pts) 1: 75 (11 pts)</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
</tr>
<tr>
<td>CPT-11</td>
<td>133</td>
<td>FTI: 24 pts</td>
<td>CPT-11 350 mg/m² q d22</td>
<td>3: 75 1g r3A N C, 1 g r3P L T</td>
<td>1 febrile ANC</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
</tr>
<tr>
<td>Decetax</td>
<td>134</td>
<td>FTI: 24 pts Capecit: 2–2.5 g/m² p.o. d1–14 q22</td>
<td>1: 150</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
<td>1 febrile ANC</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
</tr>
<tr>
<td>SCH6636</td>
<td>135</td>
<td>FTI in all studies p.o. Gem: 150 mg/m²</td>
<td>Gem: 150 mg/m²</td>
<td>n.a.</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
</tr>
<tr>
<td>Gem &amp; CDDP</td>
<td>136</td>
<td>FTI: mg bid 1: 75 2: 75 3: 100</td>
<td>CDDP Gem: (mg/m²)</td>
<td>n.a.</td>
<td>1 febrile ANC</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
</tr>
<tr>
<td>P 14 pts</td>
<td>137</td>
<td>FTI: mg bid 1: 100 (3 pts)</td>
<td>P mg/m² 1: 135 2: 175 3: 175</td>
<td>n.a.</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
</tr>
<tr>
<td>P Carbo</td>
<td>138</td>
<td>FTI: 1st cycle: d6–21 next cys: d1–21 DL mg bid</td>
<td>P mg/m² 3 h i.v./d1 Carbo: AUC 5, 30 i.v. DL mg/m²</td>
<td>n.a.</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
</tr>
<tr>
<td>Misc. solid tumors</td>
<td>139</td>
<td>FTI: 1st cycle: d3–28 next cys: d1–28 DL mg bid</td>
<td>P mg/m² 1 h i.v. d1 Gems 200 mg/m² 1: 125 2: 150 3: 175 4: 175 5: 200</td>
<td>n.a.</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
</tr>
<tr>
<td>BMS 214662</td>
<td>140</td>
<td>FTI: 80 mg/m²/wk DL: 80 (3 pts) DL: 1: 120 (3 pts)</td>
<td>P 80 mg/m² 1 h i.v.d1</td>
<td>n.a.</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
</tr>
<tr>
<td>L-778, 123</td>
<td>141</td>
<td>FTI: 1870 mg/m²/d No escalation possible</td>
<td>P 80 mg/m² 1 h i.v.d1</td>
<td>n.a.</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
</tr>
<tr>
<td>P 13 pts</td>
<td>142</td>
<td>FTI: 80 mg/m²/d DL: 280 mg/m²/d</td>
<td>P 80 mg/m² 1 h i.v.d1</td>
<td>n.a.</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
<td>1 APC (breast) 1 PR (breast) 4 PR (2 breast, 1 NSCLC, 1 UPT)</td>
</tr>
</tbody>
</table>

FTIs: PRECLINICAL AND CLINICAL INVESTIGATION RESULTS

Table 3 Phase II studies combining FTI with other drugs

No. of Pts. = number of patients; No. = number of patients; Escalated drug schedule = schedule of the escalated drug; Schedule other drug(s) = schedule of the other drug(s); RPTD = Related clinical activity described but not specified; DLT (gr 3/4 toxicities) = DLT (gr 3/4 toxicities); Responses = Responses.
ent of ras status, although ras status was assessed. None of the six patients treated at the first DL experienced DLT, and escalation to the second DL was reported for three patients. At the second DL, one episode of DLT, grade IV neutropenia, developed in a NSCLC patient. Because of the decision of the manufacturer of L-778,123 not to pursue further clinical development of the drug, the study was closed to further enrollment. None of the tumors from six NSCLC patients had a mutation in ras. Although assessing efficacy was not the primary end point of this trial, a response to the treatment was reported. Two evaluable stage IV HNC patients had CR on follow-up. One of them was alive at the time of publication of the report. The other HNC patient died of a cerebrovascular accident. The third HNC patient had a synchronous NSCLC and was taken off study. One of the six patients with NSCLC was taken off study because of DLT and distant metastatic disease and was not evaluable for response. Another NSCLC patient did not have measurable disease. Three of the four evaluable patients with NSCLC had a CR, and one patient had a PR. No evidence of local recurrence was observed for a period of 7–12 months after treatment. A separate cohort of 12 patients with locally advanced pancreatic cancer was enrolled on this trial (61). These patients received FTI and 59.4 Gy of radiation. Eight patients were treated on the first DL, and four patients were treated on the second DL. Two episodes of DLT (grade III diarrhea and grade IV hematologic toxicities) were observed at the second DL. No objective tumor responses were observed, although one patient had normalization of the tumor marker CA19-9.

Conclusions

The initial expectations of specificity of FTIs for cells with ras mutations matched the original in vitro observations. However, subsequent in vitro and in vivo studies demonstrated that FTI-mediated inhibition of tumor growth was not tightly linked to ras mutation status. It is now clear that ras mutation is not a requirement for FTI effects on tumor cells. It has not, however, been shown that Ras signaling inhibition does not play a role in these effects.

FTIs showed significant promise in preclinical studies. On the other hand, these studies also showed that there was tumor regrowth after treatment cessation in mice and that FTIs alone were not curative. The relatively poor response rates reported in clinical trials with FTIs alone might be explained by these preclinical results. However, combination of FTIs with other therapies may have a good potential, if these are applied according to biological principals of activity.

In using FTIs in combined modality treatment, the timing of FTI administration relative to chemotherapeutic or radiotherapy may be critical. As discussed above, not every chemotherapeutic drug will be suitable for combination with FTIs. The combination of FTIs with radiation is, in our experience, well tolerated and engenders no additional radiation toxicity to normal tissues. The Phase I trial of L-778,123 with radiation yielded encouraging results for this combination, consistent with our preclinical findings. This may result from a combination of FTI effects on tumor cell radiosensitivity, perhaps via inhibition of PI3K activation, and effects on the tumor microenvironment as well as the antiproliferative effects of FTIs. Much work remains to be done to fully understand the complex activity of these compounds.

References

71. Prendergast, G. C., and Oliff, A. Farnesyltransferase inhibitors: antineoplastic prop-

72. Du, W., Lefebvre, P. F., and Prendergast, G. C. Cell growth inhibition by farn-
syltransferase inhibitors is mediated by gain of geranylgeranylated RhoB. Mol. Cell.

73. Du, W., and Prendergast, G. C. Geranylgeranylated RhoB mediates suppression of
3499, 1999.

2000.

75. Ashar, H. R., James, L., Gray, K., Carr, D., Black, S., Armstrong, L., Bishop, W. R.,
and Kirschmeier, P. Farnesyl transferase inhibitors block the farnesylation of
CENP-E and CENP-F and alter the association of CENP-E with the microtubules.

76. Hussein, D., and Taylor, S. S. Farnesylation of Cenpl-F is required for G1M

77. Crespo, N. C., Ohkanda, J., Yen, T. J., Hamilton, A. D., and Sebti, S. M. The farnesyltransferase inhibitor, FTI-2153, blocks bipolar spindle formation and chro-


79. Yang, W., Tabanacy, A. P., Urano, J., and Tannan, F. Failure to farnesylate rho pathway components results in the enrichment of Ras-family GTPase in the cells in the Schizosac-

a unique nuclear protein tyrosine phosphatase, affects cell growth. Mol. Cell. Biol.,

81. Cates, C. A., Michael, R. L., Stayrook, K. R., Harvey, K. A., Burke, Y. D., Randall,
S. K., Crowell, P. L., and Crowell, D. N. Prenylation of oncogenic human PTP-

82. Berditchen, R. E., Stuhler, M. A., Blank, E., Emmer, C. A., Miller, P. J., Hundle,
J. E., Koester, J. K., Trowey, A. D., Bears, J. D., Conner, M. W., Gibbs, J. B.,
Hamilton, K., Koblan, K. S., Mosser, S. D., O’Neill, T. J., Schaber, M. D., Senderak,
tumor regression in transgenic mice harboring multiple oncogenic mutations by
mediating alterations in both cell cycle control and apoptosis. Mol. Cell. Biol., 18: 

83. Miller, M. M., Sepp-Lorenzino, L., Kohl, N. E., Oliv, A., Bolog, A., Su, D. S.,
Danieshefsky, S. J., and Rosen, N. Farnesyl transferase inhibitors cause enhanced
mitotic sensitivity to taxol and epothilones. Proc. Natl. Acad. Sci. USA, 95: 1369–1374,
1998.

84. Suzuki, N., Urao, J., and Tamanoh, F. Farnesyltransferase inhibitors induce cyto-
chrome c release and caspase 3 activation preferentially in transformed cells. Proc.


90. Parsons, J. D., Pradines, A., Visscher, J. I., Breнер, M. C., Miguel, K., Lonchampt,


92. Prendergast, G. C., et al. FTIs: PRECLINICAL AND CLINICAL INVESTIGATION RESULTS


Farnesyltransferase Inhibitors: An Overview of the Results of Preclinical and Clinical Investigations


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