Farnesyl transferase inhibitors: the next targeted therapies for breast cancer?

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Abstract

The ras family of proto-oncogenes are upstream mediators of several essential cellular signal transduction pathways involved in cell proliferation and survival. Point mutations of ras oncogenes result in constitutively active Ras and have been shown to be oncogenic. However, ras activation can occur in the absence of ras mutations secondary to upstream receptor activation. The first important step in Ras activation is farnesylation by farnesyl transferase, and inhibitors of this enzyme have been demonstrated to inhibit Ras signaling, and have anti-tumor effects. However, it is now clear that farnesyl transferase inhibitors (FTIs) have activity independent of Ras, most likely due to effects on prenylated proteins downstream of Ras, which explains their activity in several malignancies, including breast cancer, where ras mutations are rare. Several FTIs are in clinical development for the treatment of solid tumors. Preclinical evidence suggests that FTIs can inhibit breast cancers in vitro and in vivo, and a phase II trial of the FTI, R115777, in patients with advanced breast cancer produced encouraging results. Based on prior successful outcomes with agents targeting the estrogen and epidermal growth factor receptor pathways in breast cancer, the FTIs, used alone or more likely with other agents, may be the next exciting targeted therapy in breast cancer.

Introduction

In contrast to many other solid tumors, targeted therapies in breast cancer have been used successfully for over 30 years. Agents targeting the estrogen receptor (ER), in particular tamoxifen, have improved outcomes in all stages of breast cancer (Early Breast Cancer Trialists' Collaborative Group 1998) and tamoxifen is approved for the prevention of breast cancer in high-risk women (Fisher et al. 1998). More recently, trastuzumab, an antibody targeting the human epidermal growth factor receptor (EGFR), HER-2/neu, has been shown to be an effective therapy in metastatic breast cancer (Slamon et al. 2001), and is being evaluated in early stage breast cancer.

The ras family of proto-oncogenes are upstream mediators of several essential cellular signal transduction pathways (Johnston & Kelland 2001) and, as such, provide a rational target for the treatment of malignancies. Farnesyl transferase inhibitors (FTIs) are a group of agents that target the ras family and its downstream signaling pathways, and are being evaluated in phase I, II and III trials in a number of malignancies (Johnston & Kelland 2001). Unlike, many other solid tumors, breast cancers have a very low rate of ras mutations (Clark & Der 1995a). However, breast cancers have been shown to have aberrant signaling though the Ras signal transduction pathway (Clark & Der 1995b), and preclinical evaluation of FTIs in breast cancer models have produced promising results (Kelland et al. 2001). Following phase I trials, the FTI, RII5777, has been evaluated in patients with advanced breast cancer, with encouraging results (Johnston et al. 2003).

Targeted therapies in breast cancer

The selective ER modulator, tamoxifen, has been used for over 30 years in the treatment of breast cancer. In patients with advanced breast cancer, tamoxifen results in response rates ranging from 30 to 70%, depending on the ER and progesterone receptor (PgR) profile of the breast tumor (NIH Consensus Development Conference...
on Steroid Receptors in Breast Cancer 1980). As an adjuvant therapy for early stage breast cancer, tamoxifen reduces recurrence rate and improves survival (Early Breast Cancer Trialists’ Collaborative Group 1998). Tamoxifen reduces breast cancer events in patients with ductal carcinoma in situ (DCIS) (Fisher et al. 1999) and, based on the National Adjuvant Breast and Bowel Project (NSABP) Prevention (P)-1 trial (Fisher et al. 1998), was approved for the prevention of breast cancer in high-risk women in 1998. Tamoxifen is truly a targeted agent, resulting in response rates of less than 10% in patients with advanced ER- and PgR-negative breast cancer (NIH Consensus Development Conference on Steroid Receptors in Breast Cancer 1980), having no effect on outcome in ER-negative early stage breast cancer (Early Breast Cancer Trialists’ Collaborative Group 1998) or ER-negative DCIS (Allred et al 2002), and in the NSABP P-1 trial (Fisher et al. 1998) only reducing the incidence of ER-positive tumors. In addition, although tamoxifen is generally well tolerated, most toxicity appears to be due to its effects on ERs in other organs, such as the endometrium (Early Breast Cancer Trialists’ Collaborative Group 1998, Fisher et al. 1998).

Another receptor of major importance in breast cancer is the human EGFR, HER-2, which is present in approximately 20% of breast cancers. Trastuzumab is a chimeric antibody, which targets the HER-2/neu receptor. Initial studies with trastuzumab resulted in clinical benefit of approximately 13% in patients with heavily pretreated HER-2-positive advanced breast cancer (Cobleigh et al. 1999) (Table 1). However, a clinical benefit rate of about 40% was noted when trastuzumab was used as first-line treatment for HER-2/neu-positive advanced breast cancer (Vogel et al. 2002). Based on preclinical studies demonstrating marked synergy between trastuzumab and a number of chemotherapeutic agents (Pegram et al. 1999, 2000), trastuzumab combined with chemotherapy was subsequently shown to improve survival, compared with chemotherapy alone, in the first-line treatment of patients with HER-2/neu-positive advanced breast cancer (Slamon et al. 2001). It is important to recognize that the encouraging results seen with the use of trastuzumab in breast cancer are due to the fact that only patients whose tumors were HER-2/neu-positive and, therefore, had the appropriate target, were eligible for the pivotal trastuzumab trials, and that patients whose tumors did not express HER-2/neu obtained no benefit from trastuzumab therapy (Slamon et al. 2001).

Several other targeted therapies have been examined in advanced breast cancer but so far with disappointing results. The EGFR tyrosine kinase inhibitors, gefitinib and erlotinib, have demonstrated minimal activity in advanced breast cancer to date (Albain et al. 2002, Winer et al. 2002 respectively) (Table 1). Likewise, the vascular endothelial growth factor receptor antagonist, bevacizumab, did not improve outcome in patients with advanced, heavily pretreated breast cancer, when combined with capecitabine, compared with patients treated with capecitabine alone (Miller et al. 2002) (Table 1). However, unlike the trastuzumab studies, in these trials patients were not selected based on their tumors having specific markers, for example EGFR expression, which may have predicted a higher likelihood of response. In the future, it will be essential to select patients whose tumors have the rational targets for a specific targeted therapy, rather than using these agents in an unselected population. However, as described later, despite the initial belief that FTIs act solely through the Ras pathway, it is now apparent that they have other effects on several cellular signal transduction processes, which may be independent of Ras (Johnston & Kelland 2001). This hypothesis may explain why FTIs have activity in breast cancer models preclinically (Kelland et al. 2001), and may be active clinically (Johnston et al. 2003), despite the fact that breast cancers rarely have mutations of ras (Clark & Der 1995a).

Table 1 Results of phase II trials of targeted agents in patients with pretreated advanced breast cancer

<table>
<thead>
<tr>
<th>Agent</th>
<th>Response rate (%)</th>
<th>Clinical benefit (%)</th>
<th>Synergy</th>
<th>Toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trastuzumab (Cobleigh et al. 1999)</td>
<td>15</td>
<td>19</td>
<td>Yes</td>
<td>Cardiac</td>
</tr>
<tr>
<td>Bevacizumabb (Miller et al. 2002)</td>
<td>10</td>
<td>17c</td>
<td>?</td>
<td>Hypertension, proteinuria</td>
</tr>
<tr>
<td>R115777 (Johnston et al. 2000)</td>
<td>10</td>
<td>24</td>
<td>?</td>
<td>Myelosuppression, neuropathy</td>
</tr>
</tbody>
</table>

a stable disease at 8 weeks; bresults of phase I/II trial including breast cancer; cresponse rate + stable disease at 1 year.

GI, gastrointestinal.
Ras signaling

The family of ras proto-oncogenes has been demonstrated to play a role in the malignant phenotype. Point mutations in the ras proto-oncogene result in permanently active Ras and are, therefore, oncogenic. There are currently three ras proto-oncogenes (H-, N- and K-ras), which encode four proteins, known as H-Ras, N-Ras and two K-Ras proteins, K-Ras4A and K-Ras4B (Barbacid 1987). The Ras proteins transfer messages from receptors on the cell surface, such as EGFRs, to signaling systems within the cell (McCormick 1994).

In order for the Ras proteins to become activated, which involves the transfer of a phosphate group from the GDP bound form to the GTP form (Johnston & Kelland 2001), they must localize to the plasma membrane: the first step in this process is referred to as farnesylation.

Recent data from Chiu et al. (2002) and Bivona & Phillips (2003) suggest that selective trafficking of the various ras proteins on the plasma membrane, endoplasmic reticulum and Golgi apparatus is in response to critical mitogenic stimulation and that once activated they selectively activate in turn various downstream signaling pathways (Fig. 1). Ras proteins recruit Raf-1 to the membrane where it becomes activated (Leevers et al. 1994) and is then able to convert the kinases MEK1 and 2 to their activated phosphorylated forms (Fig. 1). Activation of the mitogen-activated protein (MAP) kinase pathway allows MAP kinase to move to the nucleus and results in the transcription of numerous target genes (Marshall 1996), which ultimately results in cell proliferation (Fig. 1). Raf-1-independent activation of MEK can also occur via activation of c-Jun transcription factors (Lange-Carter et al. 1993). Ras can also activate the Rho pathway, a family of proteins involved in cell adhesions and the actin cytoskeleton (Ridley & Hall 1992), and also the phosphatidylinositol 3'-kinase (PI3K) family of lipid kinases, which are involved in cell survival and apoptosis (Kennedy et al. 1997, Kodaki et al. 1994) (Fig. 1).

Farnesylation

The first step in Ras activation, or farnesylation, is called prenylation where a prenyl group is attached to a protein, in this case the Ras protein. Examples of prenyl groups are a 15 carbon farnesyl group and a 20 carbon geranylgeranyl group. Farnesyl transferase (FT) catalyses the transfer of the 15 carbon farnesyl group, while geranylgeranyl transferase catalyses transfer of the 20 carbon geranylgeranyl group. FT transfers this farnesyl group onto a cysteine moiety of the Ras protein, referred to as CAAX, where C stands for cysteine, A for an aliphatic amino acid and X for any amino acid (Fig. 2). The specific amino acid that is found in the X position (either a methionine/serine) on the CAAX moiety determines which of the Ras proteins is farnesylated (Moores et al. 1991). Once the prenyl group attaches to the CAAX moiety, the AAX part is cleaved (Fig. 2). The C-terminal farnesyl-cysteine moiety is then carboxymethylated and a fatty acid palmitate residue is

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**Figure 1** Ras signaling pathways result in activation of MAP kinase, RhoB and PI3K. MEK, mitogen-activating protein kinase (MAPK) kinase.
attached. This makes the Ras protein hydrophobic and facilitates its transfer to the cell membrane, where it becomes phosphorylated when activated by upstream tyrosine kinase signaling (Fig. 2). Since farnesylation is the most important step in Ras activation (Kato et al. 1992), and subsequent downstream signaling, an agent that blocks FT could be expected to impact cancer cell survival and proliferation.

**Effects of FTIs independent of Ras**

There is accumulating evidence to suggest that FTIs have activity independent of Ras. First, FTIs have been demonstrated to inhibit anchorage-independent growth of many human cancer cell lines, irrespective of whether they express wild-type or mutated ras (Liu et al. 1998, Feldkamp et al. 2001). Secondly, FTIs have been demonstrated to inhibit growth of tumors expressing wild-type ras in vivo (Liu et al. 1998, Feldkamp et al. 2001). Lastly, responses have been demonstrated with the FTI, R115777, in breast cancers, which have a very low rate of ras mutations (Johnston et al. 2003). The most likely explanation for these Ras-independent effects is that FTIs have effects on several other prenylated proteins involved in crucial cellular signal transduction pathways. Many potential substrates for FTIs, apart from Ras, have been identified, including lamin A and human peroxisomal farnesylated protein (PxF), both of which have been used as surrogate markers of farnesylation. RhoB, rhodopsin kinase and the γ subunit of the retinal protein transducin (Adjei 2001).

RhoB, a protein which is prenylated, and functions to regulate receptor trafficking, has been demonstrated to be altered in response to FTI treatment (Adjei 2001). RhoB can be farnesylated or geranylgeranylated, and the different prenylated forms appear to have opposing cellular effects, such that farnesylated RhoB promotes cellular transformation and geranylgeranylated RhoB suppresses the transformed phenotype (Adjei 2001). Therefore, the anti-tumor effects of FTIs may depend on the accumulation of geranylgeranylated forms of RhoB (Du & Prendergast 1999, Prendergast & Oliff 2000). However, evidence against this hypothesis is the finding that both geranylgeranyl transferase I inhibitors and dual FT/geranylgeranyl transferase have anti-tumor effects (Sun et al. 1998). Additionally, both farnesylated and geranylgeranylated forms of RhoB have been shown to suppress tumor growth in athymic mice (Chen et al. 2000). FTIs may also have effects on other members of the Rho family. Over-expression of RhoC GTPase has been demonstrated in over 90% of inflammatory breast cancers (van Golen et al. 2000). Unlike RhoB, which is primarily farnesylated and minimally geranylgeranylated, RhoC undergoes geranylgeranylation (Adamson et al. 1992, Kirshmeier et al. 2001). Interestingly, treatment of RhoC-over-expressing breast epithelial cell lines with the FTI, L-744,832, resulted in significant decreases in anchorage-independent growth, motility and invasion (van Golen et al. 2002). However, this may be explained by the finding that the FTI caused an
increase in RhoB levels, but no change in RhoC expression or activation, suggesting that the effects of the FTI on these RhoC-over-expressing cells are mediated through RhoB (van Golen et al. 2002).

Based on several observations, it appears that FTIs affect the PI3K/AKT pathway, resulting in apoptosis, which may be independent of Ras (Jiang et al. 2000, Chun et al. 2003). Jiang et al. (2000) demonstrated that FTIs inhibit the PI3K/AKT-mediated growth factor- and adhesion-dependent survival pathways, and induce apoptosis in human cancer cells that over-express AKT. Likewise, Chun et al. (2003) examined the effect of the FTI, SCH 66336, on several human head and neck squamous cancer cell lines, and noted an increase in apoptosis, which was detected by cell cycle analysis, DNA fragmentation and TUNEL assays. This increase in apoptosis was associated with a reduction in total and phosphorylated AKT, and with an increase in the ratio of pro-apoptotic to anti-apoptotic proteins, caused by a decrease in Bcl-2 and Bcl-XL, with no change in Bax (Chun et al. 2003). Therefore, it is possible that the effects of FTIs on human tumor growth may be mediated through inhibition of a farnesylated protein associated with the PI3K/AKT-mediated cell survival pathway. This hypothesis is corroborated by the finding that FTIs block the activation of p70s6k, which is downstream of PI3K (Nagasu et al. 1995).

Lastly, Ras can be activated by upstream tyrosine kinase activation (Clark & Der 1995b). The FTI, L-744,832, has been demonstrated to induce regression of mammary tumors in MMTV-TGFα transgenic mice, which is a useful model for studying the activity of anti-tumor drugs on mammary tumors with activated receptor tyrosine kinases, but without ras mutations (Norgaard et al. 1999). Tumor regression in this mouse model was associated with a reduction in MAP kinase activity and an increase in apoptosis (Norgaard et al. 1999). However, Johnston et al. (2003) did not find a correlation between response to the FTI, R 155777, and expression of HER-2/neu, in patients with metastatic breast cancer.

In summary, it is apparent that FTIs have activity on crucial components of cell signaling, independent of Ras. RhoB seems a likely target of farnesyl inhibition but, in addition, it is possible that an as yet unidentified prenylated protein involved in the PI3K/AKT pathway may be responsible for the increase in apoptosis noted with FTIs, in the absence of ras mutations.

### Ras in breast cancer

Many solid tumors express known point mutations in the ras gene, which, as stated previously, result in permanent activation of Ras. However, in contrast to many solid tumors, less than 2% of breast cancers have mutations of the ras gene (Clark & Der 1995a). However, continuous activation of Ras pathways can occur due to permanent upstream growth factor activation (Clark & Der 1995b). In fact, aberrant function of the Ras signal transduction pathway has been noted to be common in breast cancers (Clark & Der 1995b). The fact that FTIs have growth-inhibitory effects on the MCF7 breast cancer cell line, which expresses wild-type ras, in vitro and in vivo (Kelland et al. 2001), suggests that activation of the Ras pathway is present despite the absence of ras mutations, or that FTIs may act independently of Ras in certain tumors. Therefore, the presence of wild-type ras does not appear to preclude FTI activity in certain tumors.

### Classification of FTIs

Based on an understanding of the FT reaction and on the substrate specificity of the various enzymes, several different classes of FTIs have been developed. In general, the FTIs differ in their mechanisms of action, and their effects on human cancer cell lines and in xenograft models. Most FTIs inhibit ras-dependent transformation of fibroblasts (Kohl et al. 1994), and exhibit in vivo anti-tumor activity against spontaneous tumor development in ras transgenic mice (Liu et al. 1998, Norgaard et al. 1999).

In general, the more specific an FTI is for a specific FT, the more likely it is that alternative pathways producing geranylgeranylated proteins will be activated (Whyte et al. 1997), which may, in turn, increase the toxicities associated with the agent.

The initial FTIs were substrate analogs that competed for FDP. Although these agents inhibited Ras processing in H-Ras-transformed fibroblasts, they had no activity in animal models (Leonard 1997, Rowsinsky et al. 1999).

The peptidomimetic compounds are based on the C-terminal tetrapeptide CAAX sequence of Ras proteins as a determinant of enzyme recognition (Reiss et al. 1990). One such agent is L-744,832 (Fig. 3), which inhibits the growth of many tumor cell lines (Sepp-Lorenzino et al. 1995) and the growth of spontaneous tumors in H-Ras transgenic mice (Kohl et al. 1995a), with minimal systemic toxicity. FTI-277 is another peptidomimetic agent, where the central portion of the CAAX motive is replaced by a rigid spacer group (Qian et al. 1994). BMS-186511 (Fig. 3) is a bisulphate inhibitor, which has peptidomimetic properties and is an FDP analog (Kohl et al. 1995b). BMS-186511 inhibits Ras signaling and the transformed growth of spontaneous tumors in H-Ras transgenic mice, with minimal toxicity (Manne et al. 1995).

Two FTIs, currently in clinical trials, were identified through screening of natural products and compounds. SCH 66336 (Lonafarnib) and R115777 (tipifarnib)

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(Fig. 3) are two non-related FTIs identified in this manner, both of which are orally active. SCH 66336 is a tricyclic, potent inhibitor of FT. SCH 66336 inhibits the growth of several tumor cell lines and tumor growth in K-ras-transformed xenografts (Bishop et al. 1995). Additionally, SCH 66336 inhibits the growth of a number of human xenografts, including colon, bladder, lung, prostate and pancreas, in a dose-dependent manner (Liu et al. 1998). R115777 is an imidazole-containing heterocyclic compound, which inhibits the growth of several wild-type and ras-mutated tumor cell lines, and inhibits the growth of tumor xenografts in a dose-dependent manner (End et al. 2001).

Markers of inhibition of farnesylation

When using these FTIs clinically, it will be essential to identify surrogate markers of farnesylation inhibition to determine if an individual FTI is actually inhibiting farnesylation. These surrogate markers may also be used to determine the dose of an FTI required for optimal inhibition of farnesylation. Several markers of farnesylation inhibition have been evaluated preclinically. These include alterations in the Ras proteins themselves, measurements of proteins known to be farnesylated and extent of downstream signaling.

Following treatment with some FTIs, altered mobility of H-ras was seen on an SDS-polyacrylamide gel, especially in H-ras-transfected murine cells (Clark et al. 1995, Kohl et al. 1995b). However, such alterations in mobility have not been seen with the other Ras proteins after FTI treatment, partly because of activation of alternative prenylation pathways (Osman et al. 1997, Whyte et al. 1997). No change in Ras protein mobility was seen in four cancer cell lines after treatment with the FTI, SCH 66336, using a pan-Ras antibody (Adjei et al. 2000a). Additionally, simply looking at changes in the Ras proteins excludes other FT substrates, such as RhoB, which may be involved in the anti-proliferative effects seen following FTI treatment (Gibbs et al. 1997, Du et al. 1999, Lebowitz & Prendergast 1998).

Another assay of FT inhibition involves preparing extracts from FTI-treated cells, and measuring the remaining ability of FT to farnesylate a substrate polypeptide (Adjei et al. 2000a). These assays tend to underestimate the degree of FT inhibition, and are not suitable for use in multi-institutional trials.

As outlined above, several proteins, aside from the Ras family, are known to be farnesylated. These farnesylated proteins may be useful surrogate markers for determining whether a specific FTI is actually inhibiting farnesylation, by providing an indirect measure of FT inhibition. Examples of these proteins include HDJ-2, a chaperone protein (Neckers et al. 1999), Pxf (Hk33), a peroxisomal protein (James et al. 1994) and lamin A, an intranuclear intermediate filament protein (McKeon et al. 1999).
Each of these proteins has been shown to undergo mobility shifts when FT is inhibited (Beck et al. 1990, Britten et al. 1999). In the case of lamin A, this mobility shift is due to inhibition of proteolytic processing which removes a 13 amino acid peptide from the carboxy terminus of prelamin A, to yield lamin A (Beck et al. 1990). This reaction is completely dependent on farnesylation (Kilic et al. 1997) and therefore accumulation of prelamin A, containing the 13 amino acid peptide, could be a useful marker of FTI action (Sinensky et al. 1994).

Treatment of four different cancer cell lines, including MCF7 cells, with the FTI, SCH 66336, results in an increase in the amount of a slower migrating species of the chaperone protein, HDJ-2 (Adjei et al. 2000a). This increase is seen with concentrations as low as 6.25nM, and is observed in both cycling and non-cycling cells (Adjei et al. 2000a). In a phase I study, there was no change in Pxf mobility in peripheral blood mononuclear cells of patients treated with the FTI, SCH 66336 (Adjei et al. 2000a).

Lamin B mobility was not affected by SCH 66336 treatment in four cancer cell lines (Adjei et al. 2000a). In contrast, SCH 66336 treatment resulted in a change in mobility of lamin A and an increase in prelamin A levels in a dose-dependent manner (Adjei et al. 2000a). Prelamin A is expressed in very limited amounts in untreated cancer cells, but is seen at concentrations as low as 25 nM SCH 66336 (Adjei et al. 2000a). Using a probe against the 13 amino acid peptide of prelamin A, it is detectable at concentrations of 6.25 nM (Adjei et al. 2000a). As with HDJ-2, these increases in prelamin A were seen in cycling and non-cycling cells (Adjei et al. 2000a). Using an immunohistochemical antibody for prelamin A, the protein is readily detectable after SCH 66336 treatment (Adjei et al. 2000a). In a number of phase I trials, prelamin A was detected in the buccal mucosa cells of a percentage of patients treated with SCH 66336 (Adjei et al. 2000b, Awada et al. 2002). In one of these trials evaluating SCH 6636, prelamin A expression appeared dose-related, with expression of the protein in buccal mucosa specimens seen in 60% of patients treated with 200 mg, 67% of patients treated with 300 mg, 75% of patients treated with 350 mg and 100% of patients treated with 400 mg (Adjei et al. 2000b). Thus, measurements of prelamin A and HDJ-2 may be among the more useful surrogate markers of FTI activity.

Lastly, changes in downstream proteins could provide a method of determining FTI activity. Such markers include p21 and ras-1, MEK and ERK-1 (extracellular signal-regulated kinase) in their normal and phosphorylated forms. Levels of p21 increase after treatment with the FTI L744,832 (Sepp-Lorenzino & Rosen 1998). However, treatment of four cancer cell lines, including MCF7 cells, with SCH 66336, did not result in any change in p21 expression (Adjei et al. 2000a).

In summary, measurements of prelamin A and HDJ-2 seem to be the most promising intermediate markers of FTI inhibition to date. However, it is important to realize that markers may vary when different FTIs are used, as well as in different cell lines and tumor types, and that these two markers may not describe the extent of inhibition of ras-dependent signaling pathways. Nonetheless, these surrogate markers have already been shown to be valuable correlates of FTI activity in humans.

**FTIs in preclinical breast cancer models**

The FTI, R115777, has been examined *in vitro* and *in vivo* in breast cancer models (Kelland et al. 2001). The MCF7 cell line is an ER-positive breast cancer cell line which expresses wild-type ras and wild-type p53. Since breast cancers have a very low rate of ras mutation (Clark & Der 1995a) MCF7 cells are a rationale cell line to study with FTIs. R115777 inhibits MCF7 cell growth at submicromolar concentrations, with an inhibitory concentration (IC) in the midrange of sensitivity compared with other cancer cell lines (Kelland et al. 2001). In addition, prelamin A was noted to accumulate at low concentrations of R115777, and when MCF7 cells were exposed to high concentrations of the drug, prelamin A peaked at 2 h and persisted for 72 h (Kelland et al. 2001).

R115777, at doses of 25, 50 and 100 mg/kg, inhibits the growth of MCF7 breast tumors *in vivo*, compared with untreated animals (Kelland et al. 2001). R115777 is clearly cytostatic, since the MCF7 tumors regrew once the drug was stopped (Kelland et al. 2001). Tumor prelamin A levels increased in a dose-dependent manner (Kelland et al. 2001). Additionally, at the 100 mg/kg dose, there was a 1.5-fold decrease in cell proliferation based on Ki-67 levels, and a statistically significant increase in apoptosis, based on TUNEL assays (Kelland et al. 2001). These preclinical studies therefore demonstrate activity of FTIs in breast cancer models, despite a low rate of *ras* mutation, and provide a rationale for clinical trials evaluating such agents in patients with breast cancer.

**Clinical trials with FTIs**

**Phase I trials**

There are at least nine phase I trials (Johnston 2001) examining the use of FTIs in patients with solid tumors. Depending on the dosing schedule, the most common toxicities reported were myelosuppression, gastrointestinal complaints, neuropathy and fatigue.

At least four phase I trials have examined SCH 63366 in solid tumors (Hurwitz et al. 1999, Adjei et al. 2000b,
Table 2 Results of phase I trials with SCH 66336 and R115777. Doses of DLT were given twice daily (BID) or once daily (OD) depending on trial design. (x/y = x days on out of y days)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Schedule</th>
<th>Dose of DLT (mg)</th>
<th>Toxicity</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCH 66336</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hurwitz et al. (1999)</td>
<td>BID, 2 weeks only on/2 weeks off</td>
<td>400</td>
<td>GI, renal insufficiency</td>
<td>1 PR</td>
</tr>
<tr>
<td>Adjei et al. (2000)</td>
<td>BID, 7/21 days</td>
<td>400</td>
<td>GI, fatigue</td>
<td>1 PR</td>
</tr>
<tr>
<td>Eskens et al. (2001)</td>
<td>BID, continuous</td>
<td>400/300</td>
<td>GI, myelosuppression, fatigue</td>
<td>2 SD</td>
</tr>
<tr>
<td>Awada et al. (2002)</td>
<td>OD, continuous</td>
<td>400</td>
<td>GI, renal dysfunction</td>
<td>0 Resp</td>
</tr>
<tr>
<td>R115777</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zujewski et al. (2000)</td>
<td>BID, 5/14 days</td>
<td>500</td>
<td>Neuropathy, fatigue, GI</td>
<td>1 PR</td>
</tr>
<tr>
<td>Schellens et al. (2000)</td>
<td>BID, continuous</td>
<td>400</td>
<td>Skin hypersensitivity, myelosuppression</td>
<td>1 PR</td>
</tr>
<tr>
<td>Hudes et al. (1999)</td>
<td>BID, 21/28 days</td>
<td>240</td>
<td>Myelosuppression, fatigue, confusion</td>
<td>2 SD</td>
</tr>
<tr>
<td>Punt et al. (2001)</td>
<td>BID, 28/42 days</td>
<td>300</td>
<td>Neutropenia</td>
<td>1 PR</td>
</tr>
<tr>
<td>Crul et al. (2002)</td>
<td>BID, continuous</td>
<td>400</td>
<td>Myelosuppression, neuropathy</td>
<td>1 PR, 1 MR, 3 SD</td>
</tr>
</tbody>
</table>

*Dose-limiting neuropathy seen at 1300 mg BID, fatigue (not dose-limiting) at 800 mg BID; 2mean tolerated dose.

PR, partial response; SD, stable disease; MR, minor response; resp, response; DLT, dose limiting toxicity.

Eskens et al. (2001, Awada 2002). Various dosing schedules were examined, but continuous dosing was associated with intolerable toxicities (Table 2). The dose-limiting toxicity was usually gastrointestinal, especially diarrhea, which was relieved by loperamide in all cases. Dose-related nausea and vomiting which were severe at higher doses were reported but relieved by anti-emetics. Fatigue which was severe at higher doses was noted. Reversible renal dysfunction was seen, but only in patients with diarrhea and/or nausea and vomiting, and was likely to be due to dehydration. Myelosuppression, generally brief durations of leucopenia and neutropenia, was noted, but was not the dose-limiting toxicity with SCH 63366. Responses were seen in a number of patients (Table 2).

R115777 has been examined in a number of phase I trials (Hudes et al. 1999, Schellens et al. 2000, Zujewski et al. 2000, Punt et al. 2001, Crul et al. 2002). Several dosing schedules were used (Table 2) but, as with SCH 66336, continuous dosing was associated with severe toxicities. Gastrointestinal toxicities, including diarrhea, nausea and vomiting, which were severe enough at high doses to cause renal dysfunction secondary to dehydration, and myelosuppression were again seen. Additionally, neuropathy was found in several of the trials. Several tumor responses were noted (Table 2).

**Phase II trials**

A number of phase II trials evaluating R115777 have been performed in patients with various solid tumors (Table 3). Toxicities were similar to those seen in the phase I trials, including myelosuppression, fatigue and diarrhea. As noted in phase I trials, myelosuppression is more common with R115777 and gastrointestinal events with SCH 66336 (Winquist et al. 2001, MacDonald et al. 2002, Sharma et al. 2002, Adjei et al. 2003, Johnston et al. 2003). As can be seen in Table 3, efficacy rates were generally low in phase II trials examining the use of FTIs as monotherapy in patients with advanced solid tumors (Winquist et al. 2001, MacDonald et al. 2002, Sharma et al. 2002, Adjei et al. 2003). However, a phase II trial, which examined the use of R115777 in patients with advanced breast cancer, produced somewhat more encouraging results (Johnston et al. 2003). Johnston et al. (2003) treated 41 patients with advanced breast cancer with R115777, given at 400 mg twice daily continuously to the first six patients and 300 mg twice daily continuously to the next 35 patients. Patients either had ER-negative or endocrine-refractory breast tumors. Four patients had a partial response, and an additional six patients had stable disease for at least 6 months, with 24% patients obtaining clinical benefit from the FTI (Tables 1 and 3). The median duration of benefit was 11.9 months, with a median time to progression and overall survival of 3.2 months and 15.1 months respectively (Johnston et al. 2003). The drug was reasonably well tolerated, with myelosuppression being the most frequent toxicity: sensory neuropathy was seen after 12 weeks of continuous dosing (Johnston et al. 2003). Subsequently, Johnston et al. (2003) treated a further 35 patients with breast cancer with 300 mg R115777 twice daily given for 3 weeks, followed by a 1-week rest. This schedule resulted in less myelotoxicity and less neuropathy. With this schedule, five patients had a partial response and three had stable disease for at least 6 months, resulting in a clinical benefit rate of...
23% (Johnston et al. 2003) (Table 3). In these patients, the median duration of objective response and clinical benefit were 9.6 months and 8.7 months respectively (Johnston et al. 2003). Additionally, median time to progression and overall survival were 2.9 months and 10.4 months respectively (Johnston et al. 2003). There was no association in response rate based on ER or PgR status (Johnston 2003). In the initial report of this trial (Johnston et al. 2000), six of nine patients who responded to R115777 had HER-2/neu-positive tumors, suggesting an association between upstream growth factor activation of the Ras pathway and response to the FTI. However, at the final analysis, there was no significant association found between HER-2/neu status and likelihood of response to the FTI (Johnston et al. 2003). Only one patient had a tumor with a mutation in ras (Johnston et al. 2003).

In summary, phase II trials examining the use of FTIs, as single agents, in patients with advanced solid tumors are disappointing to date. It is interesting to note that minimal activity of FTIs was noted in patients with advanced pancreatic cancer and transitional cell carcinomas, which are known to have high rates of ras mutation. In contrast, a phase II trial examining R115777 in patients with advanced breast cancer resulted in encouraging clinical benefit rates, despite very low rates of ras mutation and a lack of association of benefit with expression of the upstream growth factor, HER-2/neu (Johnston et al. 2003). These results support the fact that FTIs do not work solely through Ras, and may affect downstream proteins directly.

### Phase III trials

Two phase III trials, examining R115777, have produced disappointing results in patients with gastrointestinal tumors. A trial of gemcitabine with and without the FTI demonstrated no survival advantage in patients with advanced pancreatic cancer (Van Cutsem et al. 2002). Likewise, no difference in survival was seen in patients with colorectal cancer, who had failed two prior chemotherapy regimens, treated with R115777 compared with placebo-treated patients (Cunningham et al. 2002).

### FTIs in combination with other agents

#### With chemotherapeutic agents

A number of FTIs have been examined in combination with chemotherapeutic agents in cell culture models. In general, the combination of FTIs and chemotherapeutic agents was found to be additive. However, L-744,832 exhibited synergy with paclitaxel and epothilone B in several cancer cell lines, including MCF7 (Moasser et al.

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**Table 3** Phase II trials evaluating FTIs in various solid tumors

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>FTI dose and schedule</th>
<th>No. of patients</th>
<th>Toxicity</th>
<th>Efficacy</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast (Johnston et al. 2003)</td>
<td>R115777 300 mg BID continuous</td>
<td>41</td>
<td>Myleosuppression, neuropathy</td>
<td>4PR, 6SD</td>
<td>Med. TTP 3.2 mos Med. surv. 15.1 mos</td>
</tr>
<tr>
<td></td>
<td>300 mg BID 3/4 weeks</td>
<td>35</td>
<td>Myleosuppression</td>
<td>5PR, 3SD</td>
<td>Med. TTP 2.9 mos Med. surv. 10.4 mos</td>
</tr>
<tr>
<td>TCC (Winquist et al. 2001)</td>
<td>SCH 66336 200 mg BID continuous</td>
<td>14</td>
<td>Fatigue, anorexia, nausea, vomiting, dehydration</td>
<td>1SD</td>
<td></td>
</tr>
<tr>
<td>Pancreas (MacDonald et al. 2002)</td>
<td>R115777 300 mg BID 3/4 weeks</td>
<td>47</td>
<td>Fatigue, nausea, anemia grade 5 (15%)</td>
<td></td>
<td>Med. TTP 1.3 mos Med. surv. 2.7 mos</td>
</tr>
<tr>
<td>Colorectal (Sharma et al. 2002)</td>
<td>SCH 66336 200 mg BID continuous</td>
<td>21</td>
<td>Fatigue, diarrhea, nausea, elevations in creatinine</td>
<td>3SD</td>
<td></td>
</tr>
<tr>
<td>Non-SCLC (Adjei et al. 2003)</td>
<td>R115777 300 mg BID 3/4 weeks</td>
<td>44</td>
<td>Neutropenia, anemia, anorexia</td>
<td>7SD</td>
<td>Med. TTP 2.7 mos Med. surv. 7.7 mos</td>
</tr>
</tbody>
</table>

TCC, transitional cell carcinoma; SCLC, small cell lung cancer; Med., median; TTP, time to progression; surv., survival; mos, months; PR, partial response; SD, stable disease.
With hormonal agents

Preclinical data suggest enhanced inhibitory effects of tamoxifen by FTIs on ER-positive breast cancers (Johnston et al. 2002) (Fig. 4). Mice were implanted with MCF7 tumors and treated with estrogen. Once the tumors reached a certain volume, the mice were randomized as follows: continue estrogen; continue estrogen with R115777; stop estrogen; stop estrogen and start treatment with R115777; stop estrogen and start tamoxifen; stop estrogen and start tamoxifen with R11777. Tumor growth was inhibited in the mice in which estrogen was withdrawn and in those treated with tamoxifen (Johnston et al. 2002). However, the MCF7 tumors were statistically smaller in the mice treated with R117777 in combination with tamoxifen or estrogen withdrawal, despite the finding that the FTI had minimal effect on tumor growth when given alone (Johnston et al. 2002). These data suggest that targeting the Ras pathway along with the ER pathway may be a reasonable option in clinical trials.

FTI resistance

To date little is known about resistance to FTIs. An FTI-resistant colon cancer cell line (KM12/R115P) was developed by exposing KM12 colon cancer cells to R115777 for 4 months (Smith et al. 2002). This resistant cell line did not exhibit cross resistance to chemotherapeutic agents or the MAP kinase inhibitor, U0126, the PI3K inhibitor, LY 294002 or the EGFR inhibitor, PD 153035 (Smith et al. 2002). There was, however, cross resistance to other FTIs (Smith et al. 2002). Resistance was not attributable to over-expression of membrane drug efflux pumps, but there was a lower degree of inhibition of FT, and FT activity was barely detectable in the resistant cell line (Smith et al. 2002).

Conclusions

Preclinical data examining the use of FTIs in solid tumors are encouraging. Phase II trials examining the use of FTIs as single agents in solid tumors, regardless of ras mutation rate, is to date somewhat disappointing, but results using the agents in combination with chemotherapeutic agents appear more promising. Additionally, surrogate markers of farnesylation have been identified. Despite the lack of ras mutations in breast cancer, preclinical and early clinical data suggest that FTIs may be effective therapies for breast cancer, either alone or in combination with chemotherapeutic agents or tamoxifen. Clinical results to date do not suggest an association between FTI response and expression of HER-2/neu, an upstream activator of
the Ras pathway. It seems likely therefore that FTIs, in breast cancer and other solid tumors, act independently of Ras, and may affect prenylated proteins downstream of Ras, perhaps in the PI3K/AKT pathway, resulting in apoptosis. Further preclinical and clinical studies will be needed to fully elucidate the mechanism of action of these targeted therapies.

References


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