Preclinical Antitumor Activity and Pharmacodynamic Studies with the Farnesyl Protein Transferase Inhibitor R115777 in Human Breast Cancer

Lloyd R. Kelland,[1] Vicki Smith, Melanie Valenti, Lisa Patterson, Paul A. Clarke, Simone Detre, Dave End, Angela J. Howes, Mitch Dowsett, Paul Workman, and Stephen R. D. Johnston

CRC Centre for Cancer Therapeutics, The Institute of Cancer Research, Surrey SM2 5NG, United Kingdom [L. R. K., V. S., M. V., L. P., P. A. C., P. W.]; Departments of Biochemistry [S. D., M. D.] and Medicine [S. R. D. J.], Royal Marsden Hospital, London SW3 6JJ, United Kingdom; Janssen Research Foundation, Spring House, Pennsylvania 19477 [D. E.]; and Janssen-Cilag Ltd, High Wycombe, Buckinghamshire HP14 4HJ, United Kingdom [A. J. H.]

ABSTRACT

Antitumor and pharmacodynamic studies were performed in MCF-7 human breast cancer cells and companion xenografts with the farnesyl protein transferase inhibitor, R115777, presently undergoing Phase II clinical trials, including in breast cancer. R115777 inhibited growth of MCF-7 cells *in vitro* with an IC_{50} of 0.31 ± 0.25 μM. Exposure of MCF-7 cells to increasing concentrations of R115777 for 24 h resulted in the inhibition of protein farnesylation, as indicated by the appearance of prelamin A at concentrations >1 μM. After continuous exposure to 2 μM R115777, prelamin A levels peaked at 2 h post drug exposure and remained high for up to 72 h. R115777 administered p.o. twice daily for 10 consecutive days to mice bearing established s.c. MCF-7 xenografts induced tumor inhibition at a dose of 25 mg/kg [percentage of treated *versus* control (% T/C) = 63% at day 21]. Greater inhibition was observed at doses of 50 mg/kg (% T/C at day 21 = 38%) or 100 mg/kg (% T/C at day 21 = 43%). The antitumor effect appeared to be mainly cytostatic with little evidence of tumor shrinkage to less than the starting volume. Tumor response correlated with an increase in the appearance of prelamin A, but no changes in the prenylation of lamin B, heat shock protein 40, or N-Ras were detectable. In addition, significant increases in apoptotic index and p21[WAF1/CIP1] expression were observed, concomitant with a decrease in proliferation as measured by Ki-67 staining. An increase in prelamin A was also observed in peripheral blood lymphocytes in a breast cancer patient who responded to R115777. These data show that R115777 possesses preclinical antitumor activity against human breast cancer and that the appearance of prelamin A may provide a sensitive and convenient pharmacodynamic marker of inhibition of prenylation and/or response.

INTRODUCTION

Approximately 10 years ago it was discovered that Ras oncoproteins require localization to the inner surface of the cell membrane to exert their cellular mitogenic activity (1, 2). The first and most important step in this process is a post-translational modification in which a C15 farnesyl group is transferred from farnesyl diphosphate to the sulfur atom of the cysteine residue of the COOH-terminal tetrapeptide CAAX of Ras through the activity of the enzyme, FPT.[2] Consequently, many attempts have been made to design and synthesize inhibitors of FPT.

Following the demonstration of inhibition of *ras*-dependent transformation of fibroblasts (3) and *in vivo* antitumor activity against carcinomas in *ras* transgenic mice (4), at least four FTIs recently entered clinical trials: SCH66336 (5); R115777 (6, 7); L-778,123; and BMS-214662 (8–10). Among these is the imidazole-based compound, R115777, which is a competitive inhibitor of the CAAX peptide binding site of FPT with a K_i of 0.5 nM and which inhibits the farnesylation of lamin B1 (IC_{50} = 0.8 nM) and K-ras (IC_{50} = 7.9 nM; Ref. 11). Furthermore, R115777 inhibited the proliferation of both H-ras-transformed fibroblasts (IC_{50} = 1.7 nM) and human colon and pancreatic cell lines possessing K-*ras* mutations (IC_{50} = 16–22 nM) and showed potent oral antitumor activity against human LoVo colon and human CAPAN-2 pancreatic xenografts (12–14). An initial Phase I trial with oral R115777 (25–1300 mg twice daily for 5 days every 2 weeks) identified dose-limiting toxicities of neuropathy and fatigue with some nausea, vomiting, and headache (6). Additional trials using either continuous twice-daily oral dosing or dosing for 21 days followed by 7 days of rest, reported a dose-limiting toxicity of myelosuppression (7). The recommended dose for Phase II trials based on this schedule was 300 mg p.o. twice daily.

Although the incidence of *ras* mutations in breast cancer is relatively low (<5%; Ref. 15), aberrant function of the Ras signal transduction pathway is common (*e.g.,* through upstream activation via HER2 or epidermal growth factor receptor; Ref.

[2] The abbreviations used are: FPT, farnesyl protein transferase; FTI, FPT inhibitor; PI3, phosphatidylinositol 3′-kinase; PBL, peripheral blood lymphocyte; HSP, heat shock protein; SRB, sulforhodamine B; T/C, treated *versus* control; TUNEL, terminal deoxynucleotidyl transferase (Tdt)-mediated nick end labeling.

15). Furthermore, in recent years it has become apparent that the FTIs, although originally envisaged as Ras inhibitors, may confer their antitumor properties through intracellular effects on additional proteins that require farnesylation. These include lamins A and B, Rap2, Rho B and E, and the centromere-associated proteins CENPE and CENPF (8, 10, 16). Although compelling data suggest that specific substrates such as Rho B may be the key determinant of sensitivity to FTIs (17), other data are indicative of effects on alternative proteins/pathways to the K-Ras/Raf/MEK/Erk pathway, such as the H-Ras/PI3 kinase/AKT pathway (18–20). In addition to farnesylation, some proteins are post-translationally modified by the addition of a 20-carbon geranylgeranyl moiety via the enzyme geranylgeranyl protein transferase I (21, 22). It has been reported that K-Ras may become geranylgeranylated and maintain transforming activity when FPT is inhibited in some cell lines (21, 22). These findings have led to particular challenges in the choice and design of Phase II trials with these agents and in the rational development of pharmacodynamic end points of response or toxicity.

Among various Phase II clinical trials of R115777, we have conducted a trial in women with advanced breast cancer (23). In conjunction with this trial, the aims of this preclinical study were to assess the activity of R115777 in a cell line and a companion xenograft model of human breast cancer and to investigate possible pharmacodynamic markers of response that could then be applied to PBLs or tumor biopsy specimens. On the basis of a recent report (24), particular emphasis was placed on monitoring of the appearance of unprocessed prelamin A (25) as a pharmacodynamic readout of response, although other proteins known to be prenylated (N-Ras, lamin B, and HSP40) were also investigated.

MATERIALS AND METHODS

Cell Culture. The human breast cancer cell line MCF-7 (and the A2780 human ovarian carcinoma and HT29 colon carcinoma cell lines) was grown as a monolayer in DMEM containing 10% heat-inactivated FCS, 2 mM L-glutamine, and minimal nonessential amino acids (Life Technologies, Inc., Paisley, Scotland) in a 6% CO_2-94% air atmosphere.

Cell Growth Inhibition. The sensitivity of human tumor cell lines, including MCF-7, was assessed by 96-h drug exposure. R115777 was dissolved in acidified water at 1 mM and diluted in growth medium immediately prior to its addition to cells seeded overnight in 96-well microtiter plates. Quantitation of cell numbers in treated (four replicate wells) *versus* control (four replicates) wells was assessed by SRB staining as described previously (26). Drug potency was determined in terms of IC_{50}s.

Human Tumor Xenografts. The MCF-7 line was grown as s.c. xenografts in female athymic nude mice (nu/nu) by passage of 2-mm diameter pieces of tumor. Growth was maintained by estrogen supplementation through intradermal injection of estrogen pellets (dose, 1.7 mg over 60 days; Innovative Research of America, Sarasota, FL). When tumors had reached a largest diameter of ~7 mm, mice were randomized to receive either drug vehicle [20% (w/v) β-cyclodextrin (pH 2.5)] or R115777 administered by oral gavage at doses of 25, 50, and 100 mg/kg. Dosing was twice daily for 10 consecutive days. There were 11 tumor-bearing animals in each group; 6 were used for the therapy arm, and 5 were harvested on day 11 (1 day after the final dose) for pharmacodynamic measurements. For the therapy arm, tumor growth was assessed twice weekly in control and treated groups by caliper measurements of the two largest diameters. Volumes were then calculated according to the formula: volume $= a \times b^2 \times \pi/6$, where a and b are orthogonal tumor diameters. Tumor volumes were then expressed as a percentage of the volume at the start of treatment (relative tumor volume). The effect of the drug was determined by the growth delay (difference in time, in days, for the volumes of control *versus* treated tumors to double) or % T/C on day 21, as used previously (27).

All animal procedures were performed within local and national ethics guidelines.

Western Blotting. Immunoblotting of proteins was carried out as described previously (*e.g.,* Ref. 28), using asynchronized cells in exponential growth. In addition, assessments were made using xenograft material harvested on day 11 (24 h after the final dose of drug) and PBLs taken from patients participating in the Phase II R115777 breast cancer trial (pretreatment and after 4 weeks of receiving 300 mg twice daily every day). PBLs were prepared using Lymphoprep (Nycomed) and centrifugation at 800 $\times$ g for 20 min at room temperature. PBLs were then separated, washed in PBS by centrifugation, pelleted, and snap frozen in liquid nitrogen. Xenograft samples were frozen in liquid nitrogen and homogenized, and protein was extracted. Proteins (typically 50 μg/lane) were separated by SDS-PAGE and electroblotted to nitrocellulose filters. A polyclonal antibody to the COOH-terminal domain of human prelamin A (24) was kindly provided by Drs. A. Adjei and S. Kaufmann (Mayo Clinic, Rochester, MN). Antibodies to lamin B (Santa Cruz Biotechnology C-20), N-Ras (Santa Cruz Biotechnology C-20), and HSP40 (Santa Cruz Biotechnology N-19) were used at dilutions of 1:1000, 1:1000, and 1:500, respectively. Secondary antibodies were purchased from Amersham Pharmacia.

Measurements of Proliferation and Apoptosis. Markers for proliferation and apoptosis were assessed by immunohistochemistry of formalin-fixed xenograft tissue (taken on day 11), as described previously, from MCF-7 xenografts treated with drug vehicle or with R115777 as above (29, 30). Sections (3 μM) were cut from treated and control formalin-fixed, paraffin wax-embedded xenograft tissue and placed on charged slides. Proliferation was analyzed in sections after they had been dewaxed in xylene and gradually rehydrated with water, and endogenous peroxidase activity was blocked. Antigen retrieval was performed by microwaving sections at 750 W in citrate buffer (pH 6.0) for 10 min, after which buffered sections were cooled to room temperature and a blocking antibody was applied in PBS (pH 7.4) prior to addition of the primary antibody. The sections were incubated for 45 min in 1:200 biotinylated antimouse immunoglobulins and rinsed; streptavidin ABC-horseradish peroxidase complex (Dako) was then applied for 30 min. After sections were washed in PBS, the peroxidase reaction was developed to a brown stain by the addition of 0.05% diaminobenzidine enhanced with 0.07% imidazole and hydrogen peroxide. Cell cytoplasm was counterstained blue with

Mayer's hematoxylin, and sections were dehydrated, cleared in xylene, and permanently mounted in DePex.

Proliferation was assessed by Ki-67 immunostaining using the MIB1 antibody (The Binding Site Ltd., Birmingham, United Kingdom) at a dilution of 1:50 for 1 h. Ki-67 cells positive for nuclear staining were recorded as percentages. Sections were stained in one batch together with known positive controls and scored by one individual. Ki-67 cells positive for nuclear staining were recorded as percentages. The method for immunocytochemical detection of p21 protein was the same as described above for Ki-67, except that WAF1 (Ab-1) primary monoclonal antibody (Oncogene Research Products, Calbiochem) was used at a dilution of 1:30 for 1 h. Cells with positive nuclear staining for p21 were assessed as percentages.

Apoptosis was determined by the TUNEL assay as described previously, using xenograft tumor sections (31). All sections were examined under a standard light microscope equipped with a $\times 40$ objective and a 10×10 eye piece incorporating a graticule. The apoptotic index was expressed as a percentage calculated from the number of cells that stained brown and displayed apoptotic bodies of 3000 tumor cells per section counted under high power, excluding any areas of necrosis.

RESULTS

***In Vitro* Growth Inhibition and Prelamin A Levels Induced by R115777.** R115777 inhibited growth in MCF-7 cells *in vitro* at submicromolar concentrations (Fig. 1A). The IC_{50} for growth inhibition was 0.31 ± 0.25 μM. This was in the mid-range of sensitivity for a panel of human tumor cell lines, *e.g.,* compared with IC_{50}s of 1.2 ± 0.1 μM in HT29 colon carcinoma and 0.02 ± 0.011 μM in A2780 ovarian carcinoma cells. Compared with typical dose-response curves obtained previously for cytotoxic drugs in these cell lines, *e.g.,* with cisplatin (26), the dose-response curve for R115777 was relatively flat with 25–75% inhibition occurring across almost 3 logs of concentration (10 nM to 5 μM). There was also some evidence to suggest two phases of growth inhibition: one phase at concentrations up to 100 nM, which led to ~50% growth inhibition, and a second phase at concentrations of ~5 μM, which inhibited growth up to 90%.

We next determined the dose-response relationship in MCF-7 cells for the appearance of prelamin A. Exposure for 24 h to R115777 at concentrations of 0.2–10 μM resulted in the accumulation of prelamin A at concentrations ≥ 1 μM (Fig. 1B). The kinetics of this increase was studied by exposing cells to 2 μM (*i.e.,* approximately six times the IC_{50}) and determining prelamin A levels from 1 to 72 h after the start of continuous exposure to compound. Results showed a peak in levels at 2 h, which was maintained throughout the time course up to 72 h (Fig. 1C).

***In Vivo* Antitumor Effects and Pharmacodynamic Markers of Response.** We studied the *in vivo* antitumor activity of R115777 in mice bearing s.c. MCF-7 breast cancer xenografts, using twice daily oral dosing for 10 consecutive days (Fig. 2). Dosing did not commence until tumors had reached diameters of ~7 mm. Results showed that each dose of R115777 (25, 50, and 100 mg/kg) induced some slowing in the
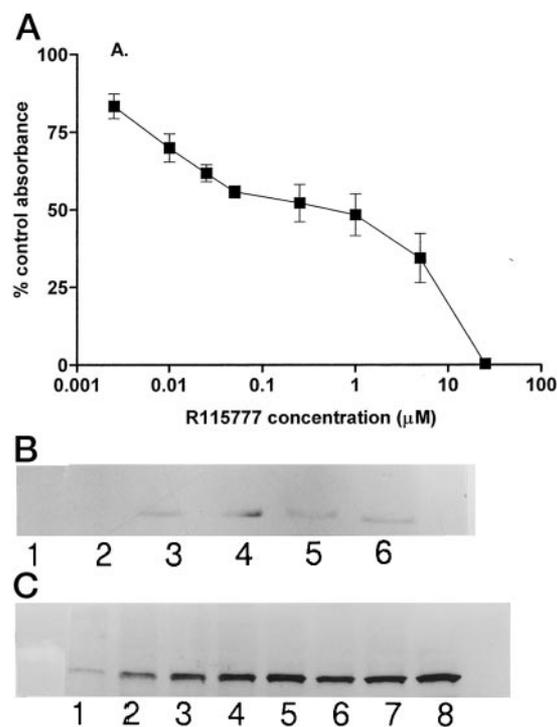

Fig. 1   In vitro effects of R115777 against MCF-7 human breast cancer cells. *A*, growth inhibition after 4 days of exposure and assessment of cell number by the SRB assay. Values are mean $\pm$ SD (*bars*); $n = 3$. *B*, induction of prelamin A as determined by Western blotting of MCF-7 cells exposed to different concentrations of R115777 for 24 h. *Lane 1*, 0.2 μM; *Lane 2*, 0.4 μM; *Lane 3*, 1.0 μM; *Lane 4*, 2.0 μM; *Lane 5*, 4.0 μM; *Lane 6*, 10 μM. *C*, induction kinetics for prelamin A following exposure of MCF-7 cells to 2 μM R115777. *Lane 1*, 1 h; *Lane 2*, 2 h; *Lane 3*, 4 h; *Lane 4*, 8 h; *Lane 5*, 16 h; *Lane 6*, 24 h; *Lane 7*, 48 h; *Lane 8*, 72 h.

rate of growth of the tumors relative to controls treated with drug vehicle alone. In contrast to our previously performed xenograft experiments with cytotoxic drugs such as cisplatin (see *e.g.,* Ref. 27), there was no evidence of tumor shrinkage to less than the starting volume with any dose of R115777. Instead there was a cytostatic effect with relatively little tumor growth over the 10 days during which the drug was administered, followed by recovery toward the control growth rate after dosing was completed. There was some evidence of a dose response in that the lowest level of activity was observed at the 25 mg/kg dose (% T/C at day $21 = 63\%$; growth delay, 3.6 days) with greater activity observed at the 50 mg/kg dose (% T/C at day $21 = 38\%$; growth delay, 9.6 days). However, there was no additional gain in inhibition with the highest dose, 100 mg/kg (% T/C at day $21 = 43\%$).

Having observed an increase in prelamin A levels *in vitro* in MCF-7 cells exposed to R115777, we also investigated prelamin A levels 24 h following the final dose (day 11) in tumors excised from mice. Data showed a clear dose-dependent increase in levels of prelamin A in treated tumors (Fig. 3). There was strong accumulation in all five tumors removed from mice treated with the highest dose (100 mg/kg), induction in four of five of tumors exposed to 50 mg/kg, and low-level induction in
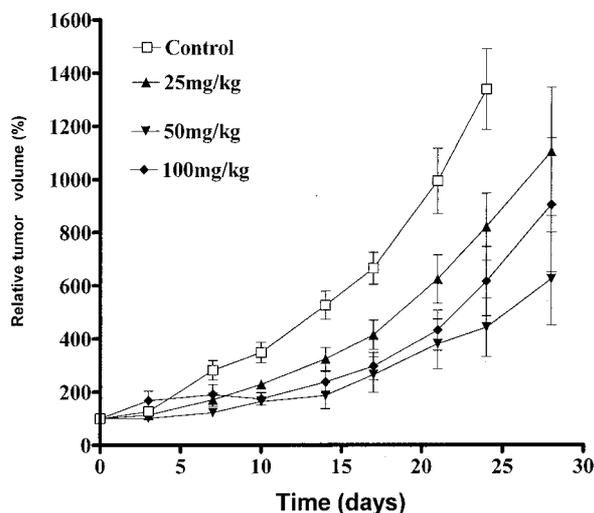
Fig. 2 Tumor growth curves for MCF-7 cells treated with either drug vehicle (*Control*; □) or with varying doses of R115777 administered p.o. twice a day for 10 consecutive days (▲, 25 mg/kg; ▼, 50 mg/kg; ♦, 100 mg/kg). Values are mean ± SD (*bars*); $n = 6$.

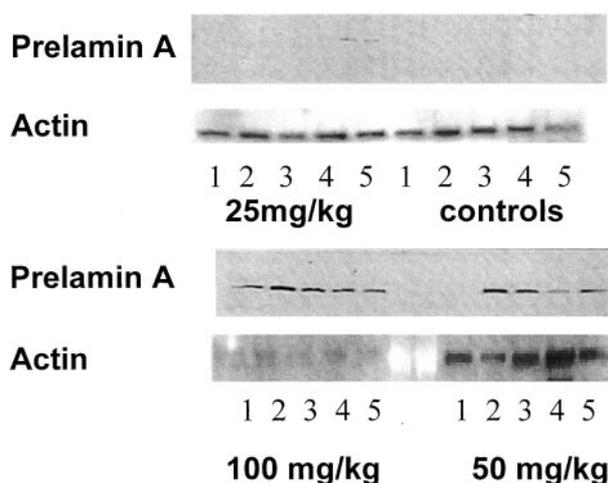

Fig. 3 Increases in prelamin A as determined by Western blotting of MCF-7 xenografts taken at day 11, one day after the final dose of either vehicle (*controls*) or R115777 administered p.o. twice daily for 10 consecutive days at dose of 25, 50, and 100 mg/kg. *Lanes 1–5* for each group refer to individual tumors/animals. Levels of *actin* are shown as loading controls.

two of five tumors exposed to the lowest dose (25 mg/kg). However, there was no prelamin A detectable in any of the five tumors removed from animals dosed with vehicle alone. In contrast, efforts to detect the inhibition of farnesylation of other proteins, such as lamin B, HSP40, or N-Ras, did not produce differences between tumors removed from treated (25, 50, and 100 mg/kg) *versus* control animals (data not shown).

In addition, we investigated markers of tumor proliferation (Ki-67 staining) and apoptosis (TUNEL staining and induction of p21), using control and MCF-7 xenografts treated for 10 days with 100 mg/kg R115777 (Fig. 4, *A–C*). The results showed that R115777 induced a significant 1.5-fold reduction in proliferation (percentage of Ki-67-positive cells; $P = 0.003$). There was also a concomitant increase in apoptosis (percentage of TUNEL-positive cells); this increase was statistically significant compared with controls ($P = 0.007$). Levels of the cyclin-dependent kinase inhibitor p21/CIP1/WAF1 also increased (percentage of positive cells) significantly ($P = 0.024$). In a dose-related pharmacodynamic marker experiment investigating apoptosis at all three R115777 doses, apoptosis increased significantly at the 50 and 100 mg/kg levels ($P = 0.027$ and 0.035, respectively) but did not attain statistical significance at the lower (25 mg/kg) dose (Fig. 4*D*).

We also determined, in a pilot study, whether any changes in prelamin A expression were detectable in the PBLs of four patients with breast cancer from our Phase II clinical trial (23). Samples were collected before the start of treatment and after patients received 300 mg of oral R115777 twice daily every day for 4 weeks. In two nonresponding patients, there was no detectable prelamin A in either pre- or posttreatment PBLs. In another nonresponding patient (Fig. 5, *Patient 2*), there were also no changes in prelamin A levels. By contrast, compared with a blood sample collected prior to the start of treatment, there was clear increase in prelamin A in the 4-week PBL sample of a patient (Fig. 5, *Patient 1*) who showed an objective response to R115777.

DISCUSSION

R115777 represents one of the first-in-class inhibitors of FPT to enter the clinic (6). In a Phase I clinical trial using oral dosing twice daily for 5 consecutive days every 2 weeks, dose-limiting toxicities of neuropathy (one patient) and fatigue were observed. Although initially developed as Ras inhibitors, it is now apparent that the *in vitro* and *in vivo* antitumor effects of drugs such as R115777 may be attributable to effects on a variety of proteins that require post-translational modification by prenylation. Our studies were performed in association with an ongoing Phase II clinical trial in patients with breast cancer (23). The aims were to determine the level of *in vitro* and *in vivo* antitumor activity of R115777 in the MCF-7 estrogen receptor-positive, wild-type Ras, wild-type p53 model of breast cancer and to identify possible pharmacodynamic markers of response mediated via inhibition of protein prenylation. Our Phase II breast cancer trial involved oral dosing with 300 mg of R115777 twice daily in a total of 41 patients; objective responses (tumor shrinkage of at least 50%) were seen in 4 patients (10%) with an additional 6 patients (15%) having stabilization of disease for at least 6 months (range, 6–12+ months; Ref. 23).

Our results reveal that submicromolar concentrations of R115777 (>50 nM) induce significant growth inhibition of MCF-7 cells *in vitro* when added to cells continuously for 4 days, as assessed by cell numbers in the SRB assay. The dose-response curve for growth inhibition is relatively flat and possibly biphasic compared with those that we obtained using MCF-7 cells and the SRB assay with cytotoxic drugs (*e.g.,* cisplatin).[3] The IC$_{50}$ of 310 nM for R115777 is somewhat higher than values reported recently for a panel of ~30 cell lines,
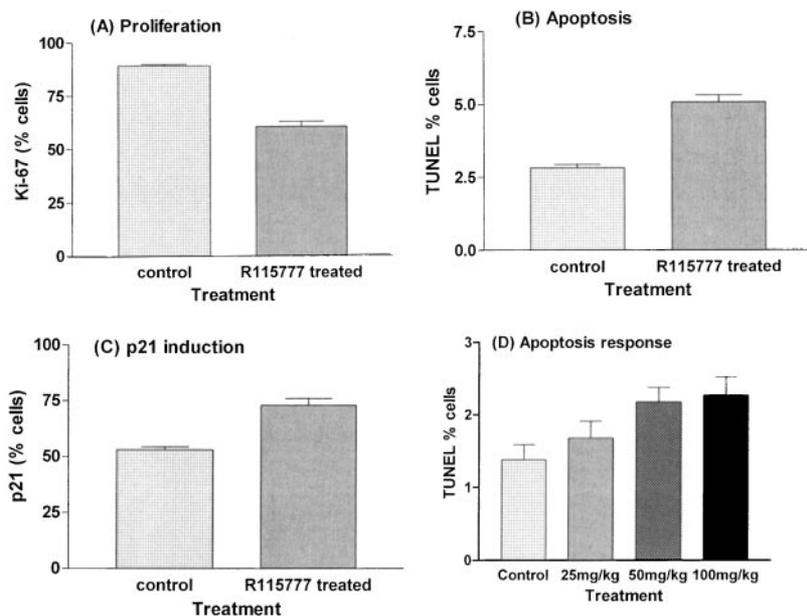
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[3] Unpublished data.

Fig. 4 Immunohistochemical analyses of proliferation (based on Ki-67 staining; *A*), of apoptosis (based on TUNEL staining; *B*), and of p21 induction (*C*) in MCF-7 xenografts removed from vehicle-treated (*control*) or R115777-treated (100 mg/kg administered p.o. twice daily for 10 consecutive days) animals. *D*, apoptosis as determined by TUNEL staining in either MCF-7 xenografts removed from vehicle-treated (*Control*) or R115777-treated (25, 50, or 100 mg/kg administered p.o. twice daily for 10 consecutive days) animals. Values are mean ± SD (*bars*); $n = 5$ tumors.
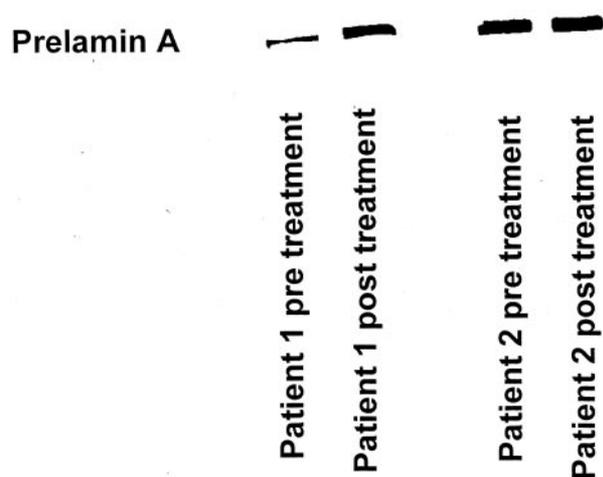

Fig. 5 Increases in prelamin A in patient PBL samples before and after treatment with R115777 (samples collected after patients had received 300 mg twice daily every day for 4 weeks) in *Patient 1* (who exhibited a partial response) and *Patient 2* (who did not respond). In two patients (nonresponders), there was no detectable prelamin A in either sample.

where IC_{50}s of 1–50 nM were obtained for a number of lines (14). However, that study used a different cell proliferation assay and a 4–7 day assay end point. It should also be noted that our *in vitro* MCF-7 studies did not use any added estrogen but relied on provision from FCS. Pharmacokinetic studies performed as part of the oral-dosing Phase I trial showed that peak plasma R115777 concentrations of ~2 μg/ml occurred 0.5–4 h post administration of 325 mg of drug (6). Similar levels were obtained after the first dose on day 1 and after the last dose on day 6.

At concentrations >1 μM (after 24 h of drug exposure), there was evidence of inhibition of prenylation, detected as the appearance of unprocessed prelamin A by an antibody that recognizes the COOH-terminal domain. This precursor of the intranuclear intermediate filament protein lamin A has been shown to require prenylation for processing. The processing involves removal of a 13-amino acid peptide from the COOH terminus of prelamin A and is dependent on farnesylation (25). Moreover, prelamin A has recently been shown to increase in cancer cells (including MCF-7) exposed *in vitro* to FTIs, including R115777 (24). In MCF-7 cells, the appearance of prelamin A occurred at concentrations similar to those required for growth inhibition of the MCF-7 cell line in continuous exposure experiments. Also of note in relation to monitoring inhibition of protein prenylation *in vivo* is that our *in vitro* data obtained from MCF-7 cells exposed to 2 μM R115777 indicate that prelamin A appeared within 2 h and remained detectable for up to 72 h, the longest time point studied.

Notably, R115777 induced a significant antitumor effect against MCF-7 tumors grown s.c. in immune-suppressed nude mice. Three significant points may be derived from this experiment. (*a*) Antitumor activity was obtained with an established s.c. (advanced stage) model of breast cancer, tumors being an average of ~7 mm in diameter at the onset of treatment. In previously reported experiments where R115777 induced an antitumor effect in s.c. human tumor xenografts (CAPAN-2 pancreatic, LoVo colon, and C32 melanoma; Ref. 14), treatment began only 3 days after tumor cell inoculation. (*b*) Compared with previously obtained data in xenograft models using cisplatin (27), the effect of R115777 was mainly to slow the rate of tumor growth relative to controls rather than to induce a cytotoxic reduction in tumor volume. These tumor inhibition data are more consistent with those that we obtained in MCF-7 xenografts with antiendocrine drugs (29, 30). As a consequence of this predominantly cytostatic antitumor effect, a probable clinical scenario is the use of R115777 in combination with cytotoxic chemotherapeutics such as paclitaxel. Of note are

preclinical studies (including in xenografts) in which, when combined, FTIs (including R115777) and paclitaxel produced additive synergistic antitumor effects (32–37). (*c*) In this model, the antitumor effect of R115777 was slightly greater at a dose of 50 *versus* 25 mg/kg but did not improve further at the highest dose, 100 mg/kg. This may be as a result of either a "threshold" effect for the inhibition of prenylation or, because of saturable drug pharmacokinetics, limited amounts of drug reaching the tumor at high doses. This was also matched by a dose-threshold effect on apoptosis. Interestingly, it has been reported that for mice bearing A549 non-small cell lung cancer xenografts treated with FTI-2148, complete inhibition of farnesyl transferase itself is not necessary to induce inhibition of tumor growth (10).

Our pharmacodynamic marker investigations indicate that, of the four proteins studied *in vivo*, the appearance of prelamin A appeared to be most sensitive to the antitumor effects of R115777. Three other proteins known to be prenylated (N-Ras, lamin B, and HSP40; Ref. 10) did not show differences between MCF-7 xenografts removed from R115777-treated and untreated control animals. This may have been because of the relative quality of the antibodies (and because an increase in prelamin A protein, not a decrease in signal, was being detected) or may represent a real biological effect whereby the effects on prelamin A are more pronounced than the effects on the remaining farnesylated proteins. These data provide evidence of inhibition of prenylation *in vivo* that broadly correlated with tumor inhibition and suggest that monitoring the appearance of prelamin A as part of clinical trials with R115777 may provide a sensitive and convenient pharmacodynamic marker of inhibition. Furthermore, our pilot study using four paired samples suggests that it may be feasible to use PBLs as a surrogate tissue; we observed an increase in prelamin A expression in a responding but not a nonresponding patient when we compared posttreatment PBL samples with pretreatment PBL samples. In this context, in a recently reported Phase I trial with the FTI SCH66336 (5), the appearance of prelamin A as an indicator of inhibition of farnesylation was observed in buccal mucosa cells of treated patients.

We have extensive prior experience determining effects on proliferation (using Ki-67) and apoptosis in xenografts, especially MCF-7 treated with antiendocrine drugs (29, 30), and in clinical breast cancer biopsies (38). The results from the TUNEL assay revealed that R115777 induces a significant increase in apoptotic index *in vivo* in s.c. MCF-7 tumors. A similar modest but significant increase in apoptosis has also been reported in mice bearing CAPAN-2 pancreatic xenografts treated with 100 mg/kg oral R115777 (14). A higher level of apoptosis was observed in another tumor (C32 melanoma) as part of the same study. Data suggest that the PI3 kinase/AKT pathway may be a critical target for FTI-induced apoptosis in cell lines (20, 39). These data suggest that the antitumor effects of R115777 observed in MCF-7 xenografts may not be entirely cytostatic in nature, but that some dose-related apoptosis may contribute as well. In addition, a significant decrease in proliferation, evident from staining for Ki-67 and concomitant with an increase in the cell cycle-dependent kinase inhibitor p21$^{CIP1/WAF1}$, was observed. By contrast, no changes in p21

levels were observed in cell lines, including MCF-7, exposed to the FTI SCH66336 (24).

Future studies will seek to identify changes in prelamin A in post- *versus* pretreatment tumor biopsy material collected as part of the breast cancer trial. In addition, gene expression profiling, as we described recently for the HSP90 inhibitor 17-allylamino-17-demethoxygeldanamycin (40), is being used with tumor biopsies from breast cancer patients receiving R115777. This may identify additional sensitive markers of response and shed further light on how R115777 induces antitumor effects (*e.g.,* the possible role of the PI3 kinase/AKT pathway). Furthermore, there is some evidence to suggest that R115777 may also induce antitumor effects *in vivo* by an antiangiogenic mechanism involving endothelial cells, vascular endothelial growth factor, and factor VIII (14).

In summary, R115777 is a potent inhibitor of cell growth in MCF-7 breast cancer cells; it also induced significant cytostatic antitumor effects in a companion s.c. advanced stage breast cancer xenograft model when administered p.o. twice a day for 10 consecutive days. The *in vivo* antitumor effect was associated with a significant dose-related induction of apoptosis. The appearance of unprocessed prelamin A may provide a sensitive pharmacodynamic marker of response for further clinical studies of R115777 in breast and other cancers.

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Lloyd R. Kelland, Vicki Smith, Melanie Valenti, et al.

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