A Phase I Trial of the Farnesyl Protein Transferase Inhibitor R115777 in Combination with Gemcitabine and Cisplatin in Patients with Advanced Cancer

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ABSTRACT

Purpose: This Phase I study was undertaken to define the toxicity, pharmacodynamics, and maximum tolerated dose of the combination of R115777, a farnesyl transferase inhibitor, with gemcitabine and cisplatin in patients with advanced solid tumors.

Patients and Methods: Thirty patients with solid tumors received a median of 2.5 cycles (range 1–30+) through five dose levels. R115777 was administered p.o. twice daily for 14 days. Gemcitabine was infused 15 min after the ingestion of R115777 on days 1 and 8. Cisplatin was administered starting 30 min after completion of the gemcitabine infusion on day 1. Cycles were repeated every 21 days. Toxicities were graded by the National Cancer Institute Common Toxicity Criteria and recorded as maximum grade per patient for each treatment cycle. At the maximum tolerated dose, accumulation of prelamin A in buccal mucosa cells of patients was evaluated as a marker of farnesyl transferase inhibition by R115777.

Results: Neutropenia and thrombocytopenia were the most common toxicities. Dose-limiting toxicity in cycle 1 was myelosuppression with thrombocytopenia alone (4 patients), neutropenia alone (1 patient), or a combination of both (3 patients). Common nonhematologic toxicities were anorexia, rash, nausea, vomiting, and fatigue, none of which was dose limiting in the first cycle. At the maximum tolerated dose, defined as R115777 300 mg twice daily p.o., 1000 mg/m² gemcitabine, and 75 mg/m² cisplatin, inhibition of prelamin A farnesylation in buccal mucosa cells of patients was demonstrated, confirming that R115777 inhibits protein farnesylation in vivo. Nine objective responses (one complete response and eight partial responses) were documented in 27 evaluable patients.

Conclusion: The combination of R115777 with gemcitabine and cisplatin was well tolerated and showed evidence of antitumor activity. The maximum tolerated dose of R115777 successfully inhibits farnesyltransferase in patients in vivo. This combination warrants further evaluation in a number of tumor types.

INTRODUCTION

The enzyme FT³ catalyzes the first step in the post-translational modification of a number of guanine nucleotide-binding proteins (G proteins) involved in cell signaling. These G proteins are synthesized as cytoplasmic precursors and require a series of post-translational modifications for conversion to membrane-bound forms. The first and obligatory step in this post-translational modification is protein prenylation, which is the covalent addition of either farnesyl (15 carbon) or geranylgeranyl (20 carbon) groups to the cysteine residue located in a tetrapeptide sequence at the COOH terminus of these proteins (1). Prenylation promotes the association of modified polypeptides with various membranes, including the plasma (Ras), peroxisomal (PxF), and nuclear (lamins A and B) membranes (1–4). In addition, prenylation can mediate protein–protein interactions (5).

FT has attracted attention because of its role in the processing of Ras proteins, which transduce receptor and nonreceptor tyrosine kinase activation to downstream cytoplasmic and nuclear effectors. Activating mutations in Ras proteins result in constitutive signaling, leading to cell proliferation and inhibition of apoptosis (6, 7). Oncogenic ras mutations have been identified in ~30% of human cancers (6).

Because farnesylation is critical for Ras maturation and function, FTIs were originally envisioned as specific and sensitive inhibitors of ras-mediated cellular proliferation (8, 9). Although FTIs clearly inhibit ras farnesylation and cause regres-

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3 The abbreviations used are: FT, farnesyl transferase; FTI, farnesyltransferase inhibitor; ANC, absolute neutrophil count; DLT, dose-limiting toxicity; MTD, maximum tolerated dose; BID, twice daily; NSCLC, non-small cell lung cancer.
sion of ras-transfected tumors in rodents, it has become clear in recent years that the critical target of FTIs may not be Ras proteins or may include other polypeptides in addition to Ras (5, 10, 11). To date, more than a hundred polypeptides possessing a “CAAX” sequence that can potentially be farnesylated have been identified (11). Theoretically, the inhibition of farnesylation of any of these polypeptides could result in the antiproliferative effects of the FTIs in human tumors. Up to 20 of these polypeptides, including Rho B, lamins A and B, transducin, CENP-E and F, rhodopsin kinase, and transducin, have been shown to actually undergo farnesylation (12). Accumulating data have identified three polypeptides whose inhibition may be the basis for the cytotoxic actions of FTIs. These are the G protein rho B, which regulates cytoskeletal organization (13); polypeptides associated with the phosphoinositide 3-OH kinase/AKT pathway (14); or the centromeric polypeptides CENP-E and CENP-F, which interact with microtubules and are necessary for the completion of mitosis (15). Another possibility is that the cytotoxicity of FTIs may be attributable to inhibition of farnesylation of several critical polypeptides, including some or all of the ras isoforms.

A number of potent and selective FTIs are in different stages of preclinical and clinical development (16). These include the oral methylquinolone R115777 (ZARNESTRA), which was the first FTI to enter human clinical trials. The most common single agent regimen for this agent is 300 mg twice daily for 21 days with 1 week off. Myelosuppression, manifested typically as neutropenia, is the most common toxicity. Thrombocytopenia and anemia are less common. A pruritic erythematous maculo-papular rash of mild to moderate severity, independent of dose, can occur but only rarely requires interruption of drug dosing. Other toxicities include fatigue, nausea, vomiting, and diarrhea, which are usually mild in severity. This agent has demonstrated activity in refractory leukemia and breast cancer in early clinical trials (17, 18).

In previous studies, we examined the effect of combining FTIs with several classes of antineoplastic agents in various human tumor cell lines using colony-forming assays. Although antagonism was demonstrated when FTIs were combined with 5-fluorouracil, the combination of FTIs and cisplatin exhibited sequence-dependent synergy, whereas the combination of FTI and gemcitabine was additive (19). As well, Sun et al. (20) demonstrated additive cytotoxicity with the combination of an FTI and gemcitabine in xenograft models. On the basis of these preclinical data, and the activity of the combination of gemcitabine and cisplatin in the treatment of NSCLC, ovarian cancer, and bladder cancer, we undertook a Phase I trial to identify the MTD, define the toxicities, and explore potential clinical activity of the combination of R115777 with gemcitabine and cisplatin. We also sought to demonstrate in vivo inhibition of FT activity at the recommended Phase II dose of R115777 in this combination.

**PATIENTS AND METHODS**

**Patient Selection.** Patients with histological or cytologic evidence of metastatic or locally advanced cancer for which there was no established life-prolonging therapy available, or who were unresponsive to conventional therapy, and had measurable or evaluable disease were eligible for this study. Eligibility criteria included age ≥ 18 years; Eastern Cooperative Oncology Group performance status ≤ 2; adequate bone mar-
row (platelets ≥ 100 × 10⁹ cells/liter and ANC ≥ 1.5 × 10⁹ cells/liter), hepatic (total bilirubin ≤ 2 mg/dl), and renal (serum creatinine ≤ 1.5 times the upper limit of normal) functions; no chemotherapy, radiotherapy, biological, hormonal, or investigational drug therapy within 28 days before study entry; and no previous nitrosourea or mitomycin C chemotherapy. Patients who had a diagnosis of leukemia; radiation therapy to >25% of the bone marrow; brain metastasis, unless disease had been resected by surgery or radiosurgery and the patient had been stable for 4 weeks; or an active infection requiring therapy were excluded from this trial. Written informed consent was obtained according to federal and institutional guidelines.

**Experimental Treatment.** R115777 (ZARNESTRA) was supplied as a 1 mg/ml aqueous solution in 50- and 100-mg vials for injection. The total dose was diluted in 750 ml of 5% dextrose in 0.45% NaCl containing 25 grams of mannitol and infused over a 2-h period. R115777 was administered p.o. twice daily for 14 days. Gemcitabine 1000 mg/m² was administered over 30 min on days 1 and 8 within 15 min of the morning dose of R115777. Cisplatin (75 mg/m²) was administered on day 1 as a 2-h infusion beginning 30 min after completion of the gemcitabine administration. Cycles were repeated every 21 days. After six cycles of therapy, gemcitabine and cisplatin were discontinued, and patients were maintained on p.o. R115777 alone. At least 3 new patients were entered at each dose level in a standard “cohorts of three” Phase I design (21). Dose escalation was not allowed in individual patients.

All toxicities were graded according to the National Cancer Institute common toxicity criteria (version II). The MTD was defined as one dose level below the dose that induced DLTs in more than one-third of patients (≥2 of a maximum of 6 patients). MTD determination was based on toxicities documented in the first cycle of treatment only. Severe or life-threatening (National Cancer Institute common toxicity criteria grades 3 or 4), nonhematologic toxicity (with the exception of nausea and vomiting, unless grade 3 or 4 occurred despite prophylactic treatment with an optimal antiemetic regimen) was considered dose limiting. An ANC < 0.5 × 10⁹/liter associated with fever or lasting for ≥ 5 days and a platelet count < 25 × 10⁹/liter of any duration were also considered dose limiting.

**Clinical Care of Patients.** Complete patient histories, physical examinations, complete blood cell counts, serum electrolytes, chemistries, urinalysis, and electrocardiograms were performed at baseline and before each course of treatment. Laboratory studies were repeated weekly while patients were on study. Ophthalmologic examination, including retinal photography, was performed at baseline and before the third cycle of treatment. Radiological studies (roentgenograms, computed axial tomographic scans, and magnetic resonance imaging) were performed at baseline and after every two cycles of therapy to assess tumor response.

**Immunohistochemistry in Buccal Mucosa Cells.** The immunohistochemical detection of prelamin A in buccal mucosa cells has been described previously (22). Briefly, buccal smears obtained before therapy and again on day 8 (12 h after the last dose of R115777) were air dried and fixed in acetone within 3 h of harvest. Samples were stored in buffer A [10% (w/v) powdered milk in 150 mM NaCl, 10 mM Tris-HCl (pH 7.4 at 21°C), 100 units/ml penicillin G, 100 μg/ml streptomycin, and 1 mM sodium azide] and subjected to the immunohistochemical assay in batches. Samples were simultaneously stained with mouse antilamin A and a rabbit antisera that detects the peptide that is removed from prelamin A in a farnesylation-dependent manner. With each batch of samples, A549 lung cancer cells treated with R115777 or diluent were included as positive and negative controls, respectively. After bound antibodies were detected with rhodamine-conjugated antimouse IgG and fluorescein-conjugated antirabbit IgG, samples were examined on a Zeiss LS310 confocal microscope. Sensitivity of the photomultiplier tubes was adjusted so that the signal for prelamin A in a farnesylated-dependent manner. All images were subsequently imported into Adobe PhotoShop 3.0, all adjustments to brightness or contrast were applied identically to paired samples harvested before and after therapy with R115777.

**RESULTS**

**Patient Demographics**

A total of 30 patients (Table 1) has received 155 assessable cycles of therapy through five dose levels (Table 2). The median number of cycles administered per patient was 2.5 (range,
1–30+). Ten patients completed six cycles of therapy and went on to receive R115777 tablets alone. One patient is still on therapy. The median age of study participants was 57.5 (range, 36–76 years). There were 15 males and 15 females enrolled. Patients had a good performance status, with only 1 patient having an Eastern Cooperative Oncology Group performance status of 2. Sixteen patients had received previous chemotherapy, and 8 had received previous radiation therapy. The most common tumor type was NSCLC, with 12 patients. There were 5 who had mesothelioma, 4 who had hepatocellular cancer, 3 with ovarian cancer, 2 with pancreatic cancer, and single patients with a variety of other solid tumors.

**Toxicities**

**Hematologic Toxicity.** The hematologic effects of R115777 in combination with gemcitabine and cisplatin and number of patients experiencing various grades of toxicity are shown in Fig. 1, and Tables 3 and 4. Reversible neutropenia and thrombocytopeny were the most common and severe toxicities. DLT in cycle 1 was thrombocytopenia alone (4 patients), neutropenia alone (1 patient), or a combination of both (3 patients), precluding dose escalation above dose level 5 (R115777 400 mg of BID, 1000 mg/m² gemcitabine, and 75 mg/m² cisplatin), where all 3 patients had dose-limiting myelosuppression in the first course. At the MTD (R115777 300 mg of BID, 1000 mg/m² gemcitabine, and 75 mg/m² cisplatin), 2 of 10 patients had dose-limiting myelosuppression (neutropenia and thrombocytopeny) in cycle 1. In general, subsequent cycles could be given with a dose reduction of 25–30% of all drugs.

**Gastrointestinal Toxicity.** Nausea, vomiting, and diarrhea occurred in the majority of patients, but the severity and frequency were not related to the dose level of R115777 (Fig. 1). The doses of gemcitabine and cisplatin were fixed after dose level 1 (Table 1). These findings therefore suggest that R115777 did not contribute substantially to the known gastrointestinal side effects of cisplatin and gemcitabine. In most instances, nausea and vomiting were controlled with aggressive prophylaxis and treatment with granisetron and dexamethasone. In 5 patients, nausea and vomiting were successfully controlled only after reduction in the doses of gemcitabine and cisplatin. Diarrhea was generally mild to moderate and easily controlled by loperamide.

**Electrolyte Abnormalities.** Electrolyte abnormalities resulting from nausea and vomiting, as well as the nephrotoxic effects of cisplatin, were relatively common (20 of 118 cycles). These were commonly manifest as hypokalemia, hypomagnesemia, and hyponatremia. Hypophosphatemia and hypocalcemia occasionally occurred.

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**Fatigue.** Fatigue was frequent, occurring in 22 patients at all dose levels. Grade 3 fatigue occurred in 2 patients, associated with nausea, vomiting, dehydration, and electrolyte abnormalities. Only 1 patient had mild fatigue while receiving R115777 after completing six cycles of chemotherapy, suggesting that fatigue was predominantly caused by the gemcitabine and cisplatin.

**Other Toxicities.** An erythematous, pruritic maculopapular rash occurred in 17 patients, unrelated to the dose of R115777, and was severe in 4 patients. This rash was distributed on the torso and had the typical appearance of a drug rash. It usually resolved after therapy with a short course of p.o. corticosteroids. One patient (treated at dose level 3) developed grade 3 peripheral neuropathy with a glove-and-stocking distribution after nine cycles of therapy and was removed from the study. Two other patients (dose levels 2 and 4) developed grade 2 peripheral neuropathy after six and eight cycles, respectively, resulting in treatment termination.

Because the visual proteins rhodopsin kinase and transducin are farnesylated (23, 24), ophthalmologic examinations were performed at regular intervals. No evidence of ocular toxicity was found. In addition, because QT prolongation occurred with
an FTI that was withdrawn from clinical testing, electrocardiograms were performed at baseline and after three cycles of therapy on all patients. No abnormalities were found.

On the basis of DLTs that occurred in cycle 1, the MTD of this combination is R115777 300 mg of BID, 1000 mg/m² gemcitabine, and 75 mg/m² cisplatin. However, with the exception of 1 patient, all patients treated at this dose level had their doses adjusted to R115777 300 mg of BID, 750 mg/m² gemcitabine, and 60 mg/m² cisplatin after the second cycle of treatment, because of nausea, vomiting, and fatigue. This dose is therefore recommended for subsequent clinical testing, because it can be administered chronically.

Antitumor Activity

Twenty-seven patients of the 30 patients were assessable for antitumor activity. Three patients were not evaluable (2 patients with NSCLC and 1 with ovarian cancer). One patient refused therapy after one cycle and did not return for reevaluation. Two other patients discontinued early because of toxicity (diffuse rash and fatigue/malaise after one cycle) and were evaluable for toxicity but not for response. Nine objective responses (one complete response and eight partial responses) were documented, with a median duration of eight cycles. The majority of these patients had evaluable (but not measurable) disease. Five of these patients (1 complete remission and 4 partial remission) had NSCLC (all but 1 treated previously); 2 had ovarian cancer that had been treated previously with multiple chemotherapy regimens, and 1 each had bile duct and hepatocellular cancer untreated previously. There was no apparent correlation of response and dose level. Eight additional patients had stable disease for three or more cycles of treatment. The ovarian cancer patient who discontinued therapy after one cycle of therapy because of toxicity also had a CA125 decrease from 8000 to 2000 ng/ml after the single cycle of treatment.

Three of the 4 NSCLC patients treated previously had progressed on a regimen of paclitaxel/carboplatin. One patient had progressed on gemcitabine. The two responders with ovarian cancer were considered platinum refractory, and each patient had received three previous regimens.

Inhibition of FT in Patients Receiving the Recommended Phase II Dose of R115777. We have described previously the development of an immunohistochemical assay for detecting prelamin A accumulation in buccal mucosa cells that could be used as a marker of FT inhibition in surrogate tissue (25). In the present study, we sought to demonstrate that the dose of R115777 defined in this combination for future studies (300 mg of BID p.o.) was adequate for inhibition of FT in patient tissues. This Phase I study was conducted concurrently with a single agent Phase II study using 300 mg of BID p.o. of R115777 in NSCLC. The Phase II study incorporated the detection of prelamin A in buccal mucosa cells as a surrogate of FT inhibition in vivo (26). We therefore chose to supplement those data by evaluating prelamin A accumulation in buccal mucosa cells from a cohort of 4 patients treated at the MTD on this study. After 7 days of treatment, prelamin A was detectable in buccal mucosa cells of 3 of these 4 patients treated at the MTD (Fig. 2).

DISCUSSION

The FTIs represent a promising class of small molecule inhibitors of cell signaling. Recently, negative studies have been reported with single agent R115777 in NSCLC (26), pancreatic cancer (27), and colorectal cancer (28). These tumors are all known to frequently harbor K-ras mutations (6). It is now known that tumors with mutant K-ras are relatively resistant to the FTIs, suggesting that the role of these agents may well be in the treatment of tumors harboring wild-type ras. This hypothesis is supported by evidence of clinical activity of R115777 in acute leukemia (17), breast cancer (18), and glioma (29). In addition, like other novel agents that inhibit key signaling proteins, these compounds may be more effective in combination with cytotoxic chemotherapy. Preclinical studies have demonstrated sequence-dependent cytotoxic synergy when cultured human cancer cell lines were exposed to cisplatin and FTIs in vitro (19). Additive effects were also demonstrated with FTIs and gemcitabine (19, 20). This trial was designed to determine whether cisplatin and gemcitabine could be safely administered with R115777 in the clinical setting. We demonstrate here that this
combination can be safely administered. The protocol-defined MTD based on toxicities documented in cycle 1 of treatment only is R115777 300 mg of BID, 1000 mg/m² gemcitabine on days 1 and 8, and 75 mg/m² cisplatin on day 1, every 21 days. However, with repeated administration, the doses of gemcitabine and cisplatin had to be reduced in all but 1 of the 10 patients treated at this dose level because of nausea, vomiting, and fatigue. This toxicity was felt to be predominantly related to the cisplatin dose. The recommended starting doses for chronic administration in future studies is, therefore, R115777 300 mg of BID × 14 days, 750 mg/m² gemcitabine on days 1 and 8, and 60 mg/m² cisplatin on day 1, every 21 days.

We have demonstrated previously that the processing of prelamin A in buccal mucosa cells of patients could be used as a marker of FTI activity in vivo (22). In this study, we observed that the R115777 dose of 300 mg of BID p.o. inhibited FT in surrogate tissue of 3 of 4 patients evaluated. Further study is required to determine whether accumulation of prelamin A in buccal mucosa correlates with inhibition of FT in tumor tissue and/or response of patients to regimens containing R115777. Studies to address these questions in the context of Phase II trials are ongoing.

The pharmacokinetics of R115777 alone, and in combination with gemcitabine, as well as gemcitabine in combination with cisplatin have been described previously (30, 31). As well, metabolism data do not suggest any potential for interaction between R115777 and cisplatin. Pharmacokinetic analyses were therefore not performed in this study. Nevertheless, not only is the recommended R115777 dose biologically effective but the gemcitabine and cisplatin doses recommended for future studies also have been shown to be therapeutic (32, 33). Thus, this study has demonstrated that clinically relevant doses of all three agents can be safely combined to treat patients.

Consistent with this finding, the current regimen was found to show signs of activity in a wide variety of tumors. There were nine objective responses. Eight additional patients had stable disease for three or more treatment cycles. Because this study is therefore not performed in this study. Nevertheless, not only is the recommended R115777 dose biologically effective but the gemcitabine and cisplatin doses recommended for future studies also have been shown to be therapeutic (32, 33). Thus, this study has demonstrated that clinically relevant doses of all three agents can be safely combined to treat patients.

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