Intermittent dosing of the farnesyl transferase inhibitor tipifarnib (R115777) in advanced malignant solid tumors: a phase I California Cancer Consortium Trial

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Tipifarnib (R115777) inhibits farnesylation of key proteins that modulate signaling pathways implicated in cell growth and proliferation, including members of the Ras and Rho families. It has broad-spectrum antiproliferative activity *in vitro* and *in vivo*. Clinical trials employing a continuous administration schedule have demonstrated dose-limiting neurotoxicity and myelosuppression. Preclinical studies have shown that intermittent oral administration can suppress tumor growth comparable to continuous administration. We conducted a National Cancer Institute-sponsored phase I trial to determine the feasibility of an intermittent dosing schedule of R115777 given orally twice daily on weeks 1 and 3 of a 28-day cycle in patients with malignant solid tumors. Starting dose was 300 mg twice daily (b.i.d.) with escalation by 300 mg b.i.d. increments over six dose levels to a maximum of 1800 mg b.i.d. Dose-limiting toxicity (DLT) was defined as any grade 3 or 4 non-hematologic toxicity, grade 4 thrombocytopenia, grade 4 neutropenia (ANC) with fever (38.3°C or above) or a documented infection. Twenty-one patients with advanced solid tumors, all of whom had prior systemic therapy, were accrued. Grade 3 fatigue was dose limiting for two of three patients at the 900 mg b.i.d. dose level. Although no responses were seen, four of six patients with stable disease remained on study for at least a year (16, 17, 13 and 12 months) before developing progressive disease. Three of these prolonged stable disease patients had non-small cell lung cancer. We conclude that intermittent dosing of R115777 is feasible and tolerable. The recommended phase II dose is 600 mg orally b.i.d. on alternate weeks. *Anti-Cancer Drugs* 16:317–321 © 2005 Lippincott Williams & Wilkins.

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Introduction

Inhibition of farnesyl transferase is a rational antineoplastic strategy. Farnesyl transferase inhibitors act by preventing a post-translational farnesylation step that key regulatory molecules require to mediate cancer cell growth and proliferation. The most commonly described target is Ras, whose function in the signal transduction of growth signals requires its farnesylation prior to insertion into the plasma membrane. Mutated Ras is constitutively activated to transduce extracellular signals that enhance tumor proliferation. Interestingly, farnesyl transferase inhibitors have been demonstrated to possess preclinical antitumor activity even in human tumor cell lines that lack Ras mutations [1]. Hence, several other farnesylated proteins have been proposed as alternative targets, including members of the Rho-B family, CENP, among many others, that can explain the antitumor activity of these inhibitors [2].

R115777 (Tipifarnib) is a novel, orally administered, selective non-peptidomimetic farnesyl transferase inhibitor that has been demonstrated to have broad-spectrum activity in a variety of *in vitro* and *in vivo* models [3–5]. Initial phase I and II trials have explored a dosing schedule of R115777 for 21 days with a 7-day break, identifying a dose of 300 mg twice daily as the recommended dose for further development. Dose-limiting toxicities (DLTs) have primarily been myelosuppression, neurotoxicity and fatigue [6–8].

Studies in nude mice of T24 F1-H-Ras-transformed 3T3 tumors showed that intermittent oral administration at 200 mg/kg/day for 5 days suppressed tumor growth for up to 21 days, similar to that seen with continuous administration at 50 mg/kg/day [9]. Tumor growth suppression was dose-related with this 5-day schedule. An intermittent schedule that dosed R115777 for 5 days with

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a 9-day rest period was initially tested at the National Cancer Institute (NCI). The protocol-defined maximum tolerated dose (MTD) was not achieved at the highest dose level (1300 mg b.i.d. for 5 days). Furthermore, no drug accumulation was noted in the pharmacokinetic studies performed as part of this trial, with steady-state concentrations achieved within 3 days of dosing [10].

We hypothesized that an every-other-week intermittent dosing schedule could potentially reduce toxicity, but may preserve efficacy. In order to plan for future efficacy trials of R115777 on an intermittent schedule, we conducted a phase I study that employed a modified intermittent schedule of R115777 given orally for 7 days followed by a 7-day break. We also collected archival tumor specimens to assess for K-Ras mutational status from patients enrolled in the trial to assess potential correlates for efficacy and toxicity.

Patients and methods

Patient selection

Patients with advanced, recurrent or metastatic malignant tumors, deemed incurable or refractory to therapy, were eligible. Measurable disease was not required. Fresh tumor tissue, paraffin tumor tissue or unstained slides were requested at protocol entry for the proposed correlative studies. Patients with brain metastases were ineligible, unless asymptomatic and controlled (i.e. after surgical resection or radiotherapy/radiosurgery). Because of potential drug interactions with R115777, patients with stable brain metastates were not allowed to be on concurrent steroids or anticonvulsants. Adequate performance status (Karnofsky score of 60% or greater) and acceptable end-organ function was required defined by pretreatment white blood cell count (WBC) $\geq 3500/\mu l$, absolute granulocyte count (AGC) ≥ 1500, platelet count $\geq 75\,000/\mu l$, creatinine clearance ≥ 60 ml/min or serum creatinine ≤ 1.6 mg/dl and serum bilirubin/AST less than or equal to 2.5 times the institutional upper limits of normal. Patients must have had at least one prior systemic therapy for their disease and must have recovered completely from all toxicities. Additionally, a minimum of 4 weeks must have elapsed since the completion of prior chemotherapy (6 weeks for prior mitomycin or nitrosourea) in order to be eligible for this study. There must have been no plans for the patient to receive concurrent hormonal, biologic or radiation therapy to measurable lesions.

Because of the limited data on the toxicity profile of this compound in the pediatric age group, patients must have been at least age 18 prior to protocol entry. It was anticipated that pediatric patients would be enrolled in future trials of this agent once safety data were confirmed.

All patients were required to be informed of the investigational nature of the study, and must have signed and given written informed consent in accordance with institutional and federal guidelines. This study was approved by the NCI Cancer Therapy Evaluation Program (CTEP) and the institutional review boards of each California Cancer Consortium site. All patients gave written informed consent to participate.

Because of the antiproliferative activity of R115777 and its unknown effects on the developing fetus or nursing infant, pregnant or lactating women were excluded from this trial and appropriate contraceptive practices were required for all patients while on protocol therapy.

Baseline evaluation

Before study entry, all patients underwent a complete history and physical examination. Baseline imaging studies of all known sites of disease were obtained within 4 weeks of initial therapy. Laboratory studies included a complete blood count with differential and platelet count, comprehensive metabolic panel (which includes electrolytes, serum creatinine, total bilirubin and AST).

DLT

DLT in a given patient was initially defined as any grade III or IV non-hematologic toxicity (except alopecia), grade IV thrombocytopenia, grade IV neutropenia with fever (defined as a temperature of 38.3°C or 101°F) or a documented infection, or any delay of therapy for more than 2 weeks due to hematologic or non-hematological toxicities. The DLT definition was later amended to delete the 'delay of therapy' provision following consultations with the NCI CTEP. DLT was based on the first cycle of treatment. Toxicity was graded according to the NCI Common Toxicity Criteria version 2.0. The length of a treatment cycle was 28 days. To be evaluable for toxicity, a patient must have received at least 1 cycle of treatment and have been observed for at least 7 days after the last day of R115777 treatment in the first cycle. All patients who were not evaluable for toxicity were to be replaced.

MTD

The MTD was defined as the highest dose level in which six patients had been evaluated for toxicity with no more than one patient experiencing DLT attributable to the study drugs, during the first cycle of therapy. The MTD is one dose level below that associated with unacceptable toxicity, i.e. the dose tested in which two or more patients experienced DLT attributable to the study drug(s). At least six patients were to be treated at the MTD.

Rules for dose escalation

Study drugs were administered twice daily on a 1-week-on/1-week-off schedule, using the dose escalation scheme

summarized in Table 1. Three patients were to be treated at each new dose level. The first patient should be entered and begin therapy for at least 1 week before the other two patients at that level are started. If none of three patients experience DLT, three patients would be treated at the next dose level. If DLT attributable to the study drug(s) was experienced in exactly one of three patients, three more patients (for a total of six) would be treated at that dose level.

If no additional DLT was observed at the expanded dose level (i.e. one of six with DLT), the dose would be escalated. Escalation would terminate as soon as two or more patients experienced any DLT attributable to the study drug(s), at a given dose level.

Closure of the trial was planned when six patients had been treated at the next lower dose level and at most one of six patients experienced DLT for each of the two strata. If more than one of six patients experienced DLT, the next lower dose was expanded.

All patients who had not experienced any DLT must have been observed for a minimum of 28 days after the start of the first cycle (i.e. the length of the treatment cycle) before the next dose level was escalated.

Patients were to receive a minimum of 1 cycle of treatment. There was no pre-defined maximum number of cycles. Patients would remain on the study as long as there was no evidence of progressive disease or unacceptable toxicity. Treatment was continued in an individual patient at the same dose level if no DLT was observed. No intrapatient dose escalation was allowed.

Treatment plan

R115777 was administered orally twice daily on a 1-week-on/1-week-off schedule (see Table 1). Cycle length was defined as 28 days, i.e. treatment was given on weeks 1 and 3, every 28 days. Patients receiving at least 2 cycles of R115777 were evaluated for response. Disease assessments were performed after every 2 cycles (or every 8 weeks). Patients with progressive disease at any time were removed from the study. Patients with a partial response or with stable disease were continued on treatment until a complete response (CR) was obtained, progressive disease was documented or unacceptable toxicity occurred. In patients achieving a CR, continuation of therapy was allowed at the discretion of the investigator until progressive disease was documented or if unacceptable toxicity occurred. 'Unacceptable toxicity' was subjectively determined by the treating physician and/or patient. Patients could choose to withdraw from the study at any time for any reason.

Table 1 Dose escalation scheme

Dose level	R115777 dose (mg)
1	300
2	600
3	900
4	1200
5	1500
6	1800

R115777 was given orally twice daily on a 1-week-on/1-week-off schedule.

Toxicity assessment and dose modifications

Patients were examined and any toxicities were graded on day 1 of each treatment cycle. Complete blood counts were obtained on days 1, 8, 15 and 22 of each cycle. Dose delays or adjustments were based on treatment day hematologic parameters and interim non-hematologic toxicities. Patients who experienced grade 3 or greater non-hematologic toxicity must have returned to baseline status before a new cycle of treatment could be started. Patients who had a treatment delay for more than 2 weeks due to hematologic toxicity, but appeared to be deriving clinical benefit, were allowed to continue on treatment at a reduced dose. All dose reductions were permanent. No intrapatient dose escalation was allowed. All severe and unexpected adverse events were reported to the NCI and the appropriate regulatory bodies.

Criteria for evaluation

All patients who were registered were accounted for in the report of the results. To be evaluable for toxicity, a patient must have received at least 1 cycle of treatment and have been observed for at least 7 days after the last day of R115777 treatment in the first cycle. Patients who completed 2 cycles and had follow-up imaging were evaluable for response. All patients who received the first cycle of treatment were included in the analysis of survival and time to failure. Tumor response was assessed using standard RECIST criteria.

Molecular correlative studies

We have adapted a highly sensitive, two-step restriction fragment length polymorphism (RFLP)-PCR assay [11] that can identify all K-Ras 12th codon mutants to examine pre-treatment, paraffin-embedded tumor tissues and serial plasma specimens [12] from patients enrolled in this trial. DNA was extracted from plasma specimens and subjected to PCR according to standard protocols. DNA from micro-dissected tumor cells was assessed by a sensitive two-step RFLP-PCR assay that detects all possible 12th codon-activating mutations in K-Ras. Mutations were confirmed by sequencing of PCR products.

Results

Patient demographics

Twenty-one eligible patients with a median age of 69 years (range 48–84) were accrued between August 2001 and March 2003. Patient characteristics are summarized in Table 2. There were nine males and 12 females. All

Table 2 Patient characteristics

	N
Patients enrolled	21
Previously chemotherapy treated	21
Prior radiation therapy	9 (43%)
Age (years) [median (range)]	69 (48–84)
Gender (male)	9 (43%)
Performance status (Karnofsky)	
90	7
80	9
70	5
Histologic types	
adenocarcinoma	16
squamous cell cancer	2
clear cell cancer	1
adenoid cystic cancer	1
large cell cancer	1
Tumor types	
NSCLC	8
colorectal cancer	6
hormone-refractory prostate cancer	3
esophageal carcinoma	1
pancreatic cancer	1
parotid cancer	1
renal cell carcinoma	1

patients had acceptable performance status: Karnofsky score was 90% in seven patients, 80% in nine and 70% in five. The tumor types enrolled were non-small cell lung cancer (NSCLC) ($n = 8$), colorectal cancer ($n = 6$), hormone-refractory prostate cancer ($n = 3$), esophageal carcinoma ($n = 1$), pancreatic cancer ($n = 1$), parotid cancer ($n = 1$) and renal cell carcinoma ($n = 1$). All patients had prior chemotherapy; nine patients had prior radiation.

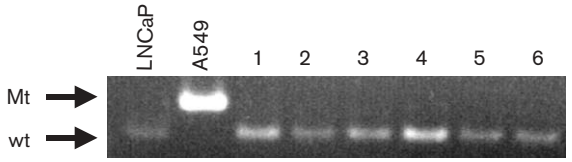
Dose escalation and toxicities

One patient in dose level 2 (600 mg orally b.i.d.) developed grade 3 seizures on day 3 of cycle 1. This patient was considered unevaluable due to inadvertent overdosing of R115777 on days 1 and 2 of therapy. An additional patient was found to have brain metastasis during cycle 1 requiring discontinuation of protocol therapy and becoming unevaluable for toxicity. Per protocol, these patients were replaced. Myelosuppression requiring a greater than 2-week delay in therapy was dose limiting in two of six evaluable patients at dose level 2. No other DLTs were observed at this dose level. Following discussions with the NCI, the DLT definition was revised to delete the 2-week delay. Two of six patients in dose level 3 (900 mg orally b.i.d.) developed grade 3 fatigue attributable to the study drug. Dose level 3 was therefore identified as the MTD level and is the recommended phase II dose for this intermittent schedule. Overall, the treatment was well tolerated. Attributable grade 3 or higher toxicities are summarized in Table 3. A median of 2 cycles of treatment was administered (range 1–16). In dose level 1, the median number of cycles delivered was 3.5 (range 2–12). In the six evaluable patients (of a total of eight enrolled) in dose level 2, the median was 4 (range 2–14), while in dose level 3, the median was 1.5 (range 1–12).

Table 3 Toxicities of grade 3 or worse attributable to R115777

Toxicity	No. patients
Fatigue (asthenia, lethargy, malaise)	2
Leukocytes	1
Lymphopenia	6
Neutrophils/granulocytes (ANC/AGC)	4
Pruritus/itching	1
Rash/desquamation	1
Seizure	1
Supraventricular and nodal arrhythmia	1

Fig. 1



K-Ras mutational analysis. This figure shows the results of the RT-PCR-RFLP analysis for six patients. A549 cells served as positive mutation control and LNCaP cells served as negative control. Mt = mutant; wt = wild-type.

Efficacy

Evaluation of response rate was not a principal objective of this study; however, objective responses were recorded. No complete or partial responses were observed. Six patients had stable disease, while 13 patients progressed. Two patients were not assessable for response.

Four patients, three of whom had NSCLC, remained on study for at least 1 year with stable disease (16, 17, 13 and 12 months).

Molecular correlates

Fourteen blood specimens and 13 paraffin-embedded tumor specimens were obtained. Molecular analysis of 12th codon K-Ras mutations was assessed in each sample, but no K-Ras mutations were found in tumors from the 13 patients. In addition, no mutations were detected in plasma DNA of 14 patients. Results of the RT-PCR-RFLP analysis for six patients are shown in Figure 1, with A549 cells as positive mutation control and LNCaP cells as negative control. Since no K-Ras mutations were found, no correlative studies with clinical outcome could be conducted.

Discussion

This phase I trial tested a novel intermittent dosing schedule for the farnesyl transferase inhibitor R115777 in an effort to define the optimal delivery and tolerability of this unique antineoplastic agent. Previous early phase trials of chronic continuous dosing have generally been unable to deliver R115777 beyond a daily total dose of 600 mg due to dose-limiting myelosuppression and

neurotoxicity. Encouraging data from intermittent dosing studies in both preclinical and clinical settings led to the development of the current trial. For example, when R115777 was administered intermittently in a preclinical model, tumor growth suppression was seen for up to 3 weeks. [9] In addition, a phase I NCI trial of R115777 on a 5-day-on/9-day-off intermittent schedule was unable to establish a DLT level when escalation was terminated at the protocol-defined ceiling of 2600 mg total daily dose without attributable DLTs occurring [10]. These studies suggested the possibility of enhancing drug exposure to tumor cells while potentially minimizing toxicity through an intermittent dosing schedule designed to exploit the 7-day persistence of farnesyl transferase inhibition post-dosing.

Our study established an MTD level of 600 mg orally b.i.d. (or a total daily dose of 1200 mg) in a cohort of patients with advanced malignant solid tumors. Fatigue was found to be dose limiting at the next higher dose level (1800 mg total daily dose). However, we found that myelosuppression—the principal DLT in previous studies—was moderate and manageable. As much of the current clinical development for R115777 has been in hematologic malignancies such as refractory myeloid leukemia where encouraging responses have already been reported [13,14], the concern for myelosuppression as a DLT may be less relevant. Therefore, further testing of this intermittent dose schedule appears warranted for hematologic malignancies to probe for a more effective higher dose to eradicate leukemic cells and to further define the non-hematologic toxicities of this schedule in that cohort. In light of this, our group is currently conducting a dose escalation study that employs this intermittent schedule in patients with refractory leukemia.

The issue of dose-limiting myelosuppression is of more relevance to solid tumors where the tolerability and activity of this compound, either alone or in combination with other cytotoxics, remains under investigation. Myelosuppression was dose limiting when R115777 was tested in the initial single-agent studies of continuous daily administration. Therefore, the feasibility of combining this agent with myelosuppressive chemotherapy in the solid tumor population would be challenging, at the very least. The results of our phase I study of intermittent dosing demonstrated manageable bone marrow toxicities, with fatigue as the principal DLT. It can be hypothesized that an intermittent dosing schedule for R115777 may serve as a more optimal platform on which to build combination regimens.

As part of this trial, tumor tissue was collected for assessment of Ras mutational status. The initial plan was to compare the results to the clinical outcomes of toxicity and efficacy in a preliminary fashion. However, because of the lack of any Ras mutants in the accrued cohort, such correlations became impossible to perform.

In conclusion, this phase I trial of R115777 identified a recommended phase II dose of 600 mg orally twice daily on a 1-week-on/1-week-off intermittent schedule. The treatment was generally well tolerated, with grade 3 fatigue as the principal DLT. The results of this trial formed the basis for the recently initiated California Cancer Consortium trial of R115777 employing this intermittent dose schedule in relapsed, refractory leukemia.

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