

Phase I Clinical and Pharmacodynamic Evaluation of Oral CI-1033 in Patients with Refractory Cancer

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Abstract **Purpose:** To determine the tolerability and pharmacokinetics of CI-1033 given daily for 7 days of a 21-day cycle. Tumor response and changes in erbB receptor tyrosine kinase activity in tumor and skin tissue were examined, and modulation of potential biomarkers in plasma was explored. **Design:** This was a dose-finding phase I study in patients with advanced solid malignancies. Patients were evaluated for safety, pharmacokinetics, and tumor response. Pharmacodynamic markers, such as Ki67, p27, and erbB receptor status, were assessed in tumor and skin tissue using immunohistochemical and immunoprecipitation methodologies. Plasma biomarkers HER2, vascular endothelial growth factor, interleukin-8, and matrix metalloproteinase-9 were evaluated using immunologic techniques. **Results:** Fifty-three patients were enrolled in the study. Dose-limiting toxicity (emesis, persistent rash, and mouth ulcer) was observed at 750 mg. The maximum tolerated dose was 650 mg. There were no confirmed objective responses. CI-1033 treatment showed down-regulation of epidermal growth factor receptor, HER2, and Ki67 in a variety of tumor tissues and up regulation of p27 in skin tissue. Plasma HER2 was reduced following CI-1033 administration, but no consistent change in vascular endothelial growth factor, interleukin-8, or matrix metalloproteinase-9 was noted. CI-1033 plasma concentrations were proportional to dose. **Conclusion:** The safety and pharmacokinetic profile of CI-1033 was favorable for multidose oral administration. Evidence of modulation of erbB receptor activity in tumor and skin tissue was accompanied by changes in markers of proliferation and cell cycle inhibition. Additional clinical trials are warranted in defining the role of CI-1033 in the treatment of cancer and further assessing the utility of antitumor markers.

The erbB receptor family of tyrosine kinases consists of four transmembrane proteins: erbB1 [epidermal growth factor receptor (EGFR)], erbB2 (or HER-2/*neu*), erbB3, and erbB4. These glycoproteins have been implicated in the development and progression of cancer (1–12). Furthermore, expression/overexpression of one or more of the erbB receptor members has been associated with more aggressive disease and poor patient prognosis (4, 5, 13–18).

Normally, ligand binding to the extracellular domain of the receptor initiates tyrosine autophosphorylation and activates various signal transduction pathways, including the phosphatidylinositol 3'-kinase/Akt and the mitogen-activated protein kinase pathways (19), ultimately resulting in DNA synthesis and cellular proliferation (20–22). Although erbB3 lacks an

active tyrosine kinase domain, it participates in signaling through formation of heterodimers with other erbB receptor family members (23).

CI-1033, a 4-anilinoquinazoline derivative, is an orally active pan-erbB tyrosine kinase inhibitor that binds irreversibly to a specific residue in the ATP receptor pocket. CI-1033 produces potent and sustained inhibitory effects on intracellular tyrosine kinase activity and downstream signaling pathways through all four erbB receptor family members (24–27). It is specific in suppressing the erbB receptor family tyrosine kinase domain with no effect on other tyrosine kinases such as platelet-derived growth factor receptor, insulin receptor, or fibroblast growth factor receptor (28).

Other erbB receptor tyrosine kinase inhibitors in clinical trials include antibodies (e.g., trastuzumab and cetuximab) and ligand-toxin conjugates, which act at the extracellular ligand-binding regions of the receptor, whereas orally active erbB inhibitors, like CI-1033, act at the intracellular domain of the receptor similar to erlotinib, PKI166, and gefitinib. The latter three agents, however, while also ATP-competitive tyrosine kinase inhibitors, are selective for EGFR and have limited or no activity against other erbB receptors (29).

In cell line and *in vivo* studies, CI-1033 has shown antitumor activity (24, 30). In murine models, CI-1033 induced dose-dependent tumor growth delays and was well tolerated in single and multidose studies (31).

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Table 1. Phase I study of CI-1033 in patients with solid tumors: baseline characteristics

Variable	
No. patients (males/females)	53 (36/17)
Median age, y (range)	53 (31-81)
Median Karnofsky performance status (range)	90% (60-100%)
Tumor types, n (%)	
Non-small cell lung	18 (34)
Head and neck	15 (28)
Squamous cell skin	4 (8)
Liver	3 (6)
Colorectal	5 (9)
Other gastrointestinal*	2 (4)
Breast	2 (4)
Other*	4 (8)
Previous treatments, n (%)	
Cytotoxic therapy	48 (91%)
Radiotherapy	36 (68)

*Pancreatic, biliary, renal, uterine, thymic, and unknown primary.

A once-daily 7-day dosing schedule at a starting dose of 50 mg was chosen for phase I clinical testing based on the irreversible nature of the binding affinity for the erbB family of receptors and the schedule dependency of observed toxicity in preclinical models. It was also felt that an intermittent dosing schedule in the earliest clinical trials would provide an added margin of safety.

This phase I study was designed to evaluate oral CI-1033 in terms of safety, pharmacokinetics, and maximum tolerated dose and to document any evidence of antitumor activity, changes in EGFR and HER2 receptor tyrosine kinase phosphorylation, and the expression of other potential biomarkers of antitumor efficacy.

Patients and Methods

Study design. This was an open-label, non-comparative, dose-finding, multicenter phase I study of CI-1033. Oral doses were administered as capsules given daily for seven consecutive days beginning on day 1 of a 21-day treatment cycle. The starting dose was 50 mg, with subsequent cohorts treated at doses based upon prior cohort toxicity. Adverse events were evaluated using the National Cancer Institute Common Toxicity Criteria, version 2.0 guidelines (32).

Eligibility requirements. Patients ≥ 18 years old with histologically documented advanced stage nonhematologic malignancies refractory to standard therapy or for which no effective therapy exists were eligible for study entry. Inclusion criteria included Karnofsky performance status ≥ 60 with the ability to swallow capsules and expected survival ≥ 12 weeks. Exclusion criteria included gastrointestinal malabsorption, creatinine clearance < 45 mL/min, bilirubin > 1.5 times upper limit of normal, alanine aminotransferase and aspartate aminotransferase > 3.0 times upper limit of normal, and absolute neutrophil count $< 1.5 \times 10^9/L$ or platelets $< 100 \times 10^9/L$. Chemotherapy or investigational agents were restricted within 4 weeks before the first CI-1033 dose. Immunotherapy, biological therapy, hormonal therapy, or radiotherapy was restricted within 2 weeks. Pregnant or nursing women or those unwilling to using adequate birth control were excluded. Patients were required to give written informed consent and be capable of adhering to study instructions. The collection of tumor biopsies was not a requirement for study entry; however, the

protocol was amended to require that patients at least have surgically "accessible" tumors. The protocol was approved by the Institutional Review Boards at The University of Texas M.D. Anderson Cancer Center and at U.S. Oncology, Inc.

Dose adjustment and escalation criteria. All patients within a cohort would receive the same milligram dose. Dose reductions due to dose-limiting toxicity (DLT) were permitted. DLT was defined as an adverse event that met any of the following criteria: absolute neutrophil count $< 0.5 \times 10^9/L$ lasting for ≥ 7 days or absolute neutrophil count $< 1.0 \times 10^9/L$ associated with a documented infection or fever $\geq 38.5^\circ C$; platelets $< 25 \times 10^9/L$ lasting for ≥ 7 days or ($< 10.0 \times 10^9/L$) of any duration, any other grade 4 hematologic toxicity, including coagulopathies, or grade ≥ 3 nonhematologic toxicity (except grade 3 hepatic toxicity lasting < 8 days, nausea/vomiting/diarrhea in the absence prophylactic intervention, tolerable skin rash); or inability to begin a subsequent treatment course within 21 days of the scheduled start date due to treatment-related toxicity. DLT necessitated immediate interruption of dosing with resumption contingent on return of neutrophils to $\geq 1.5 \times 10^9/L$, platelets to $\geq 75 \times 10^9/L$, and all other toxicities (except tolerable skin rash or alopecia) to grade ≤ 1 or baseline toxicity levels.

Three patients would be treated at 50 mg in cohort 1, with subsequent dose escalations proceeding in one patient cohorts until the observation of first-cycle grade ≥ 2 treatment-related toxicity, at which time the cohort would be expanded to three patients. If there was no recurrence of the same grade ≥ 2 toxicity during cycle 1, the next cohort would be escalated 100%. Two instances of the same first-cycle toxicity grade ≥ 2 in separate patients would end the rapid single-patient dose escalation phase. Standard dose escalations would then proceed at $\leq 40\%$ dose increments in three-patient cohorts. Any cohort with a first-cycle DLT would be expanded to up to six patients. The maximum tolerated dose was declared when ≥ 2 of 6 patients experienced DLT within the first cycle.

Treatment discontinuation. CI-1033 treatment would be discontinued if any of the following occurred: protocol criteria for progressive disease was met, adverse events remained intolerable despite dose adjustments, the patient refused further treatment, or the investigator concluded it was in the patient's best interest. Patients who discontinued study treatment were followed for a minimum of 30 days to monitor safety.

Response criteria. Patients had a radiographic assessment of index lesion(s) within 4 weeks of study entry and before each cycle. Index lesions were measured using the two longest perpendicular axes, the product of which constituted a "bidimensional measurement." The sum of the products of multiple lesions constituted the total amount of measurable tumor. Lesions assessed by radiologic methods (chest radiograph, CI, and MIR) must be ≥ 1.0 cm with clearly defined margins, not previously irradiated, and ≥ 2.0 cm if assessed by physical examination.

Complete response was defined as disappearance of all clinical evidence of tumor. Partial response was defined as $> 50\%$ decrease in the sum of the product diameters without an increase in any lesions or the appearance of new lesions. All responses were confirmed with a repeat evaluation at least 4 weeks later. Progressive disease was defined as an increase in lesion by $> 25\%$ or appearance of new lesions. The patient could achieve stable disease status if criteria for complete or partial response were not met, and progressive disease did not occur within the first 12 weeks on study.

Clinical assessments. Within 2 weeks of study entry, all patients had a physical and ophthalmologic examination, medical history, weight, performance status assessment, complete blood count, chemistries and coagulation panel, urinalysis, electrocardiogram, and pregnancy test (when appropriate). Weekly complete blood count, chemistries, and coagulation panel were completed for all cycles, and weekly urinalysis was completed for the first two cycles. Medical history and physical exams were repeated every 3 weeks, and a repeat ophthalmologic examination was conducted before the second course and every 6 weeks thereafter. Patients were required to keep a medication diary.

Pharmacokinetic procedures and methods. Blood samples were collected for pharmacokinetic analysis on days 1 and 7 of cycles 1 and 2 at pre-dose and 0.5, 1, 2, 4, and 8 h post-dose. Plasma samples were frozen until assayed for CI-1033 using a validated, specific high-performance liquid chromatography tandem mass spectrometry analytic procedure. Pharmacokinetic data would be included in a population pharmacokinetic evaluation of CI-1033 together with pharmacokinetic data from ongoing phase I studies.

Immunoprecipitation methodology for total and phosphorylated EGFR. Tumor tissue (5-20 mg) was obtained from punch biopsies pre-therapy, study day 7, and day 15. Samples were quick frozen via liquid nitrogen or dry ice block and then shipped to M.D. Anderson Cancer Center, Houston, TX for analysis. Samples were homogenized in lysis buffer [50 mmol/L HEPES (pH 7), 150 mmol/L NaCl, 1 mmol/L EGTA, 100 mmol/L NaF, 10 mmol/L Na-pyrophosphate, 10% glycerol, 1% Triton X-100, 1 mmol/L Na₃VO₄, 1 mmol/L phenylmethylsulfonyl fluoride, 10 µg/mL leupeptin, 10 µg/mL aprotinin]. Following centrifugation (18,000 × g for 15 min), samples were immunoprecipitated for EGFR (A108 monoclonal antibody) and HER2 (Oncogene Sciences; Ab-3) using 0.5 µg antibody per mg protein and 30 µL Protein A-Sepharose (A108) or Protein G-Sepharose (HER2; Pharmacia). Antibody and Protein A/G-Sepharose were incubated sequentially for 2 and 1 h, respectively. Immune complexes were washed with lysis buffer and resolved by SDS-PAGE. The supernatant from the initial precipitation was used for immunoprecipitation. The supernatant fraction after EGFR/HER2 immunoprecipitation was used for measurement of protein concentrations. Resolved proteins were transferred to NC membranes, and EGFR/HER2 immunoprecipitates were immunoblotted for phosphotyrosine (4G10, UBI) and antigen detected with secondary antibody (Bio-Rad) and enhanced chemiluminescence reagent (Amersham). The membrane was stripped of primary antibody and reprobed with anti-EGFR antibody (Transduction Labs) or HER2 (same as above). Quantitation of EGFR/HER2 expression and activation [phosphorylated EGFR (pEGFR)/pHER2] was done by scanning densitometer.

Immunohistochemistry methodology for EGFR, HER2, vascular endothelial growth factor, matrix metalloproteinase-9, Ki67, and p27. Skin punch biopsies (3 mm) and tumor were fixed in 10% buffered formalin and embedded in paraffin; 20 unstained serial sections (4 µm thickness) were mounted on poly-L-lysine-coated slides stored at room temperature. Standard avidin-biotin-peroxidase complex methodology used the following antibodies: EGFR (clone E30) and HER-2 (clone cB-11; BioGenex, Inc.), vascular endothelial growth factor (VEGF; clone c-1), matrix metalloproteinase-9 (MMP-9; clone c-20) and p27 (clone F-8; Santa Cruz Biotechnology), and Ki67 (Biomedica Corp.). Slides were deparaffinized twice in xylene and rehydrated through graded ethanol to distilled water. Antigen was retrieved by

enzymatical digestion with proteinase K (0.5 mg/mL; Boehringer Mannheim). Endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide for 15 min. Slides were then incubated with primary antibodies and visualized using 3,3'-diaminobenzidine chromogen and counterstained with Mayer's hematoxylin. Appropriate positive and negative controls for each antibody were concurrently processed. EGFR, HER2, VEGF, and MMP-9 expressions were measured according to semiquantitative criteria: 0, negative staining; 1+, 1% to 20% positive cells; 2+, 21% to 60% of positive cells; and 3+, >61% of positive cells. Ki67 and p27 nuclear staining were measured as a percentage of cells positively stained to the overall tumor area present. Biomarker data were summarized collectively over all doses, and there were no *P* value adjustments for the analysis of multiple end points; therefore, caution is advised in interpreting the results.

Plasma biomarkers. Venous blood was collected in heparinized tubes at baseline, day 8 of cycle 1, day 15 of each cycle, and at the end of the study. Following refrigerated centrifugation (1,000 × g for 15 min), the plasma was stored at -20°C until shipped to Pfizer, Inc. for analysis. VEGF immunoassay, human interleukin-8 (IL-8) chemiluminescent immunoassay, and human MMP-9 (total) immunoassay were purchased from R&D Systems. The HER-2/*neu* microtiter ELISA assay was purchased from Oncogene Science Diagnostics. Standard curves ranged from 1,000 to 31.25 pg/mL for VEGF and IL-8 following the manufacturer's instructions. Plasma was diluted 1:50 for HER2 analysis and 1:10 for MMP-9, VEGF, and undiluted for IL-8. Optical densities obtained from a Tersamax microplate reader were converted into analyte "units"/mL. Limit of sensitivities were 32 pg/mL for VEGF and IL-8, 0.156 ng/mL for MMP-9, and 1.5 ng/mL for HER2.

Results

Patient characteristics

Fifty-three patients were enrolled in the study between November 1999 and April 2001: 31 patients from Mary Crowley Medical Research Center, Dallas, TX and 22 patients from M. D. Anderson Cancer Center, Houston, TX. The majority of patients had non-small cell lung cancer (34%) or head and neck cancer (28%). Patient demographics are summarized in Table 1.

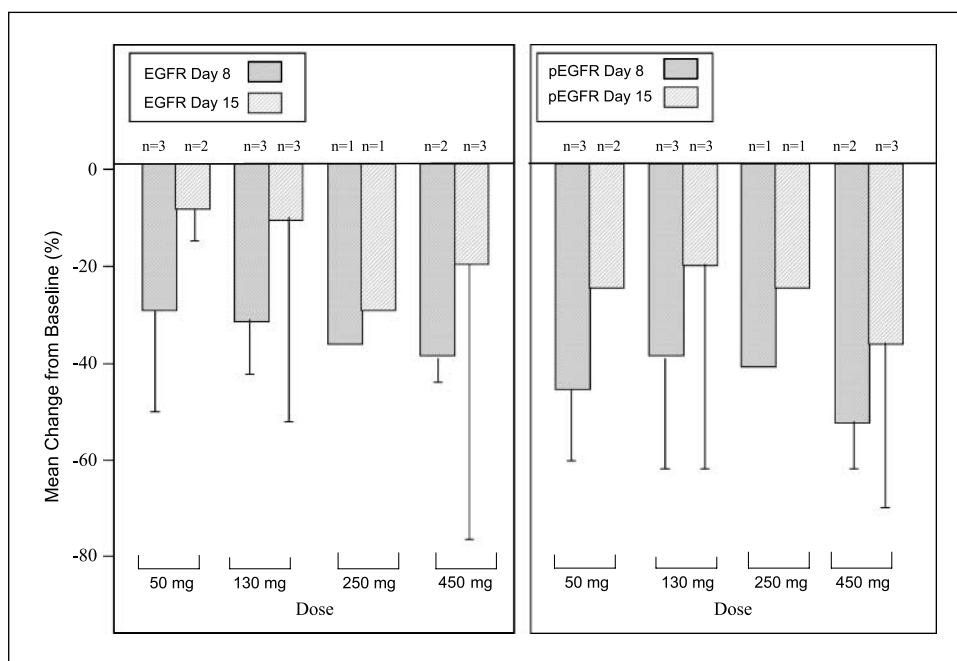
Dose escalation

There were 11 dose cohorts, from 50 to 750 mg (Table 2). At the starting dose of 50 mg, one of three patients experienced a DLT (grade 3 aspartate aminotransferase with grade 2 alanine aminotransferase and grade 3 thrombocytopenia). The cohort

Table 2. Primary adverse events and DLTs in treatment cycle 1

Dose (mg)	No. patients	Grade													
		Liver toxicity		Rash	Nausea	Emesis		Mucositis	Diarrhea		Thrombocytopenia		Hypersensitivity		
		1-2	3	1-2	1-2	1-2	3	1-2	1-2	3	1-2	3	4	1-2	3
50	7		1			1			1				1 ^{DLT}		
70	3					1							1		
95	4			2	1			1	1						
130	5			2	2	2		1	3	1					
180	3			2				2							
250	5			3	3	1			1						
350	3			3	1	1		1	3						
450	4			2	3	3		2	2						
560	8			3	3	3		3	5					1	1 ^{DLT}
650	8			7	5		1	6	6	1			1 ^{DLT}	3	
750	3			2+ (1 ^{DLT})	2	2+ (1 ^{DLT})		1 ^{DLT}	2						
	53	1		29	20	15	1	17	24	1	1	2	1	4	1

Fig. 1. Immunoprecipitation evaluation of EGFR and pEGFR in tumor tissue. Columns, mean; bar, SD.



was expanded to seven patients. Dose escalation proceeded at <40% increments for the next seven cohorts until one of eight patients at the 560 mg cohort experienced hypersensitivity reaction, manifested as angioedema, urticaria, and welts, 5 h after the first CI-1033 dose. In the next dose escalation to 650 mg, one of eight patients experienced grade 4 thrombocytopenia. At the final cohort of 750 mg, two DLTs were observed (grade 3 emesis in one patient and grade 2 emesis, rash, and mouth ulcer in one patient that was collectively dose limiting). The maximum tolerated dose was declared at 650 mg. A total of 205 courses were administered during the study (median, 2; range, 1-30; average, 3.9 courses). The median number of doses per course was 7 (range, 1-7; average, 6.6 doses).

Safety

A summary of adverse events reported during course 1 included diarrhea (25 patients, 47%); rash (29 patients, 55%); mucositis (17 patients, 32%); nausea (20 patients, 38%); vomiting (16 patients, 32%); allergic reactions, including hives, periorbital edema, tongue edema, and asymptomatic wheezing (5 patients, 9%); thrombocytopenia (4 patients, 8%); and a miscellany of other toxicities (Table 2). There were no obvious cumulative toxicities as evidenced by the small number of grade 3 treatment-associated toxicities (1 thrombocytopenia, 1 dehydration, and 1 nausea) in subsequent cycles, and no patients discontinued the study due to treatment-related adverse events. Of those patients experiencing multiple events of rash, most were considered of the same intensity within a patient, and none were reported as worsening following additional courses. Investigator descriptions of rash were consistent with an acneiform or follicular appearance that increased in frequency at higher dose levels consistent with reports from other ERGR inhibitors. Gastrointestinal toxicities were the most predominant adverse events during the study: diarrhea (62%), nausea (47%), mucositis (32%), and vomiting (30%). These events were generally of grade 1 to 2 intensity and were manageable with early intervention and standard treatment.

Hypersensitivity reaction was not evident until the higher dose levels (≥ 560 mg). One patient at the 560 mg dose level experienced angioedema of the tongue accompanied by urticaria and skin welts 5 h post-dose. There were no respiratory manifestations, and this DLT was effectively managed with antihistamine, steroids, and dose reduction. A second patient in the 560 mg dose group experienced mild wheezing on days 4 to 5 that was considered related to underlying asthma. At the 650 mg dose level, one patient experienced pruritis of the hands on day 1 and mild wheezing on days 5 to 7 that did not require dose reduction. Another patient at 650 mg experienced mild periorbital edema, hives, and chest tightness on day 1 that was successfully treated with antihistamine.

Although thrombocytopenia was not frequently reported as a clinical toxicity, an analysis of platelet levels from laboratory data showed that one or more below normal readings were noted in 22 patients (42%): grade 1 to 2 in 16 patients, grade 3 in 5 patients, and grade 4 in 1 patient. Thrombocytopenia was considered dose limiting in two separate cases at 50 and 650 mg. The duration of thrombocytopenia coincided closely with the duration of CI-1033 treatment. There was no clear evidence of a dose relationship or a cumulative dose effect, but more grade 1 to 2 events (12 of 26 patients, 47%) were recorded at doses 350 to 750 mg. However, all five episodes of grade 3 thrombocytopenia occurred in the lower dose cohorts, confounding the association of dose with the observed degree of thrombocytopenia.

Patient status and tumor response

In this end-stage patient population, the majority of patients (56.6%) developed disease progression, often within the first 12-week assessment period. Fourteen patients (26.4%) were removed from the study due to "other reasons" such as declining clinical status that was directly or indirectly related to cancer progression. Five patients discontinued the study due to adverse events that included pneumonia, deep thrombophlebitis, sepsis/acute renal failure, intestinal obstruction, and

hypoesthesia of extremities and slurred speech; none were considered related to CI-1033 treatment. Nine patients died during study or within 30 days of their last dose of CI-1033. Two of these deaths were considered adverse events (pneumonia in one patient and acute renal failure and sepsis in one patient). The other seven deaths were due to progressive disease. Post-study deaths were reported for 21 patients who died due to disease progression and 1 patient who died due to congestive heart failure, pneumonia, and pneumothorax. No deaths were associated with CI-1033 treatment.

Of 40 patients evaluable for disease assessment, there were no confirmed complete or partial responses. Three patients achieved stable disease and remained on study until disease progression (non-small cell lung cancer, 142 days; unknown primary, 373 days), or patient refusal of treatment (non-small cell lung cancer, 139 days). One 74-year-old woman with no prior chemotherapy presented with locally advanced radio-resistant squamous cell skin cancer and showed significant tumor regression after two cycles of CI-1033 at 450 mg. Although complete disappearance of lesions occurred, a partial response was not declared due to small lesion size (<2 cm). She remained on study for 10 months without recurrence before her decision to discontinue treatment for personal reasons. Notably, this patient had high levels of erbB1 and erbB2 receptor expression at baseline, and response was associated with a significant reduction in both pEGFR (-61%) and pHER2 (-41%) following a week of CI-1033 treatment (33).

Pharmacokinetics

Plasma pharmacokinetics was evaluated using population pharmacokinetic analysis on data from the 43 patients in this study combined with data from 29 patients in a second phase I study where patients were dosed once weekly for 3 weeks every month. Pharmacokinetic analysis was done using a one-compartment linear model: NONMEMV and ADVAN2 (34). Peak CI-1033 concentrations were achieved 2 to 4 h after dosing and were dose proportional. Plasma clearance (Cl/F) averaged 266 L/h, whereas the volume of distribution (V_d/F) averaged 1,330 liters, resulting in an apparent plasma elimination half-life of 4 h. CI-1033 did not accumulate with repeated dosing, and there was no evidence to suggest that adverse events were associated with atypical systemic exposure within dose groups. Post hoc analysis suggested that systemic exposure is not dependent upon age, gender, race, weight, or surface area. These findings support once a day dosing without adjustment for body weight or surface area in adult patients (35).

Tissue biomarkers

Tumor tissue EGFR, pEGFR, HER2, and pHER2 using immunoprecipitation methods. Ten patients provided tumor samples from baseline and either day 8 or day 15 that were evaluated for EGFR and pEGFR expression from the 50 to 450 mg dose cohorts. Without exception, there were decreases in both total EGFR protein expression and pEGFR activation by day 8; average decrease was -33% (range, -50% to -9%) and -44% (range, -68% to -17%), respectively. By study day 15, the inhibition of EGFR and pEGFR expression was sustained, particularly at higher-dose cohorts (Fig. 1).

HER2 and pHER2 was examined tumor tissue from seven patients dosed 50 to 450 mg. Tumor samples exhibited both inhibition of HER2 and pHER2 expression [mean reduction was -29.9% (range, -99% to 11%) and -9.1% (range, -44% to 44), respectively] that was also sustained day 15; however, there did not seem to be a strong relationship to dose. Baseline HER2 yielded a noticeably weaker staining signal than for EGFR. Densitometry ranged from 21 to 1,104 o.d. for HER2, whereas densitometry for EGFR ranged from 970 to 3308 o.d. One patient at the 130 mg dose level yielded an abnormally high day 8 HER2 expression of 338% and was considered an outlier.

Immunohistochemical evaluation of Ki67, p27, EGFR, HER2, VEGF, and MMP-9 in tumor and skin biopsies

Tumor tissue. The proliferation marker Ki67 was assessed in tumor samples from five patients on baseline and day 8 yielding an average decrease of -31% (range, -85% to 0.1%). By day 15, Ki67 expression in four patients returned to baseline or higher levels (mean change, 25%). Of interest, it was previously reported that there was high correlation between Ki67 down-regulation and a decrease in expression of pEGFR (Western blot) examined on day 8 in these five patient tumor samples (correlation coefficient = 0.97), thus reinforcing the link between down-regulation of EGFR receptor activation and reduction in an established marker of cell proliferation (ref. 33; Fig. 2).

Alternatively, the cell cycle inhibitor p27 was markedly increased in tumor tissue from seven patients on day 8, with a median increase of 139% (range, -66% to 229%) that was more pronounced at the higher dose levels and suggested a trend toward dose response (Fig. 3). The day 15 values from four patients were variable across the dose groups.

Additional tumor markers, such as EGFR, HER2, VEGF, and MMP-9, were evaluated from paired tumor sets from six patients. No consistent changes in these variables were noted except for a day 8 reduction to 0 or 1+ staining in VEGF (4 of 6 patients).

Skin tissue. Ki67, p27, EGFR, HER2, and MMP-9 expression in skin tissue was examined using immunohistochemical analysis of 23 baseline and paired day 8 posttreatment samples. Twenty-two paired samples were available for VEGF analysis.

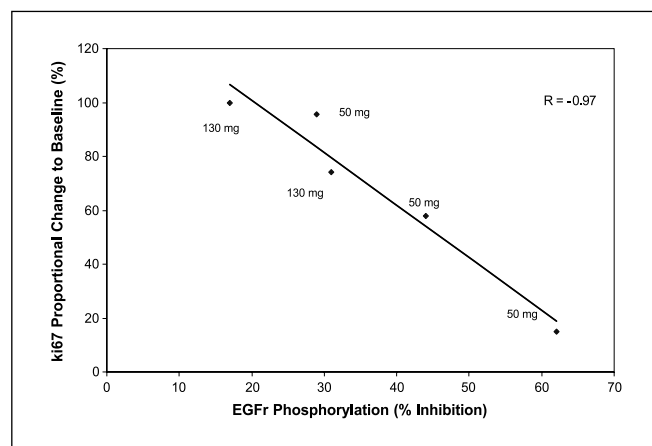


Fig. 2. Comparison of Ki67 and pEGFR in tumor tissue following a week of CI-1033 therapy.

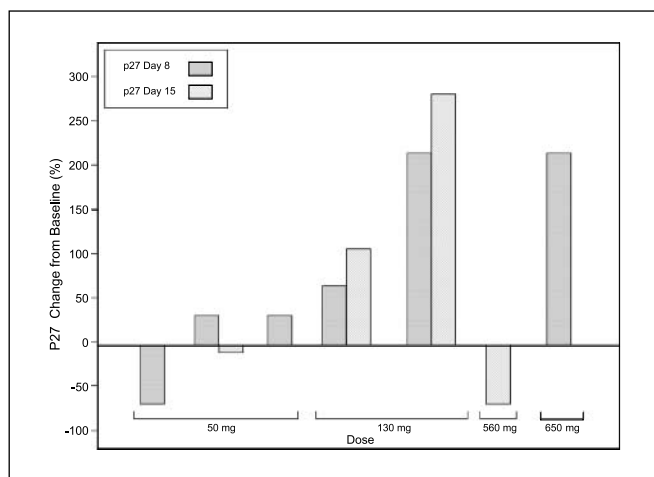


Fig. 3. Immunohistochemical evaluation of p27 in tumor tissue ($n = 7$ patients).

Ki67 expression was down-regulated in 65% of skin samples on day 8 (median, -50%; range, -94 to 322%). By day 15, median Ki67 expression was similarly reduced by -33% (range, -100% to 180%). Of note, the absolute expression of Ki67 in skin tissue was markedly lower than that observed in tumor tissue; baseline Ki67 expression median o.d. was 7% (range, 2-33%) versus 41% (range, 2-83%) in tumor tissue ($n = 12$).

p27 expression on the other hand was up-regulated in 83% of tissues by day 8; median change from baseline for all dose groups was 27% (range, -44% to 791%; $P < 0.003$) and remained elevated by day 15 (median change, 6%; range, -41% to 753%; data not shown). The up-regulation of p27 in skin tissue was more consistent at the upper end of the dose range of 130 to 650 mg, but no dose relationship was established. An examination of potential associations between changes in the intensity of p27 expression with total EGFR, HER2, or Ki67 yielded no strong correlations (36).

EGFR was overexpressed ($\geq 2+$) in almost half (48%) of baseline skin samples. On day 8, there was limited reduction in protein expression (35% increased, 43% no change, 22% decreased) nor was there a consistent change in HER2 (52% no change, 30% decreased, and 17% increased). For further examination of only those patients considered to have high HER2 expression ($\geq 2+$) at baseline, seven (58%) showed reduced expression on day 8, and all of the four tissues with 3+ staining were decreased.

A high proportion of skin tissue (74%) overexpressed VEGF ($> 2+$) at baseline. By day 8, there was no appreciable change in expression levels regardless of dose group. MMP-9 expression was also unchanged on day 8 in 70% of the 23 paired sample sets.

A direct 1:1 comparison of pharmacodynamic markers in matched skin versus tumor samples was available for only four patients due to limited tumor availability. No obvious similarities in the direction of change in biomarker expression for EGFR, HER2, VEGF, MMP-9, Ki67, or p27 could be determined, with the exception of Ki67, which was a clearly decreased in both skin and tumor tissue.

Plasma biomarkers. Plasma samples from baseline and day 8 were evaluated for HER2 (20 patients), VEGF (20 patients), MMP-9 (16 patients), and IL-8 (11 patients) at doses 50 to 650

mg. HER2 showed the most consistent change from baseline with a median decrease of 16% (range, -70% to 45%) that did not seem dose dependent. There were no consistent changes in plasma VEGF or IL-8, whereas MMP-9 showed a general increase on day 8 (median change, 55%; range, -88% to 1,973%; Fig. 4).

Discussion

This phase I trial of CI-1033 in 53 patients with advanced nonhematologic malignancies showed that oral CI-1033 administered for seven consecutive days every 21 days is safe and well tolerated over a wide range of doses up to and including the maximum tolerated dose at 650 mg. Adverse effects were similar to those observed in clinical studies of other agents with similar mechanisms, typically involving skin and gastrointestinal toxicities (37–40). Because erbB receptors have been identified in many body tissues including the skin and gastrointestinal tract, it should be of no surprise that the most common toxicities resulting from CI-1033 therapy would be skin and mucosal associated (41–44). The fact that CI-1033 is an irreversible inhibitor of erbB receptor tyrosine kinase activity, there was the possibility that toxicities could be abnormally prolonged or exacerbated due to sustained receptor inhibition. There was no indication that this was the case because no patients discontinued treatment due to drug-related toxicity, and there was no evidence of accumulation of toxicity over extended dosing intervals. In a subsequent phase I study with CI-1033, it was shown that on a more continuous dosing regimen (21-28 days on, 7 days off), the drug was well tolerated, and there was also no evidence of drug accumulation (41). Two cases of grade 3 thrombocytopenia were recorded at CI-1033 doses of 50 and 70 mg. Although no clear dose relationship was shown, the only case of grade 4 thrombocytopenia was seen at the 650 mg/d dose group. The mechanism for this reaction has not yet been determined, but there is evidence that CI-1033 and other small molecule tyrosine kinase inhibitors are not entirely selective for the erbB family of kinases and can bind to numerous off-targets that contain ATP binding sites (45), thus providing a possible further path for investigation.

Pharmacokinetic data obtained in this trial are consistent with those from other clinical CI-1033 studies (35, 46, 47). The elimination half-life of 4 h does not preclude once-a-day dosing regimen and is supported by preclinical evidence of prolonged inhibition of erbB receptor activity at low CI-1033 drug concentrations (30). CI-1033 did not accumulate with repeated dosing, and there was no evidence to suggest that adverse events were associated with atypical systemic exposure within dose groups. Post hoc analysis suggested that systemic exposure is not dependent upon age, gender, race, weight, or surface area.

Several clinical studies have reported on the modulation of pharmacodynamic end points by small molecule EGFR tyrosine kinase inhibitors; in a phase II study of gefitinib given 500 mg daily to breast cancer patients, the inhibition of pEGFR in both normal and malignant tissues was reported (48). Similarly, in a phase I study of OSI-774, there was a notable decrease in pEGFR expression in skin tissue, whereas the up-regulation of p27 in skin tissue was shown to increase in a dose-dependent manner (49). In the current CI-1033 study, down-regulation of EGFR

and HER2 receptors were observed in variety of tumor tissues using both immunoprecipitation and immunohistochemical methodology, which reinforces the mechanism of action in patient-based tumors. However, as with gefitinib and erlotinib, a dose response was not evident that could limit the utility of EGFR and HER2 as pharmacodynamic end points and raises the possibility of alternative or redundant cellular mechanisms that may be limiting further inhibition of erbB receptor activity by the drug. Inhibition of the activation status of both pEGFR and pHER2 was observed at the starting dose of 50 mg following 7 days of CI-1033 dosing with evidence of continued but somewhat diminished inhibition a week posttreatment. This would reinforce the use of an intermittent treatment schedule without significantly compromising suppression of the tyrosine kinase tumor target. The current 7-day dosing schedule also resulted in tumor response in a patient with squamous cell skin cancer whose pEGFR and pHER2 were markedly decreased following CI-1033 therapy. This limited response rate observed in the current study is very comparable with that seen with erlotinib in phase I testing where one patient with metastatic renal cancer attained complete resolution of several lesions, whereas no partial responses were reported out of 40 patients. Also in an initial phase I study with gefitinib, 4 patients (all non-small cell lung cancer) of 64 treated patients met the criteria for partial response; however, no complete responses were reported (50). Based on these preliminary studies with highly variable patient populations, there does seem to be some limited indication of efficacy in a phase I setting. Signaling

through the erbB receptor pathway requires dimerization between two members of the family. This can be in the form of homodimers or heterodimers, and the nature of subsequent signaling is thought to be dependent on the specific pairing (41). As a pan-erbB inhibitor, CI-1033 effectively conjugates and suppresses signaling across all possible pairs. The hypothesis being tested through CI-1033 is that broad suppression of ligand-mediated signaling through the erbB family may provide benefit comparable with or greater than that provided by suppressing a single member of the family (e.g., erbB2). Rigorous testing of the hypothesis, however, requires adequate, sustained suppression of signaling over the dosing regimen. This may be feasible even with a short half-life compound, such as CI-1033, due to the covalent nature of antagonism. A further requirement for proof-of-concept would be that receptor turnover is slow relative to the dosing interval, allowing continued suppression in the absence of additional, unbound drug. Recent research, however, suggests that erbB turnover is a relatively rapid and complex process of internalization followed by sorting for lysosomal degradation or recycling to the cell membrane (51). With once daily dosing, CI-1033 does not accumulate and may allow erbB-mediated signaling to resume towards the end of each dose interval and almost certainly between courses of therapy. Thus, a longer half-life analogue or more frequent dosing may be required to achieve clinically significant erbB signal suppression with an irreversible inhibitor. However, erbB4 inhibition needs to be monitored given that some reports suggest that erbB4 signaling is advantageous

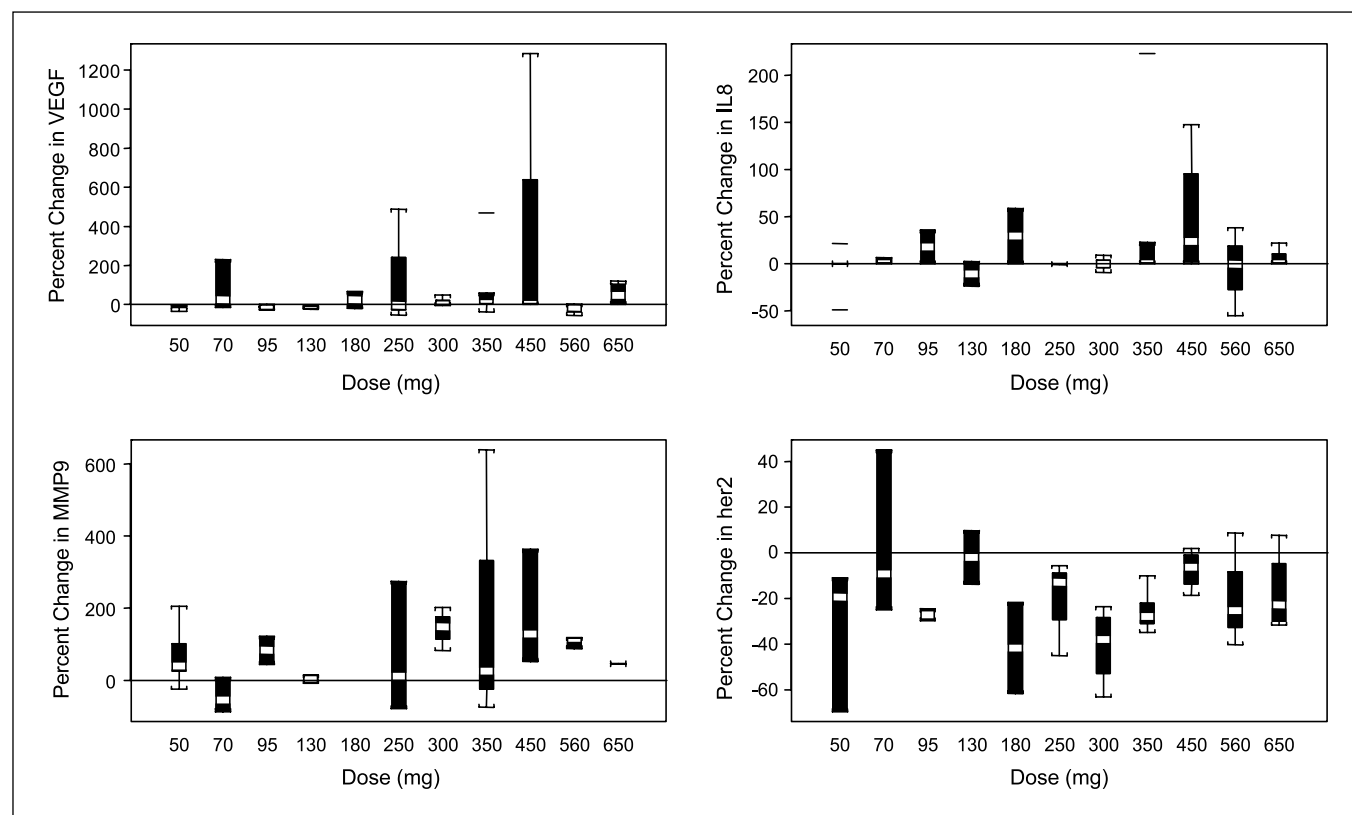


Fig. 4. Percent change in plasma biomarkers following a week of CI-1033 treatment. Bar inside box, median. Box length, 25% to 75% quartile. Whiskers are nearest value not beyond a standard span of $1.5 \times$ (interquartile range). Outliers are drawn individually. Top left, % change in VEGF. Top right, % change in IL-8. Bottom left, % change in MMP-9. Bottom right, % change in HER2.

to antitumor activity. Consequently, inhibition may deter from overall effectiveness of CI-1033.

CI-1033 treatment also resulted in modulation of other biomarkers of antitumor activity, including a reduction in the cellular proliferation marker Ki67 that correlated with a reduction in activation of the EGFR receptor in tumor tissue. In a phase II study of gefitinib, down-regulation of Ki67 and up-regulation of p27 was shown in skin tissue but was not markedly changed in tumor tissue (48). By contrast, CI-1033 yielded a marked increase in the cell cycle inhibitor p27 in both skin and tumor biopsies that seemed to be dose dependent. This raises the possibility that p27 could be used as a potential biomarker in phase II efficacy-driven studies and, if coupled with the current safety data, could be factored into the computation of future dose selection. It was anticipated that CI-1033 would show a modulation of VEGF or IL-8 in plasma based on preclinical experiments in human tumor xenograft models that showed markedly decreased VEGF and/or IL-8 in plasma when CI-1033 was given to rodents (52). There was limited indication of modulation of VEGF or IL-8 in the current phase I study, which may reflect the need to focus on selectively overexpressing tumors for further study. The use of skin tissue markers as a possible surrogate for the more invasive procedure of obtaining target tumor specimens is attractive. Evidence of modulation of pharmacodynamic markers, such as Ki67 and p27, was shown in both skin and tumor tissue, offering the possible utilization of these markers in disease assessment.

Although the modulation of antitumor markers by CI-1033 and other TKI inhibitors has been shown, a clear link to clinical

response has been elusive. Whether certain tumor types are more susceptible to agents like CI-1033 and can be evidenced by specific biomarkers remains to be determined. Currently, the utility of the pharmacodynamic markers, such as p27, that were explored in this study seem to be more applicable to reinforcing dose and schedule selection while providing encouragement in further examining their role as potential biomarkers in efficacy-oriented studies. Continued work on the clinical characterization of patients and genetic profile of tumors may also provide further insight into determining the most efficacious use of these targeted agents and markers of pharmacodynamic activity.

Conclusion

Oral administration of CI-1033 given daily for 7 days in 21-day cycles was safe and well tolerated up to a dose of 650 mg in patients with advanced solid tumors. CI-1033 has a favorable pharmacokinetic profile for daily oral administration. Results also showed evidence of biomarker modulation over a wide range of CI-1033 doses, including inhibition of EGFR and HER2 phosphorylation in tumor tissue accompanied by decreased cellular proliferation signals and an increase in markers of cell cycle inhibition in tumor and skin tissue. Additional clinical trials are warranted in further defining the role of this pan-erbB receptor tyrosine kinase inhibitor in the treatment of cancer and assessing the utility of pharmacodynamic markers in optimizing dose and schedule selection.

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