Phase I and Pharmacologic Study of PKI166, an Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor, in Patients with Advanced Solid Malignancies

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Abstract

Purpose: This phase I study was conducted to assess the tolerability, pharmacokinetics, and antitumor activity of the oral, selective epidermal growth factor receptor tyrosine kinase inhibitor PKI166 in patients with advanced solid malignancies.

Experimental Design: PKI166 was first given once daily continuously and in the second part of the study once daily for 2 weeks every 4 weeks to establish the maximum tolerated dose (MTD). Ten additional patients were studied at MTD to acquire additional safety information and characterize the effect of food intake on PKI166 pharmacokinetics. Pharmacokinetics of PKI166 were characterized after single and multiple doses at all dose levels.

Results: Fifty-four patients received a total of one hundred sixteen 28-day cycles of PKI166. Dose-limiting transaminase elevations were observed in two of seven and two of eight patients using 50 and 100 mg PKI166 continuously. In the second part with PKI166 once daily for 2 weeks every 4 weeks, MTD was set at 750 mg. Dose-limiting toxicity consisted of diarrhea, skin rash, and transaminase elevations. Pharmacokinetic analysis revealed fast absorption, a linear dose-response relationship without drug accumulation after multiple doses. At MTD, no significant influence of food intake on PKI166 pharmacokinetics was observed. Stable disease for more than two cycles was observed in 11 patients.

Conclusions: PKI166 given once daily for 2 weeks every 4 weeks is well tolerated with linear pharmacokinetics, compatible with once daily dosing, and without significant effect of food intake on absorption. The recommended dose for further studies is 750 mg once daily for 2 weeks every 4 weeks.

Amplified epidermal growth factor receptor (EGFR) signaling is frequently found in human tumors and can be caused by various mechanisms (1, 2). As amplified EGFR signaling plays a role in carcinogenesis, inhibiting this process is a rational target for anticancer drug development. Inhibition of EGFR activity can be achieved by monoclonal antibodies that bind to the extracellular domain, antisense oligonucleotides interacting with mRNA to inhibit expression of EGFR, the use of ligands as carriers of cytotoxins, and the use of small-molecule inhibitors of tyrosine kinase activity (3). A large number of these small-molecule inhibitors have been synthesized and several have shown encouraging anticancer activity in both preclinical models and clinical studies (4–7). Erlotinib, one of these small-molecule tyrosine kinase inhibitors, has recently been approved by the Food and Drug Administration for treatment of patients with locally advanced or metastasized non–small cell lung cancer after failure of at least one prior chemotherapy regimen.

PKI166 belongs to the pyrrolo-pyrimidine class of EGFR tyrosine kinase inhibitors and is active in the low nanomolar range, showing high selectivity against serine/threonine kinases and moderate selectivity against other tyrosine kinases (Fig. 1). PKI166 shows potent antiproliferative effects in various EGFR-dependent and/or EGFR-overexpressing cell lines, whereas inhibition of EGFR-independent cell lines is achieved at significantly higher concentrations. Potent growth inhibition is seen in several EGFR-dependent nude mouse tumor models following daily oral administration of doses between 10 and 100 mg/kg (8). In the 253J B-V bladder tumor model producing abundant levels of basic fibroblast growth factor, vascular endothelial growth factor (VEGF), and interleukin-8, it was shown that PKI166 was able to decrease the levels of basic fibroblast growth factor and VEGF in the tumor microenvironment by 48% and 64%, respectively (8). This finding correlated with a significant decrease of blood vessel...
PKI166 was supplied by Novartis Pharma AG (Basel, Switzerland) as hard gelatin capsules, containing either 50 or 100 mg of the active study drug. The capsules were taken once daily in the morning at least 1 hour before breakfast without interruption. A treatment cycle was defined as 28 days of treatment. At days of pharmacokinetic evaluation, PKI166 was taken 2 hours before breakfast. The starting dose was 50 mg once daily, which corresponded with one fifth of the toxic dose low in the rat, the most sensitive preclinical species. Cohorts of three patients were studied, with dose doubling between cohorts until toxicity as defined by two episodes of the National Cancer Institute Common Toxicity Criteria version 2.0 grade 2 toxicity or a single episode of DLT during the first cycle was observed. A Modified Continuous Reassessement Method was used thereafter for dose escalation decisions (9, 10). The MTD was defined as the highest dose level with no more than 25% of patients experiencing DLTs during the first cycle. DLT was defined as the National Cancer Institute Common Toxicity Criteria grade ≥3 neutropenia, thrombocytopenia, or anemia, and/or grade ≥3 nonhematologic toxicity (excluding nausea responsive to antiemetic treatment and alkaline phosphatase elevation), and/or certain grade 2 toxicities (i.e., neurotoxicity, cardiac, or renal toxicity). Intrapatient dose escalation was not allowed. Due to the onset of unexpected grade 3 transaminase elevations, as well as indications of unexpected drug accumulation following continuous treatment, an alternative dosing regimen with PKI166 once daily for 2 weeks every 4 weeks was studied. For practical reasons related to pharmacokinetic evaluation patients used the study drug 15 days instead of 14 days during the first cycle only. In addition, inclusion criteria for serum transaminases were changed to ≤2.5 times upper limit of normal irrespective of the presence of liver metastases.

**Pretreatment and follow-up studies.** Before therapy, a complete medical history was taken and a physical examination was done. A complete blood cell count, including WBC differential and serum biochemistry, which included sodium, potassium, chloride, bicarbonate, creatinine, albumin, total protein, serum transaminases, total bilirubin, calcium, phosphorus, glucose, and alkaline phosphatase were done, as were urinalysis, electrocardiogram, and chest X-ray. Weekly evaluations during the first two cycles and every other week thereafter included history, physical examination, toxicity assessment, and complete blood count including WBC differential and serum biochemistry. Tumor measurements were done every two cycles. Response was assessed using the WHO criteria (11). Patients were allowed to continue treatment in the absence of progressive disease or unacceptable toxicity.

**Pharmacokinetic sampling and data analysis.** For pharmacokinetic analyses, 5-mL blood samples were collected from an indwelling i.v. canula before dosing and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, and 24 hours after administration of the drug on days 1 and 15 of the first cycle. Blood samples were collected in precooled lithium-heparin containing tubes and plasma was separated by centrifugation. Plasma samples were stored at −80°C until analysis using a method involving liquid-liquid extraction and high-performance liquid chromatography with UV absorbance detection (320 nm). The lower limit of quantitation is 10 ng/mL when using 0.2 mL of plasma (12).

Noncompartmental analysis of the data was conducted using WINNONLIN (version 3.2). The pharmacokinetic variables area under the plasma concentration time curve (AUC) from 0 to 24 hours and 0 to infinity, peak plasma concentration (Cmax), and time to peak plasma concentration (Tmax) of PKI166 and its major metabolite PKI166-glucuronide were reported as mean values ± SD. Apparent total body clearance (CL/F) was calculated by dividing the dose by the AUC0-inf. The terminal elimination rate constant (λz) was estimated by linear regression from the terminal concentration time data. The elimination half-life (t1/2) was calculated by dividing 0.693 by λz. The apparent volume of distribution (Vz/F) associated with the terminal phase was calculated by dividing CL/F by λz.

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**Product code:** PKI166 (CSP 75166)

**Chemical name:** (R)-4-[[1-(phenylethyl)amino]-7H-pyrrrole][2,3-d]pyrimidine-6-yl]-phenol

**Molecular formula:** C29H24N4O

**Molecular weight:** 350.39

**Chemical structure:**

![Chemical structure of PKI166](https://www.aacrjournals.org/ClinCancerRes2005;11(19)October1,20056909/)

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**Patients and Methods**

**Eligibility criteria.** Patients with a histologically confirmed diagnosis of an advanced solid malignancy for whom standard therapy options did not exist were eligible. Additional eligibility criteria included age ≥18 years; WHO performance status ≤2; an estimated life expectancy of ≥3 months; adequate bone marrow function (hemoglobin > 6.2 mmol/L, granulocyte count > 1.5 x 10^9/L, platelet count > 100 x 10^9/L), hepatic function (bilirubin within normal limits, serum transaminases (alanine aminotransferase and aspartate aminotransferase) ≤2.5 times upper level of normal or ≤5 times in case of liver metastases), and renal function (creatinine within normal limits; no previous chemotherapy within 30 days (6 weeks for nitrosoureas or mitomycin C), and no surgery within 2 weeks or radiotherapy within 3 weeks. Specific exclusion criteria included impairment of gastrointestinal function that could significantly alter the absorption of PKI166 and the use of medication altering gastric pH (mild antacids were permitted if taken either 2 hours before or after drug administration). This study was approved by local ethics committees and all patients gave written informed consent.

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**Fig. 1.** Chemical structure of PKI166.
**Pharmacodynamic sampling and assays.** For analysis of VEGF, 5-mL blood samples were collected into precooled EDTA-containing tubes at baseline, immediately before dosing on days 1, 8, 15, and 22 of cycle 1; days 1 and 15 of cycle 2; day 1 of further cycles; and 10 hours after dosing on days 1 and 15 of cycle 1. After collection, samples were immediately cooled in an ice bath and plasma was separated by centrifugation within 30 minutes. Plasma samples were kept frozen at −80°C until analysis. VEGF analysis was done in duplicate according to previously described methods (13).

To determine inhibition of EGFR tyrosine kinase activity in the dermis, punch biopsies were taken from the forearm skin at baseline and at days 14 and 28 of cycle 1 in the second part of the study with PKI166 once daily for 2 weeks every 4 weeks. Immediately after removal, samples were placed in Tissue-Tek optimal cutting temperature compound (Sakura Finetek, Torrance, CA) and stored at −80°C. For determination of EGFR tyrosine kinase activity, frozen tissue sections were fixed in cold acetone (−20°C) for 10 minutes, rinsed with PBS, and incubated with a protein block consisting of 4% fish gelatin in PBS (Aurion, Electron Microscopy Sciences, Fort Washington, PA) for 10 minutes. Excess solution was removed and the samples were incubated with mouse anti-phosphoEGFR (Chemicon International, Inc., Temecula, CA; 1:100 dilution in 4% fish gelatin) and incubated overnight in a humidity chamber at 4°C. The samples were washed with PBS 3 × 3 minutes and incubated with protein block for 10 minutes followed by a 1:400 dilution of Alexa 594 conjugated goat anti-mouse IgG (Molecular Probes, Inc., Eugene, OR) for 60 minutes at room temperature. The samples were rinsed with PBS and coverslipped using a Vectashield fluorescence mounting media containing 4’,6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA) and examined in a Zeiss Axioplan microscope (Carl Zeiss, Inc., Thornwood, NY), equipped with a 100-W lamp and a Hamamatsu 5810 CCD camera (Hamamatsu, Hamamatsu City, Japan). Fluorescence images were captured at the same settings to allow for comparison of fluorescence intensities. 4’,6-Diamidino-2-phenylindole staining of nuclei was helpful in the identification of tissue architecture. Results are expressed in a qualitative way (negative, weakly positive, positive, and strongly positive for activated EGFR).

Hair follicle samples obtained at baseline and days 14 and 28 of cycle 1 to determine EGFR tyrosine kinase activity were collected from patients enrolled in the 600- and 750-mg cohorts. At each time point, five hair follicles were obtained, placed in a 15-ml polypropylene tube containing cold acetone, and stored on ice for 30 minutes. Thereafter, acetone was removed and 15 ml of cold calcium- and magnesium-free Dulbecco’s PBS was added. After gently inverting the tube several times, the Dulbecco’s PBS was removed and new Dulbecco’s PBS was added and this procedure was repeated. The hair follicles were stored in Dulbecco’s PBS at 4°C until shipment. To analyze quantitative changes in EGFR tyrosine kinase activity, automated fluorescence measurements using a Laser Scanning Cytometer (LSC, Compucyte, Cambridge, MA) were done on hair follicle samples. Immediately following the second protein block, tissue samples were incubated with a 1:100 dilution of Cy5-conjugated goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA) for 60 minutes at room temperature. The samples were rinsed with PBS and analyzed using a LSC equipped with an air-cooled 20-mW, 488-nm argon ion laser and a 5-mW 632-nm HeNe laser. Cy5 was detected through a 650-longpass filter. The LSC was directed via a computer program to sample 800 regions (otherwise known as phantom contours) within one hair follicle sample. Integrated fluorescence (Cy5 Integral) as well as fluorescence data for individual pixels within the phantom contour were collected by the LSC and expressed as fluorescence signal (Max Pixel). Data were acquired and analyzed with Wincyte acquisition software (Compucyte) and expressed quantitatively.

**Results**

Fifty-four patients, whose characteristics are summarized in Table 1, received a total of 116 cycles of PKI166. The number of patients and cycles given as a function of schedule and dose are listed in Table 2.

With PKI166 given once daily continuously, dose-limiting grade 3 transaminase elevations were observed in two of seven and two of eight patients in the 50- and 100-mg cohort, respectively. In the second part of the study with PKI166 given once daily for 2 weeks every 4 weeks, dose levels studied were 50, 200, 400, 600, 750, and 900 mg. In the 900-mg cohort, three of five patients experienced DLT during the first cycle, consisting of grade 3 skin rash (one patient), grade 3 transaminase elevation (one patient), and grade 3 diarrhea (one patient). Three additional patients were treated at the next lower dose level of 600 mg without experiencing DLT; therefore, an intermediate dose level of 750 mg was explored. Only 1 of 13 patients treated at this dose level experienced DLT during the first two cycles; therefore, 750 mg was set to be the MTD.

**Toxicity.** Occurrence of side effects, as a function of the schedule and dose, is listed in Table 3. Transaminase elevations, diarrhea, skin rash, nausea, and vomiting were the principal toxicities of PKI166. No hematologic toxicity was observed.

Dose-limiting transaminase elevations were observed in both the 50- and 100-mg dose cohorts of the first part of the study with the continuous use of PKI166. The transaminase elevations rapidly normalized after discontinuation of the study drug in three of the four patients. In the fourth patient, progression of liver metastases contributed to the lack of normalization. In the second part of the study with the use of PKI166 for 2 weeks every 4 weeks, two of nine patients in the first cohort of 50 mg had reversible grade 3 transaminase elevations, with maximum values occurring in week 3 or 4. One of these patients continued PKI166 in the same dose and developed grade 2 transaminase elevations in the second cycle, whereas in the third and fourth cycle, no transaminase elevations were recorded. In one patient in the 600-mg cohort, grade 3 transaminase elevations occurred in the fourth cycle. After returning to grade 1, PKI166 dose was reduced to 400 mg; but again, transaminase elevations occurred. In the 900-mg cohort, one patient had grade 3 transaminase elevation in the first cycle. Apart from the grade 3 transaminase elevations, mild transaminase elevations were seen in several other patients. In the dosing groups with PKI166 for 2 weeks every 4 weeks, no relationship was found between the dose of PKI166 and the occurrence of transaminase elevations. Transaminase elevations sometimes occurred in the 2-week period without PKI166 and frequently did not worsen in time with ongoing treatment and sometimes even improved with ongoing treatment. No bilirubin elevations were observed.
Diarrhea was frequently observed in both the continuous and 2 weeks every 4 weeks regimen. The diarrhea was generally mild and easily manageable with loperamide. In addition, in patients with PKI166 for 2 weeks every 4 weeks, the diarrhea was often self-limiting, with spontaneous recovery in the obligatory period of 2 weeks without study drug. Grade 3 diarrhea was only seen in one patient in the 900-mg cohort and diminished with adequate loperamide use in subsequent cycles.

Mild and transient cutaneous toxicity manifested as either dry skin, folliculitis, or skin rash was frequently observed. Skin rash was the most common manifestation and was usually located in the face and the trunk. One patient in the 900-mg cohort experienced a reversible grade 3 skin rash that was painful and itching, covering the neck, chest, back, abdomen, and buttocks and appeared from day 5 onwards. A skin biopsy revealed the classic picture of a toxic dermatitis with a perivascular lymphocytic infiltrate with several eosinophilic and polymorph nuclear cells. The rash almost completely disappeared in the 2 weeks off study drug. In the second cycle using 600 mg PKI166, the skin rash reappeared but did not exceed grade 2.

Other frequently observed toxicities were mild nausea and vomiting. These toxicities were not related to schedule or dose and could be treated effectively with antiemetics such as metoclopramide or domperidone. In those patients who used the PKI166 for 2 weeks every 4 weeks, nausea and vomiting decreased in severity or disappeared rapidly in the period without study drug. One patient had grade 3 nausea and vomiting in the third cycle, which was the reason to discontinue the PKI166 on day 12 of cycle 4. The nausea and vomiting coincided with and were probably caused by concomitant tramadol use.

**Pharmacokinetics.** The pharmacokinetic variables are summarized in Table 4 (data of metabolite PKI166-glucuronide not shown). Representative plasma concentration versus time curves of PKI166 at the MTD of 750 mg for 2 weeks every 4 weeks on days 1 and 15 are shown in Fig. 2. The plasma concentration profiles suggest a multiphasic decline consistent with preclinical findings. $T_{\text{max}}$ after oral administration was ~2 hours indicating fast absorption. PKI166 undergoes metabolism by direct glucuronidation by UDP-glucuronosyl transferase at the phenolic moiety to form the PKI166-glucuronide metabolite. Drugs that undergo metabolism by glucuronosyl transferase have been shown to undergo enterohepatic recirculation. This process was also observed for PKI166 as evidenced by the appearance of a secondary peak in plasma concentration in a number of patients. Both AUC and $C_{\text{max}}$ showed a dose-dependent increase on days 1 and 15. For PKI166, with a $t_{1/2}$ of ~12 hours, the variables on day 15 would be indicative of steady-state pharmacokinetics. The AUC$_{0-24\ h}$ following the first dose was similar to or slightly higher than the AUC$_{0-24\ h}$ on day 15, indeed showing that steady state was achieved. A similar pattern was observed for $C_{\text{max}}$. There was a high degree of variability in individual pharmacokinetic variables with a coefficient of variation in AUC and $C_{\text{max}}$ of 50% to 60%. $C_{\text{max}}$

<table>
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<tr>
<th>Administration scheme*</th>
<th>Dose level (mg)</th>
<th>No. patients</th>
<th>Total no. cycles</th>
<th>Median no. cycles (range)</th>
<th>No. of patients with DLT in cycle 1 or 2</th>
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*Administration scheme: A, PKI166 once daily continuously; B, PKI166 once daily 2 wks every 4 wks.

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**Table 1. Patient characteristics**

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<td>Colorectal carcinoma: 2/7</td>
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**Table 2. Dose escalation scheme**

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<th>Administration scheme*</th>
<th>Dose level (mg)</th>
<th>No. patients</th>
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at steady state is a good indicator of accumulation. If the $C_{\max}$ at steady state is comparable with that of the first dose, there is no drug accumulation; but if $C_{\max}$ at steady state is much higher than that of the first dose, there is significant accumulation. As shown in Table 4, there is no significant accumulation following multiple doses of PKI166 ($R \sim 1$-2). The pharmacokinetic behaviour of the glucuronide metabolite of PKI166 was very similar to the parent molecule.

Food effect evaluation. For the food effect evaluation, pharmacokinetic data were available from nine patients. In four patients, pharmacokinetic data were obtained on day 1 (fasted) and on day 2 (fed). In the other five patients, the reverse sequence was followed. Because pharmacokinetic data showed that no drug accumulation occurred at day 15, the pharmacokinetic data of the fasted and fed state of both groups were analyzed together. The results for both fasted and fed state are for $T_{\max} = 2.22 \pm 0.62$ and $3.30 \pm 0.94$ hours ($P = 0.02$), for $C_{\max} = 1,419 \pm 800$ and $1,303 \pm 837$ ng/mL ($P = 0.64$), and for $AUC_{0-24\text{h}} = 1,1433 \pm 6,530$ and $11,135 \pm 7,659$ ng hour/mL ($P = 0.84$, two-sided paired Student's $t$ tests), respectively. These results suggest that the intake of food only has a significant (but clinically not likely relevant) effect on the absorption rate of PKI166 without significantly influencing peak plasma concentrations or total drug exposure.

Pharmacodynamics. There were no significant changes in serum VEGF levels in response to PKI166 treatment (data not shown). Paired skin biopsy samples of days 1 and 14 or from days 1, 14, and 28 were available for 30 patients. In nine of these patients, no significant changes in EGFR phosphorylation status were observed. In the other 21 patients, the changes observed were relatively minor and seemed random in nature without clear correlation with the dose. In nine patients, a decrease of EGFR phosphorylation was observed on day 14. Of these nine patients with a decrease of activity on day 14, an increase was observed in five patients and a further decrease in two patients on day 28. In six patients, a decrease of EGFR phosphorylation from baseline was observed on day 28. In the remaining six patients, an increase of EGFR phosphorylation was observed either after 14 or 28 days. Paired hair follicle samples for analysis by LSC-automated fluorescence were collected from two patients in the 600-mg dose cohort and eight patients in the 750-mg dose cohort once daily for 2 weeks every 4 weeks. Paired samples of days 1 and 14 and days 1, 14, and 28 were available for 10 and six patients, respectively. The median change of fluorescence intensity from baseline to day 14 was $-29.3\%$ (range, $-58.9\%$ to $25.4\%$). On day 28, the median change from baseline was $-28.7\%$ (range, $-73.1\%$ to $7.95\%$). In general, patients who experienced a decrease in EGFR phosphorylation maintained the decrease through day 28. Exploratory analysis revealed no relationship between pharmacodynamic markers and one of the pharmacokinetic variables $AUC$, $C_{\max}$ or $t_{1/2}$.

Antitumor activity. There were no tumor responses observed. Stable disease lasting more than two cycles was seen in 11 patients (two in the continuous dosing group and nine in the intermittent dosing group). The median number of cycles in these patients was 4 (range, 3-8). There was no apparent relationship between the occurrences of stable diseases lasting more than two cycles and the dose, although 5 of 13 patients from the 750-mg cohort for 2 weeks every 4 weeks were on study for more than two cycles (range, 3-8).

Discussion

This phase I study was initially designed to evaluate the feasibility of oral administration of PKI166 in a continuous daily schedule. However, due to the occurrence of grade 3 transaminase elevations in a significant number of patients in the first two dosing cohorts, it was decided to study an alternative dosing regimen with PKI166 given once daily for 2 weeks every 4 weeks. This change of regimen was also supported by preliminary pharmacokinetic data suggesting drug accumulation. With this regimen of PKI166 for 2 weeks...
Table 4. Pharmacokinetic parameters of PKI166 (mean ± SD)

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>No. of Patients</th>
<th>AUC_{0-24} (ng h/mL)</th>
<th>(C_{\text{max}}) (ng/mL)</th>
<th>(T_{\text{max}}) (h)</th>
<th>(t_{1/2}) (h)</th>
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</tr>
<tr>
<td>50</td>
<td>7</td>
<td>304 ± 121</td>
<td>59 ± 27</td>
<td>1.2 ± 0.9</td>
<td>5.9 ± 2.3</td>
<td>180 ± 88</td>
<td>1,468 ± 855</td>
</tr>
<tr>
<td>100</td>
<td>7</td>
<td>1,126 ± 697</td>
<td>170 ± 116</td>
<td>1.6 ± 0.7</td>
<td>8.9 ± 1.3</td>
<td>104 ± 61</td>
<td>1,287 ± 676</td>
</tr>
<tr>
<td>2 wks every 4 wks</td>
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</tr>
<tr>
<td>50</td>
<td>8</td>
<td>443 ± 216</td>
<td>72 ± 41</td>
<td>1.6 ± 0.8</td>
<td>6.9 ± 3.1</td>
<td>131 ± 65</td>
<td>1,222 ± 743</td>
</tr>
<tr>
<td>200</td>
<td>3</td>
<td>2,628 ± 180</td>
<td>443 ± 220</td>
<td>1.7 ± 0.8</td>
<td>9.1 ± 1.2</td>
<td>64 ± 7</td>
<td>841 ± 45</td>
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<tr>
<td>400</td>
<td>3</td>
<td>3,662 ± 3,128</td>
<td>430 ± 249</td>
<td>5.0 ± 2.7</td>
<td>6.5 ± 2.8</td>
<td>206 ± 210</td>
<td>2,448 ± 3,245</td>
</tr>
<tr>
<td>600</td>
<td>5</td>
<td>13,356 ± 9,686</td>
<td>1,446 ± 876</td>
<td>2.2 ± 0.6</td>
<td>11.8 ± 3.1</td>
<td>54 ± 43</td>
<td>1,025 ± 1,050</td>
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<tr>
<td>750</td>
<td>7</td>
<td>12,023 ± 6,763</td>
<td>1,396 ± 908</td>
<td>3.5 ± 2.5</td>
<td>10.9 ± 3.3</td>
<td>70 ± 53</td>
<td>1,328 ± 1,418</td>
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<tr>
<td>900</td>
<td>4</td>
<td>20,238 ± 3,956</td>
<td>2,354 ± 251</td>
<td>3.0 ± 0.8</td>
<td>10 ± 1.4</td>
<td>37 ± 9</td>
<td>522 ± 104</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not available.

\[ R = \frac{C_{\text{max}}(d15)}{C_{\text{max}}(d1)} \]

Fig. 2. Plasma concentration versus time curves of PKI166, for days 1 and 15 of the 750 mg, 2 weeks every 4 weeks cohort (seven patients).

every 4 weeks, at the recommended phase II dose of 750 mg, the major toxicities were generally mild and consisted of transaminase elevations, diarrhea, cutaneous toxicity, nausea, and vomiting. The diarrhea and cutaneous toxicity are also frequently observed in other early clinical studies with small-molecule inhibitors and monoclonal antibodies of EGFR (4–6). It is suggested that these toxicities have a common underlying mechanism of action, related to EGFR inhibition. The exact pathogenesis of these toxicities is still largely unknown. The cutaneous toxicity is characterized by a rash, predominantly on the face and trunk, whereas with other small-molecule inhibitors of the EGFR frequently an acneiform drug eruption is described. Histopathologic examination in one patient showed an allergic skin reaction with eosinophylic and polymorph nuclear cell infiltrations of the dermis. The rash frequently is only mild or modest in severity and in the intermittent dosing scheme often self-limiting. There is no clear relationship with dose however, which suggests a possible allergic nature of the skin reaction. The diarrhea observed was mild and easily managed with loperamide, on an as-needed basis. After discontinuation of the study drug, a quick recovery of diarrhea was observed. It is suggested that epithelial cells of the gastrointestinal tract contain large number of EGFRs important for maintaining integrity of the intestinal mucosa. Inhibition of these EGFRs could result in damaging of the mucosa resulting in diarrhea. In addition, PKI166-glucuronide, the major metabolite of PKI166, is secreted mainly through the bile. Although the glucuronide is not active in inhibiting the EGFR, deconjugation of PKI166-glucuronide could result in free PKI166 causing EGFR inhibition of the intestinal mucosa. The fact that the diarrhea is dose independent suggests that the PKI166-glucuronide is not responsible for the diarrhea, but individual differences in the deconjugation could be. This of course is hypothetical and would require additional investigations (e.g., measurements of fecal excretion of the study drug and metabolites). The other major toxicity occurring with PKI166 is the transaminase elevation. Although in general, the elevations were quickly reversible with discontinuation of the study drug, in a number of patients, the transaminase elevations peaked in the 2-week period without study drug. Compared with other EGFR tyrosine kinase inhibitors, the hepatotoxicity is not unique, but the frequency of the hepatotoxicity seems higher. Remarkably enough, in a phase I study with OSI-774, mild to moderate hyperbilirubinemia was frequently observed without accompanying transaminase elevations (6).

Two other studies are evaluating PKI166 given once daily continuously and thrice weekly (Monday, Wednesday, and Friday; refs. 14, 15). Preliminary results from these studies support the results from this study, with PKI166 being well tolerated and with the majority of the adverse events being mild. In the once-daily regimen, the most frequently observed toxicities included rash, diarrhea, and reversible transaminase elevations (14). MTD has been defined at 450 mg, with two of four patients experiencing DLTs during the first cycle at the 600-mg cohort, including acute renal failure, fatigue, anorexia, and decrease in performance status. In the Monday, Wednesday, Friday regimen, grade 3 transaminase elevations were observed in 2 of 10 patients at the 400-mg dose level (15).

The pharmacokinetic profile revealed that PKI166 is orally bioavailable, quickly absorbed, and suitable for once daily dosing. Although in the first two dose levels of the continuous
dosing scheme drug accumulation was suggested, in the 2-weeks-every-4-weeks schedule, this could not be shown. A dose proportional increase of drug exposure was observed, and at the recommended phase II dose of 750 mg daily for 2 weeks every 4 weeks, peak plasma levels were achieved that were a thousand fold higher than the concentrations required to inhibit both in vitro EGFR phosphorylation and cellular proliferation assays. In addition, our food effect study clearly showed no influence of food intake on drug exposure.

Information obtained from the skin biopsy was not conclusive with regard to the inhibition of activated EGFR by PKI166. Although a decrease of activated EGFR was observed in a significant number of patients, in 6 of these 15 patients, the decrease was only observed at day 28 after a 2-week period without PKI166. In addition, in six patients an increase of activated EGFR was observed while using PKI166. Although a decrease of activated EGFR was observed, it was not possible to determine whether significant EGFR pathway inhibition can be achieved with PKI166, and whether this reflects clinical response or disease stabilization.

In our study, no formal tumor responses were observed among the 54 patients included. Eleven patients, however, had stable disease for more than two cycles during PKI166 therapy at doses ranging from 50 to 750 mg.

In conclusion, PKI166 is a novel agent belonging to the pyrrolo-pyrimidine class of EGFR tyrosine kinase inhibitors. PKI166 given once daily for 2 weeks every 4 weeks is well tolerated, with linear pharmacokinetics and without significant effect of food intake on absorption and at the MTD of 750 mg achieves biologically relevant plasma concentrations. The recommended dose of this schedule is set at 750 mg once daily.

**Table 4. Pharmacokinetic parameters of PKI166 (mean ± SD) (Cont’d)**

<table>
<thead>
<tr>
<th>No. patients</th>
<th>AUC&lt;sub&gt;0-24&lt;/sub&gt; (ng h/mL)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
<th>CL/F (L/h)</th>
<th>V&lt;sub&gt;f&lt;/sub&gt;/F (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>613 ± 244</td>
<td>92 ± 64</td>
<td>2.21 ± 1.8</td>
<td>12.1 ± 6.7</td>
<td>96 ± 45</td>
<td>NA</td>
</tr>
<tr>
<td>6</td>
<td>2,853 ± 1392</td>
<td>324 ± 210</td>
<td>2.17 ± 108</td>
<td>11.4 ± 2.7</td>
<td>42 ± 19</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>560 ± 331</td>
<td>62 ± 40</td>
<td>1.0 ± 0.0</td>
<td>19.3 ± 17.8</td>
<td>128 ± 101</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>3,334 ± 331</td>
<td>273 ± 116</td>
<td>4.6 ± 5.1</td>
<td>NA</td>
<td>60 ± 6</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>3,555 ± 2,433</td>
<td>403 ± 281</td>
<td>4.1 ± 1.8</td>
<td>7.2 ± 5.2</td>
<td>197 ± 194</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>11,028 ± 4,103</td>
<td>1,128 ± 405</td>
<td>2.6 ± 1.0</td>
<td>60.2 ± 20</td>
<td>60 ± 20</td>
<td>NA</td>
</tr>
<tr>
<td>7</td>
<td>13,727 ± 7,725</td>
<td>1,319 ± 399</td>
<td>1.9 ± 1.2</td>
<td>161 ± 6.7</td>
<td>58 ± 14</td>
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<tr>
<td>4</td>
<td>18,853 ± 8,688</td>
<td>2,159 ± 1,149</td>
<td>2.4 ± 1.2</td>
<td>16.2 ± 9.4</td>
<td>58 ± 32</td>
<td>NA</td>
</tr>
</tbody>
</table>

**References**

10. Goodman S, Zahrak M, Piantadosi S. Some practical improvements in the continual reassessment
Phase I and Pharmacologic Study of PKI166, an Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor, in Patients with Advanced Solid Malignancies


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