Pharmacodynamic Evaluation of the Epidermal Growth Factor Receptor Inhibitor OSI-774 in Human Epidermis of Cancer Patients


ABSTRACT

Background: OSI-774 is an inhibitor of the epidermal growth factor receptor tyrosine kinase (EGFR-TK) currently in clinical development. In preclinical models, the antitumor activity of OSI-774 was directly related to its ability to inhibit the EGFR-TK. On the basis of these data, we hypothesized that inhibition of the EGFR-TK will be required for this agent to be effective in the clinic. This study evaluated the pharmacodynamic effects of OSI-774 in normal skin tissues collected from patients treated with the agent in a Phase I study.

Methods: Patients with advanced cancer who were treated in a Phase I study of OSI-774 underwent a biopsy of normal skin epidermis at baseline and after the last dose of drug in the first course of treatment. The expression and activation of the EGFR, downstream signaling extracytoplasmatic-regulated kinase (Erk), and cell cycle regulator p27 were determined in paraffin-embedded skin tissues using an immunohistochemical method (IHC). The IHC data were analyzed using both a semiquantitative scoring system and an automatic absorbance quantitative IHC method. The number of cells with nuclear staining of p27 per 500 cells was determined. Plasma samples were collected to quantitate OSI-774 plasma concentrations.

Results: A total of 56 skin specimens was collected from 28 patients treated with OSI-774 at doses ranging from 25 to 200 mg/day. There was a significant decrease in phospho-EGFR (Tyr 1173) expression as determined semiquantitatively with OSI-774 treatment [2.75 ± 0.51 (mean ± SD) pretreatment versus 2.36 ± 0.76 after treatment, pair comparison P = 0.01]. The quantitative ratio [(phospho-EGFR/EGFR) × 100] of phospho-EGFR (Tyr1173) decreased from 64.16 ± 36.58 pretreatment to 48.87 ± 35.37 post-treatment (pair comparison, P = 0.02). No significant differences were observed in phospho-Erk (Thr202/Tyr204) expression. The mean number of cells with nuclear staining for p27 increased from 185 ± 101 (mean ± SD) pretreatment to 253 ± 111 post-treatment (pair comparison P = 0.02). A total of 12 (42.8%), 7 (25%), and 14 (50%) patients had >25% variation in the ratio of phospho-EGFR (Tyr1173), phospho-Erk (Thr202/Tyr204), and p27 expression, respectively. Only changes in p27 expression were related to the administered dose of OSI-774.

Conclusions: OSI-774 exerted pharmacodynamic effects in skin tissues of 30–50% of patients treated with the agent. Up-regulation of p27, which is a downstream effect of EGFR inhibition, was dose related. Although there was a significant decrement in phospho-EGFR (Tyr1173), it was not related to the administered dose of OSI-774. On the basis of these findings and the relatively simple and reliable method to measure p27 expression, this biomarker appears to be the most promising and is being evaluated in Phase II studies as a predictor of clinical outcome.

INTRODUCTION

The EGFR3 is a M r 170,000 plasma membrane glycoprotein composed of an extracellular ligand-binding domain, a transmembrane lipophilic segment, and an intracellular TK domain (1, 2). Binding of activating ligands such as the epidermal growth factor and transforming growth factor α to the extracellular ligand-binding site of the receptor results in receptor dimerization, activation of its intrinsic TK activity, and autophosphorylation of the receptor. Five sites of in vivo autophosphorylation have been identified in the EGFR: three major (Tyr1068, Tyr1148, and Tyr1173) and two minor (Tyr992 and Tyr1086; Refs. 1 and 2). Phosphorylation of the EGFR leads to activation of a signal transduction cascade of biochemical and physiological changes, which culminate in DNA synthesis and cell division (1, 2). Compelling data indicate that dysregulation of the EGFR signal transduction pathway plays a critical role in the process of tumor formation, growth, and metastasis, hence representing an attractive target for anticancer therapy (3). Over

Received 11/14/02; revised 2/17/03; accepted 2/25/03.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.


2 To whom requests for reprints should be addressed, at The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, 1650 Orleans Street, Room 1M88, Baltimore, MD 21231. Phone: (410) 502-9746; Fax: (410) 614-9006; E-mail: mhidalg1@jhmi.edu.

3 The abbreviations used are: EGFR, epidermal growth factor receptor; TK, tyrosine kinase; C ssmin , minimum steady-state concentration; Erk, extracytoplasmatic-regulated kinase; TBS, Tris-buffered saline.
the last few years, a number of new drugs targeting the EGFR have been synthesized and are actively being evaluated in clinical trials (4–6).

OSI-774 [6,7-Bis (2-methoxy-ethoxy)-quinazolin-4-yl]-[3-ethylphenyl]amine is a low molecular weight quinazoline derivative that inhibits the EGFR-TK and exerts antiproliferative effects (7, 8). The agent specifically inhibits EGFR-TK phosphorylation and, consequently, results in cell cycle arrest and induction of apoptosis (7). Administration of a single dose of 10 mg/kg OSI-774 to mice bearing the HN5 human head and neck tumor xenograft resulted in >50% in vivo inhibition of EGFR autophosphorylation in tumor tissues. After a single p.o. dose of 100 mg/kg maximum inhibition of EGFR autophosphorylation was evident 1 h after dosing, persisted over an inhibition range of 75–85% for >12 h, and subsequently declined with recovery to baseline function by 24 h (8). The preclinical studies conducted with OSI-774 have clearly established that the pharmacodynamic effects of the agent in the targeted receptor are plasma concentration dependent and that there is a linear relationship between the inhibition of the EGFR-TK and tumor growth inhibition. These data suggest that the evaluation of biological effects of OSI-774 may be an important end point to optimally develop this agent.

OSI-774 has successfully completed Phase I evaluation and is currently in disease-oriented Phase II–III studies (4). A Phase I and pharmacological study of OSI-774 administered on a continuous p.o. dosing schedule conducted at our institution demonstrated a maximum tolerated dose of 150 mg/day on a continuous p.o. dosing schedule. Dose-limiting toxicities consisted of diarrhea and acneforme cutaneous rash (4). Concomitant pharmacokinetic analysis indicated a dose-independent increase in plasma levels of the agent and consistent achievement of biologically active plasma concentrations at doses >100 mg/day. In that study, samples of normal skin were collected before treatment with OSI-774 and after the conclusion of the first cycle for histopathological and biological studies. The principal objective of this study was to evaluate the biological effects of OSI-774 on EGFR activation and signaling in epidermal tissues of patients treated with the agent and explore the relationship between dose and plasma concentration of the agent and biological activity.

PATIENTS AND METHODS

Patients. Patients with advanced cancer for whom no standard therapy was available enrolled and treated in a Phase I clinical trial of OSI-774 conducted at the Cancer Therapy and Research Center in San Antonio were candidates for this study. The precise eligibility criteria, clinical trial design, and clinical and pharmacological results have been published previously (4). Briefly, eligible patients were treated with escalating doses of OSI-774 in three successive schedules of administration. In Part A, patients received OSI-774 at doses ranging from 25 to 100 mg daily for three consecutive days each of three consecutive weeks followed by 1 week of rest. In Part B, patients received OSI-774 at doses ranging from 50 to 200 mg daily for three consecutive weeks followed by 1 week of rest. In Part C, the maximum tolerated dose of 150 mg determined in Part B was administered on a continuous daily basis.

Skin Tissue Acquisition. Skin biopsies were performed at baseline before treatment administration and after the last dose of the first cycle of treatment. A 5 × 5 cm area of normal appearing skin on the upper back was cleaned with an antiseptic and anesthetized with 2% lidocaine. Biopsies were obtained using an 8 mm (depth) × 4 mm (width) punch skin biopsy device. The specimen was next embedded in an orthonine carbamyl transferase gel (10.24% w/w polyvinylalcohol, 4.26% w/w polyethylene glycol, and 85.5% nonreactive ingredients) and snap frozen in a methanol and dry ice bath. Tissues were stored at −80°C until analysis. Total EGFR, total Erk, phospho-Erk (Thr202/Tyr204), and p27 were analyzed on frozen sections. Before the analysis of phospho-EGFR (Tyr1173), specimens were thawed and fixed in 10% neutral buffered formalin and embedded in paraffin.

Immunohistochemistry. Skin specimens were assessed for the expression of total and phospho-EGFR (Tyr1173), total and phospho-Erk (Thr202/Tyr204), and p27 at our institution. For total EGFR, total and phospho-Erk, and p27 staining, 4-μm frozen tissue sections were cut and fixed in acetone. Slides were rinsed sequentially in PBS and TBS-T [0.5 M Tris HCl (pH 7.6), 0.15 M NaCl, and 0.15% Tween 20]. Endogenous peroxidase activity was quenched by incubation in TBS-T containing 0.1% sodium azide and 0.3% hydrogen peroxide. Slides were incubated in primary antibody [anti EGFR (Zymed Labs), diluted at 1:80, total Erk (Santa Cruz Biotechnology), diluted at 1:500, phospho-Erk (Thr202/Tyr204; Cell Signaling Technology), diluted at 1:100, p27 (Transduction Laboratory; diluted at 1:25) in TBS-T containing 1% ovalbumin, and 1 mg/ml sodium azide (60 min) in biotinylated rabbit antismouse antibody (E-354; DAKO Corp., Carpinteria, CA)] diluted at 1:100 in 1% ovalbumin in PBS. Slides were counterstained with hematoxylin, dehydrated in graded alcohol, cleared with xylene, and mounted with xylene-based permanent mounting medium.

A specialized staining system from DAKO called catalyzed signal amplification system was used for staining with anti-phospho-EGFR (Y1173) antibody (Calbiochem, San Diego, CA). This system incorporates a signal amplification procedure based on the peroxidase-catalyzed deposition of a biotinylated phenolic compound, followed by a secondary reaction with streptavidin peroxidase. These two steps follow the streptavidin step in the procedure described above. In this procedure, 4-μm tissue sections were heated to 60°C and rehydrated in xylene and graded alcohol. Target Retrieval Solution (DAKO) was used to unmask antigenic epitopes. Endogenous biotin activity was blocked with avidin/biotin blocking kit (DAKO). Primary antibody-phosphorylated EGFR (Calbiochem) was diluted at 1:25. For each antibody used, a parallel slide was also stained as negative control where mouse isotypic IgG was used in place of the primary antibody.

Immunohistochemistry Analysis. The staining intensity was semiquantitatively assessed in each sample by scoring from 0 (no staining), 1+ (weakly positive staining of tumor cells), 2+ (moderately positive staining of tumor cells), and 3+ (strongly positive staining of tumor cells) for EGFR, phospho-EGFR...
EGFR-TK Inhibition by OSI-774

774 Cssmin and pharmacodynamic effect was explored using a test for K-unrelated samples. The relationship between OSI-774 dose and proportional change of the different variables was determined using the equation: proportional score obtained for phospho-EGFR by EGFR. Differences pre and post-treatment was determined by dividing the quantitation was performed on the CAS 2000 Image Analyzer (Becton Dickinson Corp., San Jose, CA). One color channel finds the total area of the tissue. The other channel is specifically matched to have the maximum absorption for the cromogen used to recognize the specific antigen (9). The number of keratinocytes with positive nuclear staining for p27 per 500 nuclei was also determined.

Pharmacological Studies. To study the pharmacokinetic behavior of OSI-774 blood was sampled from a peripheral vein at baseline, before drug administration and at various time points (depending on the specific schedule of administration) after drug treatment. Blood samples were centrifuged immediately after collection, and the recovered plasma was stored at −80°C until analysis. OSI-774 concentrations were quantified by a high-performance liquid chromatography method discussed previously (4). OSI-774 Cminss was calculated as the average of all predosing concentrations measured from day 8 until treatment discontinuation. Only patients treated on Part C had a sufficient number of samples to estimate the Cminss.

Statistical Analysis. Descriptive statistics were used to summarize differences in the intensity staining for EGFR, phospho-EGFR, Erk, and phospho-Erk as a function of OSI-774 dose pre and post-treatment. The proportion of activated EGFR pre and post-treatment was determined by dividing the quantitative score obtained for phospho-EGFR by EGFR. Differences between pre and post-treatment scores of the quantitative variables were compared using the nonparametric Mann-Whitney U test for two related samples. The proportional variation in the different variables was determined using the equation: proportional change = [(post-treatment value − pretreatment value)/post-treatment] × 100. The relationship between the administered dose of OSI-774 and proportional change of the different biomarkers was analyzed using the Kruskal-Wallis nonparametric test for K-unrelated samples. The relationship between OSI-774 Cminss and pharmacodynamic effect was explored using linear and nonlinear pharmacokinetic-pharmacodynamic models. In addition, differences in biomarkers were compared in patients with Cminss of OSI-774 below and above the biological relevant target concentration of 500 ng/ml using the Wilcoxon rank-sum test. The statistical analysis was conducted using the SPSS 11.0 Software.

RESULTS

General. A total of 28 patients treated in the Phase I study with OSI-774 had paired skin biopsies performed before treatment and at approximately the last administered dose of OSI-774 on cycle 1. The demographic characteristics of these patients and clinical results have been recently published (4). Eight (28.6%), 6 (21.4%), and 14 (50%) patients were treated on study Parts A, B, and C, respectively. Three patients (10.7%) each received doses of 25, 50, and 100 mg; 15 patients (53.6%) received the recommended Phase II dose of OSI-774 of 150 mg, and 4 patients (14.3%) received 200 mg on a daily p.o. administration schedule. Fourteen of the 15 patients treated on study Part C had weekly plasma samples to determine OSI-774 Cminss. The mean ± Cminss of OSI-774 in these patients was 1134 ± 799 ng/ml. Twelve patients had Cminss > 500 ng/ml.

Histological Analysis. H&E-stained sections of the skin biopsies were analyzed for the histopathologic effects of OSI-774. The thickness of the entire epidermis and granular layer did not change post-treatment as compared with pretreatment. In 5 of 28 paired patient samples, the stratum corneum was thinner, more compact, more eosinophilic, and had lost its normal basket-weave configuration. There was no significant evidence of apoptosis, and no vacuolar degeneration of the basal layer was seen. The most prominent finding was the presence of superficial perivascular and periadnexal chronic inflammatory infiltrate. Hair follicles were not present in all specimens, but where present, only chronic inflammatory changes with a lymphocytic infiltrate was seen. There was no evidence of acute folliculitis. In one specimen, a prominent follicular plug and microorganisms were found in the dilated infundibulum. No significant changes were seen in dermal sweat glands.

Table 1. Variations in phospho-EGFR and phospho-Erk intensity staining

<table>
<thead>
<tr>
<th>Dose (mg/day)</th>
<th>No. of patients</th>
<th>Pretreatment</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>phospho-EGFR</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>pretreatment</td>
<td>post-treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 + 2 + 3 + 1 + 2 + 3 +</td>
<td>1 + 2 + 3 +</td>
</tr>
<tr>
<td>25</td>
<td>3</td>
<td>0 0 0 3 2 1 0</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>3</td>
<td>0 0 0 3 0 1 2</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>0 0 0 3 0 2 1</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>15</td>
<td>0 3 12 0 4 11</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>4</td>
<td>1 2 1 2 1 1 1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>1 5 21 4 9 15</td>
<td></td>
</tr>
</tbody>
</table>

EGFR Expression and Activation

Semiquantitative Analysis. The EGFR was uniformly detected in the different epidermal strata, hair follicles, and glandular elements of the skin with a 3+ intensity score, which did not change on treatment with OSI-774. The expression of the activated phospho-EGFR was also noticed in the different epidermal structures mentioned above. With regard to the epidermal keratinocytes, the expression of phospho-EGFR (Tyr1173) was more pronounced in the basal layers of the...
epidermis and decreased in the more mature superficial strata. Table 1 summarizes the semiquantitative scores of phospho-EGFR (Y1173) in pret and post-treatment specimens. The score intensity decreased from an average intensity of 2.75 ± 0.51 pretreatment to 2.36 ± 0.76 after treatment ($P = 0.01$). Fig. 1A summarizes the changes in phospho-EGFR per individual patients, and a representative example is shown in Fig. 1B. A total of 9 patients (32%) had evidence of inhibition of EGFR activation in the post-treatment versus pretreatment specimen that was $>50\%$ in 3 of them. No association was observed between the administered dose of OSI-774 and inhibition of EGFR phosphorylation. Likewise, in the subset of patients with pharmacokinetic data, no differences were observed in OSI-774 $C_{\text{min},\text{ss}}$ concentrations in patients with and without evidence of inhibition of EGFR activation.

**Quantitative Analysis.** To obtain a more precise assessment of changes in the activation of the EGFR, the staining intensity was quantitated using an absorbance method as described in the method section in a total of 24 patients. The results of this analysis are depicted in Table 2. The average quantitative staining with the phospho-EGFR antibody decreased from $22.91 \pm 10.45$ to $16.75 \pm 10.03$ ($P = 0.001$), whereas the average quantitative score of the total EGFR increased from $35.58 \pm 10.53$ to $37.08 \pm 11.09$ ($P = 0.186$). Consequently, the ratio of activated receptor decreased from $64.16 \pm 36.58\%$ in the pretreatment samples to $48.87 \pm 35.37\%$ in the post-treatment samples ($P = 0.02$). The proportional variations in the ratio of activated receptor in individual patients as a function of dose are displayed in Fig. 1A. A total of 12 patients (42.8%) had a decrement of $>25\%$ in the ratio of activated receptor in the post-treatment specimen that was $>50\%$ in 10 of them. No relationship, however, was observed between OSI-774 dose and plasma concentration and EGFR inhibition as indicated by the quantitative analysis of phospho-EGFR pre and post-treatment (Fig. 2A).

**EGFR Signaling.** The effects of OSI-774 treatment on signaling pathways regulated by the EGFR were assessed by measuring changes in phospho-Erk (Thr202/Tyr204), a well-
EGFR-TK Inhibition by OSI-774

staining intensity had a nonsignificant decrement in staining scores as a function of OSI-774 dose. The overall score in semiquantitative analysis could be performed. Table 1 depicts the staining intensities of these structures in the different specimens, only a semiquantitative analysis could be performed. Table 1 describes the staining intensity from 2.29 ± 0.71 to 1.96 ± 0.79 (P = 0.14). A total of 7 patients had decrement of >25% in the staining intensity score (Fig. 3, A and B). No association was observed between the administered dose and plasma levels of OSI-774 and the observed effects on Erk activation.

Cell Cycle Regulation. The effects of OSI-774 on cell cycle were determined by counting the number of cells with positive nuclear staining for p27, a cell cycle inhibitor shown to be up-regulated in cells exposed to OSI-774 (7). The number of nuclei staining with the p27 antibody increased from 185 ± 101 pretreatment to 253 ± 111 post-treatment (P = 0.002). A total of 14 of 25 (56%) evaluable patients had up-regulation of p27 in the post-treatment specimen that was of >50% in 8 of them (Fig. 4A). Fig. 4B illustrates a representative patient. In contrast to the other biomarkers explored, up-regulation of p27 was related to the administered dose of OSI-774 (P = 0.02; Fig. 2B). We observed no relationship, however, with plasma concentration in the subset of patients with pharmacokinetic data.

Table 2 Quantitative analysis of the EGFR expression and activation

<table>
<thead>
<tr>
<th>Dose (mg/day)</th>
<th>No. of patients</th>
<th>EGFR Pretreatment</th>
<th>EGFR Post-treatment</th>
<th>p-EGFR Pretreatment</th>
<th>p-EGFR Post-treatment</th>
<th>p-EGFR:EGFR ratio Pretreatment</th>
<th>p-EGFR:EGFR ratio Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>3</td>
<td>25.65 ± 6.65</td>
<td>23.13 ± 11.5</td>
<td>18 ± 6.08</td>
<td>13.33 ± 5.58</td>
<td>53.76 ± 24.58</td>
<td>37.29 ± 20.90</td>
</tr>
<tr>
<td>50</td>
<td>2</td>
<td>30 ± 15.55</td>
<td>42 ± 12.72</td>
<td>19 ± 1.41</td>
<td>19 ± 7</td>
<td>71.75 ± 32.49</td>
<td>44.74 ± 3.27</td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>39.33 ± 17.03</td>
<td>51 ± 9</td>
<td>20.66 ± 12.34</td>
<td>20.33 ± 5.5</td>
<td>52.87 ± 29.71</td>
<td>41.55 ± 17.73</td>
</tr>
<tr>
<td>150</td>
<td>13</td>
<td>34.62 ± 8.80</td>
<td>34.84 ± 9.11</td>
<td>27.46 ± 10.88</td>
<td>18.76 ± 11.78</td>
<td>68.81 ± 45.36</td>
<td>58.97 ± 44.15</td>
</tr>
<tr>
<td>200</td>
<td>3</td>
<td>24.00 ± 12</td>
<td>27.66 ± 11.84</td>
<td>13 ± 4</td>
<td>6.33 ± 2.51</td>
<td>60.64 ± 21.35</td>
<td>26.77 ± 15.57</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>33.58 ± 10.53</td>
<td>37.08 ± 11.09</td>
<td>22.91 ± 10.45</td>
<td>16.75 ± 10.03</td>
<td>64.16 ± 36.58</td>
<td>48.87 ± 35.37</td>
</tr>
</tbody>
</table>

Fig. 2 Box-plot graphics of the proportional variation in the quantitative expression of phospho-EGFR (A) and p27 (B) as a function of OSI-774 dose.

defined downstream mediator in the EGFR signaling pathway. Phospho-Erk (Thr202/Tyr204) staining was not observed in the epidermal epithelium and was restricted to hair follicles and adnexal glands. Because of the variability in the contents of these structures in the different specimens, only a semiquantitative analysis could be performed. Table 1 depicts the staining scores as a function of OSI-774 dose. The overall score in staining intensity had a nonsignificant decrement in staining intensity from 2.29 ± 0.71 to 1.96 ± 0.79 (P = 0.14). A total of 7 patients had decrement of >25% in the staining intensity score (Fig. 3, A and B). No association was observed between the administered dose and plasma levels of OSI-774 and the observed effects on Erk activation.

DISCUSSION

The clinical development of targeted agents, such as the EGFR inhibitors for which a precise intracellular target is known, the agent exerts dose- and concentration-dependent inhibition of the target in preclinical models (8), and there is a correlation between target inhibition and outcome may benefit from the implementation of pharmacodynamic markers. This translational research study evaluated the effects of OSI-774, a specific inhibitor of the EGFR-TK, on the expression, activation, and signaling of the EGFR in a cohort of 28 patients with advanced solid tumors treated with escalating doses of the agent in a Phase I clinical study. The overall objectives of the study were to determine whether or not OSI-774 exerts biological effects in patients treated with the agent and develop a surrogate pharmacodynamic marker of OSI-774 effects suitable for implementation in efficacy-oriented studies and, therefore, evaluation as a surrogate of activity. Three potential markers were assessed, including inclusion of the EGFR, per se, inhibition of the EGFR-regulated downstream signaling molecule, Erk, and expression of the cell cycle inhibitor p27. The principal results of the study indicate that OSI-774 inhibits the EGFR activation and signaling and induces up-regulation of p27 in 30–50% of patients treated with the drug. Although variations in the activation of the EGFR (Tyrt1173) and Erk do not appear to be related to the dose of OSI-774, the number of nuclei with expression of p27 increased in a dose-dependent manner. This finding together with the well-established methodology for p27 staining and relative simplicity of the quantitation method for
make it an attractive biomarker that is currently being incorporated into disease-oriented clinical studies to explore its value as a surrogate of activity. It should be noted, however, that we only measured the effects of OSI-774 on the phosphorylation of residue Tyr 1173, which is one of the five autophosphorylation sites of the EGFR. Whether other phosphorylation sites can be better biomarkers of OSI-774 pharmacodynamic effects remain to be studied. There are some other considerations that could explain the observed results. Phospho-EGFR is notoriously labile and subject to the action of phosphatases; it is plausible that heterogeneity among patients or inadvertent differences in sample handling and storage could affect the quality of the data and will be important that future studies implement rigorous processes for tissues handling, storage, and preservation.

The exploration and implementation of a pharmacodynamic biomarker may have three broad applications in the development of a targeted agent such as OSI-774. These include selection of a biologically relevant dose in Phase I studies, proof of mechanism of action in early Phase I–II studies, and, ultimately, prediction of patient outcome in Phase II/III studies. Although the analysis of pharmacodynamic effects should ideally be conducted in the target tumor tissues, this is rarely possible in clinical trials. The majority of patients enrolled in clinical trials do not have accessible tumor for repetitive tissue biopsies. Furthermore, intensive clinical studies in which the acquisition of tumor tissues for biological studies is the principal objective are complex and usually do not enroll sufficient number of patients to allow correlations between pharmacodynamic parameters and indices of outcome (11, 12). For these reasons, the evaluation of normal tissues as a potential surrogate tissue for pharmacodynamic effects is a reasonable approach. In this regard, the use of normal epidermis appears to be an attractive option for the development of EGFR inhibitors. The EGFR is expressed in the epidermis, skin glandular apparatus, and hair follicle epithelium, where it plays a central role in the normal differentiation and development of the follicle as well as in growth and differentiation of keratinocytes (13, 14). Furthermore, the fact that these agents induce skin toxicity as one of the predominant side effects also suggests that the skin tissue may

![Image](https://example.com/image.png)

**Fig. 3** A, number of patients with variations in the expression of phospho-Erk after treatment; B, immunoperoxidase staining for phospho-Erk: a, normal skin, H&E staining; b, the same specimen stained with an isotypic mouse IgG (negative control); c, immunoperoxidase staining for phospho-Erk in the pretreatment specimen showing strong staining in a glandular structure (3+); d, immunoperoxidase staining for phospho-Erk in the post-treatment specimen showing marked decrement in staining (1+). Magnification: ×400.
be an appropriate tissue to explore pharmacodynamic effects (15).

The principal limitation with this approach, however, is that the relationship between pharmacodynamic effects in the skin and tumor tissues is not known, and their potential parallelism is not self-intuitive. Indeed, both pharmacological and biological factors may result in differences in the response of these tissues to inhibition of the EGFR. The distribution of drugs to tumor tissues with tortuous and disrupted vasculature is not necessarily parallel to that of a normal tissue. More importantly, even if the target receptor is equally inhibited in both tissues, the downstream effects are probably dissimilar. Tumor tissues are well known for their tremendous genetic instability and for harboring mutations that result in constitutive activation of downstream pleiotrophic and complex signaling pathways that function as a mechanism of escape in the setting of inhibition of the EGFR. In this regard, it is of interest to consider that in preclinical models, the factor that correlates with growth inhibition in cell lines exposed to inhibitors of the EGFR is not the degree of inhibition of the receptor but the repercussion of that effect on downstream proliferative and survival functions (15, 16). Therefore, additional preclinical as well as clinical studies, in which tumor tissues and normal skin tissues are collected simultaneously, are needed to validate the use of the skin as a surrogate tissue for pharmacodynamic evaluation of EGFR inhibitors. Preliminary studies, however, suggest that OSI-774 is capable of inhibiting the activation and signaling of the EGFR in tumor tissues of patients treated with the agent (12).

Despite these limitations, the evaluation of sequentially collected normal epidermis has been implemented in the development of novel EGFR inhibitors. Recently, the results of a study analyzing the effects of ZD1839, an inhibitor of EGFR-TK with a mechanism of action similar to OSI-774, have become available (17). In this study, patients enrolled in a Phase I trial of ZD1839 who received doses of the agent ranging from 150 to 1000 mg/day had a biopsy of normal skin performed before treatment and after 28 days of continuous treatment. A total of 32, 31, and 23 patients had paired biopsies evaluated for activation of the EGFR, mitogen-activated protein kinase, and p27, respectively. There was a significant reduction in the number of staining keratinocytes for each of the three markers that basically was abolished or markedly reduced in the post-

![Fig. 4 A, number of patients with variations in nuclear expression of p27 after treatment; B, immunoperoxidase staining for p27: a, normal skin, H&E staining; b, the same specimen stained with an isotypic mouse IgG (negative control); c, immunoperoxidase staining for p27 in the pretreatment specimen showing scattered staining in the epidermal layer; d, immunoperoxidase staining for p27 post-treatment showing marked increment in the number of cells staining. Magnification: ×400.](image-url)
treatment specimens. In addition to the markers mentioned above, that study also evaluated the expression of maturation markers, proliferative index Ki-67, and apoptosis and found significant variations with treatment in all of them. No association was observed between ZD1839 dose and plasma concentration and biological effects.

The results from this study complement and expand the data obtained with ZD-1839 as well as identify areas of discrepancies that will need further analysis. In this study, although the staining intensity for phospho-EGFR and phospho-Erk decreased in the post-treatment specimen, all specimens still stained positive, albeit at a reduced intensity, in the post-treatment specimen, making it impossible to develop a quantitative analysis based on the percentage of staining keratinocytes. The principal analysis of this study, therefore, focused on the evaluation of staining intensity by both manual and automatic means. At this juncture, however, which is the best method of scoring is not known, and what adds the quantitative analysis remains to be determined in future studies. Although significant differences were observed in phospho-EGFR expression in agreement with the previous report, no significant differences were observed with regard to phospho-Erk. Notwithstanding the fact that these two drugs, although similar in mechanism of action, are distinct, differences pertaining antibody used, methods of antigen retrieval, and procedures to preserve specimens may explain these discrepancies. The nuclear expression of p27 was evaluated using a similar methodology based on the percentage or absolute number of nuclei staining positive for p27. Both studies demonstrated a significant up-regulation in p27; however, the present study also found a positive correlation between p27 up-regulation and administered dose. It should be noted, however, that although skin samples were available from patients treated with the starting dose of 25 mg of OSI-774, samples were only available from patients treated with doses of ZD-1839 of 150 mg, which is two dose levels above the starting Phase I dose of 50 mg. It is also of interest to note that an additional study that evaluated the effects of C-225, a monoclonal antibody against the extracellular domain of the EGFR, also reported up-regulation of the p27 cell cycle regulator in paired skin biopsies of patients treated with the agent reinforcing the potential value of this biomarker to measure the biological effects of EGFR-interacting agents (15). Finally, the results of this study are presented considering not only global differences in biomarkers pretreatment and after treatment but also the variation in the quantitative assessment of the different markers in individual patients. This approach permits the classification of patients as a function of biological response. Interestingly, the proportion of patients noted to have pharmacodynamic effects is in the range of 30–50%, which is similar to the proportion of patients who derive clinical benefit in the preliminary results of the Phase II studies conducted with this compound (18–20). The heterogeneity of patients treated in this Phase I study precludes examining correlations between pharmacodynamic affects and outcome that will need to be the subject of prospective Phase II and III studies, in which skin biopsies are incorporated.

In summary, the results of this study indicate that OSI-774 exerts biological effects in normal epidermis of patients treated with the agent. Expression of p27 appears to be the more robust marker for reasons mentioned above and will be the principal measurement in future evaluations. Questions to be answered in future studies include validation of normal skin as a surrogate for pharmacodynamic effects and clinical outcome. The combined data from this study and the study with ZD1839 suggest, however, that the use of normal skin for biological studies could be potentially useful in the development of EGFR inhibitors and should be further evaluated. In addition, whether the other tyrosine phosphorylation sites would be useful biomarkers for EGFR inhibitors remains to be studied. To fully exploit and credential this normal tissue for biological studies, however, staining and quantitation procedures would need to be standardized, and this will require additional efforts and collaborations from investigators, corporate sponsors, and governmental organizations.

REFERENCES


Pharmacodynamic Evaluation of the Epidermal Growth Factor Receptor Inhibitor OSI-774 in Human Epidermis of Cancer Patients


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/9/7/2478

Cited articles
This article cites 18 articles, 7 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/9/7/2478.full#ref-list-1

Citing articles
This article has been cited by 30 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/9/7/2478.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.