Assessment of Erlotinib in Chemoresponse Assay

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Abstract. Background: There is a need to identify the subset of patients sensitive to epidermal growth factor receptor (EGFR) inhibition prior to using such treatments. Materials and Methods: Three non-small cell lung cancer (NSCLC) cell lines (H292, H358, and Calu3) and 34 primary human lung tumor specimens were tested for chemoresponse to erlotinib. Results: The assay distinguished responsiveness to erlotinib among NSCLC cell lines and human lung tumor explants. The H292 cells were responsive, the Calu3 cells were intermediate responsive and the H358 cells were non-responsive. These results were consistent with published tumor growth inhibition by erlotinib in xenografts derived from these cell lines. Out of the 34 patient specimens, 3 (8.8%) were responsive to erlotinib, 7 (20.6%) were intermediate responsive and 24 (70.6%) were non-responsive. Conclusion: The in vitro chemoresponse assay profile was similar to that noted for human tumors in clinical trials. Chemoresponse testing may help predict patient response to erlotinib and assist chemotherapy decision-making.

Lung cancer remains the leading cause of cancer-related deaths, with over 160,000 Americans expected to die of this disease in 2008 (1,2). Among the types of lung cancer, non-small cell lung cancer (NSCLC) accounts for 80% of all lung cancer cases and causes 3 million deaths world-wide per year. Despite the continued development of new chemotherapeutic agents, combination chemotherapy in NSCLC typically yields unacceptably low response rates of between 20% and 35% and prolongs survival by only a few months (3,4). Attention has recently been directed to drugs that target specific signaling molecules involved in cell proliferation pathways.

Activation of the epidermal growth factor receptor (EGFR) tyrosine kinase, which is overexpressed and/or present in a mutated form in many malignancies including NSCLC (5), promotes cell proliferation and malignant transformation. Small molecules have been developed that inhibit the intracellular tyrosine kinase domain, thus blocking EGFR signaling. Two of these EGFR tyrosine kinase inhibitors, gefitinib and erlotinib, have been studied in several clinical trials (reviewed in 6,7). No overall survival benefit was demonstrated in a study adding these agents to first-line platinum-based chemotherapy in patients with NSCLC (8-11). However, subgroup analyses indicated that patients who reported never smoking showed a benefit with the addition of erlotinib (10). Erlotinib showed a survival advantage when administered as monotherapy in the second- or third-line setting (12, 13, 27) and was approved for this indication by the Food and Drug Administration (FDA) in 2004. For gefitinib, subgroup analyses indicated a statistically significant survival benefit in Asians and those reporting having never smoked. However, in both of these situations, not all the patients that fitted these demographic criteria responded to these inhibitors. These findings suggested that identifying the patients who are most responsive to these drugs is clinically important and would aid the further investigation of these agents as first line and adjuvant therapy in selected responsive subpopulations.

Investigations are ongoing to identify biomarkers of response to EGFR inhibitors in NSCLC (reviewed in 14). Several somatic mutations and amplifications in the tyrosine kinase domain of EGFR, as well as increased gene number, have been associated with a likelihood of response to EGFR tyrosine kinase inhibition (15-17, 28, 29). However, a biomarker such as EGFR mutation is not associated with response in all NSCLC patients and may be better suited to provide prognostic, not predictive, information (18, 19). A chemotherapy sensitivity and resistance assay (CSRA) that could reproducibly and reliably predict tumor response to tyrosine kinase inhibition by in vitro assessment would be a valuable additional, and possibly more integrative (of the multitude of mechanisms of sensitivity and resistance), tool that might potentially enhance the selection of responsive subpopulations and might assist clinical decision making.

Response profiling using the ChemoFx® chemoresponse assay has recently been shown to correlate with progression-free interval and overall survival in patients with ovarian cancer. Patients who received a drug(s) to which they had
tested responsive by the assay had a progression-free interval nearly three times longer than patients who received a drug(s) to which they had tested non-responsive (20). Follow-up of the patients with primary ovarian cancer indicated a 33% reduction in risk of death in those receiving an assay responsive drug over those receiving an assay non-responsive drug (21). Furthermore, the assay was also shown to reproducibly and reliably predict pathological response in neoadjuvant breast cancer patients in using as little as 35 mg of tissue (22).

In the current study, the ability of the ChemoFx® assay to distinguish tumor response to erlotinib in lung carcinoma cell lines and primary lung cancer explant cultures was investigated.

Materials and Methods

Cell lines. Three human lung tumor-derived immortalized cell lines were tested in this study: H292, H358 and Calu3 (American Type Culture Collection, Manassas, VA, USA). These cell lines were seeded at 40,000 cells in T25 flasks (PGC Scientifics, Frederick, MD, USA) and allowed to grow for one week to approximately 90% confluence before testing.

Patient tumor specimens. Primary cell cultures were established using tumor specimens procured for research purposes from the following sources: National Disease Research Interchange (Philadelphia, PA, USA), Cooperative Human Tissue Network (Philadelphia, PA, USA), Forbes Regional Hospital (Monroeville, PA, USA), Jameson Hospital (New Castle, PA, USA), Saint Barnabas Medical Center (Livingston, NJ, USA), Hamot Medical Center (Erie, PA, USA) and Windber Research Institute (Windber, PA, USA). The tumors were removed from the patients at the time of surgery, placed in the supplied 125-mL bottle containing sterile McCoy’s shipping medium (Mediatech, Herndon, VA, USA) and shipped overnight to Precision Therapeutics, Inc. laboratories. Primary cultures were initiated and maintained as previously described (23). In brief, tumor specimens were minced into 1 mm³ explants which were then seeded into culture flasks. Upon near confluency, primary cultures were trypsinized and seeded into 384-well microtiter plates (Corning, Lowell, MA, USA) at 8,000 cells/mL for initiation of the assay.

Test agents and vehicles. Erlotinib hydrochloride API was kindly provided by OSI Pharmaceuticals (Melville, NY, USA) as a powder. The drug was reconstituted to 5 mM in 100% DMSO and frozen at –80°C. Drug was thawed and serially diluted in media to create 10 distinct testing concentrations immediately before treatment.

Chemoresponse assay. The cell lines and tissue specimens (and resulting primary cell cultures) were processed and tested with the ChemoFx® assay as described elsewhere (23). Ten doses of erlotinib were prepared by serial dilution. The same 10-dose concentration range was used for the cell lines and the primary cell cultures. For each dose, a cytotoxic index (CI) was calculated according to the following formula: CI=mean cell count_{one}/mean cell count_{control}, which represents the ratio of cells killed as a result of the treatment. The cell counts were the average of 3 replicates at each dose for the primary cultures and 9 replicates at each dose for each immortalized cell line tested (i.e. each assay included 3 replicates, and 3 assays were run per cell line). Dose-response curves were generated using the CI at each dose. The coefficient of variance (CoV) was calculated for each cell line using the Log EC50 values (by dose number) for each assay. Adjusted areas under the curve (aAUC) were calculated for each dose-response curve as previously described (22). The assay results were classified as responsive (R; assay score ≥7.48), intermediate responsive (IR; assay score 6.89-7.47) or non-responsive (NR; assay score ≤6.88).

Results

Cell lines. The three cell lines exhibited heterogeneous responses to erlotinib (Figure 1). The assay prediction of response for the H358 cells was NR, for the Calu-3 cells was IR, and for the H292 cells R (Table I). The CoV for the H292 cells was 7% , for the H358 cells was 9% and for the Calu-3 cells was 3% (Table II).

Patient specimens. Out of the 34 lung cancer patient specimens evaluated in this study, 22 (64.7%) were confirmed to be NSCLC by a pathologist, 11 (32.4%) were of unconfirmed lung cancer subtype and 1 (2.9%) was confirmed as not NSCLC (mesothelioma). The 34 tumor specimens exhibited heterogeneity of in vitro response to erlotinib (Figure 2). Three (8.8%) were assay responsive to erlotinib, 7 (20.6%) were intermediate responsive and 24 (70.6%) were non-responsive.

Discussion

The results of this study indicated that the ChemoFx® assay was able to distinguish tumor response to erlotinib in lung carcinoma explant cultures. The assay was first conducted on the NSCLC cell lines to determine its ability to distinguish in vitro response to erlotinib and to establish the proper range of concentrations. The responses of the three NSCLC cell lines to erlotinib in the current study were similar to the responses observed in previously published studies using other types of chemoresponse assays (24, 25). In addition, the ChemoFx assay was shown to be highly reproducible (i.e. low process variability) in assessing the chemoresponse in the three separate cell lines.

To confirm the range of responses observed, the in vitro sensitivity of these cell lines was compared to the observed outcomes of ex vivo human tumor xenografts derived from those same cell lines as an estimation of correlation with clinical response (26). The corresponding sensitivities supported the hypothesis that the in vitro response might correlate with the in vivo response (Table I).

The assay was also able to distinguish sensitivity to erlotinib among 34 human tumor specimens. The finding that 8.8% of the tumors were responsive to erlotinib was
similar to the 8.9% reported response rate in a phase 3, randomized, double blind, placebo-controlled study of previously treated NSCLC patients (13). However, whether or not the responsiveness in vitro mirrored that in the patient clinically could not be determined as the tumor specimens tested in the current study were research specimens, therefore, the patient demographics and characteristics, treatment regimens and clinical outcomes were not available. Similarly, without information on patient demographics and additional histology the sensitive specimens in the current study could not be compared with previously identified erlotinib-sensitive subpopulations, such as non-smokers (10, 13), women, adenocarcinoma subtype, those of Asian origin (13) and those more likely to develop a rash with treatment (27).

Currently, erlotinib is FDA approved only as second- or third-line treatment for advanced NSCLC. Reports from clinical trials to date have not shown a benefit from erlotinib as a first line treatment (8-11). However, as previously mentioned, subgroup analyses have shown that groups of patients differed in their sensitivity and clinical response to erlotinib (10, 13, 27). Investigators have speculated about the potential findings had the large, first-line studies been conducted on selected populations showing increased sensitivity (6). In this setting, the ChemoFx assay acts as a proxy for the biomarker to identify sensitive subpopulations of NSCLC patients. Much interest has been focused on identifying patients sensitive to EGFR inhibition using molecular profiles (reviewed in 14). The ChemoFx assay, by identifying sensitive tumor cell populations, provides a means to identify other useful biomarkers amid the multitude of mechanisms of sensitivity and resistance.

In conclusion, ChemoFx®, a cell-based chemoresponse assay can distinguish differences in sensitivity to erlotinib among human lung tumor specimens. These preliminary findings justify further investigation of the predictive ability of the assay regarding erlotinib sensitivity in patients with NSCLC.

Table I. Comparison of response to erlotinib treatment: in vitro chemoresponse assay and ex vivo human tumor xenograft outcomes on NSCLC cell lines.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>ChemoFx assay designation</th>
<th>Xenograft TGI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H292</td>
<td>R</td>
<td>85</td>
</tr>
<tr>
<td>Calu3</td>
<td>IR</td>
<td>67</td>
</tr>
<tr>
<td>H358</td>
<td>NR</td>
<td>25</td>
</tr>
</tbody>
</table>

R: Responsive, IR: intermediate responsive, NR: non-responsive; TGI: tumor growth inhibition as reported by Thomson et al. (26).

Table II. Coefficient of variance for the ChemoFx Assay in evaluating response to erlotinib in 3 NSCLC cell lines.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Mean (Log EC50*)</th>
<th>CoV</th>
</tr>
</thead>
<tbody>
<tr>
<td>H292</td>
<td>4.731</td>
<td>7%</td>
</tr>
<tr>
<td>Calu3</td>
<td>6.715</td>
<td>3%</td>
</tr>
<tr>
<td>H358</td>
<td>5.925</td>
<td>9%</td>
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*By dose number; CoV=coefficient of variance.
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References


