Second-Generation Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Non-Small Cell Lung Cancer

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LEARNING OBJECTIVES
After completing this course, the reader will be able to:
1. Describe the magnitude of benefit achieved through the use of first-generation EGFR TKIs in NSCLC.
2. Discuss the clinical obstacles of primary and secondary resistance to first-generation EGFR TKI agents.
3. Describe at least two strategies employed in improving the design of second-generation EGFR TKI agents over their predecessors.

ABSTRACT
Inhibiting epidermal growth factor receptor (EGFR) signaling has proven to be an effective strategy for treating non-small cell lung cancer (NSCLC) patients and the first generation of agents developed for this purpose, gefitinib and erlotinib, stimulated a unique escalation in both biologic and clinical research within the field. Second-generation EGFR-targeted agents that aim to further improve patient outcomes are now in preclinical and clinical trials. This review discusses four promising agents that are currently being studied in NSCLC: EKB-569, HKI-272, CI-1033, and ZD6474. The Oncologist 2007;12:325–330

INTRODUCTION
The epidermal growth factor receptor (EGFR) signaling pathway is an important mediator of cancer cell oncogenesis, proliferation, maintenance, and survival. For this reason, it has long been an attractive candidate as an anticancer drug target [1]. In the past several years, two compounds that target EGFR signaling have made a significant impact on the understanding of the biology of non-small cell lung cancer (NSCLC) and the treatment options for patients suffering from this disease. Both gefitinib (Iressa®; AstraZeneca Pharmaceuticals, Wilmington, DE) and erlotinib (Tarceva®; Genentech, Inc., South San Francisco, CA), the first-generation EGFR tyrosine kinase inhibitors (TKIs), have single-agent activity against advanced NSCLC, and erlotinib improves survival when given as salvage treatment after chemotherapy [2–5]. The subset of patients that benefit from EGFR-targeted TKIs has been partially defined, by clinical characteristics such as nonsmoking history and well-differentiated adenocarcinoma histology, and by molecular characteristics such as somatic activating

Disclosure of potential conflicts of interest is found at the end of this article.
as a result of a secondary acquired
EGFR mutation known as the T790M mutation [12, 13]. In most cases, the specific mechanism(s) of primary or acquired resistance to first-generation EGFR TKIs has not been fully elucidated; however, the general principles of signaling via parallel redundant pathways, constitutive activation of downstream mediators, altered receptor trafficking, efflux of the drug from the cell, and mutation of the drug target itself have been implicated as contributors [12–16]. The combined obstacles of primary and acquired resistance result in a modest survival advantage of 2 months when giving erlotinib as a second-line treatment to an unselected NSCLC population [5].

The second generation of EGFR TKI compounds is now emerging from the developmental pipeline and being introduced into clinical trials. This review describes four of these novel agents that are promising as NSCLC treatments, and focuses on the properties and characteristics by which these drugs attempt to improve upon their predecessors. The two most commonly employed strategies are introducing covalent (irreversible) binding of the drug to the drug target and broadening the affected receptor tyrosine kinase targets of the drug within the cell. The first-generation drugs gefitinib and erlotinib join to their target, the catalytic site in the EGFR TK domain, through classic competitive binding with ATP [17, 18]. In contrast, many of the second-generation compounds form covalent, and therefore permanent, bonds with their target, which should theoretically increase their effectiveness by prolonging the inhibition of EGFR signaling to the entire lifespan of the drug-bound receptor molecule. In cell culture systems, such irreversibly binding TKIs can effectively kill cells that have acquired resistance to first-generation TKIs [15].

The other common theme to the design of the second-generation EGFR TKIs is kinase multitargeting. Gefitinib and erlotinib are both fairly selective for the EGFR TK domain [19, 20]. However, the signaling network that emerges from the ErbB family of transmembrane TK receptors (of which EGFR is a member) is large, interconnected, and redundant, with many possible routes between the ligand at the cell surface and the message destination within the nucleus [21]. It is this diversity in possible signal transduction routes that allows a cell to have flexibility and, in the case of cancer cells treated with targeted anticancer agents, allows for the emergence of resistant cell clones that bypass the inhibited receptor [16]. Blocking multiple signaling pathways with either a combination of agents or a single multitargeted drug has been synergistic in preclinical models [22–25]. Second-generation EGFR TKIs have been developed that, in addition to blocking EGFR signaling, target additional members of the ErbB family such as HER-2 or other downstream or parallel pathways such as the vascular endothelial growth factor receptor (VEGFR) pathway. We now turn our attention to a discussion of selected second-generation EGFR TKI compounds; see Table 1 for a summary.

EKB-569
EKB-569 is a second-generation irreversibly binding inhibitor of EGFR TK activity [26]. Animal studies confirmed that even though the drug itself is rapidly cleared from the plasma, inhibition of EGFR signaling is prolonged, as expected with a covalently bound inhibitor [27]. A phase I dose-escalation study examining two different dose schedules was recently reported [28]. Thirty patients were treated daily for 14 days of a 28-day cycle and 29 patients received continuous daily dosing. The dose-limiting toxicity was grade 3 diarrhea and the maximum-tolerated dose on both schedules was 75 mg/day. Other adverse events included rash, nausea, vomiting, and asthenia. No responses were seen in this phase I study, although 24 patients had stable disease for at least 8 weeks, including one NSCLC patient with stable disease for 33 weeks. Another phase I dose-ranging study with EKB-569 in Japanese patients with advanced solid tumors was also recently completed. The full results of the study have not been published, but a case report described two patients with NSCLC, both harboring EGFR mutations and resistant to gefitinib, with clinical responses to treatment [29]. A phase II study of EKB-569 in NSCLC has been completed and results are pending.

HKI-272
HKI-272 is a second-generation TKI that uses both the strategies of covalent binding and multitargeting; it is an irreversible inhibitor of the EGFR and HER-2 receptors [30].
Animal xenograft models demonstrated activity against both EGFR-dependent and HER-2-dependent cancers, though potency was superior against HER-2-dependent cancers [30]. In a phase I dose-escalation trial including 73 patients whose tumors expressed either the EGFR or HER-2 receptor, HKI-272 was shown to be well tolerated, with primary toxicities of diarrhea, nausea, asthenia, and anorexia [31]. The dose-limiting toxicity was grade 3 diarrhea at 400 mg/day, establishing the maximum-tolerated dose as 320 mg/day. Several patients with breast cancer or NSCLC derived clinical benefit (response or stable disease) from treatment with HKI-272 in this phase I trial. Development of this compound as a breast cancer treatment was under way when preclinical research suggested that HKI-272 might be particularly effective against NSCLC that had previously been treated with first-generation EGFR TKIs and had acquired resistance, either through the T790M mutation or other mechanisms [15]. More recently, NSCLC cells that harbor rare mutations in HER-2 and are resistant to erlotinib were found to be sensitive to killing by HKI-272 [32]. Hence, development of this drug as a treatment for NSCLC is moving forward, and an international phase II trial is ongoing, including patients previously treated with gefitinib or erlotinib as well as those that are TKI naïve.

CI-1033
CI-1033 is an irreversible pan-ErbB inhibitor, meaning it inhibits EGFR and HER-2, as well as ErbB-4 [33]. The final ErB family member, ErbB-3, has no intrinsic kinase activity and signals only when dimerized with another ErbB receptor; therefore, blocking TK activity of EGFR, HER-2, and ErbB-4 precludes ErbB-3 signaling [34]. CI-1033 was designed with the hope that a pan-ErbB inhibitor would be more effective than blocking EGFR alone, because activation of EGFR signaling requires dimerization with other members of the family, and because crosstalk between pathways and parallel activation of downstream effectors are important parts of the amplification of the EGFR signal.

Several phase I studies have been performed, examining various oral dosing schedules [34–38]. These studies have shown that the maximum-tolerated dose of CI-1033 is generally lower when the consecutive number of days of treatment administration is increased; for example, 500 mg/day on a once-weekly schedule, 250 mg/day on a 7-day on/7-day off schedule, and 150 mg/day on continuous daily dosing. Diarrhea and rash have been the most common grade 3 and dose-limiting toxicities observed, and thrombocytopenia has also been reported. Patients with various solid tumors have had stable disease on treatment and one patient with squamous cell cancer of the skin had a complete response to treatment that lasted over 20 months [33]. A phase II trial of CI-1033 in NSCLC patients has completed enrollment, though results have not yet been reported.

In addition, preclinical data suggest that CI-1033 may be synergistic with chemotherapy, and a phase I study of CI-1033 with docetaxel has demonstrated the safety of this combination.

### Table 1. Summary of first- and selected second-generation Epidermal Growth Factor Receptor (EGFR) tyrosine kinase inhibitors

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Drug target and corresponding IC{50} (nM)</th>
<th>Type of binding to the target</th>
<th>NSCLC development phase</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gefitinib</td>
<td>EGFR, 30–100</td>
<td>Reversible</td>
<td>FDA approved for a restricted group of patients&lt;sup&gt;b&lt;/sup&gt;</td>
<td>AstraZeneca</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>EGFR, 2</td>
<td>Reversible</td>
<td>FDA approved for second- and third-line NSCLC</td>
<td>Genentech</td>
</tr>
<tr>
<td>EKB-569</td>
<td>EGFR, 39</td>
<td>Irreversible</td>
<td>II</td>
<td>Wyeth</td>
</tr>
<tr>
<td>HKI-272</td>
<td>EGFR, HER-2, 60</td>
<td>Irreversible</td>
<td>II</td>
<td>Wyeth</td>
</tr>
<tr>
<td>CI-1033</td>
<td>EGFR, HER-2, 19 ErbB-4, 7</td>
<td>Irreversible</td>
<td>II</td>
<td>Pfizer</td>
</tr>
<tr>
<td>ZD6474</td>
<td>EGFR, 500 VEGFR-2, 40</td>
<td>Reversible</td>
<td>III</td>
<td>AstraZeneca</td>
</tr>
</tbody>
</table>

<sup>a</sup>IC{50} is the concentration of the drug (in nanomolar) needed to inhibit the signaling of the target receptor by 50% in an in vitro assay. The lower the number, the more the drug blocks the receptor signaling. Note that biochemical IC{50}s listed here were determined using slightly different methods and are therefore not directly comparable.

<sup>b</sup>In June 2005, the labeling for gefitinib was restricted to patients previously receiving the drug and benefiting from it, or patients participating in clinical trials using gefitinib.

Abbreviations: FDA, U.S. Food and Drug Administration; NSCLC, non-small cell lung cancer.
approach [39–41]. A phase II trial of CI-1033 in combination with carboplatin and paclitaxel chemotherapy has also been completed. Finally, the strategy of administering CI-1033 i.v. has been evaluated in a phase I trial, because the bioavailability by this route is three times that of oral administration [42]. It was hoped that i.v. CI-1033 might decrease gastrointestinal side effects from treatment; however, stomatitis, nausea, vomiting, and diarrhea were still the primary toxicities observed, and the inconvenience of the three-times-a-week infusion will likely limit the further study of this approach.

ZD6474

ZD6474 is a dual-kinase inhibitor that primarily inhibits VEGFR-2, but also has moderate anti-EGFR activity [43]. VEGFR-2, also known as KDR, plays an important role in tumor angiogenesis, and its overexpression is associated with a poor prognosis in NSCLC and other cancers [44, 45]. Targeting the VEGF pathway with the monoclonal antibody bevacizumab in conjunction with chemotherapy can improve survival in NSCLC patients over chemotherapy alone [46], and there is early clinical evidence that blocking both EGFR and VEGFR together (without chemotherapy) can be active, as a phase I/II study of erlotinib plus bevacizumab demonstrated a response rate of 20% [47]. Preclinical experiments demonstrated that the dual-kinase inhibitor ZD6474 has antitumor activity, even in systems that modeled resistance to first-generation EGFR TKIs [48, 49]. In a phase I clinical study, ZD6474 was given as a daily oral treatment to 77 patients; the most common adverse events observed were diarrhea, rash, hypertension, and asymptomatic QT-prolongation, and the maximally tolerated dose was defined as 300 mg/day [50].

ZD6474 was evaluated in parallel with gefitinib in a population of 168 previously treated NSCLC patients in a randomized phase II study with the option to crossover to the other drug at the time of progression [51]. The response rate was 8% in the ZD6474 arm and 1% in the gefitinib arm, with a longer progression-free survival time for the ZD6474 arm compared with the gefitinib arm (11 weeks versus 8.1 weeks, respectively; p = .03). No survival difference was noted, perhaps because of the crossover design. A similar trial comparing ZD6474 with erlotinib is ongoing.

Given the successful outcome of combining the anti-VEGF agent bevacizumab with chemotherapy in NSCLC patients [52], a second randomized phase II trial was performed using ZD6474 or placebo in combination with docetaxel chemotherapy in 127 previously treated NSCLC patients [53]. The progression-free survival time was longer with ZD6474 than with placebo (18.7 weeks versus 12.0 weeks, respectively; p = .07, which was interpreted as significant because they had a priori established a p-value of <.2 as significant enough to warrant further study). A phase II trial of ZD6474 in combination with carboplatin and paclitaxel for first-line treatment of NSCLC and a randomized phase III trial of docetaxel with ZD6474 or placebo for previously treated NSCLC patients are currently enrolling patients.

CONCLUSION

Phase I and phase II clinical trial results of the novel second-generation EGFR TKI agents EKB-569, HKI-272, CI-1033, and ZD6474 are intriguing. Further studies are being conducted to establish the utility of these agents in treating NSCLC patients and their role within the existing armamentarium of drugs for this disease. In addition, advances in our understanding of the molecular biology of NSCLC and the effects of blocking various aspects of the oncogenic signaling network within the tumor will help guide the rational development of a third generation of targeted anticancer agents.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

REFERENCES


17 Wakeling AE, Guy SP, Woodburn JR et al. ZD1839 (Iressa): An orally active, irreversible inhibitor of the tyrosine kinase activity of 6,7-disubstituted 4-anilinoquinoline-3-carbonitriles. The design and structure-activity relationships of 6,7-disubstituted 4-anilinoquinoline-3-carbonitriles. The design of an orally active, irreversible inhibitor of the tyrosine kinase activity of the epidermal growth factor receptor (EGFR) and the human epidermal growth factor receptor-2 (HER-2). J Med Chem 2003;46:49–63.

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