1 Introduction

Recent progress in molecular biology has enabled us to better understand the molecular mechanism underlying pathogenesis of human malignancy including lung cancer. Sequencing of human genome has identified many oncogenes and tumor suppressor genes, giving us a better understanding of the molecular events leading to the formation, progression, metastasis, and the development of drug resistance in human lung cancer. In addition, many signal transduction pathways have been discovered that play important roles in lung cancer. Novel strategy of anticancer drug development now involves the identification and development of targeted therapy that interrupts one or more than one pathways or crosstalk among different signal transduction pathways. In addition, efforts are underway that combine the traditional cytotoxic (non-targeted) agents with the biological (targeted) therapy to increase the response rate and survival in patients with lung cancer, especially advanced non-small cell lung cancer (NSCLC).

2 EGFR Inhibitors

EGFR is a membrane protein that serves to transduce the growth signal from the cancer cell environment to its nucleus. It is composed of an extracellular domain that binds to growth factor and an intracellular domain that carries a tyrosine kinase which phosphorylates a variety of downstream molecules leading to cell proliferation and inhibition of apoptosis. Between 50% to 90% of NSCLC overexpress EGFR\[1\], preclinical studies have shown that the inhibition of EGFR led to the impairment of tumor growth. There are two classes of agents that inhibit the function of EGFR, monoclonal antibodies against the extracellular ligand binding domain and small molecules that inhibit the tyrosine kinase of the intracellular domain. Preclinical study suggested that the both classes of agents inhibit cell proliferation and have additive or synergistic cytotoxicities when combined with standard chemotherapy\[2\], although they differ in pharmacodynamics and pharmacokinetics clinically. The third class of EGFR inhibitor in early development is the oral small molecule inhibitors that target EGFR kinase domain. The first oral selective inhibitor of EGFR, gefitinib, was approved for the treatment of advanced NSCLC with EGFR mutations. The American Society of Clinical Oncology (ASCO) recommends the use of gefitinib for the treatment of patients with advanced NSCLC with EGFR mutations. Other small molecule inhibitors include afatinib, erlotinib, and lapatinib. These agents have shown promising activity in clinical trials and are currently being evaluated in various clinical settings.
veloped is the antibody conjugates that combine EGFR monoclonal antibody with the cytotoxin, which destroy the cancer cells that overexpress EGFR. Small molecules that inhibit both EGFR and Her2 (EGFR dur al inhibitor) or one that inhibits all EGFR’s (panErbB inhibitors) are also in early development, some of them are reversible or irreversible. At the moment, it is not clear whether there is any difference in clinical efficacy between the reversible and the irreversible EGFR irre-
hitors. Table I summarizes the EGFR targeted ther-

apy in clinical trials.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Description</th>
<th>Phase</th>
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<tbody>
<tr>
<td>Iressa® (gefitinib)</td>
<td>EGFR TK1</td>
<td>II / III</td>
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<tr>
<td>Erbitux™ (cetuximab)</td>
<td>EGFR MAb (chimeric)</td>
<td>II / III</td>
</tr>
<tr>
<td>Tarceva™ (erlotinib)</td>
<td>EGFR TK1</td>
<td>II / III</td>
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<tr>
<td>ABX-EGF</td>
<td>EGFR MAb (human)</td>
<td>II / III</td>
</tr>
<tr>
<td>PKF-166</td>
<td>EGFR TK1</td>
<td>II</td>
</tr>
<tr>
<td>C8-1033</td>
<td>ParErbBTKI (irreversible)</td>
<td>II</td>
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Table 1: EGFR targeted therapies in clinical trials

Based on the phase I study that demonstrated the safety of gefitinib at the dose of 250 mg or 500 mg daily orally, two large phase II studies were conducted in patients with advanced NSCLC who were chemotherapy naôve (IDEAL-1) or in patients who have received prior chemotherapy (IDEAL-2). The overall response of 19% and 10%, with a median survival of 8 and 6 months respectively were observed. More importantly, as high of 40% of patients achieved symptom relieve with an improvement of quality of life. Because of the preclinical activity of gefitinib in combination with chemotherapy, two large randomized phase III studies were then conducted in patients with advanced NSCLC with good performance status who are chemotherapy naôve. However, gefitinib, when added to carboplatin and paclitaxel (INTACT-1) and gemcitabine and cis-

platin (INTACT-2) failed to increase the response rate, time to tumor progression, median survival and 1-year survival when compared to chemotherapy alone. Nonetheless, gefitinib has been approved by the Food and Drug Administration (FDA) of the United States to use in patients who have failed prior chemotherapy with both platinum and taxanes because of its palliative ef-

fect. In addition, gefitinib when used alone is well toler-

ated with a few side effects such as skin rash and diar-
rhea. The initially reported high incidence of interstitial lung disease in Japan (1.5%) was found to be much less common in the two large randomized phase III trials in USA (0.2%). In addition, gefitinib have been shown to be effective in NSCLC patients with brain metastasis in a small study.

Ongoing studies are now addressing the utility of gefitinib in early stages, adjuvant setting and mainte-
nance therapy of NSCLC. Currently there is an open clinical trial by Intergroup (BR19) that tests the activ-
ity of gefitinib in patients who have received a complete resection of early stage NSCLC, and randomized to receive gefitinib 250 mg versus placebo. The study was later amended to allow chemotherapy in patients with an increased risk of relapse. Lab correlates to study the prognostic and predictive significance of EGFR expression, phosphorylation, and mutation was also incorpo-
rated. Southwestern Oncology Group (SWOG) is test-
ing the activity of gefitinib as a maintenance therapy versus placebo in patients with locally advanced NSCLC who were treated with the concurrent chemotherapy with cisplatin/etoposide and thoracic radiation followed by consolidation with single agent docetaxel. A multi-
center clinical trial of gefitinib in neoadjuvant and adju-
vant therapy is also ongoing in patients with stage III NSCLC who are treated with induction chemotherapy of carboplatin and paclitaxel with the concurrent chest thoracic radiation followed by surgery and additional adju-
vant chemotherapy with carboplatin and paclitaxel. Gefitinib was given in the beginning of the neoadjuvant chemotherapy and continued on post-operatively in con-
current with adjuvant chemotherapy.

There are many possible explanations as to why gefitinib when added to chemotherapy failed to improve the response rate and survival. The most likely explan-

ation is that the two modalities compete to inhibit the same population of proliferating cancer cells. In addi-
tion, gefitinib was given to patient unslected for the presence of EGFR expression. Currently the true preval-
ence of EGFR overexpression in NSCLC is unknown. In addition, there is no standard testing methodology. Furthermore early clinical study demonstrated a lack of correlation between the expression of EGFR and the clinical response. Finally, surrogate markers to assess tumor response in NSCLC have not been validated.
therapy was well demonstrated in another pilot study involving imatinib (Gleevec) that targets the mutated G-Kit tyrosine kinase thought to be expressed in 50% to 70% of small cell lung cancer (SCLC). Imatinib was found to have no anti-tumor activity in a phase II trial of 19 patients with SCLC. Further study demonstrated that only 28% of the tumors expressed G-Kit, none had mutated G-Kit in this patient population. Similarly, early clinical trials of herceptin in NSCLC were disappointing. In two separate phase II trials, herceptin failed to produce additional response when added to gemcitabine and cisplatin and taxanes. Herceptin failed to increase the response rate and survival when added to carboplatin and paclitaxel in another study by Eastern Cooperative Oncology Group (ECOG). Overexpression of Her2 in NSCLC was found to be very rare with 3% prevalence by FISH. Most recently, a provocative report identified somatic mutation of the tyrosine kinase domain of EGFR gene as being a strong predictor for clinical response to gefitinib in patients with NSCLC. Eight of nine patients who responded to gefitinib carried the mutated EGFR while none of the seven patients who did not respond had the mutation. Similar findings were observed in Japan. The EGFR mutations were more frequent in adenocarcinoma (21%) than in other NSCLC (2%); more frequent in women (20%) than in men (9%); and more frequent in patients from Japan (26%) than in those from USA (2%). In vitro, EGFR mutants had enhanced tyrosine kinase activity in response to EGFR and increased sensitivity to inhibition by gefitinib. At the moment, it is not clear if all patients with NSCLC who may be considered for gefitinib should be tested for EGFR mutation.

Erlotinib (Tarceva) is another small molecule EGFR inhibitor that has been well tested in NSCLC. In a phase II trial of monotherapy with 150 mg of erlotinib once a day orally involving 57 patients with advanced NSCLC who had received at least one prior platinum-based chemotherapy, an overall response rate of 15.8%, stable disease of 26.3%, a median survival of 37 weeks and 1-year survival of 48% were achieved. A phase III trial was completed in Canada in patients with advanced NSCLC whose tumor had progressed after first line chemotherapy and randomized to receive erlotinib and placebo. The result of trial is eagerly awaited.

Cetuximab (C225 or Ertibux) is a chimerized monoclonal antibody that binds to the extracellular domain of EGFR, blocking its binding to ligands. It binds to EGFR with high affinity, stimulates EGFR internalization, prevents receptor dimerization and tyrosine kinase phosphorylation. Cetuximab has been tested alone or in combination with chemotherapy in advanced NSCLC showing its clinical activity. Similarly, ABX-EGF, another monoclonal antibody against EGFR was tested in an early phase clinical trial showing activity in NSCLC. The common side effects are skin rash and diarrhea as well.

Much has been learned from the early development of EGFR inhibitors in NSCLC. In addition to the need to identify patient population who are to benefit from the EGFR inhibitors, efforts are also underway to identify patients whose tumor overexpress EGFR especially mutated EGFR and to develop surrogate clinical markers that will evaluate the response to such therapy. Subset analysis from INTACT-1 and INTACT-2 trials demonstrated that female, patients with adenocarcinoma especially bronchioalveolar carcinoma responded better to the therapy. In addition, the response to gefitinib was unrelated to performance status and to the number of prior therapy. Early clinical trial from erlotinib in NSCLC also suggested that the severity of rash may serve as a clinical response marker to EGFR inhibitor and may be used to guide treatment to obtain optimal response. In addition, nonsmoker may respond better than smokers when treated with erlotinib. Finally, research is underway to test the activity of a variety of small molecule EGFR inhibitors including: irreversible vs. reversible and specific vs. nonspecific EGFR inhibitors. GW572016 is an example of dual EGFR inhibitors that inhibits both EGFR and HER2. Table 2 summarizes the various EGFR inhibitors...
in development.

### Tab 2  Small molecule EGFRIs in development

<table>
<thead>
<tr>
<th>Specific and reversible</th>
<th>Nor specific and reversible</th>
<th>Specific and irreversible</th>
<th>Nor specific and irreversible</th>
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<tbody>
<tr>
<td>ZD1839 (Astra Zeneca; Iressa)</td>
<td>GW-572016 (Glaxo Smith Kline)</td>
<td>EKI 569 (Wyeth)</td>
<td>CI-1033 (Pfizer)</td>
</tr>
<tr>
<td>OSI774 (Genentech; Tarceva)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PKF 166</td>
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Cross-talk between different signal transduction pathways in cancer cells has been frequently demonstrated in preclinical models. Inhibition of one pathway would often result in overexpression of another pathway leading to the development of resistance to the original targeted therapy. Indeed, overexpression of EGFR and HER2 have been shown to be a predictor of poor prognosis in patients with stage I NSCLC\(^{[23]}\). Research is underway to target the cross-talk among different single transduction pathways with agents such as dual EGFR inhibitors\(^{[17, 18]}\). In addition, novel approaches are being explored to combine angiogenesis inhibitor such as bevacizumab (Avastin) that targets angiogenesis with erlotinib that targets EGFR to block the cross-talk between the two pathways in NSCLC\(^{[24]}\). ZD6474 (AstraZeneca) is a small molecule EGFR inhibitor that inhibits both EGFR and the vascular endothelial growth factor (VEGF). It is more potent than gefitinib and cetuximab in inhibiting EGFR and VEGF in preclinical experiments respectively. In addition, ZD6474 is active in gefitinib and cetuximab resistant tumor cells\(^{[25, 26]}\). ZD6474 has shown to be active in early phase clinical trial\(^{[27]}\). Randomized phase II clinical trials are ongoing in advanced NSCLC to compare the activity of ZD6474 with gefitinib, in combination with docetaxel or carboplatin and paclitaxel.

### 3 Antiangiogenesis

There are a variety of agents that inhibit angiogenesis such as matrix metalloproteinase inhibitors (MMPI) (marimastat, and BAY112-9566) and agents that target VEGF and its receptors VEGFR. Agents that inhibit the VEGF ligand include monoclonal antibodies (bevacizumab or Avastin HuMV833 and ZC3) and soluble VEGF receptors (VEGF-Trap). Agents that target the VEGF receptors include small molecule inhibitors (SU5416, SU11248, PTK787, ZD6474), antibodies (IMC-1121b), ribozymes (angiozyme) and various agents such as endostatin, thalidomide and celecoxib. However most studies of the antiangiogenesis agents in lung cancer, especially NSCLC, have been disappointing\(^{[28, 29]}\). Hypoxic cytotoxic in such as TX-1102, tirapazamine (TPZ) and TX402 that electively induce tumor cells to p53 independent apoptosis under hypoxic conditions and inhibits angiogenesis are now in early development\(^{[30]}\). ECOG is finishing a phase III trial of carboplatin with paclitaxel and radiation therapy with or without thalidomide in stage III NSCLC with survival as the endpoint. In addition, ECOG is closing a phase II trial of cisplatin plus etoposide and radiation therapy together with antiVEGF monoclonal antibody in extensive stage SCLC. ECOG also closed a trial recently in patients with advanced NSCLC treated with carboplatin and paclitaxel with or without bevacizumab. Intergroup is planning an adjuvant trial in early stage NSCLC with chemotherapy in combination with bevacizumab or placebo.

### 4 Signal Transduction Inhibitors

There are a variety of signal transduction inhibitors in development in NSCLC. A randomized phase III trial was completed recently that tested the activity of antisense protein kinase C (PKC) (Affinitak) with carboplatin and paclitaxel in advanced NSCLC. The study failed to show the benefit of additional Affinitak when combined with chemotherapy. A parallel trial of Affinitak with gemcitabine and cisplatin was discontinued based on the early negative results Bortemumab (Ve-
cide) is a small molecule that inhibits proteasome function, leading to the inhibition of multiple signal transduction pathways, decreased cell growth and survival, induction of apoptosis, reduction of cell migration, inhibition of angiogenesis, and increased cell adhesion. Initial phase I - II trials of bortezomib in NSCLC have demonstrated its activity as a single agent. A randomized phase II trial is ongoing that randomized the patients to receive taxotere versus taxotere plus bortezomib in patients who have received prior chemotherapy in advanced NSCLC. Other signal transduction inhibitors in development in lung cancer include agents that inhibit cell cycle, histone deacetylase and Hedgehog pathway (cyclopamine). Many of the early trials of signal transduction inhibitors suffered from the problem in trial design similar to EGFR inhibitors, with inadequate preclinical data, poor selection of patient population, lack of surrogate markers to assess clinical response, and therefore yielded disappointing results.

5 Apoptosis

Apoptosis plays an important role in the pathogenesis of lung cancer and the development of drug resistance. Increased antiapoptotic proteins have been associated with poor prognosis in NSCLC. Antisense against Bcl-2 (Ave3139) is now in early clinical trial in the treatment of advanced NSCLC. In addition, Apo2L/Trail (Genentech) that activates death receptors in NSCLC is entering phase I clinical trial this month.

Our laboratory has identified a tumor marker BAG-1 in NSCLC. Overexpression of BAG-1 has been identified to an important role in inhibition of apoptosis and the development drug resistance. We have performed the first retrospective analysis of BAG-1 expression in NSCLC using immunohistochemistry. Cytoplasmic expression of BAG-1 was linked to decreased risk of death in patients with NSCLC by Cox regression analysis. BAG-1 was expressed in the majority of NSCLC patients tested. In addition, overexpression of BAG-1 isoforms led to decreased sensitivity to apoptosis and increased resistance to chemotherapy. Further preclinical study of targeted therapy against BAG-1 is underway.

In summary, there are many novel targeted therapies in development that target epidermal growth factor receptors, signal transduction pathways, angiogenesis and apoptosis. Early clinical trials have demonstrated the promise and challenge in the early development of these agents. Future development of such agents requires the identification of specific patient population who will benefit from the therapy, validated testing methodology to detect the presence of the target, the development of surrogate laboratory and clinical markers to assess the response to treatment. Finally, the use of cytotoxic chemotherapy agent in combination or in sequence with the targeted therapy in a particular disease stage needs to be explored.

Reference


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