# HKI-272 in Non–Small Cell Lung Cancer

Kwok-Kin Wong

**Abstract** Somatic mutations in the kinase domain of the epidermal growth factor receptor (*EGFR*) gene are found in ~10% of lung adenocarcinomas sequenced in the United States and in ~30% sequenced in Asia. These mutations are associated with sensitivity to the EGFR inhibitors gefitinib and erlotinib. Many patients who initially respond to erlotinib or gefitinib subsequently relapse. Studies have identified EGFR T790M mutations in tumors from patients who initially responded and then relapsed. The T790M mutation, when combined *in vitro* with treatment-sensitizing EGFR mutations, permits the continued growth of tumor cells in the presence of erlotinib and gefitinib. HKI-272 is an irreversible EGFR/HER/ErbB inhibitor that has been shown to inhibit the growth of T790M mutant cells *in vitro* in human lung cancer cell lines and in murine cells transfected with sensitizing EGFR mutations. A phase I HKI-272 monotherapy trial in patients with solid tumors is close to completion. Preliminary analyses of the trial, presented at the 2006 annual meeting of American Society of Clinical Oncology, showed that HKI-272 can achieve stable disease control for over 6 months in some patients with non–small cell lung cancer that has progressed after treatment with gefitinib or erlotinib. A phase II trial of HKI-272 in non–small cell lung cancer patients has been initiated. HKI-272 might offer benefits to non–small cell lung cancer patients who have relapsed after an initial response to erlotinib.

## Lung Cancer and the Epidermal Growth Factor Receptor Signaling Pathway

The development of effective therapies for patients with advanced lung cancer continues to be a major public health imperative. Research efforts in non–small cell lung cancer (NSCLC) have been primarily focused on the development of targeted therapy directed against the epidermal growth factor receptor (EGFR). EGFR and its ligands, EGF and transforming growth factor α, are expressed in over 80% of NSCLC (1, 2). It is a member of the ErbB family of receptors that includes EGFR (ErbB1), HER2/neu (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). Upon ligand binding, EGFR homodimerizes or forms heterodimers with other members of the ErbB family, resulting in receptor phosphorylation and activation of downstream signaling events. Activated EGFR associates with multiple signaling mediators, such as Shc, Grb2, Src, JAKs, PLD, PLCγ, and PI3K, and subsequently to the activation of signaling transducers, such as ERK1/2, FAK, JNK, STATs, and Akt, resulting in increases in cellular proliferation, motility, adhesion, and invasion, as well as resistance to apoptosis and chemotherapy (3–5).

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## EGFR Kinase Domain Mutations and Lung Adenocarcinomas

The efficacy of gefitinib and erlotinib, two small molecule inhibitors of EGFR, in a subset of patients with NSCLC has further solidified the premise that EGFR is a valid target (6–10). Several groups have independently identified frequent somatic mutations in the kinase domain of the *EGFR* gene in lung adenocarcinoma. These occur in ~10% of lung adenocarcinoma specimens sequenced in the United States and ~30% sequenced in Asia. The mutations are associated with sensitivity to both gefitinib and erlotinib, explaining in part the rare dramatic clinical responses to treatment with these agents (11–13). Subsequent studies by multiple groups have now identified *EGFR* kinase domain mutations from many additional lung cancer patients (14, 15). The gefitinib and erlotinib sensitizing mutations are clustered in three groups: exon 19 deletions and point mutations at G719S and L858R. Thus far, it seems that these kinase domain mutations are more common in adenocarcinomas than in other histologic subtypes of lung cancers, such as squamous cell carcinoma (10, 15). Recent emerging data also suggest that EGFR expression assessed by immunohistochemistry and the *EGFR* gene copy number might play equally important roles in identifying patients more likely to respond and have longer survival when treated with gefitinib or erlotinib (10, 16).

Nearly all patients who initially respond to erlotinib and gefitinib subsequently relapse. Three studies identified EGFR T790M mutations in tumors from patients who relapsed (17–19). In tumor cells that have the sensitizing EGFR kinase domain mutation, these mutants permit continued tumor growth in the presence of erlotinib and gefitinib. Two irreversible inhibitors of EGFR, CL-387,785 and HKI-272, have been shown to inhibit the growth of the T790M resistance mutants *in vitro*

and offer a promising approach to treatment of tumors with acquired resistance (17, 18). Of these two agents, only HKI-272 (Wyeth Pharmaceuticals) is presently in advanced clinical development. It should also be noted that other irreversible EGFR inhibitors, such as BIBW 2992 (Boehringer Ingelheim) and PF299804 (Pfizer), are also being tested clinically in this patient population, although no data have been published regarding their efficacy against the T790M mutation *in vitro*.

## Preclinical Studies with HKI-272

HKI-272 is an irreversible pan ErbB receptor tyrosine kinase inhibitor (20). *In vitro* studies showed that it could block the receptors' tyrosine kinase enzymatic activity at the nanomolar range (20). HKI-272 has been well characterized for its biological activities in several preclinical models. It inhibits the growth of tumor cells in cell lines and xenografts that express EGFR or HER2 (20). *In vitro* studies also showed that HKI-272 can overcome the acquired resistance mutation EGFR T790M that developed in transformed cells with the sensitizing EGFR kinase domain mutations that have been chronically treated with either gefitinib or erlotinib (18). My laboratory has also shown, using the bitransgenic tetracycline–inducible mouse system, that HKI-272 can dramatically shrink murine *de novo* tumors that are driven by the EGFR kinase domain mutants (21). The same inducible bitransgenic mouse modeling approach is used to show that HKI-272 can also shrink *de novo* lung tumors driven by the EGFRvIII mutant, which is present in 5% of human squamous cell lung cancers (22). Recent data from several studies have also shown that HKI-272 can induce apoptosis in tumor cells harboring EGFR or HER kinase domain–activating mutations (23).

## Lung Cancer Clinical Trials with HKI-272

A phase I dose-escalating study sponsored by Wyeth Pharmaceutical was conducted to determine the safety and pharmacokinetics of HKI-272 in patients with solid tumors (24). HKI-272 was given orally on day 1 as a test dose and on day 8 as the start of a continuous once-daily dosing for a 28-day cycle. Additional cycles of HKI-272 were given if it was tolerated and if there was no evidence of disease progression. The primary objective of the study was to determine safety profile, tolerability, and maximum tolerated dose. The secondary objective was to study pharmacokinetics and preliminary clinical activity. The eligibility criteria for this trial stipulated that tumor samples from the enrolling patients must be positive ($\geq$1) for either HER2 or EGFR on immunostaining done by an independent testing company. The maximum tolerated dose was determined to be 320 mg from this phase I study, with diarrhea as the primary adverse effect and dose-limiting toxicity.

Although not the primary objective of the trial, encouraging clinical activity from this study was reported at the 2006 annual meeting of American Society of Clinical Oncology (24). Among the 23 evaluable breast cancer patients enrolled into the trial, there were seven confirmed partial responses and one patient with stable disease for >24 weeks. The duration of responses was 7 to 24 weeks with four patients still on study as of the American Society of Clinical Oncology presentation. Among the 12 evaluable NSCLC patients, there were no confirmed responses but five of these patients (who had progressed on erlotinib or gefitinib) achieved stable disease for >24 weeks.

A phase II HKI-272 trial in NSCLC patients has recently been initiated. This is a multiinstitution trial (funded by Wyeth Pharmaceuticals) with a planned accrual of 138 patients divided equally among three study arms (Fig. 1). For entry into two (A and B) of the three arms, eligible patients must have disease progression after at least 12 weeks of erlotinib or gefitinib therapy. Those with EGFR kinase mutations are entered on arm A, and those whose tumors are wild type for EGFR are entered on arm B. Patients with no prior erlotinib or gefitinib therapy are enrolled on arm C. Tumor sequencing of EGFR exons 18 to 24 is included in the entry criteria for all

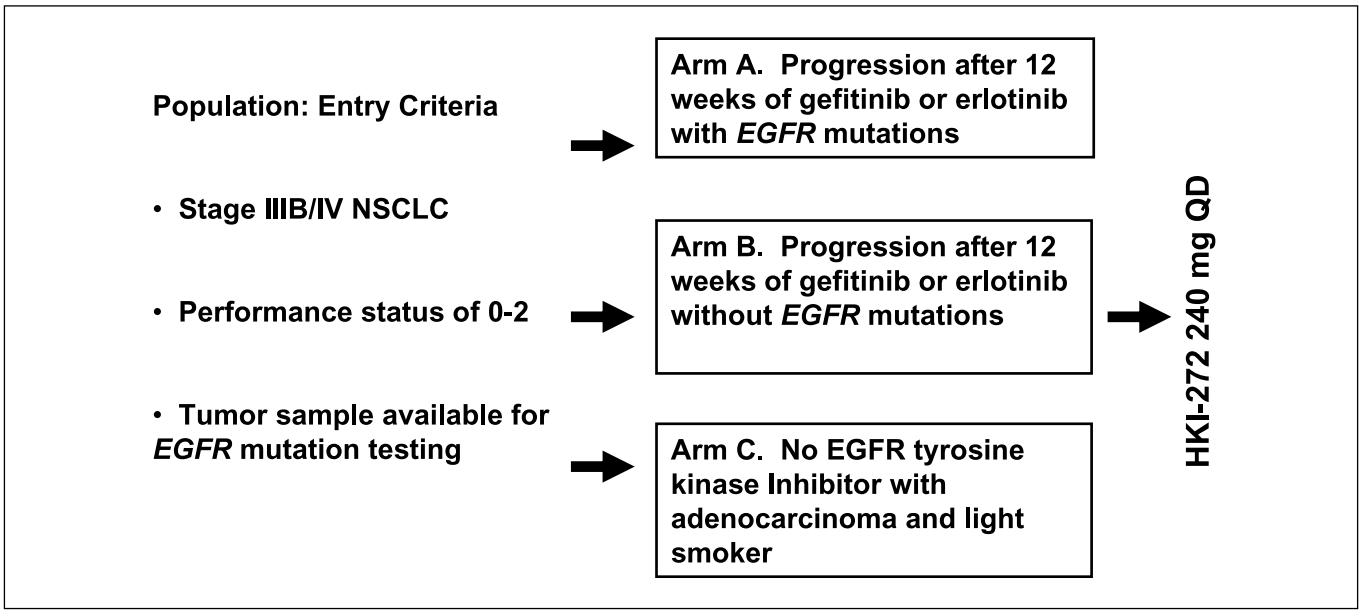


**Fig. 1.** Study design for phase II trial of HKI-272 in subjects with advanced non–small cell lung cancer.

patients and will be done by Genzyme Genetics, which is certified under the Clinical Laboratory Improvement Amendments program. All patients enrolled into the trial will receive 240 mg HKI-272/day, orally, continuously until progression of disease or development of unacceptable treatment-related toxicity. An optional tumor biopsy for patients who exhibit a response while on the trial but subsequently progress is part of the protocol. These tumors with acquired resistance to HKI-272 will then be sequenced for secondary EGFR kinase domain mutations. The EGFR mutation status data collected before and after treatment with HKI-272 will aid in defining the mechanisms of biological response to this inhibitor based on the type of EGFR kinase domain mutations that are identified in responding patients, as well as in patients with primary and acquired HKI-272 resistance.

Thus, HKI-272 will be one of the first irreversible EGFR/HER2 inhibitors to be evaluated in a phase II trial in a highly screened and selected NSCLC patient population. In addition to determining the efficacy of HKI-272 in this patient population, the correlative studies from this trial may yield novel insights in the mechanisms of acquired resistance to erlotinib. Several other agents of this class of compounds are now in phase I clinical trials. The available preclinical data suggest that these irreversible inhibitors, as a class, are effective in overcoming the acquired resistance EGFR T790M mutation *in vitro*. However, there may be differences in efficacy among the different compounds for specific EGFR mutations. In addition, these preclinical data remain to be demonstrated in lung cancer patients. It is hoped that HKI-272 and other members of this class of pan ErbB inhibitors will validate their promising preclinical data to offer additional treatment options for those patients who experience disease progression after initial response to erlotinib.

## Open Discussion

**Dr. Thomas Lynch:** Let's pursue the issue as to whether this agent is designed as a better HER1 inhibitor and not as a HER2 inhibitor. Should it have been part of the phase II clinical trial design to investigate either HER2 mutations or FISH or immunohistochemistry for HER2?

**Dr. Wong:** About 3% to 5% of patients have HER2 amplification, and HER2 mutation has also been found in non–small cell lung cancer at about 1% to 2%. I think those patients would actually derive more benefit than the T790M patients, at least with the data that we have. Looking at the preclinical data, we would predict the patients who would derive benefit from HKI-272 would be the ones with L858R plus T790M. Unfortunately, those patients never make it that far because they don't do as well as on erlotinib as those with the deletion mutations.

**Dr. Jeffrey Engelman:** These patients are not getting rashes, so do you think this agent is inhibiting wild-type EGFR in patients? It looks as though this basically a HER2 inhibitor in patients.

**Dr. Roman Perez-Soler**: There were about six responses in 30 patients with breast cancer and none in 12 patients with lung cancer.

**Dr. Lynch:** There are several pan ErbB inhibitors in various stages of development. The good thing about this study is the concept of using selection of patients based on mutation status to gain some insight. Thinking more about this class of compounds, do you think it is likely that what we will be screening people for mutations up-front and selecting patients for one versus another? Do you think that this kind of work will predict how patients will do clinically?

**Dr. Wong:** The different structures of the EGFR inhibitor compounds affect they interact with the protein. Depending on the mutation, some might go into the pocket easier and have a different potency. I believe that 5 years from now we are going to have a half dozen of these compounds in clinical development and patients will be stratified based on what particular mutations they have.

**Dr. Bruce Johnson:** The mutation sequencing on the HKI-272 trials is done at any time point during the clinical course. Very few have their biopsy done right before they get the drug. So, the numbers for which you will know the T790M status at the time they start treatment with HKI-272 are going to be small.

**Dr. Lecia Sequist:** That's another change we need to make in our paradigm in caring for patients if we are really going to use these molecular markers, because we have to rebiopsy after people progress.

**Dr. Lynch:** Dr. Socinski, you have done a large number of clinical trials in lung cancer; is it feasible for a second-line study to mandate a repeat biopsy at progression?

**Dr. Mark Socinski:** I think it is feasible and probably important, because what you are dealing with clinically at that point might not be reflected by a biopsy that was done 15 months earlier. We did some of the phase I lapatinib studies in which there were serial biopsies that needed to be done and they got done.

## References


1. Fontanini G, De Laurentiis M, Vignati S, et al. Evaluation of epidermal growth factor-related growth factors and receptors and of neoangiogenesis in completely resected stage I-IIIA non-small-cell lung cancer: amphiregulin and microvessel count are independent prognostic indicators of survival. Clin Cancer Res 1998;4:241–9.
2. Rusch V, Baselga J, Cordon-Cardo C, et al. Differential expression of the epidermal growth factor receptor and its ligands in primary non-small cell lung cancers and adjacent benign lung. Cancer Res 1993;53:2379–85.
3. Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer 2005;5:341–54.
4. Arteaga CL. ErbB-targeted therapeutic approaches in human cancer. Exp Cell Res 2003;284:122–30.
5. Mendelsohn J, Baselga J. The EGF receptor family as targets for cancer therapy. Oncogene 2000;19:6550–65.
6. Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. J Clin Oncol 2003;21:2237–46.
7. Janne PA, Gurubhagavatula S, Yeap BY, et al. Outcomes of patients with advanced non-small cell lung cancer treated with gefitinib (ZD1839, "Iressa") on an expanded access study. Lung Cancer 2004;44:221–30.
8. Kris MG, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. JAMA 2003;290:2149–58.
9. Miller VA, Kris MG, Shah N, et al. Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non-small-cell lung cancer. J Clin Oncol 2004;22:1103–9.
10. Tsao MS, Sakurada A, Cutz JC, et al. Erlotinib in lung cancer - molecular and clinical predictors of outcome. N Engl J Med 2005;353:133–44.
11. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129–39.
12. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:497–500.

**13.** Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 2004;101:13306–11.

**14.** Ji H, Sharpless NE, Wong KK. EGFR targeted therapy: view from biological standpoint. Cell Cycle 2006; 5:2072–6.

**15.** Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst 2005;97:339–46.

**16.** Hirsch FR, Varella-Garcia M, McCoy J, et al. Increased epidermal growth factor receptor gene copy number detected by fluorescence *in situ* hybridization associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes: a Southwest Oncology Group Study. J Clin Oncol 2005;23:6838–45.

**17.** Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med 2005;352:786–92.

**18.** Kwak EL, Sordella R, Bell DW, et al. Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. Proc Natl Acad Sci U S A 2005;102:7665–70.

**19.** Pao W, Miller VA, Politi KA, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2005;2:e73.

**20.** Rabindran SK, Discafani CM, Rosfjord EC, et al. Antitumor activity of HKI-272, an orally active, irreversible inhibitor of the HER-2 tyrosine kinase. Cancer Res 2004;64:3958–65.

**21.** Ji H, Li D, Chen L, et al. The impact of human EGFR kinase domain mutations on lung tumorigenesis and *in vivo* sensitivity to EGFR-targeted therapies. Cancer Cell 2006;9:485–95.

**22.** Ji H, Zhao X, Yuza Y, et al. Epidermal growth factor receptor variant III mutations in lung tumorigenesis and sensitivity to tyrosine kinase inhibitors. Proc Natl Acad Sci U S A 2006;103:7817–22.

**23.** Shimamura T, Ji H, Minami Y, et al. Non-small-cell lung cancer and Ba/F3 transformed cells harboring the ERBB2 G776insV.G/C mutation are sensitive to the dual-specific epidermal growth factor receptor and ERBB2 inhibitor HKI-272. Cancer Res 2006;66:6487–91.

**24.** Wong KK, Fracasso PM, Bukowski RM, et al. HKI-272, an irreversible pan erbB receptor tyrosine kinase inhibitor: Preliminary phase 1 results in patients with solid tumors. J Clin Oncol 2006;24:3018.

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