Afatinib (BIBW-2992): a novel dual EGFR/HER2neu inhibitor with promising activity in non-small-cell lung cancer

Lung cancer is not one disease but many diseases with specific molecular profiles and treatment alternatives. EGF receptor-activating mutations predict a high, albeit short-lived, response to reversible tyrosine kinase inhibitors (e.g., gefitinib and erlotinib). Acquired resistance mutations can result in tumor progression. Dual EGFR/HER2 irreversible inhibitors are small novel molecules that can overcome gefitinib/erlotinib resistance by potentially circumventing multiple mechanisms of acquired resistance, as shown in vitro. Afatinib is an irreversible inhibitor with potent phosphorylation inhibitory activity of both EGF receptor and HER2. Durable responses were observed in Phase I trials in advanced non-small-cell lung cancer at 50 mg once daily. In Phase II trials, reduction in tumor size was observed in 90% of patients. The objective response and disease control rate were 62 and 94%, respectively. Median progression-free survival was estimated at 12 months (95% CI: 10.0–19.2). The most common drug-related adverse events were diarrhea and rash/acne, 18% were grade 3 and none grade 4. LUX-Lung 1, one of two global Phase III studies, was presented in October 2010. At primary analysis (358 events), the study did not meet its primary end point to improve overall survival compared with placebo.

Keywords: acquired mutations activating mutations afatinib BIBW-2992 EGFR receptor HER2neu irreversible inhibitor

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insertions in exon 20 of the kinase domains of EGFR or HER2 [20]. Multiple resistance mechanisms can coexist in recurrent tumors after an initial response to gefitinib or similar reversible EGFR inhibitors. Gefitinib-resistant clones are cross-resistant to related anilinoquinazolines [21]. These observations helped guide the search for more effective therapy against a specific subset of lung cancers, based on a need for increased therapeutic efficacy of the next generation of EGFR inhibitors. The search was based on small molecules with a broader activity against ErbB receptor tyrosine kinases, but are highly selective within the human kinome, assuring an acceptable drug safety and tolerability profile.

**Irreversible inhibitors of EGFR TKI**

Signaling by the membrane-bound EGFR involves a complex pathway of ligand binding, receptor homodimerization, and heterodimerization with ErbB2 and other family members, followed by internalization and recycling of the ligand-bound receptor or ubiquitin-mediated receptor degradation [22]. In gefitinib-resistant NSCLC not containing the secondary T790M EGFR mutation, an increased EGFR internalization correlates with drug resistance. Irreversible inhibitors that covalently crosslink the receptor are effective in cell lines with the T790M mutation and in cells with altered EGFR trafficking [23]; raising the possibility that they may circumvent multiple mechanisms of acquired resistance to gefitinib and erlotinib [21]. To prove this hypothesis, initial in vitro studies generated gefitinib-resistant subclones of NCI-H1650 cells by treatment with ethyl methane sulfonate (600 µg/ml), followed by exposure to 20 µM gefitinib. Despite the resistance to gefitinib, these cells displayed persistent sensitivity to dual inhibitors of EGFR and ErbB2 (HKI-272 and -357, and EKB-569) [24–26]. All three drugs are irreversible inhibitors, most likely via a covalent bond with the Cys773 residue within the EGFR catalytic domain or the Cys805 of ErbB2. In contrast to first-generation reversible EGFR TKIs, even at high drug concentrations, investigators were unable to establish clones of cells that were resistant to these irreversible inhibitors at concentrations above 10 µM, even after ethyl methane sulfonate mutagenesis [21]. Interestingly, irreversible inhibitors were tenfold more effective than gefitinib in suppressing EGFR autophosphorylation, and AKT and MAPK phosphorylation in parental NCI-H1650 cells harboring a sensitive mutation (delE746-A750), as well as gefitinib-resistant clones (NCI-H1650(G7)).

Furthermore, the differential inhibition of EGFR signaling in gefitinib-resistant cells by reversible and irreversible inhibitors might be correlated with alterations in receptor trafficking, a well-documented modulator of EGFR-dependent signaling [21]. Gefitinib’s ability to inhibit EGFR activation is compromised in gefitinib-resistant cells, whereas the irreversible activity of inhibitors is not detectably affected, which may explain the antitumor activity of irreversible inhibitors in gefitinib-resistant tumors. Finally, the NCI-H1975 bronchoalveolar cancer cell line harbors both L858R and T790M mutations in EGFR. In in vitro experiments with this cell line, both irreversible inhibitors HKI-357 and -272 were considerably more effective than gefitinib in suppressing ligand-induced EGFR autophosphorylation and its downstream signaling, as determined by AKT and MAPK phosphorylation, and suppressed cell proliferation, under gefitinib-resistant conditions [21].

**Afatinib (BIBW-2992)**

- **Preclinical experience**

Afatinib (BIBW-2992) is an anilino-quinazoline derivative, developed by Boehringer Ingelheim Corp (Ridgefield, CT, USA) to covalently bind and modify the ATP-binding site of the kinase domains of EGFR (Cys773) and HER2 (Cys805), with a functional Michael acceptor group similar to the quinoline-derived irreversible EGFR inhibitors EKB-569 and HKI-272 [27,28]. Afatinib is an oral dual receptor TKI with potent irreversible inhibitory activity on ErbB1 (EGFR/HER1) and mutated ErbB1 receptors, including the T790M variant, and ErbB2 (HER2). Afatinib has potent phosphorylation inhibitory activity on both EGFR (half maximal inhibitory concentration \([IC_{50}]=0.5\text{ nM}\) for EGFR and \([IC_{50}]=16\text{ nM}\) for HER2) vs. gefitinib, with a potency for L858R EGFR \([IC_{50}]=0.7\text{ nM}\) and HER2 \([IC_{50}]=16\text{ nM}\) compared favorably to reference compounds in all cell types tested (human epidermoid carcinoma cell line A431, murine NIH-3T3 cells transfected with wild-type HER2, breast cancer cell line BT-474 and gastric cancer cell line NCI-N87). Afatinib effectively and selectively inhibited EGFR and HER2 total tyrosine phosphorylation in vitro and tumor cell proliferation in vivo, in EGFR wild-type as well as EGFR and HER2 mutants, including erlotinib-resistant isoforms. Afatinib is similar to gefitinib in potency for L858R EGFR \([IC_{50}]=0.7\text{ nM}\), but approximately 100-fold more active against the gefitinib-resistant L858R/T790M EGFR double mutant \([IC_{50}]=99\text{ nM}\). Afatinib suppresses transformation in isogenic cell-based assays, inhibits survival of cancer cell lines and induces
tumor regression in xenograft and transgenic lung cancer models, with superior activity over erlotinib [29]. Afatinib showed no activity toward A549 cells, which express wild-type EGFR and HER2, but simultaneously harbor an oncogenic K-RAS G12S point mutation [28]. In a xenograft model of the epidermoid carcinoma cell line, A431, expressing high levels of wild-type EGFR and detectable levels of HER2, afatinib administration resulted in dramatic tumor regression, whilst also downregulating EGFR and AKT phosphorylation. Similarly, xenograft tumor formation by the NCH1975 cell line, expressing EGFR L858R/T790M, was effectively controlled by afatinib [30]. In de novo EGFR L858R/T790M-driven erlotinib-resistant lung cancer, a more disease-relevant and challenging model, afatinib induced a greater than 50% tumor reduction after 4 weeks of treatment. The addition of rapamycin, an inhibitor of the EGFR–PI3K–mTOR axis, resulted in almost complete tumor regression in six EGFR L858R/T790M mice within 1 week of treatment, although treatment with rapamycin alone is not effective in this particular animal model [31]. Conversely, NSCLC patients with primary resistance to first-generation EGFR inhibitors caused by the previously mentioned KRAS mutations [32] or acquired resistance caused by amplification of the MET protooncogene [18], would not be expected to respond to treatment with afatinib alone. However, since MET signaling activates the PI3K pathway in a HER3-dependent manner [18], it is possible that the combination of afatinib and rapamycin would also be effective in patients with resistance to first-generation inhibitors that was acquired by this mechanism.

Clinical experience

Phase I studies

To assess tolerability, pharmacokinetics (PK), pharmacodynamics and clinical activity of afatinib, an escalating schedule of once-daily afatinib for 14 days, followed by a 14-day period off medication, was explored in patients with advanced solid tumors [28]. A total of 38 patients were enrolled. Dose levels were 10, 20, 30, 45, 70, 85 and 100 mg. At 100 mg, dose-limiting toxicity (DLT; common toxicity criteria grade 3 skin rash and grade 3 diarrhea despite treatment with loperamide) occurred in two patients. In the next-highest dose of 70 mg, DLT (grade 3 fatigue and alanine aminotransferase elevation) occurred in one of six patients. An intermediate dose level of 85 mg was studied. Here, DLT occurred in two patients (grade 3 diarrhea despite treatment and grade 2 diarrhea lasting more than 7 days despite treatment). An additional 12 patients were treated with 70 mg of afatinib. Skin biopsies did not show significant changes in EGFR-associated biomarkers. No partial or complete responses were observed; stable disease lasting more than four cycles was seen in seven patients. The recommended dose for studies with afatinib for 14 days followed by 14 days off medication is 70 mg once daily. The PK was dose proportional with a terminal elimination half-life ranging between 21.3 and 27.7 h on day 1 and between 22.3 and 67.0 h on day 27; afatinib exposure decreased after food intake. In another study, afatinib PK revealed moderately fast absorption and no deviation from dose proportionality after single and multiple doses [27].

A Phase I study of continuous once-daily oral afatinib was conducted to determine safety, maximum tolerated dose, PK, food effect and preliminary antitumor efficacy in patients with advanced solid tumors. A total of 53 patients received afatinib at 10–50 mg/day [33]. Afatinib was generally well tolerated. The most common adverse effects included diarrhea, nausea, vomiting, rash and fatigue. DLTs included grade 3 rash (n = 2) and reversible dyspnea secondary to pneumonitis (n = 1). The recommended Phase II dose was 50 mg/day. Three patients with NSCLC (two with in-frame exon 19 mutation deletions) experienced confirmed partial responses (PRs) sustained for 24, 18 and 34 months. Two other patients (one with esophageal carcinoma and one with NSCLC) had nonconfirmed PRs. A patient with a PR at 10 mg/day progressed and developed symptomatic brain metastases, which subsequently regressed with an increased dose of 40 mg/day of afatinib. A further seven patients had disease stabilization lasting at least 6 months [33]. Durable responses were observed in Phase I trials of afatinib in NSCLC patients with activating EGFR mutations and the recommended Phase II dose was 50 mg/day.

Phase II studies

Afatinib has undergone Phase II testing in patients with NSCLC, breast and prostate cancers, head and neck carcinoma, as well as glioma. In lung cancer, Boehringer Ingelheim Corp is sponsoring the LUX-Lung 2 program, which is part of the comprehensive LUX-Lung clinical trial program, comprising over ten trials conducted across the globe. As part of the program, an open-label, single arm, two-stage Phase II study was conducted in Taiwan and the USA [101]. Patients with stage IIIB/IV adenocarcinoma of
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the lung, whose tumors harbor activating mutations within exon 18–21 of the EGFR receptor, with Eastern Cooperative Oncology Group (ECOG) 0–2, who progressed or relapsed after one prior cytotoxic chemotherapy regimen, as well as chemotherapy naïve patients (only in stage 2), are being treated with afatinib 50 mg or 40 mg once daily until progressive disease. The protocol was amended (17 December 2008), to a lower starting dose of afatinib, with two possible dose reductions if needed after discontinuation caused by drug-related adverse events. This study is examining effectiveness of this irreversible dual inhibitor of EGFR and HER2 kinases in patients carrying EGFR-activating mutations. The primary end point is response rate (complete response [CR] and PR) according to RECIST criteria. Secondary outcome measures include safety of afatinib, clinical benefit (CR, PR and stable disease) determined by response evaluation criteria in solid tumors (RECIST), duration of objective response (OR), time to OR, progression-free survival (PFS) time, overall survival (OS) time and PK evaluation. The study began in August 2007 and the estimated primary completion date is January 2012 (final data collection date for primary outcome measure). At the time of the first interim data report in May 2008, 174 patients (156 in Taiwan and 18 in the USA) were screened for EGFR mutations. Mutations were present in 41% of patients from Taiwan (n = 64) and none from the USA. A total of 27 patients (42%) were identified with EGFR L858R, 26 (41%) with exon 19 deletion, five (7.8%) with an exon 20 insertion, and one patient each with G863V, L861P, K860E, L861Q, G719S+S768I and G724S+S768I. PRs were seen in one patient with brain metastases, and two patients with the G719S+S768I and L861Q mutations [34,35]. At the last annual meeting of the American Society of Clinical Oncology (ASCO) in June 2010, updated data were presented. A total of 444 patients were tested for EGFR. L858R mutation was seen in 54 (42%), deletion 19 in 52 (40%) and other mutations in 23 (18%) patients. In this study, a patient with the T790M mutation did not respond to BIBW-2992 [36]. The last update of this trial was presented at the annual meeting of the European Society of Clinical Oncology (ESMO), in October 2010 [36]. RECIST was used to assess response at 4, 8 and 12 weeks, and at 8-weekly intervals thereafter. A total of 129 patients received at least one dose of afatinib. Among the 129 patients, the investigators reported an OR rate (ORR) of 67% (confirmed ORR of 60%), disease control rate (DCR) of 86%, median PFS of 14 months and median OS of 24 months. Comparable efficacy was observed in the first- and second-line settings. A similar confirmed ORR, DCR and median PFS were seen in the 54 patients with L858R mutations (59%, 83%, and 16.1 months, respectively) and in 52 patients with deletion 19 mutations (69%, 93% and 13.7 months, respectively). The most common drug-related adverse events were diarrhea and rash/acne in 95 and 91% of patients, respectively, with a Common Terminology Criteria for Adverse Events (CTCAE) grade of 3 in 19 and 21% of patients, respectively [37]. Another Phase II trial in advanced (EGFR-FISH+) lung cancer, is currently recruiting 70 participants in Italy [102]. This is a single-arm, nonrandomized, open-label Phase II trial exploring the ORR (CR and PR) by the RECIST criteria as the primary end point, of patients with an ECOG performance score of 0, 1 or 2, pathologically confirmed stage IIIB or IV, adeno- or broncho-alveolar carcinoma type, stratified by line of therapy (first- vs second-line treatment). The secondary end points are evaluation of safety profile and PK analysis of afatinib. Presence of K-RAS and EGFR mutation is evaluated during screening for selected patients [103]. Although beyond the scope of this article, it is worth mentioning another irreversible inhibitor, neratinib (HKI-272), which has also entered Phase I and II clinical trials at 240 mg/day. A total of 167 patients were treated. The response rate was 3% in patients carrying sensitive EGFR mutations and 0 for wild-type. No patient with known T790M responded. Notably, three out of the four patients with an exon 18 G719X EGFR mutation had a PR and the fourth had stable disease lasting 40 weeks [38,39]. A Phase II, three-arm trial included stage IIIB/IV recurrent NSCLC, stratified by EGFR mutation or wild-type, following erlotinib, gefitinib or chemotherapy failure [40]. The study examined 165 patients accrued (median age 60 years, 30% male, 96% performance status [PS] 0 or 1 and 64% with prior chemotherapy), the ORR was 2% (four out of 165), with a median PFS of 13.1 weeks for those previously treated with EGFR TKIs and 7.4 weeks for those with prior chemotherapy exposure.

Phase III studies

On 15 February 2008, the US FDA granted fast-track designation (FDA Modernization Act of 1997 [FDAMA]) for Boehringer Ingelheim to conduct a pivotal trial program studying afatinib (BIBW-2992) in late-stage NSCLC patients. Afatinib is the first orally administered, irreversible dual inhibitor of EGFR and HER2 to
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Afatinib (BIBW-2992) in tumors with HER2neu mutations

HER2 mutations are found in 2–4% of lung adenocarcinomas and, similar to EGFR mutations, are more common in females, nonsmokers and patients with an Asian background. An exploratory Phase II study in demographically
and genetically selected NSCLC is being conducted. Patients with stage IIIB/IV lung adenocarcinoma who have never smoked or are light ex-smokers, and whose tumors harbor EGFR or HER2 mutations, or are demonstrated by FISH analysis to overexpress EGFR, are eligible. Patients received 50 mg afatinib daily until disease progression. The primary end point is response rate. As of August 2009, three patients with a HER2 mutation in exon 20 have been included. All three patients are female non-smokers with stage III/IV adenocarcinoma of the lung and failed chemotherapy (up to five lines). A preliminary analysis shows significant improvement in patients’ symptoms and PS, as well as tumor size reduction amounting to PR in all three patients. Diarrhea and skin rash were the prominent adverse events. This is the first report of the use of afatinib in pretreated patients with NSCLC and activating HER2 mutations in exon 20. This mutation characterizes a subgroup of NSCLC dependent on the HER2 pathway for survival, making afatinib, an irreversible inhibitor of EGFR/HER2, a potential new treatment option for these patients.

**Conclusion**

Contrary to common misconception, lung cancer of the non-small-cell type is not just one disease. Subtypes of NSCLC have recently been characterized by molecular parameters. The molecular make-up of certain lung cancers make them particularly sensitive to inhibitors of tyrosine kinase activity in the intracellular domain of the EGFR. The two so-called reversible inhibitors in the market are gefitinib and erlotinib. Despite an overall initial response of approximately 70%, secondary resistant mutations in the cancer cells (T790M accounts for approximately 50%) lead to unresponsiveness to gefitinib and erlotinib after an average of 8.0–10.8 months. Rebiopsy is highly recommended to show new mutations responsible for the acquired resistance of tumors, especially to guide treatment decisions as new agents become available. Investigators have recently developed new small molecules that bind irreversibly to the tyrosine kinase of the receptor, overcoming resistance in vitro, with potential significant clinical benefit. Afatinib is one such compound as it has proven to be safe and have durable anti-tumor activity. LUX-Lung 1, a recently presented Phase III study (October 2010), did not meet its primary end point to improve OS, even when significant advantages favoring afatinib were seen for PFS, DCR and ORR.

**Future perspective**

Cancer is an extremely complex molecular condition. In lung cancer, genes encoding for growth factors/growth factor receptors (e.g., EGF/EGFR and VEGF/VEGFR), modulators of cell growth response (e.g., K-RAS and BRCA1) and enzymes involved in nucleotide excision repair pathways (e.g., ERCC1 and RRM1) have prognostic and predictive value of response and survival with certain therapeutic agents. EGFR is overexpressed in 40–80% of NSCLC patients and 10–20% carry somatic mutations of the receptor’s tyrosine kinase domain, which predict a 65–81% response rate and 96% DCR to the EGFR TKIs erlotinib and gefitinib, with a median OS of 33 months. However, the response is short lived (average: 8.0–10.8 months), at which point cancer cells develop multiple acquired mechanisms of resistance, including specific new mutations. It would be naïve to expect to win the battle against cancer by targeting just one molecule in the immensely complex molecular machinery of the cancer cell, made even more complex under natural genetic pressure and added genetic abnormalities associated with treatment. New targeted approaches in cancer management aim at blocking the signaling translational pathway at various key checkpoints downstream from the surface receptor or key molecular players, in two or more pathways, in a concurrent or sequential fashion. BIBW-2992 is a novel molecule with multiple molecular targets, which may account for its broader anticancer activity when compared with the first-generation EGFR TKIs. However, the positive data presented in this article should not be taken as the culmination but the beginning of a long and arduous process of understanding many current unknowns. It is possible that, in the near future, BIBW-2992 may become part of a rational combination of targeted agents that not only block a few target molecules, but also inhibit the whole cancer machinery by strategic targeting of the complex molecular network responsible for the cancer phenotype.

**Financial & competing interests disclosure**

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Executive summary

- Erlotinib and gefitinib are reversible EGFR tyrosine kinase inhibitors with cytotoxic activity against lung cancer cells harboring somatic mutations in the kinase domain of EGFR. Even when these two drugs produce initial dramatic clinical responses, cells develop acquired drug resistance caused by new mutations.
- Irreversible inhibitors are effective in cell lines resistant to EGFR tyrosine kinase (erlotinib/gefitinib), raising the possibility that they may circumvent multiple mechanisms of acquired resistance. BIBW-2992 is an oral dual receptor tyrosine kinase inhibitor with potent irreversible inhibitory activity on ErbB1 (EGFR/HER1) and mutated ErbB1 receptors including the T790M variant, as well as ErbB2 (HER2).
- In Phase II, the clinical activity of 50 mg/day BIBW-2992 was explored in lung, breast, prostate and head and neck cancer, as well as glioma. In lung cancer, 129 patients were treated with BIBW-2992, and had a objective response and disease control rate of 62 and 94%, respectively, and median progression-free survival estimated at 12 months. The most common adverse effects included diarrhea, nausea, vomiting, rash and fatigue.
- In February 2008, the US FDA granted fast-track designation to Boehringer Ingelheim to conduct pivotal Phase III studies in advanced non-small-cell lung cancer patients failing erlotinib or gefitinib, or as first-line treatment versus chemotherapy in tumors harboring EGFR mutations.
- If BIBW-2992 is approved by the FDA, Boehringer Ingelheim will market the compound under the trade name TOVOK™.

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Papers of special note have been highlighted as:

* of interest
** of considerable interest


** First paper to describe somatic mutations in the intracellular domain of the EGF receptor (EGFR), in exons 19 and 21, to explain the selective sensitivity of lung cancer cells carrying such mutations, to the reversible EGFR tyrosine kinase inhibitors gefitinib and erlotinib.

10 Very important publication on systematic profiling of patients with advanced non-small-cell lung cancer in a large European population and outcome of treatment with first-line erlotinib in patients with tumors harboring sensitive mutations.

** Important paper explaining the mechanism of action of irreversible EGFR inhibitors.
Seminal paper explaining modulation of EGFR/HER2neu membrane trafficking as possible mechanism of resistance to first generation EGFR tyrosine kinase inhibitors.

Describes BIBW-2992 mechanism of action in patients with specific mutations in the tyrosine kinase intracellular domain of HER2neu, similar to what we know for EGF receptor tyrosine kinase-specific mutations.

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