Review: Clinical Trial Outcomes

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CLINICAL INVESTIGATION

Afatinib: rationale for use in non-small-cell lung cancer based on clinical trial data

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Afatinib is an irreversible ErbB family blocker approved for use as first-line therapy in EGF receptor (EGFR)-positive metastatic non-small-cell lung carcinoma. Its preclinical evaluation showed a higher affinity than reversible tyrosine kinase inhibitors to EGFR-mutated cell lines. This affinity was significantly higher (100-fold) for cell lines expressing the gatekeeper mutation *T790M* in comparison with EGFR wild-type clones. This article reviews the Phase I studies used to ascertain its safety profile, appropriate dosing and recommended scheduling. A Phase IIb/III trial compared afatinib with placebo in patients who had failed treatment with an EGFR-tyrosine kinase inhibitor and demonstrated an improved response rate and progression-free survival, although no improvement in overall survival. Combination therapy with afatinib and cetuximab has shown promising results. This article also reviews the evidence to support the use of afatinib as a first-line therapy in patients with non-small-cell lung cancer positive for the EGFR mutation exon 19 deletion or *L858R* point mutation.

Keywords: EGF receptor • non-small-cell lung cancer • tyrosine kinase inhibitors

The EGF receptor (EGFR) belongs to the ErbB family of tyrosine kinase (TK) receptors composed of EGFR (ErbB1), EGFR-2 (HER2/neu), HER3 (ErbB3) and HER4 (ErbB4) [1]. EGFR mutations have been detected in approximately 17% of North American patients diagnosed with non-small-cell lung cancer (NSCLC) [2]. Approximately 90% of these mutations are exon 19 deletions or exon 21 *L858R* point mutations. The EGFR TK inhibitor (TKI) erlotinib is US FDA approved for first-line therapy in patients with EGFR-mutant-positive advanced NSCLC [3]. Trial BR21 compared erlotinib with placebo in an unselected NSCLC patient population who had progressed after platinum-based chemotherapy. This seminal trial reported an improvement in progression-free survival (PFS) and overall survival (OS; 6.7 vs 4.7 months) for erlotinib compared with the placebo and resulted in the FDA approval of erlotinib as second- or third-line therapy in patients with stage IV NSCLC [4]. It was after this trial was completed that the EGFR mutation was discovered and found to correlate with a response to the EGFR TKIs, erlotinib or gefitinib. In the past 5 years, several large, randomized, Phase III trials have compared first-line EGFR TKIs with chemotherapy in patients with EGFR-mutation-positive NSCLC and demonstrated improved response rate and PFS for patients treated with an EGFR TKI [3,5–7]. These trials have failed to demonstrate a significant improvement in OS, which is most likely due to the crossover effect on those studies.

Unfortunately, all patients with EGFR-mutant tumors treated with an EGFR TKI eventually developed acquired resistance. Disease progression usually occurs around 12 months [3,5,6]. Approximately 50% of patients have tumors that harbor a second site mutation, most commonly the *T790M* mutation occurring within

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exon 20 [8]. Other mechanisms of acquired resistance have been described and include Met amplification in 5% of the patients, HER2 mutations and/or overexpression [9], *PIK3CA* mutation [10], small-cell lung cancer transformation [10], epithelial–mesenchymal transition [10] and the EGFRvIII mutation [11].

De novo resistance to reversible EGFR inhibitors has been observed clinically and *in vitro*. Greulich *et al.* demonstrated that exon 20 insertions are more resistant to gefitinib *in vitro* [12], and these mutations occur in approximately 1–6 % of all EGFR-mutant patients [13]. *T790M* is called the 'gatekeeper mutation' because it is located within the catalytic cleft of EGFR and causes steric hindrance of the reversible EGFR inhibitors. This mutation also increases the catalytic turnover of EGFR by increasing its affinity to ATP [14]. There is some evidence that *T790M* mutations are present as a germline mutation that coexist in the background of heterogeneous tumors harboring *L858R* mutations and/or exon 19 deletions [15]. These tumors are not thought to respond to EGFR TKIs. In this setting, *T790M* driven resistance would develop as a function of clonal selection after EGFR blockade.

In an attempt to overcome resistance to reversible EGFR inhibitors, several irreversible EGFR inhibitors have been formulated and are being evaluated in clinical trials. Irreversible EGFR inhibitors contain a reactive Michael-acceptor group that binds covalently with cysteine groups within ERBB kinases. This bond is of a higher affinity than ATP to EGFR (wild-type [WT] and *T790M*) in a specific manner since some of the other ERBB kinases do not contain cysteine groups within the catalytic subunits [16]. Irreversible EGFR inhibitors are designed to overcome the steric interference conferred by *T790M* mutations at the catalytic cleft of EGFR [17]. On the other hand, they also irreversibly inhibit WT EGFR, which can potentially increase toxicity. Preclinical data suggest that irreversible inhibition of any ERBB kinase is more profound and efficacious [17]. *In vitro* studies demonstrate that lower concentrations of irreversible TKIs have longer ERBB signaling suppression on EGFR WT and mutated cell lines. Furthermore, acquired resistance *in vitro* also develops slower when compared with reversible inhibitors [17]. However, whether this translates into a clinically meaningful benefit remains to be determined.

There are several irreversible EGFR inhibitors that have been tested clinically with promising results. They include, but are not limited to, neratinib (HK1–272), dacomitinib (PF-002998040), afatinib (BIBW2992), EXEL-7647 (XL-647), CO-1686, BMS-690514, AP-26113 and AZD9291. They differ on their binding affinity to the EGFR and their specificity to other TK receptors such as HER2, ERBB4, VEGF receptor, RET and so forth. From these compounds, afatinib is the furthest along in clinical development. This article reviews the preclinical and clinical data for afatinib in NSCLC patients.

Preclinical data

Afatinib (BIBW2992) is an anilino-quinazoline irreversible EGFR/HER2/HER4 inhibitor, designed to covalently bind to the ATP-binding site of the kinase domains of EGFR (Cys773) and HER2 (Cys805) [18]. Cell free *in vitro* assays showed that BIBW 2992 was similar to reversible EGFR TKIs in its binding affinity to WT EGFR and its mutant forms. However, it was 100-fold more active with EGFR double-mutant *T790M*/*L858R* found in EGFR TKI-resistant tumors [18]. *In vitro* cell-based transformation assays demonstrated that BIBW2992 inhibited colony formation of the clinically relevant acquired double mutant *T790M*/*L858R*. In addition, it was effective against cells that harbored the highly resistant exon 20 insertion (primary resistance) as well as cells with other mutations, such as *D770–771insNPG* and the *R108K* extracellular domain point mutation. Interestingly, activated EGFR WT cell lines were highly sensitive to this agent after ligand-induced stimulation with EGF *in vitro* [18].

In vivo, afatinib has been shown to be active in xenografts from cell lines with oncogenic addiction to the mutated *L858R*/*T790M* EGFR or HER2 overexpression. Cell lines derived from the murine lung cancer model expressing the *L858R*/*T790M* EGFR mutation were also very sensitive to this compound [17]. On the other hand, there was very little activity of BIBW2992 against tumors expressing *KRAS* mutations or MET amplification.

Phase I studies

Several Phase I studies were conducted in patients with solid tumors, evaluating different dose escalation regimens for safety, pharmacokinetics, tolerability and maximum-tolerated dose [19–22]. These included cycles of 2 weeks on and 2 weeks off [19], 3 weeks on and 1 week off [20] and continuous daily dosing in a 4-week cycle [21,22]. In these trials, the majority of dose-limiting toxicities occurred with doses higher than 20 mg daily. Dose-limiting toxicities included, but were not limited to, grade 3 diarrhea, stomatitis, acneiform rashes and dyspnea secondary to pneumonitis. The most common grade 1 and 2 toxicities reported are common to this class of agents and included diarrhea (84%), rash (95%) and fatigue (75%).

The highest antitumor activity was noted in trials that included continuous daily dosing [21,22]. A Phase I

trial with continuous dosing (afatinib 40 mg daily) for patients with advanced solid tumors showed promising results in three NSCLC patients [22], two of whom had deletion 19, an EGFR mutation. These patients had confirmed partial responses sustained for 24, 18 and 34 months, respectively. From these studies, the recommended daily dose for pretreated patients was afatinib 50 mg daily.

The Lux-Lung 4 trial was an open-label Phase II trial designed to assess the efficacy of afatinib in Japanese patients with advanced NSCLC [23]. Inclusion criteria included patients with advanced NSCLC who had received prior chemotherapy and treatment with erlotinib or gefitinib or who were not amenable to treatment with established therapies. Patients were treated with afatinib 50 mg daily. In the 62 Japanese patients enrolled, the tolerability profile of afatinib was found to be similar to the findings from non-Japanese studies. Diarrhea and rash were the most frequent side effects, occurring in 50% of patients. In total, 45 (72.6%) of patients enrolled were EGFR-mutation positive and 51 (81.3%) had acquired resistance to EGFR TKIs per Jackman criteria [24]. Efficacy was observed in five out of 61 patients treated with evidence of tumor shrinkage. Median PFS was 4.4 months and OS was 19.0 months [23].

Phase II & III studies

The Lux-Lung 1 study was a randomized, double-blinded, multicenter, Phase IIb/III trial comparing afatinib (50 mg daily) with a placebo in patients with advanced adenocarcinoma of the lung, after progression on one to two lines of chemotherapy and at least 12 weeks of therapy with reversible EGFR TKIs [25]. The primary end point of this trial was OS. In total 585 patients were randomized. Of those, 58% of the patients were of Asian origin, 60% were female and the majority were never smokers. Of these patients 81% had been previously treated with a reversible EGFR TKI for more than 24 weeks and over half of those had confirmed partial or complete responses. It should be noted that the presence of a known EGFR mutation was not required for enrollment on the study. Of patients enrolled, 141 had tissue available for EGFR mutation testing of which 96 were positive for the EGFR mutation, the majority of which were *L858R* point mutations or exon 19 deletions (79%).

The primary end point was not met, there was no statistically significant difference in OS between afatinib-treated patients compared with placebo – 10.8 months (95% CI: 10–12) versus 12 months (95% CI: 12.2–14.3) hazard ratio (HR): 1.08 (95% CI 0.86–1.35). However, there was a benefit in independently assessed PFS for patients treated with afatinib compared with placebo (3.3 months vs 1.1 months; HR: 0.38; $p < 0.0001$). This benefit was not seen in the 45 EGFR WT patients [24]. Confirmed objective responses were seen in 7.4% of afatinib-treated patients compared with 0.5% of placebo ($p < 0.01$). Additional secondary end points included disease-control rates lasting longer than 8 weeks, which was higher for afatinib compared with placebo (58 vs 19%; $p < 0.0001$). Lastly, there was an improvement in cancer-related symptoms in afatinib-treated patients. Afatinib appeared to have a manageable safety profile with a low discontinuation rate of 5% (21/390). The major grade 3 toxicities were diarrhea (17%) and rash or acne (14%). The lack of improvement in OS for afatinib-treated patients compared with placebo may be explained by the higher percentage of placebo-treated patients receiving therapy beyond study; 79% compared with 68% for afatinib-treated patients. Moreover, 24% of patients in the placebo arm were started back on an EGFR TKI at the time of progression compared with only 12% of patients in the afatinib treatment arm.

The Lux-Lung 2 trial was a single-arm, multicenter, Phase II trial for EGFR TKI naive patients with advanced NSCLC, adenocarcinoma histology harboring EGFR mutations, who had progressed on up to one prior chemotherapy regimen and – as amended – who were chemotherapy-naive [26]. The primary end point was efficacy measured, confirmed by objective responses (complete response or partial response based on RECIST criteria). Initially patients started with 50 mg of afatinib, which was amended to a starting dose of 40 mg for better tolerability. In total, 129 patients were treated with afatinib; 99 patients received a starting dose of 50 mg daily and 30 patients a starting dose of 40 mg daily. In total 48% (61/129) of the patients received afatinib as a first-line therapy, and 52% (68/129) of patients had received at least one prior regimen of chemotherapy. Overall, a response as assessed by independent review was noted in 61% of patients. Patients with *L858R* mutations and deletions on exon 19 were the most sensitive to afatinib with 66% of those patients demonstrating a response in comparison with 39% of patients harboring other types of EGFR mutations. Two patients had a complete response to therapy. Response rates were similar for patients taking 40 (60%) and 50 mg daily (62%). 90% of patients receiving afatinib as a second-line of therapy received the higher dose (50 mg daily). The median OS, irrespective from treatment line, was 25 months with a median PFS of 14 months. Patients with *L858R* mutations and deletion 19 had similar PFS (15.8 and 15.5 months, respectively). Grade 3 toxicities were significantly higher in patients taking

50 mg daily (diarrhea 22% and rash 28%) in comparison with those patients taking 40 mg daily (7% with experienced grade 3 diarrhea and rash). However, efficacy was maintained at the lower dose while decreasing the frequency of grade 3 toxicities [26].

Lux-Lung 3 was a randomized Phase III trial comparing afatinib with chemotherapy with cisplatin and pemetrexed in treatment-naive EGFR mutation-positive patients with stages IIIb and IV NSCLC [7]. In this study, 345 patients were randomized in a 2:1 fashion to either afatinib 40 mg daily (230/345) until disease progression or cisplatin (75 mg/m^2) plus premetrexed (500 mg/m^2; 115/345) every 3 weeks for up to six cycles [7]. There was an improvement in the independently assessed PFS for afatinib-treated patients (11.1 vs 6.9 months in the chemotherapy arm; HR: 0.58 [0.43–0.78]; p = 0.0004). The response rate, also by independent reviewer, was significantly higher with afatinib (56 vs 23%; p < 0.001). In addition, a significant delay in time to deterioration of the prespecified cancer-related symptoms of cough (HR: 0.60, p = 0.0072) and dyspnea (HR: 0.68, p = 0.0145) was seen with afatinib compared with chemotherapy. The most common drug-related adverse effects were diarrhea (95%), rash (62%) and paronychia (57%) with afatinib; and nausea (66%), anorexia (53%) and vomiting (42%) with chemotherapy. Drug-related adverse events led to treatment discontinuation in 8% of afatinib-treated patients and 12% of patients receiving chemotherapy. A similar benefit in PFS was seen in the Lux-Lung 6 trial comparing afatinib to cisplatin and gemcitabine chemotherapy, (median PFS: 11.0 vs 5.6 months; HR: 0.28 with p < 0.0001) [27]. These trials, similar to other studies with erlotinib and gefitinib in this patient population, demonstrate that afatinib is a reasonable first-line treatment option for EGFR-mutant NSCLC patients. There is an ongoing trial (Lux-Lung 7) comparing afatinib with gefitinib in this patient population.

Her2/neu mutations

Afatinib is an inhibitor of mutated and/or overexpressed HER2 [28]. Preclinical models showed efficacy in HER2/neu mutated lung cancer cell lines and Ba/F3 cells expressing an artificial HER2 mutant [29]. HER2/neu mutations occur in 1–4% of adenocarcinomas with a similar phenotype to tumors with EGFR mutations [30].

Afatinib is currently being evaluated in previously treated HER2-positive breast cancer patients [31,32]. In NSCLC, a case report from a Phase II afatinib trial performed in Belgium reported objective remissions in three out of five patients with HER2/neu mutations [33]. These responses occurred even after a lack of response to reversible EGFR TKIs (erlotinib) and other HER2-targeted treatments (trastuzumab). In the two patients with HER2/neu mutations that did not respond, treatment was discontinued due to grade 3 rash and diarrhea. There are no prospective NSCLC studies using Her2 as a biomarker predictive of response to ErbB blockade. However, we should learn more about Her2 in NSCLCs from retrospective analysis of some of the Lux-Lung series such as Lux-Lung 8 [101]. Accrual is expected to finish in 2015.

Safety & tolerability

The dose-limiting toxicities observed with afatinib in Phase I clinical trials occurred with doses higher than 20 mg daily. The majority of serious adverse events occurred in patients treated at the 50 mg dose in the Lux-Lung 1 and 2 studies. In the Lux-lung 1 trial, 38% of patients required a dose reduction, 21% due to diarrhea and 17% due to rash or acne. In the Lux-Lung 1 trial, 0.02% discontinued afatinib therapy due to diarrhea and 7 patients due to rashes. Overall, 17% of patients had to have their treatments discontinued due to toxicity. To date, there have been two deaths attributed to afatinib therapy, both occurred in the Lux-Lung 1 trial; one patient developed heart failure and the other hepatic failure. Pneumonitis has been rarely reported.

Future perspective

Afatinib has shown promising results as first- and second-line therapy for patients with advanced NSCLC. Activity has primarily been demonstrated in patients with known EGFR mutations who have not been treated with a prior EGFR TKI. There has been limited efficacy demonstrated when afatinib was given alone to patients who have progressed on prior erlotinib or gefitinib. More promising are the results from a Phase I trial combining afatinib 40 mg daily, with cetuximab 500 mg/m^2 every two weeks in patients with acquired resistance to EGFR TKIs [2,34]. In this heavily pretreated patient population a response rate of 30% was observed in the first 100 patients entered on the trial with a PFS of 4.7 months. Stable disease was observed in an additional 45% of patients. The most common grade 3/4 toxicities from combination therapy were rash (18%), diarrhea (7%), fatigue (9%) and xerosis (3%). In total, 19% of patients discontinued therapy due to an adverse event. This is higher than what has been reported with erlotinib and cetuximab, cetuximab alone or afatinib alone in the Lux-Lung 1 trial [25]. Based on these results, a randomized Phase III trial (ECOG1513) comparing afatinib with afatinib and cetuximab in patients with acquired resistance to EGFR TKIs is currently under development. In

Table 1. Ongoing and completed clinical trials assessing afatinib in non-small-cell lung cancer patients.

Trial	Population	Phase	Treatment	End point	Results	Ref.
Lux-Lung 4	Asian advanced NSCLC, s/p doublet platinum-based chemotherapy and/or reversible TKI	I/II	Afatinib	I = Safety II = ORR	MTD = 50 mg/q.d. DCR = 66%	[101]
Lux-Lung 1	Advanced NSCLC, s/p Chemotherapy, refractory to reversible TKI	IIb/III	Afatinib vs placebo	OS	Negative OS, PFS, ORR, DCR improved	[102]
Lux-Lung 2	Advanced EGFR-positive NSCLC, s/p Chemotherapy, TKI naive	II	Afatinib 50 mg vs afatinib 40 mg	ORR	ORR 61% for both doses; Del19/L858R RR 66% vs 39% other	[103]
Lux-Lung 3	Advanced NSCLC EGFR-positive treatment naive	III	Afatinib vs chemotherapy	PFS	Afatinib > chemotherapy PFS 11 vs 6.9 months; ORR 56 vs 23%	[7]
Lux-Lung 5	Advanced NSCLC s/p chemotherapy, reversible TKI with initial afatinib response	III	Afatininb followed by afatinib and paclitaxel vs any chemotherapy	PFS	Ongoing, recruiting	[104]
Lux-Lung 6	Advanced NSCLC, first-line, EGFR-positive	III	Afatinib vs chemotherapy	PFS	Median PFS: 11 vs 5.6 months	[105]
Lux-Lung 7	Advanced NSCLC first-line, EGFR-positive	IIb	Afatinib vs gefitinib	PFS OS	Ongoing, recruiting	[106]
Lux-Lung 8	Advanced NSCLC s/p chemotherapy	III	Afatinib vs erlotinib	PFS	Ongoing, recruiting	[107]
Lux-Lung 8	Advanced NSCLC s/p reversible TKI (clinically acquired resistance)	I/II	Afatinib and cetuximab	I = Safety II = ORR	Interim: ORR 35% T790M+	[107]

DCR: Disease control rate; MTD: Maximum tolerated dose; NSCLC: non-small cell lung cancer; ORR: Overall response rate; OS: Overall survival; PFS: Progression free survival; RR: Response rate; s/p: Side population of cells; TKI: TK inhibitor; q.d.: Once daily.

addition to its use in EGFR-mutation-positive patients, we should continue to explore this compound in HER2/neu mutation positive patients. This group of patients might represent a select cohort of lung adenocarcinomas that would benefit from HER2/neu and EGFR blockage by a dual TKI inhibitor such as afatinib.

The side-effect profile of afatinib in patients with previous exposure to reversible EGFR TKIs appears to be manageable at 50 mg daily with standard symptomatic management. However, the standard dose of 40 mg/daily of afatinib to be used in EGFR-TKI naive patients maintains its efficacy. There are a multitude of ongoing Lux-Lung clinical trials (Table 1) comparing afatinib with chemotherapy and other EGFR TKIs that will provide us with more data in relation to safety and efficacy for this agent, moving forward.

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Executive summary

- Afatinib is an irreversible ErbB family blocker that targets EGF receptor (EGFR), Her2/neu and ErbB4.
- Phase I data show doses below 50 mg daily are safe and efficacious for most patients. Based on Lux-Lung 2, 40 mg daily would be the recommended dose for future clinical uses.
- The most common side effects are diarrhea, skin rashes and fatigue.
- Afatinib therapy improved progression-free survival and overall response rates in patients with metastatic and resistant non-small-cell lung cancer.
- Patients with specific EGFR mutations such as exon 19 deletions and exon 21 *L858R* point mutations seem to have the major clinical benefit.
- Afatinib studies in combination with other targeted agents should be undertaken for non-small-cell lung cancer EGFR-mutated patients as first- or second-line therapeutic options.

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