Review: Clinical Trial Outcomes

CLINICAL INVESTIGATION

The potential role of new targeted therapies in the treatment of advanced non-small-cell lung cancer

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Platinum-based doublet chemotherapy is the standard treatment for advanced non-small-cell lung cancer, with a median survival and 1-year survival of 8–10 weeks and 30–40%, respectively. A better knowledge of cancer biology and mechanisms of oncogenesis has allowed the identification of several potential molecular targets for cancer treatment, such as *VEGF*, *EGFR*, *ALK* and *c-MET*. The identification of several factors, including both the genetic profile of the patients and the biological characteristics of the disease, remains crucial to the overall success of such targeted therapies. Targeted molecular therapeutic approaches have already become an integral part of modern state-of-the-art cancer therapy. This review will focus on the well-characterized, therapeutically relevant molecular events in non-small-cell lung cancer patients and several inhibitors used in clinical practice and/or in development.

Keywords: bevacizumab • c-MET inhibitors • cetuximab • crizotinib • erlotinib • gefitinib • non-small-cell lung cancer • targeted therapy

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Non-small-cell lung cancer (NSCLC), accounts for 80–85% of all lung cancer cases and is a major cause of death worldwide, generally with advanced disease (locally advanced or metastatic stage) at diagnosis [1]. The palliative cytotoxic chemotherapy, the standard of care for these patients, has added limited advances in terms of efficacy in the last few years [2], with considerable toxicities, including neuropathy and fatigue, which may limit dosing. The major understanding of cancer biology and mechanisms of oncogenesis have allowed the identification of several potential molecular targets for cancer treatment, such as *VEGF*, *EGFR*, and more recently, *ALK* rearrangement and *c-MET* activation. An effective targeted therapy requires the appropriate patient population to be selected, with identification of several factors, including both the genetic profile of the patients and the biological characteristics of the disease. Therefore, molecular analysis should now be considered an essential part of pretreatment, diagnostic procedures and every effort should be made to obtain sufficient tissue to allow testing. This review will focus on the well-characterized, therapeutically relevant molecular events in NSCLC patients, and several inhibitors used in clinical practice and/or in development.

Bevacizumab

Bevacizumab, a humanized monoclonal anti-VEGF antibody, has demonstrated significant efficacy in combination with first-line platinum-based doublet chemotherapy in advanced NSCLC. First, in the E4599 trial, the combination of bevacizumab (15 mg/kg every 3 weeks) with carboplatin and paclitaxel in 878 patients with recurrent or advanced nonsquamous NSCLC (excluding patients with a higher risk of pulmonary bleeding: squamous cell carcinoma, brain metastases, clinically significant hemoptysis, history of documented hemorrhagic diathesis

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or coagulopathy, therapeutic anticoagulation, agents known to inhibit platelet function) demonstrated a significant benefit in terms of overall survival (OS; primary end point: 12.3 vs 10.3 weeks; p = 0.003), a significant improvement in progression-free survival (PFS; hazard ratio [HR]: 0.66; p < 0.001), and in objective-response rates (ORR; 35 vs 15%; p < 0.001) [3]. Subsequently, in the explorative analysis of survival and safety outcomes based on histology, the addition of bevacizumab to chemotherapy was associated with a benefit of approximately 4 weeks in term of OS in patients with adenocarcinoma (14.2 vs 10.3 months; HR: 0.69; 95% CI: 0.58–0.83) [4].

Second, in the AVAiL trial, bevacizumab at two different doses (7.5 and 15 mg/kg) in association with cisplatin plus gemcitabine statistically improved PFS (median PFS was 6.1, 6.7 and 6.5 months in the chemotherapy-alone, chemotherapy plus bevacizumab 7.5 mg/kg, and chemotherapy plus bevacizumab 15 mg/kg arms, respectively) in 1043 patients with advanced nonsquamous NSCLC (selected criteria according with the E4599 trial) [5]. HRs of progression compared with the control were 0.75 (95% CI: 0.62–0.91) and 0.82 (95% CI: 0.68–0.98) for the lower and higher doses of bevacizumab, respectively. However, the PFS benefit did not translate into a significant OS benefit, most likely due to the large proportion of patients (61–65%) who received post-progression treatments, confounding the potential difference between arms [6]. In E4599 and AVAiL, treatment with bevacizumab was associated with higher incidence of hypertension, proteinuria and bleeding than in the control groups, and lower rate of severe pulmonary bleeding than that observed in the previous Phase II trial [7].

Based on these results, bevacizumab in combination with carboplatin plus paclitaxel was approved by the US FDA for the first-line treatment of patients with unresectable, locally advanced, recurrent or metastatic nonsquamous NSCLC. Subsequently, the European Medicines Agency has approved bevacizumab to be used in combination with any platinum-based chemotherapy, in the same setting of patients.

Recently, a panel of expert oncologists, pulmon-ologists and radiologists reviewed the available data to identify predictive factors for pulmonary bleeding, confirming only squamous histology and/or a history of grade ≥2 hemoptysis (≥2.5 ml per event) as exclusion criteria for bevacizumab, while eligibility is not affected by patient age, performance status (PS), anticoagulation or antiplatelet therapy, cavitation and central tumor location, brain metastases [8].

For treatment optimization, potential molecular biomarkers were investigated in order to select patients who are more likely to benefit from bevacizumab-based chemotherapy. A prospective correlative study, including measurements of several biomarkers (VEGF pretreatment, bFGF, ICAM, E-selectin) in baseline and at week 7 was conducted in the E4599 trial [9]. In this analysis, baseline VEGF levels were predictive of response to bevacizumab: patients with high baseline levels of plasma VEGF had an increased probability of response to the bevacizumab in the paclitaxel–carboplatin arm, while those with low baseline VEGF levels had similar response rates in both arms. However, baseline VEGF levels were not predictive of the survival benefit afforded by the addition of bevacizumab to chemotherapy. None of the baseline candidate biomarkers levels explored in the BO21015 trial (bFGF, E-Selectin, ICAM, PIGF, VEGFA, VEGFR1, VEGFR2), statistically significantly correlated with best overall response to bevacizumab combined with carboplatin–gemcitabine or carboplatin–paclitaxel in chemotherapy-naive patients with advanced/recurrent nonsquamous NSCLC [10]. To date, there are no validated biomarkers that predict response to bevacizumab. Further identification of blood, tissue and imaging biomarkers in relation with clinical outcome are under investigation in exploratory trials.

The manageable safety profile and efficacy of first-line bevacizumab (7.5 or 15 mg/kg every 3 weeks) in combination with various standard chemotherapy regimens (generally carboplatin and cisplatin doublets), was confirmed in a Phase IV study (Safety of Avastin in Lung), enrolling a broad range of patients, including those receiving concomitant treatment, elderly patients and patients with a PS of 2 [11]. The efficacy of bevacizumab-based therapy was generally similar across chemotherapy regimens, with the exception of patients who received non-platinum doublets or monotherapy, who had slightly lower median OS than patients receiving other regimens. In addition, patients receiving taxanes in Safety of Avastin in Lung had slightly longer median time to progression (TTP) and OS than did those receiving nontaxanes. Median OS (14.6 months), disease control rate (DCR: 89%) and ORR (52%) were higher than data previously reported in clinical trials of NSCLC (E4599 and AVAiL). However, the limitations of this study should be considered during interpretation of the data: any information about subsequent lines of therapy, nonstandard methods and timing of tumor assessments and the absence of centralized independent efficacy evaluation [11]. Subsequently, a preplanned subgroup analysis demonstrated a similar clinical benefit (OS, TTP, DCR and ORR) from first-line bevacizumab-based therapy in elderly patients (aged >65 years) with nonsquamous NSCLC as their younger counterparts experienced without increased toxicity [12].

To date, several trials on maintenance treatment in advanced nonsquamous NSCLC have been performed

or are ongoing. Based on pemetrexed benefit as continuation maintenance in the AVAPERL trial, bevacizumab and pemetrexed as maintenance in 253 patients with advanced nonsquamous NSCLC, without progression after first-line induction therapy (cisplatin + pemetrexed + bevacizumab), improved median PFS by almost 50% versus bevacizumab alone (median PFS from start of first-line was 10.2 vs 6.6 months; HR: 0.50; 95% CI: 0.37–069; p < 0.001; median PFS from randomization was 7.4 vs 3.7 months; HR: 0.48; 95% CI: 0.35–066; p < 0.001) [13]. OS was among secondary end points of the trial: a preliminary analysis demonstrated a median time of 15.7 months with bevacizumab maintenance, with the median survival not yet reached in patients randomized to bevacizumab + pemetrexed (HR: 0.75; 95% CI: 0.47–1.20; p = 0.23) [13].

A Phase III study ('Point Break' study) randomized 939 untreated advanced nonsquamous NSCLC patients to receive pemetrexed + carboplatin + bevacizumab induction followed by pemetrexed + bevacizumab maintenance (arm A) or paclitaxel + carboplatin + bevacizumab induction followed by bevacizumab maintenance (arm B). This trial did not meet the primary end point of superior OS: 12.6 months in arm A versus 13.4 months in arm B (HR: 1.00; p = 0.949). Among secondary end points, there was only a statistically significant improvement in PFS (6.0 vs 5.6 months; HR: 0.83; p = 0.012) in arm A, while any differences were showed in terms of ORR (34.1 vs 33.0%) and DCR (65.9 vs 69.8%). Toxicity profiles differed between regimens: more drug-related grade 3/4, thrombocytopenia and fatigue in arm A, while more grade 3/4 neutropenia, febrile neutropenia, sensory neuropathy and grade 1/2 alopecia in arm B [14].

An ongoing randomized Phase IIIb study is recruiting patients with advanced nonsquamous NSCLC in progression after four to six cycles of first-line treatment (bevacizumab + a platinum-based doublet) and a minimum of two cycles of bevacizumab (monotherapy) maintenance treatment. These patients will be randomized to receive second-line chemotherapy alone (limited to pemetrexed, docetaxel or erlotinib) or in combination with bevacizumab. The primary end point of this study is OS [15].

In conclusion, the addition of bevacizumab to first-line chemotherapy should be considered among treatment options for selected patients with advanced NSCLC. Moreover, several open questions are waiting responses: currently, data of direct efficacy comparisons between platinum-based chemotherapy plus bevacizumab with newer treatment options, such as cisplatin plus pemetrexed, are not available; the efficacy of bevacizumab as maintenance therapy is still unclear; the identification of molecular biomarkers defining groups of patients potentially benefiting from the drug are under investigation.

EGFR inhibitors in clinical practice

■ Monoclonal antibody: cetuximab

Cetuximab is a chimeric human/murine IGg1 monoclonal antibody that selectively binds to the extracellular domain of EGFR on the tumor cell, thereby inhibiting receptor-associated tyrosine kinase activation. After promising data of a randomized Phase II trial [16], a large randomized Phase III trial (FLEX), assessed the efficacy of cetuximab in addition to first-line platinum-based chemo-therapy (cisplatin/vinorelbine) as first-line treatment in 1125 advanced NSCLC patients with EGFR-detectable by immunohistochemistry (IHC) [17]. The primary end point was OS. The combination of chemotherapy and cetuximab demonstrated a small but statistically significant benefit in OS (median 11.3 vs 10.1 months; HR: 0.871; 95% CI: 0.762–0.996; p = 0.044) with an increase in ORR (36 vs 29%; p = 0.010), without a difference in PFS (median 4.8 months in both groups; HR: 0.943; 95% CI: 0.825–1.077). Prolongation of survival was achieved with an acceptable safety profile: the grade 3 acne-like rash was the main cetuximab-related adverse event (AE) and it occurred in 10% of patients. At the subgroup analyses, the benefit associated with cetuximab was independent of sex, performance status, tumor histology and smoking status. Interestingly, the best predictor of clinical benefit for the addition of cetuximab to chemotherapy appeared to be treatment-related early acne-like skin rash: the development of a skin rash of any grade was related to a significantly longer survival (median 15.0 vs 8.8 months; HR: 0.63; 95% CI: 0.52–0.77; p < 0.001) [18].

Subsequently, cetuximab was tested in combination with carboplatin plus a taxane in 676 chemotherapy-naive patients with advanced NSCLC enrolled in the randomized Phase III trial BMS 099, without restrictions by histology or *EGFR* expression [19]. As with the FLEX study, the addition of cetuximab to chemotherapy was associated with a statistically significant benefit in ORR (25.7% and 17.2%, respectively; p = 0.007) without any improvement in the primary study end point, PFS (median PFS 4.4 vs 4.24 months; HR: 0.902; 95% CI 0.761–1.069, p = 0.24). However, the difference in OS was similar in both studies (approximately 1.3 month increase in median OS and 11–13% reduction in the death risk), although in BMS099 trial was not statistically significant [19].

In addition to *EGFR* expression, the predictive use of other candidate molecular biomarkers including *KRAS* mutation status, *EGFR* mutation status, and *EGFR* gene copy number have been investigated in retro-spective

analyses of tissue from patients enrolled in both the FLEX study and Phase III, BMS099 study. None of these biomarkers seemed to have a predictive role in clinical benefit associated with the addition of cetuximab to chemotherapy [18]. However, in a further analysis of the FLEX-study, authors calculated an IHC score (H score) to provide a more detailed assessment of EGFR protein expression and how this affected response to treatment with cetuximab in patients with advanced NSCLC. The H score takes into account the percentage of cells (0–100%) in each intensity category (0–3+) and computes a final score, on a continuous scale between 0 and 300. High EGFR expression according to a tumor IHC score of 200 or more seems to be the only effective pretreatment biomarker so far identified for the prediction of clinical benefit from chemotherapy plus cetuximab in the first-line treatment of advanced NSCLC [20].

Finally, a meta-analysis of four randomized Phase II/III studies involving 2018 patients (1003 patients treated with chemotherapy + cetuximab and 1015 patients treated with chemotherapy alone) was performed. This meta-analysis demonstrated a significant benefit for cetuximab combination over chemotherapy alone, irrespective of which platinum doublet was used and in all histological subtypes of NSCLC in term of OS (HR: 0.878; 95% CI: 0.795–0.969; p = 0.010), PFS (HR: 0.899; 95% CI: 0.814–0.993; p = 0.036), and ORR (odds ratio: 1.463; 95% CI: 1.201–1.783; p < 0.001) with a favorable safety profile [21].

Despite these positive results, both the FDA and the European Medicines Agency rejected the licensing of cetuximab in combination with chemotherapy for first-line therapy of advanced NSCLC in consideration of the small OS benefit and no significant prolongation of PFS or improvement in health-related quality of life.

■ *EGFR* tyrosine kinase inhibitors

Gefitinib

Gefitinib is a small molecule, orally active, selective and reversible *EGFR* tyrosine kinase inhibitor (TKI) that blocks the signal transduction pathways implicated in the proliferation and survival of cancer cells. Despite promising preclinical results, showing that *EGFR*-TKIs can enhance the antitumor activity of chemotherapy, the concomitant addition of gefitinib to first-line platinum-based chemotherapy (gemcitabine and cisplatin [22] or paclitaxel and carboplatin [23]) of advanced NSCLC, followed by single-agent gefitinib until disease progression, produced negative results in two large randomized Phase III trials.

Furthermore, gefitinib when administered as single-agent in pretreated patients, demonstrated a non-inferiority to docetaxel in terms of OS [24]. Of note, patients enrolled in these trials were not selected for any clinical or molecular characteristic. In 2004, specific activating mutations within the *EGFR* tyrosine kinase domain (deletion of exon 19 or L858R amino acid substitutions in exon 21) were correlated with the dramatic responses to gefitinib. These mutations were found more frequently in a subpopulation of NSCLC patients with characteristics associated with a better treatment outcome: Asian origin, female gender, history of never or light smoking, as well as adenocarcinoma histology [25,26].

Based on this evidence, four randomized Phase III clinical trials evaluated the role of gefitinib as first-line therapy of patients with advanced NSCLC, selected based on clinical or molecular features [27–30].

In the first randomized Phase III trial (IPASS), gefitinib demonstrated not only the noninferiority but also the superiority when compared with carboplatin–paclitaxel in terms of PFS (HR: 0.74; 95% CI: 0.65–0.85; p < 0.001) in 1217 Asian patients with advanced NSCLC selected on clinical, but not on molecular, markers [27]. In all clinical subgroups, PFS was significantly longer with gefitinib than chemotherapy, but there was a statistically significant interaction with *EGFR*-mutation status (p < 0.001): gefitinib was significantly better than chemotherapy in patients with *EGFR*-mutated tumors (HR for PFS: 0.48; 95% CI 0.36–0.64; p < 0.0001), whereas chemotherapy was significantly better in *EGFR* wild-type patients (HR for PFS: 2.85; 95% CI: 2.05–3.98; p < 0.0001). Similarly, in the mutation-positive subgroup, gefitinib demonstrated a higher ORR than chemotherapy (71.2% with gefitinib vs 47.3% with carboplatin–paclitaxel; p < 0.001), in contrast to the mutation-negative subgroup (1.1 [one patient] vs 23.5%; p = 0.001). However, the significant treatment-related differences for PFS and ORR according to *EGFR*-mutation status were not observed for OS, most likely due to the subsequent treatments [31]. Gefitinib was superior to chemotherapy in terms of quality of life and demonstrated a more favorable toxicity profile. *Post hoc* analyses of PFS by *EGFR*-mutation type, confirmed exon-19 deletions and exon-21 point mutations as the strongest predictive biomarker for gefitinib efficacy, independently of *EGFR* gene copy number, with a slightly greater advantage in the exon 19 deletions subgroup.

The First-SIGNAL study, similar to the IPASS, compared the efficacy of gefitinib with standard chemotherapy as first-line treatment (gemcitabine–cisplatin) in clinically selected Asian patients. While OS (primary end point) was similar in both groups, failing to show the hypothesized superiority of gefitinib compared with chemotherapy, PFS at 1 year was superior in the gefitinib compared with chemotherapy group (20.3 and 5.0%,

respectively) and also quality of life improved in the gefitinib group. Moreover, a subgroup analysis showed an OS of 30.6 months in *EGFR*-mutation-positive patients and 18.4 months in those without mutations (HR: 0.845; p = 0.643) treated with gefitinib and a PFS of 8.4 and 2.1 months, respectively (HR: 0.394; p = 0.0006); the ORR was also dramatically better in this subgroup of patients (84.6 and 25.9%, respectively) [28].

Two randomized Phase III studies have been performed in *EGFR*-mutated patients with advanced NSCLC, to compare the efficacy of gefitinib versus chemotherapy in the first-line setting [29,30]. In the WJTOG3405 study, gefitinib conferred longer PFS and higher ORR than first-line chemotherapy (cisplatin + docetaxel) in a molecularly defined group of patients with NSCLC (median PFS: 9.2 vs 6.3 months; HR: 0.489; 95% CI: 0.336–0.710; p = 0.0001; ORR: 62.1 vs 32.2%, respectively). So, the presence of *EGFR* mutations, and not the clinical background of patients, was related with clinical efficacy [29]. Similarly, in the NEJ002 trial, gefitinib compared with carboplatin plus paclitaxel demonstrated a significant superiority in terms of PFS (median PFS: 10.8 vs 5.4 months; HR: 0.30; 95% CI: 0.22–0.41; p < 0.001) and of ORR (73.7 vs 30.7%; p < 0.001) independently of type of *EGRF* mutation (exon 19 deletion or L858R point mutation), without any difference in OS between the two treatment groups (median OS: 30.5 months with gefitinib vs 23.6 months with chemotherapy; p = 0.31) [30]. Therefore, these studies highlighted the role of molecularly based selection of patients on the basis of *EGFR* mutation status.

Based on these results, in July 2009 gefitinib was approved by European Medicines Agency for the treatment of locally advanced or metastatic NSCLC, harboring *EGFR*-activating mutations, across all lines of therapy.

Erlotinib

Erlotinib is another selective and reversible EGFR-TKIs that demonstrated a significant improvement in OS when compared with placebo in pretreated patient with advanced NSCLC [32]. In a subsequent analysis, the detection of *EGFR* mutations (exon 19 deletion and L858R) in the BR.21 study was associated with a significantly better response to erlotinib compared with the wild-type, but the mutation status was not predictive of OS [33]. Recently, to assess the role of erlotinib in *EGFR* wild-type patients as second line, a prospective Phase III trial compared erlotinib with docetaxel, demonstrating a clear superiority of chemotherapy in term of PFS [34].

After the evidence of efficacy in previously treated patients with advanced NSCLC [32], several studies have also evaluated erlotinib in a first-line setting. In the TRIBUTE [35] and TALENT [36] Phase III randomized trials, the combination of erlotinib and concurrent platinum doublets (carboplatin–paclitaxel and cisplatin–gemcitabine, respectively) did not demonstrate a survival benefit over chemotherapy alone in chemotherapy-naive unselected advanced NSCLC patients. In another Phase III trial in untreated advanced NSCLC patients with a poor PS (ECOG PS 2/3 or PS 0/1 unfit for platinum chemotherapy), erlotinib plus best supportive care did not improve OS versus best supportive care alone (HR: 0.98; p = 0.77), but in subgroup analyses OS and PFS were significantly longer for females (HR: 0.75; p = 0.04 and HR: 0.64; p < 0.001, respectively) and also PFS for adenocarcinoma histology (HR: 0.74; p = 0.03) [37]. Similar to gefitinib, the patient selection process is essential to identify which could gain interesting clinical benefit by erlotinib as front-line therapy.

The first Phase III trial conduced on chemotherapy-naive *EGFR*-mutated patients with advanced NSCLC (OPTIMAL study) showed that erlotinib provides significantly longer PFS than chemotherapy (gemcitabine and carboplatin) in this preselected population, prolonging median PFS of 8.5 months (13.1 vs 4.6 months; HR: 0.16; 95% CI: 0.10–0.26; p < 0.0001) [38]. Activating *EGFR* mutations were the most important factor for therapeutic benefit with TKI in terms of PFS, irrespective of clinical characteristics (age, PS, tumor histology or smoking status). In addition, the result in term of ORR was higher in the erlotinib group (82 vs 36%), similar across clinical subgroups.

Although this study included Asian patients only, the efficacy of erlotinib was independent of ethnic origin. In particular, in the Phase III study EURTAC, erlotinib compared with platinum-based chemotherapy improved PFS significantly (primary end point) also in non-Asian chemotherapy-naive *EGFR*-mutated patients: median PFS of 9.7 months (95% CI: 8.4–12.3) with erlotinib compared with 5.2 months (95% CI: 4.5–5.8) with chemotherapy (HR: 0.37; 95% CI: 0.25–0.54; p < 0.0001). In the multivariable analysis only treatment group and PS were significant factors for PFS. Patients treated with erlotinib also had a higher ORR (64 vs 18%), with milder side-effects than did those treated with standard chemotherapy [39]. In contrast to OPTIMAL trial, former smokers seemed to benefit less from erlotinib than did current smokers in the subgroup analyses, but these results must be interpreted with caution for the small number of patients in each group. In the EURTAC, OS did not differ significantly between treatment groups (median OS: 19.3 months with erlotinib vs 19.5 months with chemotherapy group). These data confirmed the efficacy of erlotinib in PFS and ORR in European patients with *EGFR*-mutation-positive NSCLC compared with standard chemotherapy, as in previous studies in Asian patients [39].

Based on these results, erlotinib, already approved as second- or third-line without molecular restrictions, has also recently been approved as first-line in patients with *EGFR* mutations.

Irreversible EGFR-TKIs

Multiple strategies, including the development of agents that bind irreversibly and/or inhibit multiple targets simultaneously, are being investigated to treat NSCLCs that are resistant to first-generation EGFR TKIs. Unlike reversible TKIs, irreversible TKIs bind covalently at the ATP-binding site of mutant EGFR, overcoming the competition with ATP that becomes unfavorable to reversible TKIs in the presence of the T790M mutation. Among several irreversible multitargeted HER family TKIs, neratinib (no further clinical development in NSCLC), dacomitinib (PF00299804) and afatinib are under clinical investigation.

Afatinib is an oral irreversible HER family inhibitor, that binds to EGFR (HER-1), HER-2 and HER-4. The role of afatinib (50 mg/day) in patients with NSCLC (pretreated with one or two chemotherapy treatments and progressed following treatment with reversible TKIs) has been explored in a Phase IIb/III randomized trial, LUX-Lung 1. The study did not meet its primary end point (OS: median OS 0.8 months for afatinib and 12.0 months for placebo), but afatinib arm demonstrated significantly better results in terms of PFS (3.3 vs 1.1 months), DCR at 8 weeks (58 vs 19%) and ORR (7.4 vs 0.5%) than with placebo. Diarrhea and rash/acne were the two most common side effects of afatinib, effectively managed by supportive care and dose reduction [40].

Recently, similar data in terms of PFS (3.3 months) and ORR (8%) were reported in an interim analysis of part of a Phase III trial that assessed afatinib monotherapy (50 mg/day) in 1154 patients who had previously failed to respond to chemotherapy and reversible EGFR TKIs. After progression, patients with clinical benefit (≥12 weeks) were eligible to continue afatinib (40 mg/day) plus paclitaxel or receive investigator's choice chemotherapy (Part B). Results of part B are pending [41].

Interestingly, Afatinib demonstrated activity in the first- and second-line treatment of 129 EGFR-TKIs-naive patients with lung adenocarcinoma harboring *EGFR* mutations (especially deletion 19 or L858R mutations) [42].

LUX-Lung 3 is the largest prospective trial conducted in 345 untreated patients with *EGFR* activating mutations advanced adenocarcinoma, comparing afatinib (40 mg/day) with pemetrexed–cisplatin. Treatment with afatinib significantly prolonged PFS (median PFS 11.1 vs 6.9 months, with an improvement of 6.7 months in presence of deletion 19/L858R mutations) with a significant improvement in ORR (56 vs 23%) and clinical benefit (delay in time to deterioration of cancer-related symptoms of cought and dyspnea). Most common drug-related AEs were diarrhea (95%), rash (62%) and paronychia (57%) in the afatinib arm [43].

Ongoing trials are evaluating afatinib as a first-line treatment in patients with advanced NSCLC with *EGFR* mutations enrolled in a Chinese Phase III trial (LUX-Lung 6, investigating the efficacy and safety of afatinib compared with standard chemotherapy [201]) and in a Phase IIb trial (LUX-Lung 7, a investigating afatinib head-to-head vs gefitinib; [202]). Another ongoing Phase III trial (LUX-Lung 8 [203]) is evaluating afatinib head-to-head versus erlotinib in second-line treatment of squamous cell carcinoma of the lung.

Dacomitinib (PF00299804) is a highly selective irreversible small-molecule inhibitor of all catalytically active members of the HER family of tyrosine kinases. In preclinical studies, it has demonstrated greater anticancer activity in gefitinib- and erlotinib-sensitive and -resistant cell lines and xenograft NSCLC models [44]. Dacomitinib demonstrated antitumor activity in Phase I and II trials, in NSCLC patients after progression with an EGFR TKI and one or more chemo-therapy regimens [45–47]. Subsequently, the first trial that directly compared an irreversible pan-HER TKI with a reversible EGFR-selective TKI in 188 patients who had failed at one or two chemotherapy regimens (no prior HER-directed therapy), demonstrated improved PFS (median PFS: 2.86 vs 1.91 months; $p = 0.012$) after treatment with dacomitinib (45 mg/day) over treatment with erlotinib (150 mg/day). Some imbalance between treatment arms may have influenced these results. In the subgroup analysis, patients with *KRAS* wild-type/*EGFR* any-status tumors treated with dacomitinib had a two-fold improvement in PFS over erlotinib (PFS: 3.71 vs 1.91 months; $p = 0.006$); however a significant benefit in PFS was also observed in *KRAS*/*EGFR* wild-type tumors (PFS: 2.21 vs 1.84 months; $p = 0.043$), with no difference in *EGFR* mutant patients (PFS: 7.44 months; $p = 0.098$). A trend toward improved OS with dacomitinib relative to erlotinib was observed that did not reach statistical significance (9.53 vs 7.44 months). Common treatment-related AEs were dermatologic and gastrointestinal, predominantly grade 1 to 2, and more frequent with dacomitinib [48].

These results have to be confirmed by an ongoing Phase III trial (ARCHER 1009) in advanced NSCLC patients previously treated with at least one prior regimen. The PFS in all unselected population and in wild-type *KRAS* patients (independently of *EGFR* status) are the co-primary end points of the study, in order to evaluate dacomitinib in all patients and, prospectively,

the potential correlation between clinical outcome and *KRAS* molecular status [49].

An ongoing Phase II trial will explore the safety and efficacy of dacomitinib in chemotherapy-naive patients with adenocarcinoma, former light or nonsmoking history, *EGFR* mutation or *HER2* amplification or mutation [204].

c-MET inhibitors

Despite an initial response to the treatment of EGFR-TKIs in responsive patients, most patients inevitably acquire resistance after a progression-free period of approximately 9–13 months [50]. Different mechanisms have been reported to be associated with acquired resistance to EGFR-TKIs such as the T790M mutation, activation of IGF1R and particularly mesenchymal-epithelial transition (MET) amplification and overexpression of HGF [51]. The MET–HGF signaling pathway can be activated by overexpression of the ligand HGF (autocrine or paracrine stromal secretion) or by *MET* overexpression, *MET* genomic amplification or its activating mutations. *MET* amplification rarely occurs in untreated NSCLC and is related with poor prognosis; however, it is a resistance mechanism in 5–20% of patients with *EGFR* mutations progressing after initial response to TKI therapy [52]. Inhibiting *c-MET* signaling is emerging as a promising strategy for a new class of targeted lung cancer therapies and several c-MET inhibitors are in various stages of clinical development.

Tivantinib (ARQ 197) is an oral selective, non-ATP-competitive c-MET inhibitor. Based on interesting results in term of PFS in *EGFR* wild-type, nonsquamous cell and *KRAS* mutant patients, there are several Phase II and III trials of ARQ 197 in combination with erlotinib currently being planned or performed in this cohort of subjects [205–208].

MARQUEE is a Phase III, randomized, double-blind, placebo-controlled trial that evaluated the efficacy of tivantinib plus erlotinib versus erlotinib alone in chemotherapy pretreated (EGFR-TKs and c-MET inhibitors-naive) patients with advanced nonsquamous NSCLC [207]. The trial started in November 2010 but was stopped early following a planned interim analysis, when they found that the trial would not meet its primary end point of improved OS. Although the interim analysis demonstrated a statistically significant improvement in PFS in the intent-to-treat population, this benefit did not carry over to OS.

An ongoing Phase III trial (ARQ197–006) is evaluating if the combination regimen of ARQ 197 with erlotinib will improve OS compared with erlotinib monotherapy in a similar setting of patients enrolled in the MARQEE trial, but with wild-type *EGFR* status. This study started in July 2011, and the final data collection date for the primary outcome measure is December 2013 [208].

MetMAb (Onartuzumab, OA-5D5) is a potent anti-c-MET monovalent antibody that blocks HGF binding to c-MET and HGF-induced dimerization, receptor activation and downstream activity. MetMAb was recently studied in a randomized double-blind Phase II study (OAM4558g) comparing MetMAb plus erlotinib with placebo plus erlotinib in 137 patients with advanced NSCLC as second- or third-line therapy. In ab intention to treat population, the combination of MetMAb and erlotinib failed to significantly improve median TTP over erlotinib (2.2 vs 2.6 months; p = 0.69) or OS (8.9 vs 7.4 months; p = 0.34). However, in the Met-diagnostic positive (Met Dx+) group (defined as ≥50% of tumor cells staining 2+ or 3+ intensity for c-MET by IHC, Met Dx+), MetMAb plus erlotinib resulted in a statistically and clinically significant improvement in both PFS (2.9 months in combination arm vs 1.5 months in control arm; p = 0.04; HR: 0.53) and OS (12.6 months in combination arm vs 3.8 months in control arm; p = 0.002; HR: 0.37) resulting in a near threefold reduction in the risk of death. As tivantinib, the therapy was well tolerated and the toxicity profile was comparable between treatment arms [53].

MetMAb is currently being evaluated in Phase II–III studies [209–211]. Therefore, an ongoing Phase III randomized, multicenter, double-blind, placebo-controlled study (MetLUNG study, [209]) is recruiting pretreated patients with Met Dx+ (defined as ≥50% of tumor cells staining 2 or 3+ intensity for c-MET by IHC, Met Dx+) advanced NSCLC to evaluate efficacy and safety of onartuzumab in combination with erlotinib. The primary end point is OS. Secondary end points include PFS, response rates, safety, patient-reported outcomes and pharmacokinetics [209].

ALK inhibitors: crizotinib

ALK is a transmembrane protein, identified in 1994 in anaplastic large-cell lymphoma with t(2;5) chromosomal translocation as a fusion protein to nucleo-phosmin. EML4 is a cytoplasmic protein essential for the formation of microtubules and microtubule binding protein [54].

The *EML4-ALK* fusion gene results from intrachromosomal rearrangement within chromosome 2 containing the amino-terminal half of *EML4* and the intracellular catalytic domain of *ALK*. This region of *EML4* results in constitutive dimerization of the kinase domain of *ALK* with aberrant activation of downstream signaling such as Akt, STAT3 and ERK1 and 2 involved in the inhibition of apoptosis and the promotion of

cellular proliferation [55]. To date, at least 11 variants of *EML4-ALK* have been reported with unclear clinical significance: the most common variants were variant 1 (detected in 33% of NSCLC patients), which leads to the juxtaposition of exon 13 of *EML4* to exon 20 of *ALK* (E13;A20) and variant 3a/b (29% of NSCLC patients), in which exon 6 of *EML4* was joined to exon 20 of *ALK* (E6a/b;A20) [56]. In *ALK* rearranged NSCLC, *EML4* is not the exclusive fusion partner with *ALK*. Two other fusions (*mTFG* and *KIF5B*) have been identified as an *ALK*-fusion partner from NSCLC tumor samples [57,58]. The presence of these non-*EML4* fusion partners for *ALK* has implications for the method used for clinical detection of *ALK* translocated NSCLC.

Currently, a major issue is defining the best way to assess for the presence of *ALK* gene rearrangements (*ALK* positivity) and the resulting aberrant ALK expression: fluorescent *in situ* hybridization (FISH) is considered the 'gold standard'. However, IHC and reverse transcriptase PCR have advantages and are widely used in ongoing research [59,60]. In the clinic, the distinction between *EML4-ALK* and *EGFR* mutant tumors has important therapeutic implications. Whereas *EGFR* mutation confers sensitivity to EGFR TKIs, *EML4-ALK* was strongly associated with resistance. Therefore, in these analyses *ALK* rearrangements were identified as a poor predictive marker for the EGFR TKI response. These data suggested that patients with activating *EGFR* mutations, objective responses to previous *EGFR* could be excluded from future *ALK* screening and this could be an effective enrichment strategy for *ALK*-positive cases. This result validated the assertion that effective targeted therapy requires the appropriate patient population to be selected [61].

ALK rearrangements in lung cancer can potentially be targeted using specific drugs. However, *EML4-ALK* is a relatively rare event in unselected NSCLC population, closest to 2–7% in adenocarcinoma NSCLC population [62]. Therefore, the identification of the appropriate patients for therapy remains key to the overall success of such targeted therapies. Several trials have investigated associations between *ALK* fusion status and clinicopathological variables in NSCLC, showing that *ALK* fusion gene appears to occur more frequently in adenocarcinoma histology, in light (≤10 pack years) or never smokers, younger age, but rarely (<1%) in those with squamous cell carcinoma; also the *ALK* fusion gene tends to occur independently from *EGFR* and *KRAS* mutations [63,64].

At the maximum tolerated dose (MTD; 250 mg twice a day), in patients with *ALK*-positive advanced NSCLC, crizotinib demonstrated a marked efficacy, with more than 60% of patients having an objective response, with responses seemingly rapid (median time to first documented objective response was 7.9 weeks) and durable (median duration of response was 49.1 weeks).

Visual effects, nausea, diarrhea, constipation, vomiting, and peripheral edema were the most common AEs, which occurred early and seemed to improve over time, with the exception of the treatment-emergent edema that seemed to be a late-onset cumulative AE. The grade 3/4 AEs were neutropenia, raised alanine aminotransferase, hypophosphatemia and lymphopenia [65]. Recently, rapid-onset hypogonadism and lower total serum testosterone levels have been noted in male patients treated with crizotinib, probably correlated to a central (hypothalamic or pituitary) effect [66].

The marked activity of crizotinib observed in a Phase I study [65] has led to Phase II–III trials. PROFILE 1005 is a Phase II, open-label single-arm study of the efficacy and safety of crizotinib in 901 patients with advanced NSCLC harboring translocation or inversion involving the *ALK* gene locus detected by FISH. Crizotinib demonstrated a high response rate with an ORR of 59.8% (four complete responses [CRs] and 151 partial responses [PRs]) in 259 response-evaluable patients. The responses occur within the first 8 weeks of treatment in 71% of patients with a CR or PR (median time to response: 6.1 weeks). Median PFS for the mature population was 8.1 months (95% CI: 6.8–9.7) [67].

Recently, data on the registration Phase III trial PROFILE 1007 were presented. Crizotinib was superior to standard single-agent chemotherapy (pemetrexed or docetaxel) in terms of response (ORR: 65 vs 20%; $p < 0.0001$) and PFS (median 7.7 vs 3.0 weeks; $p < 0.0001$) in 347 *ALK*-positive advanced NSCLC patients pretreated with first-line, platinum-based chemotherapy. To date, data on the OS rate with the two drugs are still immature. Interestingly, despite side effects (diarrhea, nausea, vomiting and elevated transaminases), patients still reported improved quality of life on crizotinib compared with chemotherapy [68].

Among ongoing trials, the Phase III PROFILE 1014 trial is evaluating efficacy and safety of crizotinib versus pemetrexed–cisplatin or pemetrexed–carboplatin in previously untreated patients with *ALK* rearranged nonsquamous NSCLC, with PFS as primary end point. Another ongoing Phase I trial is testing the combination of oral/ALK inhibitor (PF-02341066) and PAN-HER inhibitor (PF-00299804) in patients with advanced NSCLC [69].

In addition, the combination of erlotinib with crizotinib seems to be well tolerated with no unexpected AEs, and shows signs of activity in a pretreated advanced NSCLC enrolled in Phase I/II study [70].

Recent retrospective analyses have indicated that *ALK*-positive patients had a prolonged PFS when treated with pemetrexed-based therapies, compared with other molecularly defined subtypes of NSCLC patients (e.g., *EGFR* mutant, *KRAS* mutant, triple negative) [71]. The

low level of thymidylate synthase expression, associated with *EML4-ALK*-positive NSCLC, may have contributed to the long-term response to pemetrexed-based chemotherapy [72].

Interestingly, clinical activity of crizotinib was also showed in a distinct subpopulation of NSCLC patients, with chromosomal rearrangements of the *ROS1* receptor tyrosine kinase gene detected by FISH assay, but negative for *ALK* rearrangement; the ORR was 50% (10/20), with nine PRs and one CR [73].

In addition to crizotinib, new molecules continue to be described, and several clinical trials are in progress. Some examples include CH5424802 (AF802) [74], Hsp90-inhibitors, such as IPI-504 [75], AUY922 [76] and LDK378 [77]. Among Hsp90 inhibitors, IPI-504 was the first molecule with a demonstrated clinical activity in NSCLC patients with *ALK* rearrangements enrolled in a Phase I/II study. However, additional research is required to prospectively evaluate the efficacy of Hsp90 inhibition in this setting of patients and other oncogenic driver mutations [78]. Another highly potent HSP90 inhibitor (AUY922), tested in an ongoing Phase II trial, registered the greatest median PFS rate at 18 weeks (45%) in *EGFR*-mutated patients in progression with TKIs [76].

As has been seen with other targeted therapies, resistance will emerge in many, if not all, patients who demonstrate initial response to ALK inhibition. The acquired resistance to crizotinib was due to two mechanisms: *ALK*-dominant mechanism (novel *ALK* kinase domain mutation such as L1196M and C1156Y, and/or *ALK* copy number gain) that preserve the dominance of ALK signaling in the crizotinib-resistant state [79–81]; *ALK*-nondominant mechanisms (different second oncogenic drivers coexisting in the same cell with the *ALK* rearrangement, including *EGFR* and *KRAS* mutations, *CNG* of *KIT*, and ligand-driven activation of wild-type *EGFR* and *HER2*) [79–81]. So, in order to overcome resistance, in *ALK*-dominant situations, second-generation ALK-TKI (LDK378), Hsp90 inhibitors (STA-9090 and IPI-504) are being explored in ongoing early-phase clinical trials. Meanwhile, in a non--dominant situation, combination therapy with agents directed against different drivers or nonmolecularly focused cytotoxic chemotherapy may be required [82]. Of note, in the pre-crizotinib setting, preliminary data suggested that pemetrexed, alone or in combination, may be particularly effective in *ALK*-positive NSCLC [67]. Finally, in patients with isolated CNS progression, local CNS therapy (e.g., radiotherapy) and continuing crizotinib to maintain extracranial control should be considered, while in isolated extracranial progression (so-called 'oligoprogressive disease') local ablative therapy (e.g., with stereotactic body radiation therapy or metastasectomy) with continuation of crizotinib should be suitable [83].

On 26 August 2011 the FDA granted accelerated approval of crizotinib (Xalkori®) and on 19 July 2012 the European Medicines Agency adopted a positive opinion, recommending the granting of a conditional marketing authorization for crizotinib, for the treatment of adults with previously treated *ALK*-positive advanced NSCLC.

Novel potential molecular targets in NSCLC

HER2 (or ERBB-2) is a member of the EGFR family of receptor tyrosine kinases. The homodimerization or heterodimerization with other members of the HER family activates various kinases, including the PI3K pathway, MAPK pathway and the JAK/STAT pathway. In NSCLC, HER2 is overexpressed in approximately 20% of patients, although *HER2* mutations occur in only 2%, with a similar phenotype as tumors with *EGFR* mutations (adenocarcinoma, never- or light-smokers, women and Asian patients). *HER2* mutations involve in-frame insertions in exon 20, leading to constitutive activation of the receptor, and are not presented in tumors harboring *EGFR* or *KRAS* mutations [84,85]. *In vitro*, cells harboring these mutations are sensitive to TKIs targeting HER2 and EGFR but are resistant to TKIs targeting EGFR alone [86]. A monoclonal antibody that targets HER2, trastuzumab (herceptin) has been tested in advanced NSCLC patients overexpressing HER2 in a Phase II trial, in combination with cisplatin and gemcitabine [212], and failed to show survival benefit in all HER2 IHC-positive lung cancers. Furthermore, HER2 3+/FISH-positive patients may benefit from trastuzumab (response rate: 83% and median PFS: 8.5 weeks) [87]. Trastuzumab has been tested alone, in IHC-positive or, respectively, HER2-mutated or -amplified NSCLC [213,214] and in combination with carboplatin and paclitaxel, with a feasible toxicity profile [88]. Results are pending. Lapatinib has been tested in molecularly unselected advanced NSCLC patients, including one trial that has been stopped for futility after interim analysis [215]. Pertuzumab has been tested in a Phase II trial in advanced NSCLC patients with *HER2* activation [216]. Results are pending. Unlike a potent irreversible ErbB family blocker, Afatinib has showed more promising results in *HER2*-mutation-positive NSCLC.

B-RAF is a serine threonine kinase enzyme that links RAS GTPase to enzymes of the MAPK family that are known to be involved in the control of cell proliferation. *BRAF* mutations cause increased kinase activity and constitutive activation of MAPK2 and MAPK3. In NSCLC, 1–3% of adenocarcinoma show *BRAF* mutations, which are most commonly the non-Val600Glu mutations [89]. Interestingly, mutations in *BRAF* are most likely to occur in adenocarcinomas and in former

or current smokers, and are mutually exclusive with *EGFR* and *KRAS* mutations, as well as *ALK* rearrangements [90]. Several BRAF inhibitors are in early clinical development, such as XL281 (BMS-908662; Bristol-Myer Squibb [217]) AZD6244 (AstraZeneca [218,219]) and GSK2118436 (GlaxoSmithKline [220]), with pending results.

PI3Ks are a family of intracellular, heterodimeric lipid kinases that phosphorylate the 3′hydroxyl group of phosphatidylinositols and phosphoinositides. The PI3K pathway regulates diverse cellular processes including cell proliferation, survival, metabolism, apoptosis and cell migration. PIK3 signaling is negatively regulated by an important tumor suppressor, PTEN. Therefore, the PIK3 pathway is frequently overactivated in NSCLC due to three mechanisms: mutation/amplification of *EGFR*, mutation or loss of *PTEN* and mutation of *PIK3CA*.

In NSCLC, *PIK3CA* mutations most frequently affect the catalytic domain encoded in exon 9 and are found in approximately 2% of NSCLC, as frequently in adenocarcinoma as in squamous cell carcinoma, inducing oncogenic cellular transformation. Amplification of *PIK3CA* has also been observed in NSCLC, particularly in squamous cell carcinoma and male patients who smoke; however, the oncogenic potential of *PIK3CA* amplification alone has not yet been shown [91]. Preclinical data suggests that coexisting *KRAS* and *PIK3CA* mutations are associated with resistance to PI3K/AKT/mTOR inhibitors [92].

Several inhibitors of the PIK3CA/AKT/mTOR pathway have been tested or are currently under investigation in clinical trials (BKM120, GDC-0941, NVP-BEZ235, XL765, XL147, PI103 and PX-866). BKM120 is currently being evaluated for use as a single agent in a Phase II trial in pretreated advanced NSCLC patients with activated PI3K pathway [221] and in combination with erlotinib in EGFR-TKI resistance patients [222].

Recently, lung adenocarcinomas were reported to harbor novel in-frame fusion transcripts of *KIF5B* and the *RET* oncogene (1–2% of lung adenocarcinomas from people from Japan and the USA), involved in cell proliferation, neuronal navigation, cell migration and differentiation [93].

The tumorigenic potential of the *RET* gene rearrangement (*KIF5B-RET*, and others, such as *CCDC6-RET* and *NCOA4-RET*) is linked to their constitutive ligand-independent kinase activity, similar to *ALK*. Interestingly, *RET* fusion occurs in 1.4% (13/936) of Chinese surgically resected NSCLCs (nine patients had *KIF5B-RET*, three patients had *CCDC6-RET*, and one patient had a novel *NCOA4-RET* fusion) and 1.7% of lung adenocarcinomas (11/633) with identifiable clinicopathologic characteristics (younger age, never-smoker status, early lymph node metastases, poor differentiation, and a solid-predominant subtype). In the subcohort of 633 lung adenocarcinomas, all known mutations and gene fusions (*EGFR*, *KRAS*, *HER2*, *BRAF* mutations and *ALK* rearrangements) were mutually exclusive, indicating the role of *RET* rearrangements as driver mutations [94].

Vandetanib is a multitarget TKI (VEGFR, EGFR and RET tyrosine kinase) with very promising results in hereditary medullary thyroid cancer, and currently in development in NSCLC. In several Phase II studies vandetanib was tested in different doses as monotherapy (MTD: 300 mg) and as a combination with chemotherapy (MTD: 100 mg). The promising results, in terms of PFS, of vandetanib in combination with docetaxel in pretreated NSCLC [95], led to the design of the ZODIAC Phase III study, in which the addition of vandetanib to second-line regimen with docetaxel increased PFS by 26%, meeting its primary end point (4 vs 3.2 weeks; $p < 0.001$) [96].

Patients in the vandetanib plus docetaxel group also had a higher ORR and longer time to deterioration of lung-cancer symptoms than those in the placebo group. Similar outcomes have been shown in the smaller Phase III ZEAL study with combination of vandetanib and pemetrexed versus pemetrexed alone as second line, but there was not a significant PFS prolongation (HR: 0.86; 97.54% CI: 0.69–1.06; $p = 0.108$), most likely due to the smaller size of the ZEAL trial. Vandetanib was well tolerated with the side effects known from both EGFR and VEGFR kinase inhibitors; rash, diarrhea and hypertension [97].

Vandetanib in combination with gemcitabine versus gemcitanine alone has been tested in a Phase II study enrolling 124 elderly (aged ≥70 years) chemotherapy-naive patients. Despite a marginally statistically significant improvement in PFS, the study did not meet the primary (PFS: 80% power to detect a HR ≤ 0.667) and secondary end points (OS, ORR and DCR) [98].

Two large Phase III trials evaluated vandetanib 300 mg as a monotherapy. In the ZEST trial, vandetanib did not improve PFS in comparison with erlotinib in 1240 pretreated patients with advanced NSCLC; however, in a preplanned noninferiority analysis, vandetanib and erlotinib deomonstrated equivalent efficacy for PFS and OS [99]. In addition, the ZEPHYR study did not meet its primary objective of demonstrating an OS benefit with vandetanib versus placebo (HR: 0.95; 95.2% CI: 0.81–1.11; $p = 0.527$)in patients with advanced NSCLC who had previously failed chemotherapy and received treatment with an EGFR TKI, although PFS was better with vandetanib (HR: 0.63; 95.2% CI: 0.54–0.74; $p < 0.0001$) [100].

Future perspective

The discovery of multiple molecular mechanisms underlying the development, progression and prognosis of lung cancer, has created new opportunities for targeted therapy. Several molecular aberrations have been identified in NSCLC, with subsequent development of drugs targeted to these aberrations. Some examples include gefitinib, erlotinib, and cetuximab for the treatment of NSCLC harboring *EGFR* mutation or overexpression, and crizotinib for the treatment of NSCLC with the *EML4-ALK* fusion translocation oncogene. A more recent actionable target is MET, a multifaceted receptor tyrosine kinase within the human kinome. In addition, monoclonal antibody bevacizumab binding VEGF improved outcomes in association with chemotherapy platinum based on first-line treatment of nonsquamous NSCLC patients, while fewer therapeutic options are actually available for squamous histology patients who could be treated with chemotherapy containing platinum plus a third generation cytotoxic agent.

Although targeted therapies have increased survival, this increase has remained small. This is most likely due to the difficulty of identifying the subset of patients for whom targeted treatment will provide a dramatic improvement in survival. A greater understanding of tumor heterogeneity at the molecular level and tumor-resistant mechanisms, both intrinsic and acquired, should provide further targeted therapeutic opportunity. Therefore, clinical trials that investigate the activity of novel agents, and incorporate patient selection based on clinical and molecular factors, are required.

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Executive summary

Bevacizumab

- The addition of bevacizumab to first-line chemotherapy should be considered among treatment options for selected patients with advanced non-small-cell lung caner (NSCLC). However, the identification of molecular biomarkers defining groups of patients potentially benefiting from the drug are under investigation.

Reversible EGFR tyrosine kinase inhibitor

- Gefitinib is approved for the treatment of locally advanced or metastatic NSCLC with sensitizing mutations of the *EGFR* gene, across all lines of therapy, while erlotinib, already approved as second- or third-line without molecular restrictions, has also recently been approved as first-line treatment in patients with *EGFR* mutations.

Overcome resistance to reversible EGFR tyrosine kinase inhibitor

- Multiple strategies are being investigated to treat NSCLCs that are resistant to first-generation EGFR tyrosine kinase inhibitors, such as irreversible EGFR tyrosine kinase inhibitors (afatinib, dacomitinib) and MET inhibitors (tivantinib, MetMAb).

ALK-inhibitors

- In consideration of the superiority of crizotinib to standard single-agent chemotherapy in terms of response and progression-free survival in pretreated *ALK*-positive advanced NSCLC patients, the European Medicines Agency adopted a positive opinion, recommending the granting of a conditional marketing authorization for crizotinib in this setting of patients.

Novel potential molecular targets in NSCLC

- New molecular pathways and their targeted inhibitors are under investigation, such as HER2, B-RAF, PI3K and RET.

References

Papers of special note have been highlighted as:

- ▪ of interest

1 Ferlay J, Shin HR, Bray F *et al.* Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int. J. Cancer* 127, 2893–2917 (2010).

2 Brenner H, Gondos A, Arndt V. Recent major progress in long-term cancer patient survival disclosed by modeled period analysis. *J. Clin. Oncol.* 25, 3274–3280 (2007).

3 Sandler A, Gray R, Perry MC *et al.* Paclitaxel-carboplatin alone or with bevacizumab for non-small cell lung cancer. *N. Engl. J. Med.* 355, 2542–2550 (2006).

4 Sandler A, Yi J, Dahlberg S *et al.* Treatment outcomes by tumor histology in Eastern Cooperative Group Study E4599 of bevacizumab with paclitaxel/carboplatin for advanced non-small cell lung cancer. *J. Thorac. Oncol.* 5(9), 1416–1423 (2010).

▪ **The E4599 trial evaluated the combination of bevacizumab with carboplatin and paclitaxel in first-line treatment of advanced nonsquamous non-small-cell lung cancer (NSCLC): the addition of bevacizumab to chemotherapy was associated with a benefit of approximately 4 months in terms of overall survival in patients with adenocarcinoma, leading to approval by the US FDA.**

5 Reck M, von Pawel J, Zatloukal P *et al.* Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer: AVAil. *J. Clin. Oncol.* 27, 1227–1234 (2009).

- **The AVAiL trial tested bevacizumab at two different doses in association with chemotherapy (cisplatin plus gemcitabine) as first-line therapy for nonsquamous NSCLC. The combination statistically improved progression-free survival (PFS), without any significant overall-survival benefit.**

6 Reck M, von Pawel J, Zatloukal P *et al.* Overall survival with cisplatin – gemcitabine and bevacizumab or placebo as first-line therapy for nonsquamous non-small-cell lung cancer: results from a randomized Phase III trial (AVAiL). *Ann. Oncol.* 21, 1804–1809 (2010).

7 Johnson DH, Fehrenbacher L, Novotny WF *et al.* Randomised Phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. *J. Clin. Oncol.* 22, 2184–2191 (2004).

8 Reck M, Barlesi F, Crino L *et al.* Predicting and managing the risk of pulmonary haemorrhage in patients with NSCLC treated with bevacizumab: a consensus report from a panel of experts. *Ann. Oncol.* 23(5), 1111–1120 (2012).

9 Dowlati A, Gray R, Sandler AB *et al.* Cell adhesion molecules, vascular endothelial growth factor, and basic fibroblast growth factor in patients with non-small cell lung cancer treated with chemotherapy with or without bevacizumab – an Eastern Cooperative Oncology Group Study. *Clin. Cancer Res.* 14(5), 1407–1412 (2008).

10 Mok T, Gorbunova V, Juhasz E *et al.* Biomarker analysis in BO21015, a Phase II randomised study of first-line bevacizumab (BEV) combined with carboplatin–gemcitabine (CG) or carboplatin–paclitaxel (CP) in patients (pts) with advanced or recurrent non-squamous non-small cell lung cancer (NSCLC). Presented at: *16th European Multidisciplinary Cancer Congress.* Stockholm, Sweden, 23–27 September 2011.

11 Crinò L, Dansin E, Garrido P *et al.* Safety and efficacy of first-line bevacizumab-based therapy in advanced non-squamous non-small-cell lung cancer (SAiL, MO19390): a Phase IV study. *Lancet Oncol.* 11, 733–740 (2010).

12 Laskin J, Crinò L, Felip E *et al.* Safety and efficacy of first-line bevacizumab plus chemotherapy in elderly patients with advanced or recurrent non-squamous non-small cell lung cancer: safety of avastin in lung trial (MO19390). *J. Thorac. Oncol.* 7(1), 203–211 (2012).

13 Barlesi F, de Castro J, Dvornichenko V *et al.* AVAPERL (MO22089): final efficacy outcomes for patients with advanced nonsquamous non-small cell lung cancer randomised to continuation maintenance with bevacizumab or bevacizumab + pemetrexed after first-line bevacizumabcisplatin-pemetrexed treatment. Presented at: *The European Multidisciplinary Cancer Congress.* Stockholm, Sweden, 23–27 September 2011.

14 Patel JD, Bonomi P, Socinski MA *et al.* Treatment rationale and study design for the pointbreak study: a randomized, open-label Phase III study of pemetrexed/carboplatin/bevacizumab followed by maintenance pemetrexed/bevacizumab versus paclitaxel/carboplatin/bevacizumab followed by maintenance bevacizumab in patients with stage IIIB or IV nonsquamous non-small-cell lung cancer. *Clin. Lung Cancer* 10(4), 252–256 (2009).

15 Gridelli C, Bennouna J, de Castro J *et al.* Randomized Phase IIIb trial evaluating the continuation of bevacizumab beyond disease progression in patients with advanced non-squamous non-small-cell lung cancer after first-line treatment with bevacizumab plus platinum-based chemotherapy: treatment rationale and protocol dynamics of the AvaALL (MO22097) trial. *Clin. Lung Cancer* 12(6), 407–411 (2011).

16 Rosell R, Robinet G, Szczesna A *et al.* Randomized Phase II study of cetuximab plus cisplatin/vinorelbine compared with cisplatin/vinorelbine alone as first-line therapy in *EGFR*-expressing advanced non-small-cell lung cancer. *Ann. Oncol.* 19, 362–369 (2008).

17 Pirker R, Pereira JR, Szczesna A *et al.* Cetuximab plus chemotherapy in patients with advanced non-small cell lung cancer (FLEX): an open-label randomised Phase III trial. *Lancet* 373, 1525–1531 (2009).

18 O'Byrne KJ, Gatzemeier U, Bondarenko I *et al.* Molecular biomarkers in non-small-cell lung cancer: a retrospective analysis of data from the Phase 3 FLEX study. *Lancet Oncol.* 12(8), 795–805 (2011).

19 Lynch TJ, Patel T, Dreisbach L *et al.* Cetuximab and first-line taxane/carboplatin chemotherapy in advanced non-small-cell lung cancer: results of the randomized multicenter Phase III trial BMS099. *J. Clin. Oncol.* 28, 911–917 (2010).

20 Pirker R, Pereira JR, von Pawel J *et al.* EGFR expression as a predictor of survival for first-line chemotherapy plus cetuximab in patients with advanced non-small-cell lung cancer: analysis of data from the Phase 3 FLEX study. *Lancet Oncol.* 13, 33–42 (2012).

21 Thatcher N, Lynch TJ, Butts CA *et al.* Cetuximab plus platinum-based chemotherapy as 1st-line treatment in patients with nonsmall cell lung cancer: a meta-analysis of randomized Phase II/III trials. Presented at: *13th World Conference on Lung Cancer (WCLC).* CA, USA, 31 July–4 August 2009.

22 Giaccone G, Herbst RS, Manegold C *et al.* Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a Phase III trial – INTACT 1. *J. Clin. Oncol.* 22, 777–784 (2004).

23 Herbst RS, Giaccone G, Schiller JH *et al.* Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a Phase III trial – INTACT 2. *J. Clin. Oncol.* 22, 785–794 (2004).

24 Kim ES, Hirsh V, Mok T *et al.* Gefitinib versus docetaxel in previously treated non-small cell lung cancer (INTEREST): a randomised Phase III trial. *Lancet* 372, 1809–1818 (2008).

25 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.* 350, 2129–2139 (2004).

26 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.* 97, 339–346 (2005).

27 Mok TS, Wu YL, Thongprasert S *et al.* Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N. Engl. J. Med.* 361, 947–957 (2009).

28 Lee JS, Park K, Kim SW *et al.* A randomized Phase III trial of gefitinib (IRESSA) versus standard chemotherapy (gemcitabine plus cisplatin) as a first-line treatment for never-smokers with advanced ot metastatic adenocarcinoma of the lung. *J. Thorac. Oncol.* 4s, 283–284 (2009).

29 Mitsudomi T, Morita S, Yatabe Y *et al.* Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised Phase III trial. *Lancet Oncol.* 11, 121–128 (2010).

- **In the randomized Phase III WJTOG3405 study, gefitinib was compared with first-line chemotherapy (cisplatin + docetaxel) in a molecularly defined group of patients with NSCLC, harboring *EGFR* mutation: the presence of *EGFR* mutations was related with clinical efficacy. These results lead to the approval of gefitinib in this molecularly defined set of patients, across all lines of therapy.**

30 Maemondo M, Inoue A, Kobayashi K *et al.* Gefitinib or chemotherapy for non-small-cell lung cancer with mutated *EGFR*. *N. Engl. J. Med*. 362, 2380–2388 (2010).

- **In the NEJ002 trial, gefitinib was compared to carboplatin plus paclitaxel, confirming the clinical benefit, independently of type of *EGRF* mutation (exon 19 deletion or L858R point mutation), and highlights the role of molecularly based selection of patients on the basis of *EGFR*-mutation status. These results lead to the approval of gefitinib in this molecularly defined set of patients, across all lines of therapy.**

31 Fukuoka M, Wu YL, Thongprasert S *et al.* Biomarker analyses and final overall survival results from a Phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). *J. Clin. Oncol.* 29(21), 2866–2874 (2011).

32 Shepherd FA, Pereira JR, Ciuleanu TE *et al.* Erlotinib in previously treated non-small cell lung cancer. *N. Eng. J. Med*. 353, 123–132 (2005).

33 Zhu CQ, da Cunha Santos G, Ding K *et al.* Role of KRAS and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada Clinical Trials Group Study BR.21. *J. Clin. Oncol.* 26, 4268–4275 (2008).

34 Garassino MC, Martelli O, Bettini A *et al.* TAILOR: a Phase III trial comparing erlotinib with docetaxel as the second-line treatment of NSCLC patients with wild-type (wt) EGFR. *J. Clin. Oncol.* 30(Suppl.), Abstract LBA7501 (2012).

35 Herbst RS, Prager D, Hermann R *et al.* TRIBUTE: a Phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small-cell lung cancer. *J. Clin. Oncol.* 23, 5892–5899 (2005).

36 Gatzemeier U, Pluzanska A, Szczesna A *et al.* Phase III study of erlotinib in combination with cisplatin and gemcitabine in advanced non-small-cell lung cancer: the Tarceva Lung Cancer Investigation Trial. *J. Clin. Oncol.* 25, 1545–1552 (2007).

37 Lee S, Rudd R, Khan I *et al.* TOPICAL: randomized Phase III trial of erlotinib compared with placebo in chemotherapy-naive patients with advanced non-small cell lung cancer (NSCLC) and unsuitable for first-line chemotherapy. *J. Clin. Oncol.* 28(Suppl.), Abstract 7504 (2010).

38 Zhou C, Wu YL, Chen G *et al.* Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, Phase III study. *Lancet Oncol.* 12(8), 735–742 (2011).

39 Rosell R, Carcereny E, Gervais R *et al.* Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised Phase III trial. *Lancet Oncol.* 13, 239–246 (2012).

- **The EURTAC trial confirmed the efficacy of erlotinib in PFS and objective-response rates in European chemotherapy-naive patients with *EGFR* mutation-positive NSCLC compared with standard chemotherapy, as previous studies in Asian patients. Based on these results, erlotinib, already approved as second- or third-line without molecular restrictions, has recently also been approved as first-line treatment in patients with *EGFR* mutations.**

40 Miller VA, Hirsh V, Cadranel J *et al.* Phase IIB/III double-blind randomized trial of afatinib (BIBW 2992, an irreversible inhibitor of EGFR/HER1 and HER2) + best supportive care (BSC) versus placebo + BSC in patients with NSCLC failing 1–2 lines of chemotherapy and erlotinib or gefitinib (LUX-LUNG 1). *Ann. Oncol.* 21(Suppl. 8), Abstract LBA1 (2010).

41 Schuler MH, Planchard D, Yang JC-H *et al.* Interim analysis of afatinib monotherapy in patients with metastatic NSCLC progressing after chemotherapy and erlotinib/gefitinib (E/G) in a trial of afatinib plus paclitaxel versus investigator's choice chemotherapy following progression on afatinib monotherapy. *J. Clin. Oncol.* 30(Suppl.), Abstract 7557 (2012).

42 Awada A, Dumez H, Wolter P *et al.* A Phase I dose finding study of the 3-day administration of BIBW 2992, an irreversible dual EGFR/HER2 inhibitor, in combination with 3-weekly docetaxel in patients with advanced solid tumors. Presented at: *45th ASCO Annual Meeting*. FL, USA, 26 May–2 June 2009.

43 Yang JC-H, Schuler MH, Yamamoto N *et al.* LUX-Lung 3: a randomized, open-label, Phase III study of afatinib versus pemetrexed and cisplatin as first-line treatment for patients with advanced adenocarcinoma of the lung harboring EGFR-activating mutations. *J. Clin. Oncol.* 30(Suppl.), Abstract LBA7500 (2012).

44 Engelman JA, Zejnullahu K, Gale CM *et al.* PF00299804, an irreversible pan-ERBB inhibitor, is effective in lung cancer models with EGFR and ERBB2 mutations that are resistant to gefitinib. *Cancer Res*. 67, 11924–11932 (2007).

45 Jänne PA, Boss DS, Camidge DR *et al.* Phase I dose-escalation study of the pan-HER inhibitor, PF299804, in patients with advanced malignant solid tumors. *Clin. Cancer Res.* 17, 1131–1139 (2011).

46 Janne P, Reckamp K, Koczywas M *et al.* Efficacy and safety of PF-00299804 (PF299) in patients(pt) with advanced NSCLC after failure of at least one prior chemotherapy regimen and prior treatment with erlotinib (E): a two-arm, Phase II trial. Presented at: *45th ASCO Annual Meeting*. FL, USA, 26 May–2 June 2009.

47 Park K, Seog Heo D, Chul Cho B *et al.* Updated safety and efficacy results of a Phase I/II study of PF299804 in Korean patients with NSCLC who experienced disease progression on platinum based chemotherapy plus gefitinib or erolotinib. *J. Thorac. Oncol*. 5(Suppl.), S371–S423 Abstract O-018 (2010).

48 Ramalingam SS, Blackhall F, Krzakowski M *et al.* Randomized Phase II study of dacomitinib (PF-00299804), an irreversible pan-human epidermal growth factor receptor inhibitor, versus erlotinib in patients with advanced non-small-cell lung cancer. *J. Clin. Oncol.* 30, 3337–3344 (2012).

49 Boyer MJ, Jänne PA, Mok T *et al.* ARCHER: dacomitinib (D; PF-00299804) versus erlotinib (E) for advanced (adv) non-small cell lung cancer (NSCLC) – a randomized double-blind Phase III study. *J. Clin. Oncol.* 30 (Suppl.), Abstract TPS7615 (2012).

50 Jänne PA. Challenges of detecting EGFR T790M in gefitinib/erlotinib resistant tumours. *Lung Cancer* 60(Suppl. 2), 3–9 (2008).

51 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.* 2, e73 (2005).

52 Engelman JA, Zejnullahu K, Mitsudomi T *et al.* MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 316, 1039–1043 (2007).

53 Spigel D, Ervin TJ, Ramlau R *et al.* Final efficacy results from OAM4558g, a randomized Phase II study evaluating MetMAb or placebo in combination with erlotinib in advanced NSCLC. *J. Clin. Oncol.* 29(Suppl.), Abstract 7505 (2011).

54 Houtman SH, Rutteman M, De Zeeuw CI, French PJ. Echinoderm microtubule-associated protein like protein 4, a member of the echinoderm microtubule-associated protein family, stabilizes microtubules. *Neuroscience* 144(4), 1373–1382 (2007).

55 Chiarle R, Voena C, Ambrogio C *et al.* The anaplastic lymphoma kinase in the pathogenesis of cancer. *Nat. Rev. Cancer* 8, 11–23 (2008).

56 Sasaki T, Rodig SJ, Chirieac LR *et al.* The biology and treatment of EML4-ALK non-small cell lung cancer. *Eur. J. Cancer* 46, 1773–1780 (2010).

57 Rikova K., Guo A, Zeng Q *et al.* Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. *Cell* 131, 1190–1230 (2007).

58 Takeuchi K, Choi YL, Soda M *et al.* Multiplex reverse transcription-PCR screening for EML4-ALK fusion transcripts. *Clin. Cancer Res.* 14, 6618–6624 (2008).

59 Perner S, Wagner PL, Demichelis F *et al.* EML4-ALK fusion lung cancer: a rare acquired event. *Neoplasia* 10, 298–302 (2008).

60 Rodig SJ, Mino-Kenudson M, Dacic S *et al.* Unique clinicopathologic features characterize ALK-rearranged lung adenocarcinoma in the western population. *Clin. Cancer Res.* 15, 5216–5223 (2009).

61 Shaw AT, Hayes DN, Martins R. The importance of histology and molecular testing (EGFR and EML4-ALK) in the initial evaluation of advanced non-small cell lung cancer. Presnted at: *Educational ASCO 2011*. IL, USA, 3–7 June 2011.

62 Yung-Jue B. The potential for crizotinib in non-small cell lung cancer: a perspective review. *Adv. Med. Oncol*. 3(6), 279–291 (2011).

63 Takahashi T, Sonobe M, Kobayashi M *et al.* Clinicopathologic features of non-small-cell lung cancer with EML4-ALK fusion gene. *Ann. Surg. Oncol*. 17, 889–897(2010).

64 Zhang X, Zhang S, Yang X *et al.* Fusion of EML4 and ALK is associated with development of lung adenocarcinomas lacking EGFR and KRAS mutations and is correlated with ALK expression. *Mol. Cancer* 9, 188 (2010).

65 Camidge DR, Bang Y-B, Kwak EL *et al.* Activity and safety of crizotinib in patients with ALK-positive non-small-cell lung cancer: updated results from a Phase I study. *Lancet Oncol.* 13, 1011–1019 (2012).

66 Weickhardt AJ, Rothman MS, Salian-Mehta S *et al.* Rapid-onset hypogonadism secondary to crizotinib use in men with metastatic nonsmall cell lung cancer. *Cancer* 118(21) 5302–5309 (2012).

67 Kim DW, Ahn M-J, Shi Y *et al.* Updated results of a global Phase II study with crizotinib in advanced ALK-positive non-small cell lung cancer (NSCLC). Presented at: *European Soc. Med. Oncol. 2012*. Vienna, Austria, 28 September–2 October 2012.

68 Shaw AT. Phase III trial shows crizotinib superior to single-agent chemotherapy for ALK-positive advanced NSCLC. Presented at: *European Soc. Med. Oncol. 2012*. Vienna, Austria, 28 September–2 October 2012.

■ **The registration Phase III trial PROFILE 1007 compared crizotinib with standard single-agent chemotherapy (pemetrexed or docetaxel) in *ALK*-positive advanced NSCLC patients pretreated with first-line, platinum-based chemotherapy: the significant benefit in terms of PFS and objective-response rates with crizotinib allowed it to a conditional marketing authorization by European Medicines Agency for these patients.**

69 Jänne PA, Shaw AT, Giaccone G *et al.* Phase I trial of irreversible Pan-Erbb inhibitor Dacomitinib (DAC) in combination with ALK/MET inhibitor crizotinib (CRIZ) in previously treated advanced non small cell lung cancer. Presented at: *European Soc. Med. Oncol. 2012*. Vienna, Austria, 28 September–2 October 2012.

70 Ou S-HI, Govindan R, Eaton KD *et al.* Phase I/II dose-finding study of crizotinib (CRIZ) in combination with erlotinib (E) in patients (pts) with advanced non-small cell lung cancer (NSCLC). Presented at: *European Soc. Med. Oncol. 2012*. Vienna, Austria, 28 September–2 October 2012.

71 Camidge DR, Kono SA, Lu X *et al.* Anaplastic lymphoma kinase gene rearrangements in non-small cell lung cancer are associated with prolonged progression-free survival on pemetrexed. *J Thorac. Oncol*. 6, 774–780 (2011).

72 Takeda M, Okamoto I, Sakai K *et al.* Successful long term treatment with pemetrexed of NSCLC associated with EML4-ALK and low thymidylate synthase expression. *Clin. Lung Cancer* 13(2), 157–159 (2012).

73 Ou SI, Camidge DR, Engelman JA *et al.* Clinical activity of crizotinib in patients with advanced non-small cell lung cancer harboring ROS1 gene rearrangement. Presented at: *European Soc. Med. Oncol. 2012*. Vienna, Austria, 28 September–2 October 2012.

74 Nishio M, Kiura K, Nakagawa K *et al.* A Phase I/II study of ALK inhibitor CH5424802 in patients with ALK-positive NSCLC; safety and efficacy interim results of the Phase II portion. Presented at: *European Soc. Med. Oncol. 2012*. Vienna, Austria, 28 September–2 October 2012.

75 Sequist LV, Jänne PA, Sweeney J *et al.* Phase I/II trial of the novel Hsp90 Inhibitor, IPI-504, in patients with relapsed and/or refractory stage IIIb or stage IV non-small cell lung cancer (NSCLC) stratified by EGFR mutation status. Presented at: *AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics*. CA, USA, 22–26 October 2007.

76 Felip E, Carcenery E, Barlesi F *et al.* Phase II activity of the Hsp90 inhibitor AUY922 in patients with ALK rearranged (ALK+) or EGFR-mutated advanced non-small cell lung cancer (NSCLC). Presented at: *European Soc. Med. Oncol. 2012*. Vienna, Austria, 28 September–2 October 2012.

77 Shaw AT, Camidge DR, Felip E *et al.* Results of a first-in-human Phase I study of the ALK inhibitor LDK378 in advanced solid tumors. Presented at: *European Soc. Med. Oncol. 2012*. Vienna, Austria, 28 September–2 October 2012.

78 Sequist LV, Gettinger S, Senzer NN *et al.* Activity of IPI-504, a novel heat-shock protein 90 inhibitor, in patients with molecularly defined non-small-cell lung cancer. *J. Clin. Oncol.* 28, 4953–4960 (2010).

79 Camidge DR, Doebele RC *et al.* Treating ALK-positive lung cancer – early successes and future challenges. *Nat. Rev. Clin. Oncol.* 9(5), 268–277 (2012).

80 Doebele RC, Pilling AB, Aisner DL *et al.* Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. *Clin. Cancer Res.* 18(5), 1472–1482 (2012).

81 Katayama R, Shaw AT, Khan TM *et al.* Mechanisms of acquired crizotinib resistance in ALK-rearranged lung cancers. *Sci. Transl. Med*. 4 (120), 120ra17 (2012).

82 Doebele RC, Aisner DL, Le AT *et al.* Analysis of resistance mechanisms to ALK kinase inhibitors in ALK+ NSCLC patients. *J. Clin. Oncol.* 30(Suppl.), Abstract 7504 (2012).

83 Weickhardt AJ, Scheier B, Burke JM *et al.* Local ablative therapy of oligoprogressive disease prolongs disease control by tyrosine kinase inhibitors in oncogene-addicted non-small-cell lung cancer. *J. Thorac. Oncol.* 7(12), 1807–1814 (2012).

84 Shigematsu H, Gazdar AF. Somatic mutations of epidermal growth factor receptor signaling pathway in lung cancers. *Int. J. Cancer* 118(2), 257–262 (2006).

85 Stephens P, Hunter C, Bignell G *et al.* Lung cancer: intragenic ERBB2 kinase mutations in tumours. *Nature* 431, 525–526 (2004).

86 Wang SE, Narasanna A, Perez-Torres M *et al.* HER2 kinase domain mutation results in constitutive phosphorylation and activation of HER2 and EGFR and resistance to EGFR tyrosine kinase inhibitors. *Cancer Cell* 10, 25–38 (2006).

87 Gatzemeier U, Groth G, Butts C *et al.* Randomized Phase II trial of gemcitabine cisplatin with or without trastuzumab in HER2-positive non-small-cell lung cancer. *Ann. Oncol.* 15, 19–27 (2004).

88 Schiller J, Langer CJ, Thor A *et al.* Trastuzumab in combination with paclitaxel/carboplatin advanced non-small cell lung cancer: final report of ECOG 2598. *Proc. Am. Soc. Clin. Oncol.* 22, Abstract 2606 (2003).

89 Naoki K, Chen TH, Richards WG et al. Missense mutations of the BRAF gene in human lung adenocarcinoma. *Cancer Res.* 62, 7001–7003 (2002).

90 Marchetti A, Felicioni L, Malatesta S *et al.* Clinical features and outcome of patients with non-small-cell lung cancer harboring BRAF mutations. *J. Clin. Oncol.* 29, 3574–3579 (2011).

91 Yamamoto H, Shigematsu H, Nomura M *et al.* PIK3CA mutations and copy number gains in human lung cancers. *Cancer Res.* 68(17), 6913–6921 (2008).

92 Di Nicolantonio F, Arena S, Tabernero J *et al.* Deregulation of the PI3K and KRAS signaling pathways in human cancer cells determines their response to everolimus. *J. Clin. Invest.* 120, 2858–2866 (2010).

93 Kohno T, Ichikawa H, Totoki Y *et al.* KIF5B-RET fusions in lung adenocarcinoma. *Nature Med.* 18(3), 375–377 (2012).

94 Wang R, Hu H, Pan Y *et al.* RET fusions define a unique molecular and clinicopathologic subtype of non-small-cell lung cancer. *J. Clin. Oncol.* 30 (2012).

95 Heymach JV, Johnson BE, Prager D *et al.* Randomized, placebo-controlled Phase II study of vandetanib plus docetaxel in previously treated non small-cell lung cancer. *J. Clin. Oncol.* 25, 4270–4277 (2007).

96 Herbst RS, Sun Y, Eberhardt WEE *et al.* Vandetanib plus docetaxel versus docetaxel as second-line treatment for patients with advanced non-small-cell lung cancer (ZODIAC): a double-blind, randomised, Phase III trial. *Lancet Oncol.* 11(7), 619–626 (2010).

97 de Boer R, Arrieta O, Gottfired M *et al.* Vandetanib plus pemetrexed versus pemetrexed as second-line therapy in patients with advanced non-small cell lung cancer (NSCLC): a randomized, doubleblind Phase III trial (ZEAL). *J. Clin. Oncol.* 27(Suppl.), Abstract 8010 (2009).

98 Gridelli C, Novello S, Zilembo N *et al.* Final results of a randomized, double-blind, Phase II study of gemcitabine plus vandetanib or plus placebo in the treatment of advanced (stage IIIB/IV) non-small cell lung cancer (NSCLC) elderly patients (ZELIG study NCT00753714). *J. Clin. Oncol.* 30(Suppl.), Abstract 7550 (2012).

99 Natale RB, Thongprasert S, Greco FA *et al.* Vandetanib versus erlotinib in patients with advanced non-small cell lung cancer (NSCLC) after failure of at least one prior cytotoxic chemotherapy: a randomized, double-blind Phase III trial (ZEST). *J. Clin. Oncol.* 27(Suppl.), 8009 (2009).

100 Lee J, Hirsh V, Park K *et al.* Vandetanib versus placebo in patients with advanced non-small cell lung cancer (NSCLC) after prior therapy with an EGFR tyrosine kinase inhibitor (TKI): a randomized, double-blind Phase III trial (ZEPHYR). Presented at: *European Soc. Med. Oncol. 2010.* IL, USA, 4–8 June 2010.

Websites

201 ClinicalTrials Database: NCT01121393. www.clinicaltrials.gov/show/NCT01121393

202 ClinicalTrials Database: NCT01466660. www.clinicaltrials.gov/show/NCT01466660

203 ClinicalTrials Database: NCT01523587. www.clinicaltrials.gov/show/NCT01523587

204 ClinicalTrials Database: NCT00818441. www.clinicaltrials.gov/show/NCT00818441

205 ClinicalTrials Database: NCT01395758. www.clinicaltrials.gov/show/NCT01395758

206 ClinicalTrials Database: NCT01580735. www.clinicaltrials.gov/show/NCT01580735

207 ClinicalTrials Database: NCT01244191. www.clinicaltrials.gov/show/NCT01244191

208 ClinicalTrials Database: NCT01377376. www.clinicaltrials.gov/show/NCT01377376

209 ClinicalTrials Database: NCT01456325. www.clinicaltrials.gov/show/NCT01456325

210 ClinicalTrials Database: NCT01519804. www.clinicaltrials.gov/show/NCT01519804

211 ClinicalTrials Database: NCT01496742. www.clinicaltrials.gov/show/NCT01496742

212 ClinicalTrials Database: NCT00016367. www.clinicaltrials.gov/show/NCT00016367

213 ClinicalTrials Database: NCT00004883 www.clinicaltrials.gov/show/NCT00004883

214 ClinicalTrials Database: NCT00758134 www.clinicaltrials.gov/show/NCT00758134

215 ClinicalTrials Database: NCT00073008 www.clinicaltrials.gov/show/NCT00073008

216 ClinicalTrials Database: NCT00063154 www.clinicaltrials.gov/show/NCT00063154

217 ClinicalTrials Database: NCT01086267. www.clinicaltrials.gov/show/NCT01086267

218 ClinicalTrials Database: NCT00888134. www.clinicaltrials.gov/show/NCT00888134

219 ClinicalTrials Database: NCT00372788. www.clinicaltrials.gov/show/NCT00372788

220 ClinicalTrials Database: NCT01336634. www.clinicaltrials.gov/show/NCT01336634

221 ClinicalTrials Database: NVT01297491. www.clinicaltrials.gov/show/NVT01297491

222 ClinicalTrials Database: NCT01487265. www.clinicaltrials.gov/show/NCT01487265