New targeted therapies for non-small-cell lung cancer

Advances in the understanding of non-small-cell lung cancer biology have led to the clinical development of biological therapies targeting molecular mechanisms underlying cancer growth and survival of this disease. In some cases, such strategies have significantly improved the outcome of advanced non-small-cell lung cancer in combination with platinum-based chemotherapy, as for the monoclonal antibodies cetuximab and bevacizumab directed against the EGF receptor and VEGF, respectively. In others, they have found a place in therapy in the management of pretreated patients, as for the EGF receptor tyrosine kinase inhibitors gefitnib and erlotinib. Importantly, the recognition that certain clinical or biological characteristics predict for increased sensitivity to treatment has highlighted the importance of patient selection when designing clinical trials with these agents. This review is structured in order to summarize the targeted therapies currently used for advanced non-small-cell lung cancer. In the latter part, biological agents under investigation are discussed.

KEYWORDS: bevacizumab ■ cetuximab ■ EGF receptor ■ erlotinib ■ gefitinib ■ multitargeted agents ■ non-small-cell lung cancer ■ targeted therapies ■ tyrosine kinase inhibitors ■ VEGF

Giulio Metro[1] & Federico Cappuzzo[2†]

[†]Author for correspondence
[1]Regina Elena Cancer Institute, Rome, Italy
[2]Istituto Clinico Humanitas IRCCS, via Manzoni 56, 20089 Rozzano, Italy
Tel.: +39 028 224 4097
Fax: +39 028 224 4590
federico.cappuzzo@humanitas.it

Lung cancer continued to lead cancer-related death worldwide in 2008 [1]. Although a slight decline has recently been registered in the overall incidence of this disease in western countries, its incidence in developing countries is rising. Despite therapeutic advances, the prognosis of lung cancer remains poor, and the overall cure rate is less than 15%. Chemotherapy and radiation therapy, used in the management of advanced non-small-cell lung cancer (NSCLC), are associated with significant therapeutic and safety limitations. These limitations can cause poor outcome in terms of disease control and overall survival, thus emphasizing the need for treatment approaches that demonstrate efficacy in targeting tumor cells. Given the rapid advances in the molecular and biological understanding of the disease process, carcinogenesis, angiogenesis and cell growth regulation, several new strategies have emerged for the treatment of NSCLC. Over the last 5 years, agents targeting the EGF receptor (EGFR) or VEGF have significantly prolonged survival when used alone or in combination with chemotherapy, as illustrated in Table 1 [2–4]. Although these agents are offering new hope for NSCLC patients, definitive cure is not achievable in cases of metastatic disease, and survival outcome is still disappointing, thus highlighting the urgent need for more effective strategies.

Existing treatments

Since the publication of a meta-analysis in 1995, platinum-based chemotherapy has been regarded as the standard of care for advanced NSCLC [5]. In the 1990s, several trials evaluated the role of new cytotoxics, such as taxanes, gemcitabine and vinorelbine, in combination with platinum. These studies demonstrated that combinations of a new drug with a platinum derivative produce better results when compared with single-agent chemotherapy, an older two-drug combination, or an older three-drug regimen, at least in terms of response rate [6–13]. For these reasons, the combination of cisplatin or carboplatin with a new cytotoxic became the standard treatment for advanced NSCLC patients. Subsequently, several Phase III trials compared these new platinum-based doublets, in order to determine the best regimen for advanced NSCLC [14–16]. These trials demonstrated a substantial equivalence of the new regimens, with a median survival of 8–9 months, and differences only in terms of costs and toxicity profile. Such discouraging results led to the design of new trials incorporating novel cytotoxics such as pemetrexed, or targeted therapies, such as anti-EGFR and anti-VEGF drugs.

■ Anti-EGFR agents

Since its identification, EGFR has emerged as a crucial factor in the development and growth

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Table 1. Applicability of existing targeted therapies currently in use for advanced non-small-cell lung cancer.

	Setting	Preferential combination chemotherapy regimen	Clinical predictors of increased sensitivity	Biological predictors of increased sensitivity	Exclusion
Cetuximab	First line	Cisplatin/vinorelbine	–	–	–
EGFR-TKIs*	Second and successive lines	None	Never smoking status Adenocarcinoma histology Female gender Asian ethnicity	Activating mutations of the *EGFR* gene (exon 18 to 21) Increased *EGFR* gene copy number (FISH+)	–
Bevacizumab	First line	Carboplatin/paclitaxel	–	–	Squamous-cell histology Presence of brain metastases

Gefitinib or erlotinib
EGFR: EGF receptor; FISH: Fluorescence in situ *hybridization; IHC: Immunohistochemistry; TKI: Tyrosine kinase inhibitor.*

of human malignancies. The EGFR signal transduction network plays an important role in multiple tumorigenic processes contributing to proliferation of cancer cells, angiogenesis and metastasis [17]. The EGFR family includes four distinct receptors: EGFR/erbB-1, HER2/erbB-2, HER3/erbB-3 and HER4/erbB-4. Each extracellular domain of EGFR, HER3 and HER4 interacts with a specific set of soluble ligands, whereas no ligand has been identified for the orphan HER2 receptor. Binding of ligands to the extracellular domain of EGFR, HER3 and HER4 leads to the formation of homo- and hetero-dimeric complexes, activation of the intracellular intrinsic tyrosine kinase activity with subsequent recruitment of second messengers, eventually leading to intensification of the antiapoptotic signaling. The main strategy aimed at inhibiting the EGFR pathway includes agents directed against the extracellular domain of the receptor, such as monoclonal antibodies, or small molecules interfering with the tyrosine kinase activity of the intracellular domain, such as tyrosine kinase inhibitors (TKIs).

Monoclonal antibodies

The most widely tested anti-EGFR antibody in NSCLC is cetuximab, a human–murine chimeric anti-EGFR IgG monoclonal antibody that binds to the extracellular domain of EGFR. In preclinical studies, cetuximab inhibited the growth of lung cancer cell lines and mouse xenografts, particularly in combination with chemotherapy [18,19]. In NSCLC, a Phase II study of cetuximab monotherapy in pretreated patients with advanced disease showed a response rate of 4.5% with an overall survival comparable to that achieved with other drugs approved for second-line treatment, such as pemetrexed, docetaxel or erlotinib [20]. Early Phase I–II trials of cetuximab plus chemotherapy demonstrated encouraging response rates and median survival, leading to further investigations of combination regimens [21,22]. More recently, two small Phase II trials evaluated the combination of cetuximab with carboplatin–paclitaxel or carboplatin–docetaxel [23,24]. These studies, conducted respectively in 53 and 80 chemonaive NSCLC patients, demonstrated once again the activity and feasibility of the cetuximab and chemotherapy combination treatment. In order to investigate the best way to combine cetuximab with chemotherapy, the Southwest Oncology Group (SWOG) conducted a randomized Phase II trial (S0342) comparing chemotherapy (carboplatin–paclitaxel) and cetuximab versus sequential treatment (the same chemotherapy followed by cetuximab) in untreated advanced NSCLC [25]. In this study, in which 106 patients were assigned to concurrent treatment and 117 to the sequential approach, no difference in response rate and progression-free survival (PFS) was observed. Nevertheless, median survival was 11 months in both arms, suggesting that adding cetuximab to chemotherapy had the potential to improve survival compared with chemotherapy alone. The Lung Cancer Cetuximab Study further supported the role of cetuximab in NSCLC [26]. This study was an open-label, randomized, Phase II trial of cisplatin and vinorelbine versus the same combination plus cetuximab conducted in 86 NSCLC patients who were positive for EGFR expression by immunohistochemistry. Although the trial was not designed to formally compare the two arms of treatment, a 1-month improvement in survival was observed in favor of the cetuximab arm (8.3 vs 7.3 months), thereby suggesting that cetuximab could improve the efficacy of

cisplatin–vinorelbine. Recently, the results of two large Phase III trials comparing standard chemotherapy versus chemotherapy plus cetuximab have been presented [4,27]. The FLEX trial was a large Phase III study randomly assigning EGFR-expressing patients to cisplatin–vinorelbine or the same regimen plus cetuximab. A total of 1688 patients were screened, of whom 1442 (85%) were EGFR positive by immunohistochemistry, and 1125 were enrolled into the trial. In this study, the addition of cetuximab to chemotherapy led to a significant improvement in response rate (36 vs 29%; p = 0.012) with a significant survival benefit (11.3 vs 10.1 months; p = 0.044), even though the benefit in survival was marginal (hazard ratio [HR]: 0.87) and was associated with an increased risk of side effects, particularly febrile neutropenia [4]. These results have been confirmed in another large Phase III study (BMS099) randomly assigning 676 chemonaive NSCLC patients to carboplatin plus a taxane versus the same chemotherapy regimen plus cetuximab [27]. Importantly, patients were enrolled into the study regardless of EGFR expression. Although the primary end point of PFS was not met (4.4 vs 4.2 months; p = 0.2), response rate (25 vs 17%; p = 0.007) and survival (9.6 vs 8.3 months) favored the cetuximab arm, with a reduction in the risk of death comparable to the FLEX trial (HR: 0.89), although this was not statistically significant (p = 0.17). The survival results observed in the FLEX and BMS099 trials clearly indicated that there is a consistent portion of NSCLC patients deriving no or little benefit from cetuximab therapy, thus highlighting the importance of proper patient selection. Presence of *EGFR* mutations, a critical factor for response to EGFR-TKIs, does not seem to be relevant for cetuximab sensitivity [28]. Data on colorectal cancer demonstrated that increased *EGFR* gene copy number as detected by fluorescence *in situ* hybrydization (FISH) might be associated with increased sensitivity to cetuximab, at least in terms of response and time to progression [29,30]. Identifying increased *EGFR* gene copy number as a predictive marker for anti-EGFR therapy would be crucially important in NSCLC, given recent data showing that EGFR positivity as assessed by FISH is not associated with poor prognosis in patients with resected NSCLC [31]. In lung cancer, Hirsch *et al.* analyzed the impact of *EGFR* gene copy number detected by FISH on survival of NSCLC patients enrolled into the S0342 trial [25,32]. In this analysis, PFS and survival were significantly longer in *EGFR* FISH-positive patients treated with cetuximab and chemotherapy than in the *EGFR* FISH-negative patients receiving the same treatment (PFS: 6 vs 3 months, p = 0.0008; survival: 15 vs 7 months; p = 0.04) [32]. More recently, Kambata-Ford *et al.* presented an extensive biomarker analysis conducted among individuals participating in the BMS099 trial [33]. In this study, no difference in PFS was observed in the FISH-positive group, irrespective of the treatment. Surprisingly, median survival was significantly longer among *EGFR* FISH-positive patients treated with chemotherapy alone versus *EGFR* FISH-positive patients treated with chemotherapy plus cetuximab (12.5 vs 8.6 months; p = 0.03). In colorectal cancer, the strongest biomarker useful for selection of patients for cetuximab therapy is *Kras* [34]. Colorectal cancer patients harboring a *Kras* mutation derive no benefit from cetuximab, and *Kras* testing is now used in clinical practice for selection of patients for cetuximab therapy [34]. By contrast, in the analysis conducted by Kambata-Ford in the BMS099 study, patients with *Kras* mutation treated with cetuximab and chemotherapy had longer PFS (5.6 vs 2.8 months) and longer survival (16.8 vs 10.8 months) than individuals treated with chemotherapy alone, even if the difference was not statistically significant (PFS p-value = 0.3; survival p-value = 0.93) [33]. Therefore, based on available data, at the present time there is no reliable biomarker for selection of NSCLC patients for cetuximab therapy in clinical practice.

Tyrosine-kinase inhibitors

Gefitinib and erlotinib are selective EGFR-TKIs that, in early Phase I and II trials, demonstrated activity in pretreated NSCLC [34–40]. Two Phase III studies comparing erlotinib or gefitinib to placebo in pretreated NSCLC showed a survival improvement for individuals receiving the EGFR-TKI, statistically significant for patients with certain clinical or biological characteristics [2,42–44]. When compared with chemotherapy in a large Phase III study, gefitinib demonstrated noninferiority versus docetaxel in the second-line setting [45]. On the other hand, four large Phase III trials failed to show an improvement in survival when an EGFR-TKI was administered concomitantly with first-line chemotherapy [46–49]. The Iressa Non-small-cell lung cancer Trial Assessing Combination Treatment (INTACT) 1 & 2, Tarceva Responses in Conjunction with Paclitaxel and Carboplatin (TRIBUTE) and Tarceva Lung Cancer Investigation (TALENT) trials randomly assigned more than 4000 chemonaive NSCLC patients to standard chemotherapy (cisplatin plus

gemcitabine or carboplatin plus paclitaxel) versus the same combination plus gefitinib (INTACT 1 & 2) or erlotinib (TALENT and TRIBUTE). Although no differences in survival were observed between the two arms of treatment, some benefit was noted at the end of chemotherapy, particularly for never-smokers, suggesting that a sequential approach could be more effective than a concomitant strategy [50]. Three recent Phase III studies strongly supported the use of an EGFR-TKI as maintenance treatment after chemotherapy. The West Japan Thoracic Oncology Group randomly assigned 598 chemonaive NSCLC patients to platinum-based chemotherapy up to six cycles versus three cycles of chemotherapy followed by gefitinib [51]. Although the primary end point of survival was not reached, PFS was significantly prolonged in the gefitinib arm (4.6 vs 4.2 months; $p < 0.001$), with a modest but statistically significant survival improvement in the adenocarcinoma population (15.4 vs 14.3 months; $p = 0.03$; HR: 0.79). The Sequential Tarceva in Unresectable Non-small-cell Lung Cancer (SATURN) trial was a large Phase III study of erlotinib as maintenance therapy in nonprogressing NSCLC patients treated with four cycles of platinum-based chemotherapy. Even though no data have been presented yet, Roche (Basel, Switzerland) has recently announced that the study met its primary end point of PFS prolongation [201]. On the other hand, Mok *et al.* recently presented the results of a large Phase III study (Iressa Pan–Asia Study [IPASS]) of gefitinib versus carboplatin–paclitaxel in chemonaive Asiatic NSCLC patients with adenocarcinoma histology [52]. The study demonstrated that in a selected patient population in which females and never-smokers were over-represented, gefitinib significantly prolongs PFS versus chemotherapy (HR: 0.74). Importantly, in the whole population and in patients harboring an *EGFR* mutation, PFS improvement was observed after 4–5 months of treatment, further supporting a sequential approach. Another important aspect of the IPASS trial was the lack of efficacy in terms of response rate and PFS in patients without *EGFR* mutations. In this group of patients both response and PFS were significantly better in the chemotherapy arm, clearly indicating that even in a population enriched with clinical characteristics predicting sensitivity to EGFR-TKIs (Asiatic race, adenocarcinoma histology, female gender, never-smoking status) the targeted agent is ineffective when the disease is not EGFR dependent, thus highlighting the importance of biological as opposed to clinical selection.

Since their identification in 2004, activating *EGFR* gene mutations have emerged as the most important predictor of response to gefitinib or erlotinib [53–55]. Several retrospective and prospective studies confirmed that patients carrying an *EGFR* mutation were particularly sensitive to EGFR-TKIs, with responses observed in up to 90% [56–72]. Since these early reports, it clearly emerged that a significant fraction of patients with *EGFR* mutations do not respond to EGFR-TKIs, thus suggesting that other mechanisms are involved in drug sensitivity [42,58,73]. Importantly, increased *EGFR* gene copy number as assessed by FISH emerged as another relevant method for patient selection [66,73]. However, although indirect comparison indicates that *EGFR* mutations could be better than *EGFR* gene copy number in predicting response to treatment, no direct comparison has ever been made between the two methods. Nevertheless, in clinical practice, particularly for patients with limited therapeutic options, such as those with pretreated NSCLC, response to therapy may not be the best end point, since improvement in survival is not only confined to patients with tumor shrinkage [2]. Therefore, the question of whether mutation analysis is better than FISH for patient selection should be investigated in prospective randomized clinical trials with a control arm, which is the only way to assess the impact of such biomarkers on patient survival. Three randomized studies comparing gefitinib (Iressa Survival Evaluation in Lung Cancer [ISEL], Iressa Non-Small-Cell Lung Cancer Trial Evaluating Poor Performance Patients [INSTEP]) or erlotinib (BR21) versus placebo demonstrated that *EGFR* FISH-positive patients treated with an EGFR-TKI have a significant improvement in survival when compared with *EGFR* FISH-positive patients treated with placebo [2,42–44,74]. The impact on survival of *EGFR* gene mutations was explored only in the BR21 study, in which erlotinib produced a substantial survival improvement in both *EGFR*-mutated and wild-type patients [2]. Importantly, although it is not possible to exclude that some mutations reported in the BR21 were artefacts, survival results were not different when the analysis was confined to patients harboring a 'classic' *EGFR* mutation [75]. Although it is now clear that *EGFR* FISH or mutation analyses are useful for patient selection, with FISH the best predictor for survival, in clinical practice it is not possible to deny an EGFR-TKI to *EGFR* FISH-negative or *EGFR* wild-type individuals. Results from the BR21 trial suggest that some survival benefit cannot be excluded in any patient subgroup, and there is no single EGFR test able to

detect patients with no benefit at all from EGFR-TKIs, even if the expected survival improvement is minimal. Therefore, in the absence of any other valid therapeutic option, the treatment should also be considered for patients with EGFR-negative NSCLC. Additional biomarkers or a combination of multiple tests might allow us to identify the 30% of NSCLC patients in whom a TKI should not be offered.

Targeting the VEGF: bevacizumab

A dominant process regulating angiogenesis is the interaction between VEGF and its receptor (VEGFR). VEGFR is specifically expressed on the surface of endothelial cells and, like VEGF, is regulated by hypoxia [76]. Three different forms of VEGFR have been identified: VEGFR-1 (Flt-1) has the highest binding affinity for VEGF-A, but generates relatively little kinase activity; VEGFR-2 (KDR or Flk-1) is the isotype mostly associated with endothelial cell proliferation and chemotaxis; VEGFR-3 (Flt-4) seems to regulate lymphangiogenesis [77]. Disruption of cellular signaling through the VEGF/VEGFR pathway represents an attractive target for therapy. There are two main ways through which VEGFR activity can be blocked, either by anti-VEGF or -VEGFR monoclonal antibodies or by molecules that inhibit VEGFR tyrosine-kinase activity.

A recombinant humanized monoclonal antibody to VEGF, namely bevacizumab, was the first angiogenesis inhibitor to demonstrate efficacy in solid tumors [78]. In NSCLC, a randomized Phase II trial of chemonaive patients treated with standard platinum-based chemotherapy plus placebo or bevacizumab (at either 7.5 or 15 mg/kg) demonstrated a higher response rate and increased survival in favor of the higher dose of bevacizumab as compared with placebo [79]. Importantly, patients with squamous-cell histology, as well as those with tumor cavitation and disease location close to major blood vessels, were found to be at higher risk for fatal tumor-related bleeding events. Based on these findings, the Eastern Cooperative Oncology Group (ECOG) conducted a large Phase III trial comparing the standard doublet of carboplatin–paclitaxel versus the same regimen plus bevacizumab at a dose of 15 mg/kg in 878 untreated advanced NSCLC [3]. In order to reduce the risk of side effects, the study excluded patients with squamous histology, gross hemoptysis or brain metastases. The trial demonstrated that the addition of bevacizumab to standard chemotherapy significantly prolongs survival versus chemotherapy alone ($p = 0.003$; HR: 0.79), and for the first time in advanced NSCLC median survival exceeded 1 year (12.3 vs 10.3 months). However, there was a higher incidence of grade 3–4 bleeding events with bevacizumab (4.4 vs 0.7%), as well as increased rates of grade 3–4 hypertension 3 (7 vs <1%) and grade 4 neutropenia (26 vs 17%). More recently, a large Phase III trial conducted in Europe (Avastin In Lung Trial [AVAIL]) randomly assigned untreated NSCLC patients to cisplatin–gemcitabine or the same regimen plus two different doses of bevacizumab (7.5 and 15 mg/kg) [80]. The study excluded patients with squamous histology, gross hemoptysis, brain metastases, tumor invading major blood vessels or uncontrolled hypertension. Although the primary end point of the study was reached in that a significant improvement in PFS was demonstrated for patients receiving bevacizumab, no benefit in overall survival was observed. PFS was 6.2 months in the cisplatin–gemcitabine arm versus 6.8 months in the bevacizumab 7.5 mg/kg arm ($p = 0.0003$) and 6.6 months in the bevacizumab 15 mg/kg arm ($p = 0.045$). Median survival was 13.1 vs 13.6 (HR: 0.93) versus 13.4 months (HR: 1.03) in each arm, respectively. Although a confounding effect of second- and third-line therapies could explain the lack of survival benefit in the AVAIL trial, it is not possible to exclude the possibility that cisplatin–gemcitabine might not the best regimen with which bevacizumab should be combined. Available data suggest that carboplatin–paclitaxel is the preferable regimen to be used in combination with bevacizumab at the dose of 15 mg/kg [3]. However, there are several unanswered questions regarding the use of bevacizumab in metastatic NSCLC, including chemotherapy regimen, dose, duration and patient selection. In the ECOG and AVAIL trials, bevacizumab was given until disease progression. Preclinical data suggest that early withdrawal of anti-VEGF therapy results in rapid vessel regrowth, indicating that bevacizumab should be given at least until disease progression [81]. Ongoing trials are currently evaluating the potential of using bevacizumab beyond progression, as well as the efficacy and safety of bevacizumab in patients previously excluded from large Phase III trials, particularly individuals with brain metastases and squamous histology [202]. Another unsolved issue is whether bevacizumab should be combined with other targeted agents. At the present time, the negative results of the β-lung trial, a Phase III study comparing erlotinib plus placebo versus erlotinib plus bevacizumab in pretreated NSCLC patients, as well as the negative results

observed in colorectal cancer with the combination of bevacizumab plus cetuximab, discourage this approach in clinical practice [82,83].

In conclusion, despite the fact that bevacizumab, similarly to cetuximab, has improved survival when added to first-line chemotherapy [3,4], the choice of treatment between these two monoclonal antibodies depends only on tumor characteristics such as histologic subtype and clinical factors such as patient comorbidities. In fact, no biomarkers of sensitivity have been identified so far for either bevacizumab or cetuximab.

New targeted agents under investigation

Targeted therapies in patients resistant to EGFR-TKIs

Although some NSCLCs initially respond to EGFR inhibitors, all patients invariably become resistant and develop progressive disease [84]. In approximately 50% of patients, acquired resistance is caused by a secondary mutation in exon 20 (T790M) or exon 19 (D761Y) [85–87]. More recently a novel mechanisms of acquired resistance to EGFR-TKIs has been described by Engelman *et al.* [88]. They isolated gefitinib-resistant clones from HCC827 lung cancer cells harboring *EGFR*-activating mutations and found that resistant cells maintained HER3 and Akt activation in the presence of gefitinib owing to focal amplification of the *MET* proto-oncogene. Importantly, inhibition of MET signaling in these cells was able to restore sensitivity to gefitinib or erlotinib, indicating that the concomitant use of an EGFR-TKI and a MET inhibitor has the potential to revert resistance to EGFR-TKIs. In addition, studies on NSCLC specimens obtained from human material found that *MET* amplification occurs in approximately 20% of patients with acquired resistance to EGFR-TKIs [88,89]. By contrast, the same phenomenon occurs in 3–7.2% NSCLCs not treated with TKIs, thus confirming that MET could also be a relevant therapeutic target for some individuals with acquired resistance to EGFR-TKIs [89,90].

Currently, there is no standard treatment for patients with acquired resistance to EGFR-TKIs. Clinically, some patients might keep benefiting from continued EGFR inhibition with erlotinib or gefitinib [91]. However, preclinical data suggest that new compounds that are named second-generation EGFR-TKIs may have the ability to prevent or overcome acquired resistance to EGFR-TKIs, having shown antitumor activity in the presence of the T970M mutation [92–95]. Among these new drugs, BIBW2992 is the most promising agent. BIBW2992 is an irreversible EGFR-TKI that also inhibits HER2. Recently, a Phase I study tested BIBW2992 in 53 patients with advanced solid tumors [96]. Durable responses (≥12 months) were observed in three out of 15 NSCLC patients (20%), two of which were reported in patients with an activating *EGFR* mutation. On this basis, a Phase II study was carried out in NSCLC patients with activating *EGFR* gene mutations [97]. Out of 24 evaluable

Table 2. Trials currently investigating BIBW2992 in advanced non-small-cell lung cancer.

Protocol IDs	Type of study (planned accrual)	Previous chemotherapy	Previous gefitinib or erlotinib	Patient selection	Primary end point
1200.33 NCT00711594	Phase I/II (72 patients)	Yes*	Yes‡	None	OR
1200.23 NCT00656136	Phase IIb/III§ (400 patietnts)	Yes*	Yes	None	OS
001264-37 NCT00796549	Phase II (70 patients)	No (40 patients) Yes (30 patients)	No	*EGFR* FISH+	OR
1200.41 NCT00730925	Phase II (40 patients)	Yes¶	No	Never smokers# and *EGFR* FISH+ or *EGFR* mut+ ** or *HER2* mut+	OR
1200.22 NCT00525148	Phase II (120 patients)	Chemonaive and pretreated‡‡	No	*EGFR* mut+ **	OR

*No more than two lines; at least one platinum-based.
‡As the most recent treatment; prior documented clinical benefit (response or stable disease) from treatment with gefitinib or erlotinib.
§Randomized, double-blind Phase IIb/III study of BIW2992 + best supportive care versus placebo + best supportive care.
¶No more than three lines.
#Or patients having smoked 15 or fewer pack-years or patients having stopped smoking for at least 1 year before diagnosis (except for HER2 mut+).
**In exon 18 to exon 21.
‡‡No more than one line.
EGFR: EGF receptor; FISH: Fluorescence in situ hybridization; HER: Human epidermal receptor; mut: Mutation; OR: Overall response; OS: Overall survival.

patients, 12 (50%) responded to treatment, with an additional nine (37.5%) achieving disease stabilization. Currently, ongoing trials with BIBW2992 are aiming to test this drug both as an upfront treatment of advanced NSCLC and in patients who have had prior treatment with documented resistance to the first-generation EGFR-TKIs gefitinib and erlotinib (TABLE 2). PF-00299804 is another second-generation irreversible pan-erbB TKI under clinical development for NSCLC [98]. A recently presented Phase I study in heavily pretreated advanced NSCLCs showed that this drug is active in patients with prior exposure to gefitinib or erlotinib [98]. XL647 is another compound with the ability to overcome acquired resistance to first-generation EGFR-TKIs [99]. This agent simultaneously inhibits EGFR, HER2, VEGFR 2, Flt-4 and EphB4 [99]. In untreated NSCLC patients enriched with characteristics that predict response to gefitinib or erlotinib, XL647 produced responses in 17% of patients with a disease control of 53% [100]. Interestingly, another Phase II study tested XL647 in a population of patients with acquired resistance to gefitinib or erlotinib or with documented T790M mutations [101]. Of note, in this resistant population, XL647 produced a disease control rate of 51%, thus supporting further testing of this agent in patients with disease relapse after prior benefit from gefitinib or erlotinib. HKI-272 is an irreversible TKI of EGFR, HER2 and HER3, which has been tested in a Phase II trial of advanced NSCLC patients [102]. Patients with previous treatment with erlotinib or gefitinib for more than 3 months were allocated to one of two arms based on the presence or absence of drug-sensitizing *EGFR* mutations. A third arm accrued patients who had received no prior therapy for NSCLC but had clinical characteristics associated with response to gefitinib or erlotinib, namely adenocarcinoma histology and current nonsmokers or patients who had smoked fewer than 20 pack-years. Disappointingly, the results showed no significant differences in response rate across the treatment arms: arm A: 2%; arm B: 2%; and arm C: 4%. Similar results were observed for patients with stable disease (47, 46 and 39%, respectively) and PFS (11.6, 14.7 and 7.4 weeks, respectively). Given the limited efficacy shown by this agent, at the present time this drug is not being developed further in NSCLC.

MET inhibitors represent another class of drugs under clinical development for the treatment of NSCLC patients with acquired resistance to EGFR-TKIs [103]. Importantly, because *MET* amplification and T790M mutation often occur in the same patient, probably the best strategy is to combine a second-generation irreversible EGFR-TKI with MET inhibitors [88,89]. There are several ways to inhibit the MET signaling pathway, including anti-MET antibodies, inactivation of the MET ligand, namely HGF, or inhibition of MET kinase activity. Currently, the latter strategy is being tested in advanced NSCLC, with a Phase I–II study investigating the MET-TKI XL184 with or without erlotinib in subjects with advanced NSCLC who have progressed after responding to treatment with erlotinib [203].

Multitargeted agents

Vandetanib is a multitargeted inhibitor of VEGFR-2 and -3, EGFR and RET kinases. This drug was developed based on the assumption that dual EGFR/VEGFR inhibition would prove more beneficial than blocking a single pathway [104]. In preclinical studies, vandetanib was found to be a potent inhibitor of the growth of multiple epithelial malignancies including lung cancer [105]. In Phase I and multiple randomized Phase II studies, vandetanib was established as a promising novel targeted agent for the treatment of patients with advanced NSCLC, also supporting its potential role when administered in addition to chemotherapy [106]. Recently, the preliminary results of three Phase III studies investigating vandetanib in advanced NSCLC have been released by the company manufacturer of the drug [204]. The Zactima in Combination with Docetaxel in Non-small-cell Lung Cancer (ZODIAC) and Zactima Efficacy with Alimta in Lung Cancer (ZEAL) trials investigated whether the addition of vandetanib to single-agent chemotherapy would improve the efficacy of docetaxel or pemetrexed, respectively, in pretreated patients. Importantly, both trials showed that the addition of vandetanib to chemotherapy improves responses and PFS as compared with chemotherapy, although PFS prolongation reached statistical significance only in the ZODIAC study. The third Phase III study (Zactima Efficacy Study versus Tarceva [ZEST]) compared vandetanib with erlotinib in pretreated advanced NSCLCs. This study did not meet the primary objective of demonstrating a statistically significant prolongation of PFS for the vandetanib arm. However, vandetanib and erlotinib showed equivalent efficacy for PFS and survival in a pre-planned non-inferiority analysis. Taken together, these data show that vandetanib is able to potentiate the efficacy of chemotherapy and

holds the potential of improving clinical outcome in combination with first-line platinum-based chemotherapy [107].

Sunitinib and sorafenib are two small molecules inhibiting several members of the receptor tyrosine kinase family. On one hand, sunitinib blocks VEGFR-1 and -2, PDGF receptors (PDGFR) α and β, CSF-1R, c-KIT, FLT3 and RET, while on the other, sorafenib acts against VEGFR-2, raf-kinases, PDGF-β and c-KIT. In two Phase II studies of pretreated advanced NSCLC, sunitinib demonstrated a response rate of 11.1 and 2.1%, with a disease control rate of 39.7 and 21.2%, respectively, depending on whether the drug was administered with an intermittent or continuous schedule [108,109]. Importantly, severe adverse events appeared to be more common with the intermittent schedule, consisting mainly of fatigue, pain or myalgia, dyspnea and nausea or vomiting. For this reason, the majority of ongoing trials with sunitinib in NSCLC have adopted a continuous dosing strategy. Table 3 lists ongoing trials investigating sunitinib in combination regimens for advanced NSCLC.

Sorafenib was tested as monotherapy in chemonaive advanced NSCLC patients, demonstrating an activity of 12% and a disease control rate of 40% [110]. In another Phase II trial of previously treated NSCLC patients, sorafenib demonstrated a more limited activity [111]. However, although no responses were observed in pretreated patients, 48% of patients achieved disease stabilization [111]. Recently, a Phase III study (Evaluation of Sorafenib, Carboplatin and Paclitaxel Efficacy in Non-small-cell Lung Cancer [ESCAPE]) testing carboplatin–paclitaxel with or without sorafenib as first-line treatment for advanced NSCLC suffered from early closure after the independent Data Monitoring Committee concluded that the study would not meet its primary end point of improved overall survival for the sorafenib arm [205]. The reason for this failure is attributed to the greater mortality registered in the sorafenib arm for patients with squamous-cell histology. This observation probably implies that, similarly to bevacizumab, multitargeted TKIs with a predominant antiangiogenetic action might induce an excess of toxicity in the squamous subtype of NSCLC. At the present time, the majority of trials investigating sorafenib in pretreated advanced NSCLC are focusing on dual EGFR/VEGFR blockade with the 'chemotherapy free' combination of sorafenib–erlotinib (Table 4).

Axitinib is another multitargeted kinase that specifically inhibits VEGFR-1, -2 and -3. This drug has shown single-agent activity in a Phase II study of advanced NSCLC [112]. On this basis, three Phase II studies are currently testing axitinib in combination with platinum-based chemotherapy for the first-line treatment of advanced NSCLC [206].

Anti-IGF receptor strategies

The IGF-1 receptor (IGF-1R) is a transmembrane heterotetrameric protein, encoded by a gene located on chromosome 15q25-q26, which is implicated in promoting oncogenic transformation, growth and survival of cancer cells [113]. IGF-1R activation triggers a cascade of reactions involving two signal transduction pathways [114,115]. One activates Ras, Raf and MAPK, and the other involves phosphoinositol-3-kinase (PI3K). Preclinical models showed

Table 3. Ongoing Phase II and III studies testing sunitinib in combination regimens for stage IIIB/IV non-small-cell lung cancer.

Protocol IDs	Type of study (planned accrual)	Design	Pretreatment	Primary end point
CDR0000589102 NCT00698815	Phase II* (225 patients)	Pemetrexed vs pemetrexed + sunitinib versus sunitinib	Yes‡	18 weeks PFS
CDR0000613264 NCT00748163	Phase II (72 patients)	Nab-paclitaxel + sunitinib	No	OR
A6181058 NCT00265317	Phase II§ (155 patients)	Erlotinib + sunitinib versus erlotinib	Yes¶	Radiographic progression of disease
A6181087 NCT00457392	Phase III# (956 patients)	Erlotinib + sunitinib versus erlotinib	Yes¶	OS

**Randomized Phase II study.*
‡No more than one line of chemotherapy (either platinum or non-platinum-based therapy).
§Randomized, double-blind, placebo-controlled Phase II study.
¶No more than two lines of chemotherapy; at least one platinum-based.
#Randomized, double-blind, placebo-controlled Phase III study.
OR: Overall response; OS: Overall survival; PFS: Progression-free survival.

Table 4. Ongoing Phase II and III studies testing sorafenib in combination regimens for stage IIIB/IV non-small-cell lung cancer.

Protocol ID	Type of study (planned accrual)	Design	Pretreatment	Primary end point
002688-26 NCT00449033	Phase III* (350 patients)	CDDP/GEM versus CDDP/GEM + sorafenib	No	PFS
SR06-1015 NCT00600015	Phase II‡ (168 patients)	Erlotinib + sorafenib versus sorafenib	Yes§	OR
CDR0000618003 NCT00801385	Phase II (47 patients)	Erlotinib + sorafenib	Yes¶	OR
SCRI LUN 162 NCT00609804	Phase II# (94 patients)	Erlotinib + sorafenib versus sorafenib	Yes**	PFS
004625-14 NCT00722969	Phase II (48 patients)	Erlotinib + sorafenib	No	Rate of nonprogression at 6 weeks
CDR0000536546 NCT00454194	Phase II# (104 patients)	Pemetrexed + sorafenib versus pemetrexed	Yes‡‡	PFS

**Randomized, double-blind, placebo-controlled Phase III study.*
‡Randomized, double-blind, placebo-controlled, Phase II study.
§No more than two lines of chemotherapy.
¶No more than two lines of chemotherapy; at least one platinum-based.
#Randomized Phase II study.
***No more than two lines of chemotherapy; prior documented clinical benefit (response or stable disease) from treatment with erlotinib.*
‡‡No more than one line of chemotherapy.
CBDCA/PAC: Carboplatin/paclitaxel; CDDP/GEM: Cisplatin/gemcitabine; OR: Overall response; OS: Overall survival; PS: Performance status; PFS: Progression-free survival.

that IGF-1R expression could be implicated in resistance to anti-EGFR strategies [116,117]. Jones *et al.* showed that in breast and prostate cancer cells increased signaling via the IGF-1R pathway leads to acquired resistance to the EGFR tyrosine kinase inhibitor gefitinib [116]. However, Morgillo *et al.* found that the simultaneous use of gefitinib with an IGF-1R inhibitor prevents the development of gefitinib resistance in NSCLC cell lines [117]. These data strongly suggest that dual EGFR and IGF-1R blockade could be more effective than blocking EGFR alone. However, in two studies evaluating whether IGF-1R expression and gene copy number could affect response to gefitinib or cetuximab in NSCLC and colorectal cancer, respectively, no relationship was observed between IGF-1R and response to anti-EGFR strategies either at a protein or genomic level, even though a longer survival for IGF-1R overexpressing patients was reported [30,118]. Importantly, recent data support the use of agents targeting IGF-1R in combination with chemotherapy for advanced NSCLC [119,120]. In fact, in a Phase II study of the anti-IGF-1R monoclonal antibody CP-751871 in combination with carboplatin–paclitaxel, a response rate of 48% was observed in untreated patients. Interestingly, tumors with high levels of IGFR protein expression as assessed by the automated quantitative analysis (AQUA) technique were found to have a trend toward improved PFS [120]. Moreover, a response as high as 71% was observed in a patient with squamous-cell histology, likely reflecting the higher expression of IGF-1R in this subpopulation as compared with other histotypes [119]. On this basis, a large Phase III study investigating carboplatin–paclitaxel with or without CP-751871 is being conducted [207].

Mammalian target of rapamycin inhibitors

Mammalian target of rapamycin (mTOR) is a serine/treonine kinase involved in checkpoint regulation of the cell cycle, DNA repair and cell death [121]. Its abnormal activation, which occurs following signaling of the PI3K/Akt pathway, has been frequently reported in human cancers including lung cancer, where mTOR is often found co-activated with Akt [122]. Among the mTOR inhibitors, CCI-779 (temsirolimus) and RAD001 (everolimus) are currently under clinical development in NSCLC. Temsirolimus was tested as front-line therapy in a two-stage Phase II study of advanced NSCLC patients, and demonstrated a response of 8% and a disease stabilization of 30% [123]. Toxicity was manageable, with dyspnea and fatigue being the most common (≥10%) severe adverse events. Although this study did not meet the predefined success criteria, temsirolimus showed a good tolerability with an activity similar to that of other signal transduction inhibitors. Everolimus was investigated in a Phase II study of platinum-refractory advanced NSCLC patients [124]. Patients were divided into two cohorts on the basis of whether they had been previously exposed to

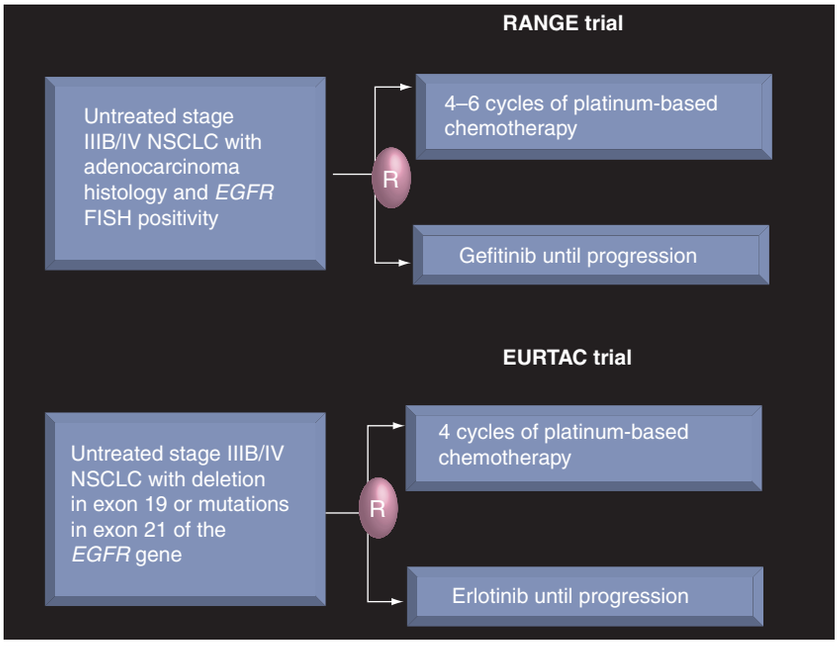

Figure 1. Phase III studies in advanced NSCLC that are prospectively validating the use of *EGFR* FISH positivity (RANGE trial) or *EGFR* mutations (EURTAC trial) in the first-line treatment of advanced NSCLC with an EGFR-TKI.

EGFR: EGF receptor; EURTAC: European Randomized Trial of Tarceva vs Chemotherapy; FISH: Fluorescence *in situ* hybridization; NSCLC: Non-small-cell lung cancer; R: Randomization; RANGE: Randomized Gefitinib Trial.

chemotherapy only (arm 1) or chemotherapy and an EGFR-TKI (arm 2). The results were similar for both arms of treatment. Response in arm 1 was 4.8%, with a disease stabilization of 47.6%, whereas the corresponding values for arm 2 were 2.3 and 37.2%, respectively. Again, treatment was very well tolerated, with fatigue being the most common (≥10%) grade 3–4 toxicity.

Based on the hypothesis that a multi-targeted approach may be the better choice for patients whose tumors present with simultaneous activation of the EGFR and the PI3K/Akt/mTOR pathway, everolimus was evaluated in combination with gefitinib in a NSCLC study whose results have been recently reported [125]. In this trial, patients with untreated or platinum-based pretreated NSCLC were enrolled. Also, only patients who were current or former smokers were eligible. In untreated patients a response of 18% was observed, while 13% of pretreated patients responded to treatment. Similarly, another study found that everolimus plus erlotinib is associated with a response of 13.8% in a population of advanced NSCLC patients pretreated with chemotherapy [126]. Collectively, these data suggest that dual mTOR/EGFR inhibition is a promising strategy in NSCLC. Currently, several trials are being conducted and others are planned with mTOR inhibitors in NSCLC [208].

Future perspective

Non-small-cell lung cancer is a heterogeneous tumor whose growth depends on the dysregulation of multiple signaling pathways. The introduction in the clinic of several biological therapies, each one targeting specific key cancer molecular profiles, represents a major step forward in the treatment of this disease. It is clear that not all targeted therapies are the same, which is best exemplified by the fact that their use in combination with standard chemotherapy has not always led to an improvement in clinical outcome. The identification of patients who will benefit from such treatment used either alone or in combination regimens would allow physicians to deliver effective treatments to sensitive patients, while preventing others from suffering the side effects of inactive drugs. In addition, biomarkers of response to a certain biological agent may differ according to the type of malignancy. For instance, in colorectal cancer, the presence of wild-type *Kras* was found to be a strong predictor of sensitivity to cetuximab. By contrast, wild-type *Kras* does not appear to have the same predictive value for sensitivity to cetuximab in NSCLC. For this reason, ongoing trials are often designed to attempt to elucidate what biomarkers could best predict sensitivity to treatment. To this end, the lesson learned from EGFR-TKIs may represent a proof of concept. In fact, after the accumulation of clinical data demonstrating that *EGFR* mutation or increased *EGFR* gene copy number were able to select a population with a high likelihood of benefiting from such treatments, two Phase III trials have been designed and are currently recruiting patients in order to prospectively validate these biomarkers (Figure 1) [209]. Once the results of these and other studies become available, they will contribute to a better understanding of the role of targeted therapies in NSCLC with regard to optimal dose, schedule, combination strategies and, above all, patient selection.

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Executive summary

Introduction

- Non-small-cell lung cancer (NSCLC) is the leading cause of cancer-related deaths in western countries.
- For patients with advanced disease, chemotherapy with third-generation platinum-based doublets represented the standard of care until recently, when major breakthroughs in the knowledge of cancer biology has resulted in the appearance of numerous targeted therapies for NSCLC treatment.

Existing treatments

- The EGF receptor (EGFR) is among the most studied targets for the development of biological therapies in NSCLC.
- Cetuximab, an anti-EGFR monoclonal antibody, has recently been found to improve the clinical outcome of untreated advanced NSCLC in combination with platinum-based chemotherapy. However, at the present time, no biomarker has proven able to predict sensitivity to treatment with cetuximab in NSCLC.
- Gefitinib and erlotinib are two EGFR-tyrosine kinase inhibitors (TKIs) currently approved for use in pretreated NSCLC. However, there is evidence suggesting that these agents might be more effective than chemotherapy in patients selected on the basis of certain clinical (never-smoking status, adenocarcinoma histology, female gender, Asian ethnicity) or biological (activating mutations of the *EGFR* gene or increased *EGFR* gene copy number) characteristics.
- Among biomarkers, increased *EGFR* gene copy number seems to be the best predictor for improved survival from treatment with EGFR-TKIs. In fact, it allows for the identification of both responding patients and individuals who derive prolonged disease stabilization, as opposed to *EGFR* mutations, whose predictive value appears to be confined to the identification of responders only.
- The anti-VEGF monoclonal antibody bevacizumab is currently approved in combination with platinum-based chemotherapy for the first-line treatment of advanced disease. However, its use is limited to patients with nonsquamous histology, since an excess of fatal hemoptysis was observed in individuals with squamous-cell subtype.
- Several issues still need to be defined with regard to bevacizumab in NSCLC, such as its use in patients with brain metastases or beyond progression.

New targeted agents under investigation

- Second-generation EGFR-TKIs are irreversible inhibitors of the EGFR under clinical development for NSCLC, particularly for patients who develop clinical resistance to gefitinib or erlotinib as a result of specific secondary mutations in the *EGFR* gene. On the other hand, MET inhibitors represent another appealing strategy for overcoming resistance to EGFR-TKIs, especially in individuals whose acquired resistance is the result of the amplification of the *MET* proto-oncogene.
- Multitargeted agents are compounds that simultaneously block several receptor tyrosine kinases, including EGFR and VEGF receptors. Among these, vandetanib has proven active in the second-line setting either in combination with chemotherapy or as single-agent.
- Drugs targeting the IGF-1 receptor (IGF-1R) hold great potential in the treatment of NSCLC, particularly for patients with squamous-cell histology, where IGF-1R is often found expressed at high levels. Preliminary evidence also suggests that the degree of IGF-1R expression might predict for the efficacy of treatment with IGF-1R inhibitors.
- mTOR inhibitors are agents that target a serine/treonine kinase that plays a crucial role in proliferation and survival of cancer cells. These agents are being investigated in patients pretreated with chemotherapy plus or minus an EGFR-TKI, with encouraging results in both cases.

Future perspective

- Ongoing research is focusing on the identification of patients who are more likely to benefit from targeted agents. While potential predictive markers have been identified for some targeted therapies such as EGFR-TKIs, their true value remains to be confirmed in prospective studies.
- Importantly, only rationally designed clinical trials can contribute to our understanding of the role of targeted therapies in NSCLC. In fact, the future of biological strategies no longer lies on empirical drug administration, but rather on patient selection on the basis of key cancer molecular profiles.

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