The place of targeted therapies in the management of non-small cell bronchial carcinoma

Target therapies in lung cancer management

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Résumé

Les thérapies ciblées ont été largement utilisées chez les patients atteints de cancer bronchique non à petites cellules (CBNPC) avancé, avec pour certaines une réelle efficacité. Les inhibiteurs tyrosine-kinase (TK) de l’EGFR (epidermal growth factor receptor), gefitinib et erlotinib, sont efficaces chez les patients prétraités par chimiothérapie. Des taux de réponses plus élevés sont observés chez les patients asiatiques, non fumeurs, de sexe féminin, et porteurs d’adénocarcinome. Ces résultats peuvent s’expliquer par le taux significativement plus élevé de mutations du gène de l’EGFR dans ces sous-groupes de patients. Par contre, l’association d’inhibiteurs TK de l’EGFR avec une chimiothérapie de première ligne n’a pas permis d’obtenir d’amélioration de la survie, et une meilleure sélection des patients qui pourraient bénéficier de ces molécules semble essentielle dans le développement de futures stratégies. Parallèlement, l’utilisation d’inhibiteurs de l’angiogenèse comme traitement anticancéreux est finalement entré dans la pratique clinique. Le VEGF (vascular endothelial growth factor) et son récepteur sont des régulateurs-clés de l’angiogenèse, ce qui en fait des cibles thérapeutiques intéressantes. De nombreux agents ciblant VEGF sont en cours d’investigation et une étude de phase III vient de montrer un bénéfice significatif à la combinaison de bevacizumab à une chimiothérapie de première ligne dans le CBNPC avancé. Cette revue fait le point sur l’utilisation actuelle des thérapies ciblées dans le CBNPC.

Mots-clés : EGFR • VEGF • Cancer bronchique non à petites cellules • Erlotinib • Gefitinib • Bevacizumab • Angiogenèse • Thérapie ciblée.
Targeted therapies have been largely employed in patients with advanced Non-Small Cell Lung Cancer (NSCLC), and some of them have demonstrated consistent activity. Epidermal growth factor receptor (EGFR) inhibitors, gefitinib and erlotinib, are effective in patients previously treated with chemotherapy. Responses are higher in Asians, non-smokers, females and subjects with adenocarcinoma. These findings can be explained by the substantially higher incidence of EGFR mutations in such subgroups. EGFR inhibitors in combination with first-line chemotherapy however, failed to improve survival, and better selection of patients to be offered these agents will be essential in the development of future strategies. At the same time the use of angiogenesis inhibitors as anticaner therapy has finally reached the daily practice. Vascular endothelial growth factor (VEGF) and its receptors are key regulators of the process of angiogenesis, which makes them attractive therapeutic targets. A multitude of VEGF-targeted inhibitory agents are currently being investigated for the treatment of NSCLC and a phase III study proved a significant benefit when bevacizumab was added to first-line chemotherapy in advanced NSCLC. This review focuses on the actual scenario of targeted therapies in NSCLC.

**Key-words:** EGFR • VEGF • Non-small cell lung cancer • Erlotinib • Gefitinib • Bevacizumab • Angiogenesis • Targeted therapy.

### Introduction

Lung cancer has the highest mortality rate for all cancers among both men and women, accounting for almost one third of all cancer deaths [1]. Although lung cancer mortality among men decreased steadily from 1990 to 2000 an inverse trend was registered among women from 1950 to 2000 [2]. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer cases and the majority of patients presents with locally advanced or metastatic disease. Overall 5-year survival rate of 15% is a disappointing figure [3].

Cell proliferation and differentiation are regulated by a number of growth factors, hormones and cytokines that interact with cellular receptors and interact with the nucleus through a network of intracellular signaling pathways. In cancer cells, oncogenes might alter key steps of these pathways through overexpression or mutation, leading to deregulated cell signaling, inhibition of apoptosis and uncontrolled cell proliferation. Continuous progress in the understanding of tumor biology has led to the identification of many of such molecular pathways that drive tumor growth. Each step in the abnormal signaling pathways represents a potential unique target for new anticanter therapies. Targeted therapies have the potential advantage of considerably reduced toxicity compared with standard cytotoxic agents.

### Epidermal growth factor receptor (EGFR) inhibitors

EGFR is a member of the ErbB family of transmembrane tyrosine-kinase receptors, which includes ErbB1 (or HER-1, or EGFR), ErbB2 (or HER-2/neu), ErbB3 (or HER-3), and ErbB4 (or HER-4). Overexpression of EGFR is commonly seen in several solid tumors [4]. Upon ligand binding the receptor undergoes either homo or hetero-dimerization with subsequent activation of the intrinsic tyrosine-kinase activity. This step triggers signal transduction pathways that lead to uncontrolled cell proliferation, inhibition of apoptosis, and angiogenesis [5]. Overexpression of EGFR is reported in 40-80% of NSCLC cases [6]. Several studies showed that levels of EGFR expression correlate with poor prognosis and shorter survival [7, 8]. However, a recent meta-analysis on almost 3,000 cases did not show a negative prognostic impact in NSCLC overexpressing EGFR [9].

Several EGFR inhibitors have been developed in recent years, which can be categorized into two classes: monoclonal antibodies to the extracellular domain of the EGFR, or small molecules that inhibit the intracellular tyrosine-kinase domain by interfering with autophosphorylation by adenosine triphosphate (ATP). Two oral agents targeting EGFR were approved by the Food and Drug Administration (FDA) initially for use in NSCLC: gefitinib (Iressa, AstraZeneca) in May 2003 and erlotinib (Tarceva, Roche) in November 2004. The use of gefitinib was recently limited, however, to those cancer patients who, in the opinion of their treating physician, are currently benefiting or have previously benefited from gefitinib treatment. Erlotinib continues to be investigated in a number of tumor types and was recently approved for the treatment of pancreatic cancer in combination with gemcitabine. FDA also approved the intravenous administered monoclonal antibody Cetuximab (Erbitux, ImClone Systems) for the use in EGFR-expressing metastatic colorectal cancer in patients who are refractory to irinotecan-based therapy.

### Gefitinib

Gefitinib was the first molecularly targeted agent to be registered in Japan, United States and other countries in the world for the treatment of patients with advanced NSCLC.
who experienced disease progression after one or more lines of chemotherapy. Registration has been based on two large randomized phase II studies, the Iressa Dose Evaluation in Advanced Lung Cancer (IDEAL)-1 and IDEAL-2 studies, in patients with advanced NSCLC previously treated with at least one platinum-based chemotherapy regimen (Table I).

IDEAL-2 was performed in the United States on 221 patients randomly assigned to receive either 250 mg or 500 mg gefitinib daily [10]. Patients were heavily pre-treated with chemotherapy (including a platinum compound and docetaxel), with 58% of cases receiving three or more regimens. There was no significant difference in response rate (approximately 10%) or survival between the two dosages. Symptoms of NSCLC improved in 43% of patients receiving 250 mg gefitinib and in 35% of those receiving 500 mg gefitinib. Symptomatic improvement correlated with clinical response: it approached 100% in patients with major responses and was 60% in patients with stable disease, suggesting that benefit could also be reached in absence of response. The 500 mg gefitinib dose was more toxic causing more acneiform rash and diarrhea. Overall grade 3-4 toxicities were uncommon, but more frequent in the 500 mg dosing. Antitumor activity in 10% of patients was an important achievement compared with standard second-line chemotherapy, far more toxic and even less active.

IDEAL-1 was conducted mainly in Europe and Japan, on patients previously treated with one or two prior chemotherapy regimens, including a platinum compound, also randomly assigned to receive a daily dose of gefitinib at 250 or 500 mg [11]. Response rate reached 20% and was similar in both arms with a symptom improvement rate of 40%, which was higher in patients who had an objective response. Again, adverse effects were more frequent and severe with the 500-mg dose.

Overall, disease control rate (responses plus stable disease) was achieved in 50% of patients in both trials: radiographic tumor responses were rapid and long lasting. In the IDEAL-1 trial, 68% of responses were seen within 1 month after randomization, while in the IDEAL-2 trial, the median duration of response was 7 months. Responses were not related to the number of prior therapy regimens, age, gender, or performance status. In IDEAL-2, 56% of patients who had symptom improvements did so in the first week of treatment and 75% had improvements by 3 weeks from start of gefitinib.

Symptom improvement was predictive of longer survival: in the IDEAL-2 trial, patients with symptom improvement had a median survival of 13 months, compared to 5 months in patients without symptom improvement.

Among the stratification factor, ethnicity played a role with response rates of 27.5% in Japanese vs 10.4% for non-Japanese. In addition, at the multivariate analysis female sex, adenocarcinoma, and previous hormonal or immunologic therapies carried significantly higher chances of response.

Gefitinib has since been available in many countries through extended access programs, and several reports have been published based on this experience. Besides results in advanced NSCLC patients who relapsed after chemotherapy, data on a number of chemonaive patients, either unfit or unwilling to receive chemotherapy, were reported. Gefitinib was administered at the standard 250 mg daily dose. Results and toxicity profile mirrored those achieved in the IDEAL studies. However selection criteria were all but rigid. Gefitinib showed activity also as first-line therapy, although not higher than in the second or third line setting. In particular patients with brain metastases and elderly patients did receive some benefit. Patients with performance status 2 are also good candidates for gefitinib treatment.

Single-agent gefitinib is effective in patients with bronchioloalveolar carcinoma (BAC) [12]. A peculiar expression of biomarkers of EGFR pathways could be the rationale for such sensitivity [13]. Southwest Oncology Group investigated gefitinib in BAC in a phase II study on 136 patients (101 untreated, 35 previously treated) [14]. The response rate was 17%, with 6% complete responses among 69 previously untreated patients, and 9% among 22 pretreated patients. Median survival was 13 months for both groups, with 3-year survival of
23%. Subset analyses revealed improved survival among women, patients with rash, never-smokers and a PS of 0 or 1. Gefitinib might therefore represent a viable first-line option in BAC.

Interstitial lung disease is a potentially fatal side effect in patients receiving gefitinib. Worldwide the incidence of interstitial lung disease is about 1% with a peak of 2% in the Japanese population and only 0.3% in the US. One-third of cases resulted in death from respiratory failure.

A large randomized study compared gefitinib 250 mg daily versus placebo in patients relapsing after chemotherapy [15] (table I). The ISEL (Iressa Survival Evaluation in Lung Cancer) study included 1,692 patients and failed to demonstrate an improvement of median survival with gefitinib in either overall population (5.6 vs 5.1 months) or in adenocarcinomas (6.3 vs 5.4 months). Subgroup analyses suggested a statistically significant survival benefit in patients of Asian origin (median survival of 9.5 vs 5.5 months) and in patients who never smoked (median survival of 8.9 vs 6.1 months). A statistically significant higher response rate was observed for gefitinib-treated patients compared with placebo. These results are disappointing and surprising in view of the positive results obtained with erlotinib vs placebo. Different dose-intensity could be an explanation as well as patient characteristics. The 150 mg daily dose of erlotinib should correspond to 600-700 mg of gefitinib, but the two IDEAL studies could not show a difference in outcome between the 250 mg and the 500 mg dose. The ISEL study included a larger number of heavily pre-treated and worse performance status patients. In addition, selection of patients, based on molecular markers and patient characteristics, should probably be considered for future studies.

A new line of clinical research with gefitinib is investigating the role of maintenance therapy after primary treatment. At our institution we are currently running a phase III randomized study in patients not progressing after first-line chemotherapy in stage IV NSCLC. Patients are randomized to gefitinib or placebo after standard first-line chemotherapy. A large adjuvant study (BR.19) that randomized radically resected NSCLC patients to receive gefitinib or placebo as adjuvant therapy was prematurely stopped after the data release from a planned interim analysis of the SWOG 0023. In this study patients with locally advanced disease treated with concurrent chemo-radiotherapy followed by maintenance docetaxel for three cycles were subsequently randomized to receive gefitinib or placebo. The above mentioned interim analysis revealed the absolute impossibility to reach the planned 33% increase in survival in the gefitinib arm and, consequently, the study was prematurely closed for accrual.

### Gefitinib plus chemotherapy

Two large randomized studies explored the combination of two doses of gefitinib (250 or 500 mg daily) or placebo, with different first-line chemotherapy regimens: INTACT-1 used cisplatin and gemcitabine (cisplatin 80 mg/m² on day 1 and gemcitabine 1,250 mg/m² on days 1 and 8 every 3 weeks) [16]; INTACT-2 used carboplatin and paclitaxel (carboplatin given at area under the curve (AUC) of 6 and paclitaxel at 225 mg/m² in 3-hour infusion every 3 weeks) [17] (table II). Chemotherapy was given for up to six cycles and gefitinib or placebo were continued in responders or stable patients until progression. A total of 1,093 and 1,037 patients were entered respectively in the two studies but both failed to demonstrate a survival advantage with gefitinib when added to standard chemotherapy in first-line treatment of advanced NSCLC. A subset analysis of patients with adenocarcinoma who received 90 days of chemotherapy or more in the INTACT-2 study demonstrated statistically significant prolonged survival, suggesting a gefitinib maintenance effect. As expected, gefitinib at the 500 mg dose was associated with more toxicity.

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It is also interesting to note that in the initial part of the survival curves, the EGFR tyrosine-kinase inhibitors (TKIs) arms in all of these studies did worse than the placebo arms. EGFR inhibitors act by reducing proliferation in wild-type EGFR tumor cells, which are mostly affected by chemotherapy, therefore an antagonistic effect between EGFR TKIs and chemotherapy is possible. The impact of Ras mutations on prognosis of patients treated with EGFR TKIs and chemotherapy could also explain the failure of the association.

**Erlotinib**

Erlotinib received approval from FDA based on the results from a randomized double-blind, placebo-controlled phase III trial of 731 patients treated for second-line and third-line advanced NSCLC [18]. In this trial, patients receiving 150 mg of erlotinib daily had a response rate of 8.9% with a median survival of 6.7 months, resulting in a 42% improvement in median survival compared with patients receiving placebo that had a median survival of 4.7 months. Additionally, 31% of patients receiving erlotinib in the study were alive at 1 year versus 21% in the placebo arm. Cancer-related symptoms (cough, dyspnea, and pain) were significantly improved in the erlotinib arm compared with placebo.

Toxicity was acceptable, consisting mainly of skin toxicity and diarrhea, in the range observed with the higher doses of gefitinib in the IDEAL studies. Grade 3-4 rash and diarrhea occurred in 9% and 7% of patients resulting in discontinuation of therapy in 1% of patients. Dose reductions because of rash occurred in 6% and were caused by diarrhea in 1%. The incidence of rash was higher with erlotinib compared with gefitinib in phase II and III studies; rash seems to be dose dependent. Plasma exposure, as measured by area under the plasma concentration-time curve, is 7.5-fold higher with erlotinib than gefitinib when comparing FDA-approved daily doses of 150 and 250 mg, respectively [19]. Rash onset (on the face, neck, and upper chest) occurs usually within 8 days of starting therapy with maximal intensity in the second week. It usually subsides by week 4 in most patients despite continued therapy, although it might persist for longer periods. Etiology of the rash is unclear, and no clinical trials have specifically addressed the issue of its management, despite sporadic successes with retinoids, steroids, and antibiotics.

Interstitial lung disease occurred in 0.8% patients receiving erlotinib in the pivotal NSCLC trial, similar to the incidence among patients receiving placebo. Erlotinib should be discontinued if dyspnea or severe cough develop. Minor side effects include transient grade 2 elevations in liver function tests and uncommon gastrointestinal bleeding, conjunctivitis and keratitis.

**Erlotinib plus chemotherapy**

As previously reported with gefitinib, erlotinib was administered in combination to standard platinum-based first-line regimens in advanced NSCLC in two large randomized trials.

Again, both studies failed to demonstrate any benefit from the addition of erlotinib in terms of survival. The TALENT study randomized 1,172 patients randomly assigned between cisplatin/gemcitabine given at the same doses as in the INTACT-1 study plus erlotinib 150 mg daily or placebo [20]. The TRIBUTE study randomized 1,079 patients to carboplatin/paclitaxel (same schedule as in INTACT-2 study) plus erlotinib or placebo. Maintenance with erlotinib was allowed in responding or stable patients [21].

**Determinants of response**

The relatively low response rates observed in the trials with both erlotinib and gefitinib were in sharp contrast to the clinical evidence of sporadic but dramatic and long-lasting responses seen in a minority of patients. Females, Asians, patients with adenocarcinoma, and never-smokers were the best candidates for response to erlotinib and gefitinib than the rest of patients. Clinical responses to gefitinib and erlotinib were highly dependent on the presence of EGFR somatic mutations that affect critical amino acids in the ATP-binding cleft of the TK domain of the receptor on exons 18-21. In these three studies, out of 60 NSCLC patients treated with EGFR TKIs, EGFR somatic mutations were identified in 25 (80.6%) of 31 responding patients, as compared with none of the 29 patients who did not respond [22-24]. Several studies have reported mutational analyses in over 3000 cases of NSCLC with an overall mutation rate in unselected cases of NSCLC around 15%, displaying wide variations according to ethnicity, sex and smoking habit. Asians have a higher prevalence of mutations compared to Caucasians, as well as non-smokers and women, in the proportion of roughly 30% vs 7% [25]. Reasons at the basis of such data are as yet unclear. Furthermore, mutations appear at a higher frequency in adenocarcinomas (23%) or BAC (18%) histology when compared to others (2%).

In gefitinib trials mutations in the EGFR tyrosine-kinase domain, specifically on exons 18 through 21, seemed to be associated with sensitivity to the drug [26]. In the erlotinib trial immunohistochemical analysis of tumor biopsy samples showed a correlation between overexpression of EGFR and the response to erlotinib, not mutational status. This was particularly true in never-smokers. In this trial mutations in EGFR did result in a trend toward higher response rates compared with patients whose tumors lacked mutations. In the TRIBUTE trial with erlotinib combined with chemotherapy EGFR mutations were associated with a statistically significant
increased response rate but no improvement in survival [27]. In the INTACT trials with gefitinib plus chemotherapy the presence of EGFR mutations was not predictive for response.

In fact, 12% of NSCLCs carrying mutations still progress on TKIs and, to complicate the issue, not all responders carry mutations. Separate reports described the finding of a secondary mutation in exon 20 inducing tumor resistance to gefitinib [28, 29].

In the subset analysis of BR.21 study, erlotinib significantly prolonged survival for patients whose tumors were EGFR positive with little effect on those with EGFR-negative tumors, but with wide confidence intervals. Erlotinib continued to be superior to placebo even in squamous tumors and in patients without mutations.

Prospective evaluation by screening for EGFR mutations was approved by FDA (EGFR Mutation Assay, Genzyme Corp., Cambridge, MA) to select patients for therapy with TKIs.

Development of a rash could be a potential predictor of response and survival in patients treated with erlotinib [30], while the relationship between rash and clinical outcome is less consistent for gefitinib [31]. In trials of erlotinib combined with chemotherapy in NSCLC, a subset analysis showed a correlation between the development of rash and longer survival [32].

Other molecular mechanisms have been explored to predict for resistance. KRAS mutations are the most frequently reported alteration in NSCLC, occurring in ∼20% of NSCLC especially in adenocarcinomas and smokers, and are mutually exclusive of EGFR mutations [33]. A lack of activity of the tyrosine-kinase inhibitors in NSCLC was associated with KRAS mutations [34]. Interestingly, patients whose tumors had KRAS mutations (approximately 20%) almost never harbored EGFR mutations, and their prognosis was particularly bad when erlotinib was given with chemotherapy in the TRIBUTE trial.

Routine screening for mutations remains a major challenge, timely and expensive to set up in everyday clinical practice.

Patients with BAC and increased EGFR copy number assessed by either quantitative PCR [35] or by fluorescence in situ hybridization (FISH) [36] had a significant increase in partial response rates, median progression-free survival, and overall survival. However no clinical characteristics were associated with increased copy number of EGFR gene. It must also be remembered that patients treated with both gefitinib and erlotinib can have clinical benefit without having a partial response but just obtaining a stable disease. Another paper showed that an increased EGFR gene copy number determined by FISH was most closely associated with longer survival in patients with NSCLC, following treatment with gefitinib [37]. EGFR mutations and increased copy number are probably not separate molecular events.

Most retrospective analyses of phase II trials of EGFR inhibitors, have also reported superior survival for patients with classical mutations and/or increased gene copy number. However, superior survival could not necessarily be a direct result of treatment with the EGFR inhibitor, because there is increasing evidence to suggest that the presence of EGFR mutations is not a predictive factor for longer survival but merely prognostic [38]. In fact significantly longer survival has been reported recently for untreated mutation-positive patients from surgical series [39]. Moreover, in the placebo arm of BR.21, classical mutations predicted longer median survival than wild-type or novel EGFR variants suggesting a more favorable prognosis as a result of the presence of mutations per se and not as an effect of treatment with TKIs.

In the near future the challenge will be how to use EGFR mutations, EGFR copy number, EGFR immunohistochemistry or a combination of these tests to select those patients which are most likely to respond to therapy with TKIs [40].

Monoclonal antibodies (MoAb)

MoAb have been developed to specifically target the extracellular component of the EGFR receptor. They compete with natural ligands for EGFR, such as TGF-alpha and EGF for the extracellular binding site thus preventing autophosphorylation of the intracellular region [41]. As a result, the TK domain remains inactive and downstream signaling does not occur.

The chimeric human-murine IgG antibody cetuximab is currently approved for treatment of colorectal carcinoma. Panitumumab (ABX-EGF, human IgG2), pertuzumab (HER dimerization inhibitor), nimotuzumab (humanized Ig anti-EGFR) and matuzumab (EMD-72000, humanized IgG1) are antibodies in earlier stages of development in ongoing phase II trials.

In a phase I trial in EGFR-positive NSCLC patients matuzumab doses up to 800 mg weekly with paclitaxel every 3 weeks were well tolerated, with no apparent drug interactions and with evidence of antitumor activity [42].

Cetuximab

Cetuximab has antitumor activity against a variety of solid tumors, including NSCLC, and can be safely combined with cisplatin and radiotherapy.

To date, only one single-agent study with cetuximab has been performed in advanced NSCLC, with two partial responses out of 29 patients who experienced treatment failure after one or more regimens of chemotherapy [43]. The main adverse effects of cetuximab are skin rash and acneiform skin toxicity, and up to 4% of hypersensitivity reactions. In contrast...
to TKIs treatment diarrhea is not a common adverse effect of monoclonal therapy. Kim et coll. reported a response rate of 28% with the combination of cetuximab and docetaxel as second-line in 47 patients with EGFR-expressing NSCLC [44].

In a phase II study in chemonaïve patients with advanced NSCLC 86 patients were randomized to receive cisplatin/vinorelbine with or without cetuximab. The response rate (31% vs 20%) and the time to progression (4.7 vs 4.2 months) favored the cetuximab-based regimen [45]. Leukopenia was the most common reported toxicity. A phase III trial based on this experience is ongoing (FLEX study) with the same schedule as in phase II. Another first-line phase II trial of cetuximab in combination with gemcitabine and carboplatin in 35 patients with EGFR-positive, stage IV non-small-cell lung showed mild to moderate toxicities with a response rate of 28.6% [46].

A similar study examined the safety profile of Cetuximab, when added to paclitaxel and carboplatin in 31 untreated patients with EGFR positive stage IV NSCLC. The most common cetuximab toxicity was rash in 84% of patients (grade 3 in 13%). Response was observed in 26% of cases with median survival of 11 months [47].

An ongoing phase II feasibility study is testing the combination of cetuximab plus radiotherapy in patients with stage III NSCLC (the NEAR Protocol).

**Other EGFR Inhibitors**

CI-1033 is an orally available highly potent and selective pan-erbB tyrosine-kinase inhibitor that irreversibly blocks intracellular signaling through all members of the EGFR family and recently completed a randomized phase II study of three different schedules of administration. Toxicities seem to be similar to other EGFR inhibitors (skin rash and diarrhea) but thrombocytopenia and allergy were described. Results are currently being analyzed.

Lapatinib (GW572016, GlaxoSmithKline) is one of several tyrosine-kinase inhibitors currently under evaluation in clinical trials. It is designed to selectively inhibit the tyrosine-kinase activity of EGFR and HER-2 and is therefore classified as a dual tyrosine-kinase inhibitor. Data from preclinical and Phase I/II clinical trials indicate that lapatinib has activity in patients with solid tumors, especially breast cancer. A phase II study was originally conducted in untreated NSCLC with two different dose levels, then amended to include only never-smokers or adenocarcinomas with BAC features or pure BAC, and allowed one line of prior chemotherapy [48].

EBK-569 is a potent, irreversible inhibitor of the EGFR tyrosine-kinase. Based on phase I data two phase II, single-agent studies with EKB-569 are being conducted in patients with advanced colorectal cancer or NSCLC at an oral dose of 50 mg [49].

**Angiogenesis**

As a general rule a tumor cannot grow beyond 1-2 mm³ in diameter without the development of a vascular supply [50]. Angiogenesis depends from a delicate balance between local pro-angiogenic and antiangiogenic factors, which are released by both tumor and host cells. To date the most promising target is vascular endothelial growth factor (VEGF), a pro-angiogenic molecule.

The VEGF family of molecules currently consists of six growth factors, including VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor, and three VEGF receptor (VEGFR)-1, VEGFR-2, and VEGFR-3. VEGF-A, which is commonly referred to as VEGF, is the most studied member of the VEGF family and is composed of six isoforms. The three VEGFRs are transmembrane tyrosine-kinases that are predominantly found on endothelial cells. VEGF expression is upregulated in virtually all types of cancer, and tumor-associated VEGF levels frequently correlate with microvascular density and predict for disease recurrence and decreased survival [51].

High VEGF levels have been correlated with poor prognosis in patients with lung cancer and represent an independent prognostic factor [52].

Antiangiogenetic agents act either by preventing VEGF-receptor binding or inhibiting downstream receptor signaling. Since antiangiogenetic agents often exert an indirect, cytostatic effect, many are being evaluated in combination with conventional chemotherapies in order to optimize the anticancer effects of both strategies.

The inhibition of tumor angiogenesis is a key therapeutic strategy that holds great promise for the management of lung cancer. The most promising agents fall into two main categories: monoclonal antibodies and small-molecule tyrosine-kinase inhibitors (TKIs).

**Bevacizumab**

Bevacizumab (avastin; Genentech) is a humanized monoclonal antibody that acts by binding and neutralizing all VEGF-A isoforms. Bevacizumab is currently the only clinically available antiangiogenic agent and is licensed for use in combination with fluorouracil (FU)-based chemotherapy as first-line treatment of metastatic colorectal cancer in the United States and Europe in 2004.

The addition of bevacizumab to paclitaxel/carboplatin produced an increase in response rate, particularly in patients with a non-squamous histology [53]. In a phase II trial, 99 patients were randomly assigned to bevacizumab 7.5 or 15 mg/kg plus carboplatin (AUC 6) and paclitaxel (200 mg/m²) every 3 weeks or carboplatin and paclitaxel alone. On disease
progression, patients in the control arm had the option to receive single-agent bevacizumab 15 mg/kg every 3 weeks. Treatment with carboplatin and paclitaxel plus bevacizumab (15 mg/kg) resulted in a higher response rate (31.5% vs 18.8%), longer median time to progression (7.4 vs 4.2 months) and a modest increase in survival (17.7 vs 14.9 months). Bleeding was the most important adverse event either as minor mucocutaneous hemorrhage or as major hemoptysis, which was associated with squamous carcinomas, mass cavitation and central location. Six patients experienced life-threatening pulmonary hemorrhage, four of whom died. This event did not appear to be dose-dependent.

Eastern Cooperative Oncology Group conducted a phase III trial (study E4599) to evaluate the efficacy of the addition of bevacizumab to a standard platinum-based chemotherapy in chemotherapy-naive patients with advanced non-squamous NSCLC [54]. A total of 878 patients were randomized to receive either paclitaxel (200 mg/m²) plus carboplatin (AUC of 6), with or without bevacizumab (15 mg/kg) up to six cycles; patients in the chemotherapy arm alone were not allowed to cross over to bevacizumab. Median survival was significantly longer in the bevacizumab arm (12.5 months vs 10.2 months; p<0.0075). Also, response rate favored the bevacizumab arm (27% vs 10%; p<0.0001) with a significantly longer progression-free survival time (6.4 months vs 4.5 months; p<0.0001). A higher incidence of bleeding was associated with bevacizumab administration (4.5% vs 0.7%): of the 10 treatment-related deaths five resulted from hemorrhage. This was the first phase III trial to demonstrate a survival advantage obtained from a first-line treatment combining a targeted biologic with chemotherapy in advanced NSCLC. However, some bevacizumab-associated adverse effects warrant special attention, including hypertension, proteinuria and hemorrhage. In addition it should be noted that overall survival was significantly increased in men but not in women. This is really surprising considering the good prognostic factor represented by female gender in most of the previously preformed chemotherapy trials.

Results from a phase I/II study of bevacizumab plus erlotinib in previously treated stage IIIb/IV NSCLC patients showed a response rate of 20% and stable disease 65% of patients. The median overall survival time was 12.6. Both agents could be given at the full dose of 150 mg/d of erlotinib and 15 mg/kg bevacizumab every 3 weeks [55].

**PTK787**

PTK787 (vatalanib) is an oral inhibitor of VEGFR-1, -2, and -3 tyrosine-kinases, related kinases such as platelet-derived growth factor receptor-β (PDGFR-β) and c-kit with good tolerability and promising efficacy in colorectal, renal, prostate cancer and glioblastoma multiforme. The role of PTK787 in patients with lung cancer is being evaluated in a phase II study (the GOAL Study) in France and Germany.

**ZD6474**

ZD6474 is an orally bioavailable inhibitor of VEGF receptor-2 tyrosine-kinase activity that in preclinical studies has been shown to inhibit both VEGF-induced signaling in endothelial cells and tumour-induced angiogenesis. ZD6474 also has activity against EGFR tyrosine-kinase. ZD6474 is undergoing large randomized phase II studies in advanced NSCLC [56]. ZD6474 plus docetaxel provided a response rate of 18% in NSCLC patients who had previously failed first-line platinum-based chemotherapy [57]. ZD6474 combined with carboplatin/paclitaxel as a first-line therapy in NSCLC (IIIB-IV) demonstrated a response rate of 39% [58]. The safety and tolerability of ZD6474 has been evaluated in two phase I studies on Western and Japanese populations with refractory tumors, including NSCLC. Adverse events included diarrhea, rash, and asymptomatic QTc prolongation. Over 40% of patients in the Western study had disease stabilization and in the Japanese study, four of nine patients with refractory NSCLC had partial responses. A phase II randomized trial compared ZD6474 with gefitinib in 160 patients with relapsed advanced NSCLC patients. ZD6474 produced a statistically significant longer time to progression than gefitinib (11.9 weeks vs 8.1 weeks) [59].

**Miscellaneous agents**

SU11248 is an oral, multtargeted TKI that targets VEGFR, PDGFR, Kit, and Fli-1. A phase II study of this agent is currently ongoing in NSCLC [60].

AG-013736 inhibits VEGFR-2, VEGFR-3, and VEGFR-1. In a phase I study in 36 patients with advanced solid tumors, the main dose-limiting toxicities observed were hypertension, proteinuria, and seizures [61]. A phase II study of AG-013736 is currently being planned for patients with metastatic or locally advanced NSCLC.

AZD2171 is a highly potent and orally active inhibitor of VEGF-2 tyrosine-kinase activity, and demonstrates excellent selectivity versus a range of cancer xenograft models [62]. Phase I evaluation of AZD2171 for the treatment of various malignancies is ongoing, both as monotherapy and in combination with chemotherapy.

**Other antiangiogenic agents**

Another class of angiogenesis inhibitors currently in development is the small-molecule tyrosine-kinase inhibitors (TKIs). VEGFR TKIs currently in development include vatalanib (PTK787/ZK-222584), SU11248, and ZD6474.
BAY 43-9006

The Ras/Raf signaling pathway is an important mediator of tumor cell proliferation and angiogenesis. BAY 43-9006 (sorafenib) is a potent inhibitor of Raf-1 and also active against VEGFR-2, VEGFR-3, and PDGFR-β. Once-daily oral dosing of BAY 43-9006 has demonstrated evidence of tumor regression in multiple tumor types, including renal, colorectal, melanoma, thyroid, sarcoma, and pancreas [63]. The most common adverse events were skin rash, hand and foot syndrome, and diarrhea, which was the dose-limiting toxicity. BAY 43-9006 (400 mg twice daily) is currently in phase III clinical testing for the treatment of advanced NSCLC in combination with chemotherapy.

Hypertension and bleeding are two common adverse events that have been observed with several angiogenesis inhibitors and should be considered as a “class” effect. In cases of hypertension, management may require dose interruption or reduction, as well as an anti-hypertensive therapy.

Ras inhibitors

Oncogenic K-ras mutations are frequent in NSCLC, colorectal and pancreatic carcinomas; interrupting the Ras signaling pathway has been a major focus of new drug developments through inhibition of Ras protein expression by antisense oligonucleotides, prevention of membrane localization of Ras, inhibition of ras function by blocking downstream Ras effectors [64].

Oligonucleotides that are complementary to mRNA transcripts of the activated ras oncogene, have been utilized to decrease ras protein expression. An investigational antisense oligodeoxynucleotide, ISIS 2503 has completed testing in phase I and phase II clinical trials: efficacy seem to be limited [65].

Farnesyltransferase inhibitors (FTIs)

Mutations in one family member K-ras occur in 50% of non-small cell lung cancer and have been associated with poor prognosis. Because farnesylation is critical for Ras maturation and function, FTIs were originally developed as specific and sensitive inhibitors of ras-mediated cellular proliferation. Only one phase II study has been completed with single-agent FTI in NSCLC with tipifarnib in advanced NSCLC [66]. There were no objective responses in 43 evaluable patients with 19% of stable disease and median survival of 7.7 months and a low incidence of severe toxicity. Tipifarnib might be considered for evaluation in NSCLC in combination with chemotherapy or other targeted agents.

In a phase I study, 12 patients with advanced solid tumors received escalating doses of erlotinib and tipifarnib [67]. No objective responses were reported but the combination appears well tolerated and a phase II evaluation is planned.

m-Tor inhibitors

mTOR (mammalian target of rapamycin) is a serine/threonine kinase which belongs to the family of phosphatidylinositol kinase–like kinases (PIK) involved in the regulation of a wide range of growth-related cellular functions [68]. mTOR inhibitors in clinical development include rapamycin and the structurally related compounds CCI-779, RAD001, and AP23573.

The role of rapamycin in tumor vascularization is becoming particularly interesting because of preclinical studies that show potent antiangiogenic effect [69]. CCI-779 and rapamycin demonstrated similar antitumor profiles and potencies. phase II studies evaluating the feasibility of administering CCI-779 either alone with the weekly dosing or in combination with cytotoxic chemotherapeutics are ongoing [70].

Conclusions

Targeted therapies offer a unique chance to strike cancer cells in a selective way. One of the major challenges remains the selection of patients more likely to benefit from each drug. The correlation of EGFR mutations with responsiveness to small-molecule inhibitors of the EGFR in NSCLC further supports this idea. However, issues of testing availability in everyday practice and costs of both genetic tests and new targeted therapies is a fundamental issue. Approaches that can measure early changes in the whole tumor as functional imaging studies like positron emission tomography, may be one of the few ways to predict early responses to therapy. New directions of development of targeted therapies should include maintenance treatment based on responses to chemotherapy or by implementing pharmacogenomics profile in chemotherapy trials.

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