Targeted therapy for nonsmall cell lung cancer: focusing on angiogenesis, the epidermal growth factor receptor and multikinase inhibitors

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Chemotherapy used to be the only available option to fight advanced nonsmall cell lung cancer. Platinum-based medication combined with taxanes, vinca alkaloids, and antimetabolites improved patient survival rates. Unfortunately, neoplasmatic diseases remain a global killer because chemotherapy benefits have reached a plateau and most patients are diagnosed at the metastatic stage. The urgent need for therapeutic agents, along with advances in the knowledge of the molecular events of oncogenesis, has resulted in the development of medication that specifically targets processes and pathways critical for tumor growth, such as angiogenesis and the epidermal growth factor receptor. Initially, inhibiting these pathways managed to prolong patient survival, although not to the extent desired. Moreover, targeted therapy combined with conventional cytotoxic agents has shown no superiority to chemotherapy alone in terms of patient survival. Hence, numerous multidynamic agents have appeared in the hope that they might help fight nonsmall cell lung cancer. However, no group of patients who will hopefully gain maximum benefit from such interventions has been clearly identified yet. This paper presents current evidence with regard to such novel agents and angiogenesis and epidermal growth factor inhibitors. *Anti-Cancer Drugs* 21:151–168 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

In recent years we have witnessed a rapidly increasing incidence of nonsmall cell lung cancer (NSCLC) across the globe. The disease could well be characterized as an epidemic of our times. Conventional therapy, despite its adequate symptom control, cannot significantly alter the natural course of this particular type of neoplasia. Prognosis and overall survival remain dismal. The majority of patients are diagnosed with stage IV disease, which means a 5-year survival for only 1% of them [1]. Therefore, the need for novel therapeutic agents is imperative. With the advancement of new molecular techniques and better understanding of biological events implicated in carcinogenesis, drugs may selectively target cancer cells and their evolution pathways, vitally affecting tumor growth and progression with minimal toxicity. The aim of this paper is to present the latest developments in regard to targeted agents; these are studied alone or in combination in clinical trials against NSCLC, which focus on inhibitors of angiogenesis, inhibitors of the epidermal growth factor receptor (EGFR) and multikinase inhibitors.

Inhibitors of angiogenesis

The ability of malignant tumors to grow, invade surrounding tissues and eventually achieve systemic dissemination is dependent upon neoangiogenesis, that is, the formation of new blood vessels through the proliferation of vascular endothelial cells [2]. Vascularization is essential for a tumor's survival, providing nutrients, oxygen supply and a waste-removal route. It is known that without a blood supply the dimensions of a tumor cannot exceed 2–3 mm^3 owing to hypoxia leading to the death of tumor cells [3]. In healthy individuals, activator (proangiogenic) and inhibitor (antiangiogenic) molecules regulate angiogenesis to maintain a state of equilibrium. Disturbance of this balance in favor of proangiogenic molecules is the turning point leading to tumor vascularization. Cancer angiogenesis is stimulated by a number of molecules and cytokines, which can serve as positive regulators; these are secreted from cancer cells, as well as inflammatory and stromal cells [4]. Such regulators include fibroblast growth factor (FGF), hepatocyte growth factor, tumor necrosis factor-α, and hypoxia inducible factor-1, a key transcription factor that affects the expression of genes related to angiogenesis and the vascular endothelial growth factor (VEGF). These molecules play a major role in endothelial proliferation and migration, in extracellular matrix degradation and in tube formation [5]. Tumor blood vessels look fragile when compared with normal vessels. They are distended, abnormal in shape and perivascularly detached, allowing cancer cells to enter the circulation and

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Table 1 Main trials of angiogenesis inhibitors

Study	Treatment	PFS (months)	OS (months)	RR %	Setting	Reference
Phase III	C/P + Bevacizumab	6.2	12.3	35	1st line	[18]
	C/P + Placebo	4.5	10.3	15	Non-squamous	
Phase III	Cis/G + Bevacizumab 7.5 mg	6.7	N/A	34.1	1st line	[19]
(AVAiL)	Cis/G + Bevacizumab 15 mg	6.5	N/A	30.4	Nonsquamous	
	Cis/G + Placebo	6.1	N/A	20.1		
Phase III	C/D/RT + Thalidomide	8.0	16.0	N/A	1st line	[30]
	C/D/RT	7.6	15.3			
Phase III	C/G + Thalidomide	N/A	8.4	N/A	1st line	[31]
	C/G + Placebo		8.9			
Phase III	Cis/V + Endostatin	6.3	N/A	35	1st line	[36]
	Cis/V + Placebo	3.6		19.5	Chinese patients	

C, carboplatin; Cis, cisplatin; D, docetaxel; G, gemcitabine; N/A, not available; OS, overall survival; P, paclitaxel; PFS, progression-free survival; RR, response rate; RT, radiotherapy; V, vinorelbine.

metastasize. Targeting the pathways responsible for the newly formed blood vessels opens up new prospects for the treatment of various solid tumors, including NSCLC [6] (Table 1).

Vascular endothelial growth factor inhibitors

A group of signaling proteins that regulate neoangiogenesis in NSCLC is VEGF, and its tyrosine kinase (TK) receptors, that is, the VEGFRs. VEGF was described in the early 1980s as a protein secreted from tumor cells with a capacity to increase permeability *in vivo* [7]. The VEGF family comprises VEGF-A (major representative), VEGF-B (embryonic angiogenesis), VEGF-C, VEGF-D (lymphangiogenesis), and placental-like growth factor [8]. VEGF-A, commonly referred to as VEGF, is a homodimeric heparin-binding protein and the most potent regulator of angiogenesis. It acts mainly on VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1) receptors, which are expressed on neoplastic and endothelial cells. VEGFR-2 seems to mediate almost all known cellular responses to VEGF. The function of VEGFR-1 is less clearly defined, although it is thought to modulate VEGFR-2 signaling. Another function of VEGFR-1 may be to act as a dummy/decoy receptor, sequestering VEGF from VEGFR-2 binding. VEGF specifically recruits endothelial cells into hypoxic and avascular areas and stimulates their proliferation and cell migration while also increasing vascular permeability [9]. VEGF expression is observed in all types of NSCLC, and is associated with increased microvessel count. Today, there are conflicting reports as to whether VEGF overexpression may be considered a prognostic factor. Nevertheless, many trials report that VEGF overexpression is correlated with early post-operative relapse, poor prognosis and overall survival in the advanced stages of the disease. From a clinical point of view, tarteting the VEGF ligand-receptor system is a very promising treatment modality, as the VEGFRs are chiefly localized on malignant endothelium [10–16].

Bevacizumab (Avastin; Genentech/Roche, San Francisco, California, USA) is a recombinant humanized monoclonal antibody that blocks VEGF binding to its receptors. Bevacizumab at a dose of 15 mg/kg showed promising results in a pivotal phase II trial, resulting in a significant increase in time to progression (TTP): 7.4 versus 4.2 months ($P = 0.023$), compared with chemotherapy alone, when administered in combination with carboplatin and paclitaxel to untreated patients with advanced or recurrent NSCLC disease. However, when tumors of squamous histology – centrally located close to large blood vessels with necrosis or cavitation – were treated with bevacizumab, they showed a tendency to cause bleeding presented as hemoptysis. This resulted in four fatal episodes of major hemoptysis [17].

The official integration of bevacizumab into our clinical practice against NSCLC at a first-line setting was achieved in 2006 during a phase III US study (E4599): 878 chemonaive patients with stage IIIB or IV NSCLC were administered chemotherapy treatment with paclitaxel and carboplatin plus bevacizumab at 15 mg/kg ($n = 434$) or paclitaxel and carboplatin alone ($n = 444$). Serious bleeding events during the phase II trial mentioned above had led to the exclusion of patients with squamous cell carcinoma, hemoptysis and brain metastases, taking into account the possibility of brain hemorrhage. Response rates were 35% in the bevacizumab arm and 15% for paclitaxel and carboplatin alone. This finding was reflected by a significant increase in progression-free survival, (PFS) 6.2 versus 4.5 months ($P < 0.001$), and also in overall survival, (OS) 12.3 versus 10.3 months ($P < 0.003$), for patients treated with bevacizumab. Fifteen deaths related to bevacizumab occurred compared with two associated with chemotherapy only. Pulmonary hemorrhage, gastrointestinal bleeding, febrile neutropenia, and cerebrovascular events were responsible for the fatal outcomes [18]. Nevertheless, the advantages gained through the use of bevacizumab far outweigh the risk involved.

A European phase III trial (Avastin in Lung Cancer) including 1043 patients with advanced nonsquamous NSCLC and no prior treatment confirmed the advantages resulting from adding bevacizumab to platinum-based chemotherapy. The cisplatin/gemcitabine doublet was administered and bevacizumab at 7.5 ($n = 345$) or at 15 mg/kg ($n = 351$) or with placebo ($n = 347$) every

3 weeks until progression. Bevacizumab extended PFS by 6.7 months at 7.5 mg/kg ($P = 0.002$) and by 6.5 months at 15 mg/kg ($P = 0.03$) compared with 6.1 months for placebo, which also showed that the antibody was equally effective at both doses. Generally speaking, the combination of cisplatin and gemcitabine plus bevacizumab was well tolerated, showing a safe profile of administration. Slightly higher rates of pulmonary hemorrhage of all grades were observed (7% for bevacizumab in the low-dose group and 9.7% in the high-dose group), compared with 4.9% for chemotherapy alone [19]. Analyzing the data from these trials, patients with squamous tumors, cavitation or history of hemoptysis faced the highest risk of developing major pulmonary hemorrhage, usually within the first two cycles of treatment with bevacizumab. Other adverse events expected are mainly hypertension and proteinuria, and, more rarely, thromboemolism, congestive heart failure, and gastrointestinal perforation [20].

Currently, bevacizumab is under evaluation when combined with other chemotherapeutic agents, such as docetaxel, oxaliplatin and pemetrexed, showing promising results at advanced NSCLC stages. In particular, the combination of bevacizumab with carboplatin and pemetrexed, followed by maintenance pemetrexed and bevacizumab, has led to impressive results, with an overall response rate of 55%, [95%, confidence interval (CI) 41–69%], a median PFS and OS of 7.8 and 14.1 months, respectively, and acceptable toxicity, as initial treatment for untreated patients with stage IIIB and IV disease and nonsquamous histology [21]. This regimen could become our primary option when treating patients with the characteristics described above, while also considering that pemetrexed is more effective for adenocarcinoma patients.

To date, receiving bevacizumab has been contraindicated for patients with brain lesions or squamous tumors owing to the risk of cerebrovascular bleeding and hemoptysis. Recently announced data, however, attempt to set a new pattern for bevacizumab use, claiming that it can be safely administered to individuals with brain metastases treated with neurosurgery or whole brain radiation therapy. Leukoencephalopathy, mental status changes, cerebral arteriosclerosis, ataxia, and cases of grade II central nervous system bleeding have been reported as adverse events. Bevacizumab seems to be safe, with a low incidence of central nervous system hemorrhage [22,23]. Forthcoming studies will clarify this perspective. As for squamous cancers, the ongoing AVAstin in SQuamous tumors trial will probably provide answers to the question of whether radiation therapy before chemotherapy combined with bevacuzumab might be an approach to minimize the probability of bleeding. At the moment, bevacizumab is the standard of care for chemonaive, advanced, nonsquamous NSCLC, with the potential to expand its benefits to other patients as well.

VEGF Trap (Aflibercept; Sanofi-Aventis/Regeneron, Tarrytown, New York, USA) is a soluble receptor made from the extracellular domains of VEGFR1 and VEGFR2, and binds to all forms of VEGF. This protein was found to have a significant anticancer effect as an antiangiogenic factor when tested in ovarian cancer and neuroblastoma xenograft models [24,25]. Analysis of data from 33 patients with platinum resistant adenocarcinoma confirmed two partial responses (PRs) and 63% of patients with stable disease. When administered intravenously, this agent seems to be well tolerated, causing side effects expected from VEGF inhibition [26]. More trials are in progress for VEGF Trap as a second-line or third-line treatment scheme.

IMC-1121b (ImClone Systems) is a humanized monoclonal antibody that binds to the extracellular domain of VEGFR-2. An ongoing trial aims at assessing the PFS rate when this agent is combined with paclitaxel and carboplatin for patients with stage IIIB-IV NSCLC.

Thalidomide

Thalidomide is an old drug with an unclear mechanism of action; it was developed in the late 1950s to relieve patients from insomnia and pregnant women from morning sickness, but proved to have teratogenic effects. In 2006, under the brand name Thalomid (Celgene Corporation, Summit, New Jersey, USA), thalidomide was granted approval for the treatment of multiple myeloma. Thalidomide interacts in numerous ways: it acts as an inhibitor of angiogenesis; it downregulates VEGF; it acts as an immunomodulator that suppresses cytokines and tumor necrosis factor-α; and it modifies extracellular matrix components [27,28]. In a phase II trial including 43 patients with advanced NSCLC and no earlier chemotherapy, thalidomide was administered *per os* with carboplatin and irinotecan. However, this regimen led to a poor median OS of 8.1 months (95% CI: 5.0–12.9) [29]. The results released from a phase III study of thalidomide or placebo, plus gemcitabine and carboplatin, on 722 patients enrolled with stages IIIB and IV, have not indicated any improvement in survival rates (8.5 months for thalidomide and 8.9 months for placebo, $P = 0.12$). Yet, individuals in the thalidomide arm had a predisposition to sustain thrombotic events [30]. Recent data from a phase III study of carboplatin, docetaxel, and thoracic radiation with ($n = 272$) or without ($n = 277$) thalidomide administered to stage III patients also failed to provide a clinical benefit from the addition of thalidomide. The median PFS and OS were 8.0 ($P > 0.05$) and 16.0 months ($P = 0.88$), respectively, for those treated with thalidomide and 7.6 and 15.3 months for those patients that did not receive thalidomide [31].

Endogenous inhibitors (endostatin)

Endostatin is a fragment of the human XVIII collagen gene with an inhibitory effect on VEGF-induced endothelial

cell proliferation and migration [32]. In the Lewis lung carcinoma model, it led to tumor regression in dormant lesions [33]. However, there is no clear picture, as yet, of the biological mechanisms of endostatin. Reverse transcription-PCR analysis and genome-wide expression profiling have indicated that various complex signaling pathways are regulated. A rate of 12% of all cellular genes is affected by endostatin [34]. This discovery, along with the fact that phase II trials on several types of cancer showed minimal or no tumor response to endostatin, halted clinical trials in the USA in 2005. At present, endostatin is approved in China under the name Endostar (recombinant form) for the treatment of NSCLC [35]. In a phase III trial, 486 Chinese chemonaive patients with stage IIIB and IV NSCLC were administered cisplatin and vinorelbine plus rh-endosatin ($n = 326$) or cisplatin and vinorelbine plus placebo ($n = 167$). The patients in the experimental arm showed a response rate of 35.4 versus 19.5%, ($P = 0.0003$) for placebo. TTP was 6.3 months for chemotherapy in conjunction with rh-endostatin versus 3.6 months for chemotherapy plus placebo. No differences in toxicity were recorded between the two arms [36]. Despite these encouraging results in a Chinese population, endostatin still has many hurdles to overcome before it can be reconsidered as a serious candidate for targeted therapy in Western countries.

Epidermal growth factor receptor family inhibitors

EGFR is a family of four closely related transmembrane receptors (ErbB-1, ErbB-2/HER-2, ErbB-3/HER-3, ErbB-4/HER-4). EGFR receptors can form heterodimers or homodimers owing to ligand activation; they can create an intercellular network of TK signaling pathways, responsible for multiple cellular function. The identification of the EGFR family has been a landmark in anticancer therapy [37,38] (Table 2). ErbB-1, known as EGFR, is capable of extracellular ligand binding, while also having an intracellular domain with TK activity. Its activation leads to downstream signaling cascades including the mitogen-activated protein kinase, the signal transducer and activator of transcription proteins and the phosphatidylinositol 3-kinase (PI3-K/AKT) pathways, which instigate cell growth, proliferation, and survival [39]. Gene amplifications, TK domain mutations, EGFR overexpression – encountered in 40–80% of NSCLC tumors – and the ensuing perpetual stimulation of intracellular pathways are substantially involved in NSCLC pathogenesis, while promoting motility, cell immortality, invasion, and metastatic behavior [40,41].

EGFR and EGFR/HER-2 tyrosine kinase inhibitors

Gefitinib (Iressa; Astra Zeneca, London, UK) is a synthetic anilinoquinazoline agent administered *per os*,

Table 2 Main trials of EGFR family inhibitors

Study	Treatment	PFS (months)	OS (months)	RR %	Setting	Reference
Phase II (IDEAL I)	Gefitinib 250 mg	2.7	7.6	18.4	2nd and 3rd line with prior platinum chemotherapy	[45]
	Gefitinib 500 mg	2.8	8.0	19		
Phase II (IDEAL II)	Gefitinib 250 mg	N/A	7.0	12	3rd line with prior docetaxel or platinum chemotherapy	[46]
	Gefitinib 500 mg		6.0	9 $P=0.51$		
Phase III (INTACT I)	Cis/G + Gefitinib 250 mg	5.8	9.9	50.3	1st line	[48]
	Cis/G + Gefitinib 500 mg	5.5	9.9	49.7		
	Cis/G + Placebo	6.0 $P=0.76$	10.9	44.8		
Phase III (INTACT II)	C/P + Gefitinib 250 mg	N/A	9.8	30.4	1st line	[49]
	C/P + Gefitinib 500 mg		8.7	30.0		
	C/P + Placebo		9.9 $P=0.64$	28.7		
Phase III (ISEL)	Gefitinib 250 mg	N/A	5.6	8	2nd and 3rd line with prior platinum chemotherapy	[47]
	Placebo		5.1	1		
Phase III (I-PASS)	Gefitinib 250 mg	5.7	18.6	43	1st line, Asians	[62]
	C/P	5.8	17.3	32.3	Adenocarcinomas Never or light smokers	
Phase III (INTEREST)	Gefitinib 250 mg	N/A	7.6	9.1	2nd line after platinum chemotherapy	[63]
	Docetaxel		8.0	7.6		
Phase III (BR21)	Erlotinib 150 mg	2.2	6.7	9	2nd and 3rd line with prior platinum chemotherapy	[66]
	Placebo	1.8	4.7	1		
Phase IIIb	Erlotinib 150 mg	N/A	6.3	8.3	2nd line	[69]
Phase III (TRIBUTE)	C/P + Erlotinib 150 mg	N/A	10.6	21.5	1st line	[70]
	C/P + Placebo		10.5 $P=$N/S	19.3 $P=$N/S		
Phase III (TALENT)	Cis/G + Erlotinib 150 mg	N/A	10.0	31.5	1st line	[71]
	Cis/G + Placebo		10.3 $P=$N/S	29.9 $P=$N/S		
Phase III (BeTA)	Erlotinib 150 mg + Bev 15 mg	3.4	9.3	12.6	2nd line	[181]
	Erlotinib 150 mg	1.7	9.2 $P=$N/S	6.2		
Phase III (SATURN)	Erlotinib 150 mg	12.3 weeks	12	12	Maintenance after platinum chemotherapy	[77]
	Placebo	11.1 weeks	11	5		
Phase III (ATLAS)	Bev 15 mg + Erlotinib 150 mg	4.8	N/A	N/A	Maintenance after platinum chemotherapy + Bev	[182]
	Bev 15 mg + Placebo	3.7				
Phase III (FLEX)	Cis/V + Cetuximab	4.8	11.3	36	1st line	[117]
	Cis/V	4.8	10.1	29	EGFR (+) tumors	

Bev, bevacizumab; C, carboplatin; Cis, cisplatin; EGFR, epidermal growth factor receptor; G, gemcitabine; N/A, not available; N/S, not significant; OS, overall survival; P, paclitaxel; PFS, progression-free survival; RR, response rate; V, vinorelbine.

which binds to the TK region of EGFR intracellular domain, thus preventing signaling transduction. It belongs to the class of reversible inhibitors that bind noncovalently to the enzyme with rapidly formed bonds without permanent effects [42]. As ZD1839, gefitinib delayed tumor growth in various series of neoplasms *in vitro* [43,44]. In 2005, it was approved for the treatment of patients with recurrent or refractory NSCLC who had failed in earlier chemotherapy with cisplatin or carboplatin with docetaxel. The Iressa Dose Evaluation Dose Evaluation in Advanced Lung Cancer (IDEAL 1) and IDEAL 2 phase II trials showed a response rate for gefitinib of 19 and 12%, respectively, lesser toxicity and a 40% rate of symptom control. Patients from IDEAL 1 (50% were Japanese) and IDEAL 2 were administered gefitinib at doses of 250 or 500 mg/m^2, respectively. Skin reactions and diarrhea were the main adverse events [45,46]. However, the positive results for gefitinib from the IDEAL 1 and 2 studies were not confirmed by the Iressa Survival Evaluation in Lung Cancer phase III randomized placebo-controlled study with 1692 demographically balanced patients. This study revealed that gefitinib was not superior to placebo as a second-line or third-line therapy scheme in stage IIIB and IV NSCLC. The median OS was 5.6 months for gefitinib and 5.1 months for placebo ($P = 0.087$) [47]. At the first-line setting against advanced NSCLC – as indicated by the INTACT-1 (Iressa NSCLC Trial Assessing Combination Treatment) and INTACT-2 phase III randomized placebo-controlled trials with a total of 2130 patients – no extension in OS was observed when gefitinib was combined with chemotherapy [48,49]. These alarming data led to gefitinib being banned in Europe and the USA.

Subgroup analyses indicated that Asians, women and never smokers with adenocarcinoma seemed to respond better. Retrospectively, it was discovered that such patients were more frequently carriers of somatic mutations of the TK domain in exon 19 and L858R mutation in exon 21 [50,51]. Mutations may alter TK activity and inhibitor binding. Patients with mutations, who were included in the Iressa Survival Evaluation in Lung Cancer study, showed a response rate of 37.5%, while that of those without mutations was 2.6%, but the higher response rate was not associated with a survival benefit [52]. The EGFR staining lacked any predictive value [53]. Prospective studies support that patients with a high EGFR gene copy number evaluated by fluorescence, in situ hybridization (FISH) have benefited from gefitinib treatment [54,55].

In Asian countries, in individuals harboring EGFR mutations, gefitinib is an accepted and convincing option for second-line treatment, with proven efficacy in several studies [56–58]. Surprisingly, a recently conducted phase III noninferiority trial (V-15-32) of gefitinib compared with docetaxel in previously treated Japanese patients regardless of their EGFR mutational status missed its goal, but questions arise about the trial design [59]. As a first-line treatment in patients with mutations, gefitinib showed an impressive response rate (75%) and a median PFS of 9 months in phase II trials, even though it was not compared with chemotherapy [60,61]. For this purpose, the phase III IPASS (IRESSA Pan-ASia Study) randomized 1217 patients with adenocarcinoma never or light smokers (10 packets/year) to gefitinib ($n = 609$) or carboplatin with paclitaxel ($n = 608$). PFS was superior for gefitinib compared with chemotherapy. Among patients in the gefitinib arm, 24.9% of them did not progress after 1 year of treatment, compared with 6.7% in the chemotherapy arm, ($P = 0.001$). Patients with mutations in both arms achieved even better PFS. The overall response rate was 43 and 32% for gefitinib and chemotherapy, respectively ($P = 0.0001$). The 71.2% response rate of mutation-positive patients in the gefitinib group – as was the case in earlier first-line studies – had a great impact versus only 1% in the mutation-negative patients. Remarkably, the response rate for the mutation-positive tumors in the chemotherapy group was 47.3 versus 23.5% for the mutation-negative patients, ($P = 0.0013$). In addition, patients with EGFR mutations presented a longer PFS than those whose tumors did not show the mutation (9.5 vs. 1.5 months) [62]. In any case, these results place gefitinib in the front line of treatment against lung adenocarcinoma within a limited group of patients.

The impact of gefitinib on NSCLC progression is being re-evaluated worldwide. The efficacy of gefitinib in nonselected patients has been re-assessed in a large phase III multinational randomized study (INTEREST-IRESSA NSCLC Trial Evaluating REsponse and Survival against Taxotere), in which 50% of the tumors were adenocarcinomas and 20% of the patients were Asian. In this study, gefitinib (250 mg per day, $n = 723$) proved its noninferiority vis-à-vis docetaxel (75 mg/m^2 every 21 days, $n = 710$) with a median OS of 7.6 versus 8.0 months [hazard ratio (HR) = 1.020, 96% CI: 0.905–1.150]. These findings will presumably support an argument in favor of gefitinib's return to the European and US markets, as an alternative second-line-of-treatment agent. However, it should be noted that patients with EGFR mutations, high EGFR copy number or Asian origin have had the same response to gefitinib and docetaxel, which contradicts what we have known thus far [63]. Therefore, instead of shedding new light, the INTEREST study has raised more questions. Gefitinib seems very effective when used among specific Asian populations, but among Western patients its role is expected to be clarified by numerous scheduled trials of selected cohorts.

Erlotinib (Tarceva; Roche, Basel, Switzerland) is also a reversible wild-type EGFR TK inhibitor that binds to the adenosine triphosphate site of the receptor. As a result,

the signal ceases owing to autophosphorylation inhibition [64]. Erlotinib (OSI-774) induced antitumor activity in patients with solid malignancies and had acceptable side effects (skin rash and diarrhea) at a dose of 150 mg daily [65]. The BR.21 placebo-controlled randomized trial led to the inclusion of erlotinib into our therapeutic armamentarium against NSCLC. Seven hundred and thirty-one patients received erlotinib at 150 mg daily or placebo as a second-line or third-line regimen. Erlotinib achieved a response rate of 8.9% that added 2 months to OS compared with placebo (6.7 vs. 4.7, HR = 0.70, $P = 0.001$). Non-smokers with EGFR-expressing tumors showed better response rates ($P = 0.0007$). Bronchoalveolar carcinomas also had an impressive response rate of 25% [66,67]. As expected, rash and diarrhea were the main toxicity expressions. It must also be highlighted that the presence and severity of rash were strongly associated with higher survival rates. Patients without a rash had a 3.3 month survival rate, whereas the equivalent period was 11.1 months for patients with a grade 2+ rash ($P < 0.001$) [68]. Similar results for erlotinib (response rate equal to 8.3% and 6.3-month OS) have been published in another phase III study with 229 enrolled patients [69].

As in the case of gefitinib, the concurrent administration of erlotinib with platinum doublets in the first-line setting examined under the Tarceva Responses in conjunction with paclitaxel and carboplatin (TRIBUTE) and TALENT (Tarceva Lung Cancer Investigation) trials – numbering 2231 patients – did not prolong survival. It should be noted that never smokers in the TRIBUTE study achieved an OS of 22.5 versus 10.1 months for those with a smoking history [70,71]. As for nonselected populations, the probability that cytotoxic agents and oral TKIs target the same cancer cells may partially explain the failure of gefitinib and erlotinib when combined with chemotherapy. Nevertheless, according to the data available, erlotinib seems more effective than gefitinib as a monotherapy, offering pretreated patients a significant benefit advantage.

With regard to the role of EGFR mutations, in retrospective molecular analyses on BR.21, patients presenting mutations on exon 19 and exon 21 (L858R) showed increased response rates, when compared with those without mutations (27 vs. 7%), but this did not improve their survival rates. The EGFR gene copy number prognostic importance on BR.21 is dubious [72,73]. In the TRIBUTE study the high EGFR copy number (100 out of 245 analyzed samples), although retrospectively assessed, was not correlated with longer survival [74]. Patients with mutations (13%) had improved life expectancy, irrespective of the treatment received ($P < 0.001$). These subgroups of patients may also respond to conventional chemotherapy, making EGFR sequencing an important prognostic factor for the successful outcome of their treatment [75,76].

The SequentiAl Tarceva in UnResectable NSCLC placebo-controlled phase III trial with 889 enrolled patients is about to attribute a new role to erlotinib against NSCLC, as maintenance therapy. Four hundred and thirty-eight patients received erlotinib after four cycles of platinum chemotherapy without evidence of disease progression compared with 451 patients who received placebo. The trial has met its primary endpoint, that is, to extend PFS [HR=0.71 (95% CI: 0.62–0.82), $P = 0.000003$]. This was a minimal PFS prolongnation from 11.1 weeks to 12.3 for the general patient population [77]. The OS was significantly longer for those on erlotinib than for those on placebo (12 vs. 11 months). In a 3-year follow-up there was an absolute difference of 10% between the treatment and placebo groups (OSI Pharmaceuticals, press release).

Recently, the Spanish Lung Cancer Group presented a prospective trial of erlotinib administered as first-line or second-line therapy to patients ($n = 217$) with EGFR mutations and advanced NSCLC. This single-arm trial showed a response rate of 70.6% for the patients, which is similar to the response achieved by gefitinib in the IPASS study. TTP and OS were 14 and 27 months, respectively. However, the efficacy of erlotinib was not compared against that of chemotherapy. This issue is a task for the randomized trial of first-line erlotinib versus cisplatin and a taxane agent or gemcitabine for stage IV NSCLC patients with mutations, also launched by the Spanish Lung Cancer Group. The results are anticipated with intense interest [78].

A significant issue is resistance to erlotinib and gefitinib. Resistance is often a result of mutations to the downstream molecule K-ras (occurs in 20–30% of NSCLC patients), located in exon 2, codons 12 and 13 [79,80]. The mutated K-ras allows for continuous aberrant activation of the underlying pathways, despite the silencing of the EGFR receptor. In BR.21, patients with K-ras mutation responded at a ratio of 5% compared with 10% among patients with wild-type K-ras [73,81,82]. In the TRIBUTE, 21% of participants detected with mutated K-ras showed worse TTP and prognosis [75]. In contrast, in the SequentiAl Tarceva in UnResectable NSCLC trial erlotinib had a similarly beneficial effect in both K-ras-mutant and K-ras wild-type patients [83]. Gefitinib and erlotinib help approximately 10% of nonselected patients to improve their outcomes. However, resistance to EGFR inhibition is inevitable even for these patients at some point in their treatment. The known key factors implicated for secondary resistance are a point mutation (T790M) in exon 20, amplification of the TK receptor cMET that induces downstream signaling cascades, persistent activity of PI3K/AKT and ERK pathways, and loss of the binding proteins of the insulin growth factor receptor, the result of which is depression of insulin growth factor receptor signaling that may

contribute to TKI resistance [84–87]. Neither gefitinib nor erlotinb can circumvent such obstacles. This illustrates the inability of patients with acquired resistance to gefitinib to respond to erlotinib [88,89].

Lapatinib (Tykerb/GSK) is a reversible dual EGFR and HER-2 oral inhibitor approved in 2007 for the treatment of HER-2-expressing breast cancer [90]. The HER-2 receptor has no known ligand, yet heterodimerization between HER-2 and other EGFR family members enables the activation of a signaling repertoire related to cell proliferation, DNA synthesis and survival [91]. HER-2 overexpression (3+) in NSCLC, determined by immunohistochemistry, does not exceed 5–10%, and is noted predominately in cases of adenocarcinoma [92,93]. Tumors with HER-2 overexpression or gene amplification tend to be associated with shorter survival time [94]. The efficacy of lapatinib in NSCLC was tested in 131 patients divided into a target group of never smokers or with BAC tumors and another with no specific parameters. Patients were randomized into 1500 mg-once and 500 mg-twice daily groups. Toxicity symptoms, including rash, diarrhea, and nausea, were manageable for both schedules, but the study had to be stopped owing to the very low response rates, although one of the two patients with increased HER-2 gene copy number showed 51% tumor shrinkage [95]. This negative result may be interpreted as owing either to the nonselected population or the incapability of lapatinib to by-pass the T790M mutation [96]. Ongoing trials on lapatinib combined with cytotoxic agents will clarify its usefulness in NSCLC.

HKI-272 (Neratinib; Wyeth Pharmaceuticals, Collegeville, Pennsylvania, USA) is a potent oral EGFR and HER-2 irreversible inhibitor with notable success in HER-2-expressing breast cancer tumors. Irreversible inhibitors are enzyme inactivators producing permanent chemical changes to HER-2. Similar to all irreversible inhibitors, HKI-272 is innovative because it impedes the EGFR signaling of NSCLC tumors with T790M mutation through covalent binding [97–99]. In a phase II trial, 167 patients were divided into three arms. Arm A included patients with EGFR mutations and disease progression after 12 weeks of erlotinib or gefitinib treatment, arm B included patients receiving the same treatment scheme but without mutations, and arm C included patients with adenocarcinoma and no prior EGFR treatment. There were two PR in arm A, one in arm B, and one in arm C. Furthermore, 42, 22, and 11 patients presented a stable disease in arms A, B, and C, respectively. The overall response rate was not significant across treatment arms: 2% for arm A, 2% for arm B, and 4% for arm C. Molecular analysis will certainly explain the unexpected reduced HKI-272 activity, particularly in the erlotinib/gefitinib arm [100].

CI-1033 (Carnetinib; Genentech/Roche) is a novel irreversible TK inhibitor of EGFR, HER-2 and ErbB-4 receptors. HER-3 needs to be dimerized with another ErbB receptor to acquire signaling activity. This makes CI-1033 a pan-ErbB inhibitor [101]. In a phase II trial with 166 previously treated patients, CI-1033 was administered as monotherapy at three different doses, of 50 or 150 mg daily for 21 days, or 450 mg daily for 14 days. Response rates were 2, 2 and 4% for the three arms; however, the 450 mg arm was correlated with unacceptable toxicity (rash and diarrhea). Patients with rash and HER-2 expression presented prolonged survival [102]. CI-1033 showed modest efficacy, but it is essential that it should be evaluated among selected populations in future studies.

EKB-569 (Wyeth Pharmaceuticals, Madison, New Jersey, USA) and CL-387 785 (Alexis Biochemicals, Farmingdale, New York, USA) are also irreversible EGFR and HER-2 inhibitors against NSCLC and a variety of tumors at early development stages. EKB-569 at a maximum tolerated dose of 75 mg daily for 14 days in a 21-day cycle caused toxicity presented as gastrointestinal disorders, rash, and asthenia. In this phase I trial, 24 participants including NSCLC patients presented a stable disease for 2 months [103]. The results from phase II studies of EKB-569 combined with chemotherapy in NSCLC are not yet available. CL-387 785 *in vivo* inhibits EGFR-2 and HER-2-expressing cells [104].

BIBW 2992 (Tovok; Boehringer Ingelheim, Ingelheim, Germany) is a new oral dual EGFR and HER-2 inhibitor found to suppress tumor growth in cancer lines and transgenic lung cancer models. BIBW 2992, an irreversible inhibitor, is quite effective against wild-type, mutated or even resistant EGFR carcinomas [105,106]. For the time being, BIBW 2992 is under investigation among select target groups. Data from a phase II trial in NSCLC refer to PR in 29 of 55 evaluable patients (53%) with adenocarcinoma and EGFR mutations and progressive disease treated with BIBW 2992 at a dose of 50 mg daily for at least 28 days. Twenty-three patients (42%) achieved SD. The side effects were diarrhea and rash [107].

PF-00299804 (Pfizer, New York, New York, USA) is an irreversible oral pan-Erb inhibitor with significant antitumor activity in xenograft models presenting elevated EGFR expression. It also has the advantage of affecting T790M- or ErbB2-mutated tumors [108]. In heavily pretreated NSCLC patients, including those who received reversible EGFR inhibitors, PF-0029804 has thus far achieved SD in nine of 18 evaluable adenocarcinomas. The most commonly observed toxicity events were diarrhea and skin-related symptoms [109].

Epidermal growth factor receptor monoclonal antibodies

Cetuximab (Erbitux/ImClone; Bristol Myers, New York, New York, USA) is a chimeric human-mouse IgG1

monoclonal antibody that neutralizes the extracellular EGFR domain by attaching to specific proteins. Cetuximab is indicated against metastatic colorectal cancer and squamous cell head-and-neck carcinoma. Cetuximab showed modest activity in phase II trials at the first-line setting when combined with chemotherapy against advanced NSCLC. In these trials no differences in response rates were observed between tumors with positive or negative EGFR expression. The most commonly described adverse events were febrile neutropenia, allergic-type infusion reactions as a consequence of the activation of the complement pathway that leads to antibody-dependent cellular toxicity, acne-like rash, and hypomagnesemia [110–115]. Cetuximab, as a monotherapy for refractory NSCLC, resulted in a disappointing response rate of 4.5% [116]. Satisfactory news has come from the phase III First-Line Erbitux (FLEX) in Lung Cancer study results: 1125 untreated patients with positive EGFR staining and advanced NSCLC had been administered cisplatin and vinorelbine on day 1 with or without cetuximab, initially administered at a dose of 400, and then 250 mg/m^2 weekly in a 21-day cycle. The PFS (4.8 months) was the same for both groups. Clinical activity was evident, as patients treated with cetuximab presented an OS of 11.3 months compared with 10.1 months in those who had received chemotherapy alone ($P = 0.044$). The response rates were 29 and 36% for chemotherapy and cetuximab, respectively ($P = 0.12$). Acne-like rash and incidents of febrile neutropenia increased in patients in the cetuximab arm [117]. Patients treated with cetuximab who developed rash of any grade presented longer OS periods than those without rash (15.0 vs. 8.8 months, $P < 0.001$) [118]. However, another phase III study, the BMS-099, did not indicate a statistically significant improvement in overall survival, although it had not been designed to detect survival benefit as precisely as the FLEX trial. A total of 676 patients with advanced NSCLC (irrespectively of their EGFR status) were randomized into two groups that received taxane plus carboplatin with or without cetuximab as first-line therapy. The median OS was 9.7 months for chemotherapy plus cetuximab and 8.4 months for chemotherapy alone, with an HR of 0.89 (95% CI: 0.75–1.05, $P = 0.17$) (ImClone Systems Inc., New York, New York USA; press release). Recently, the results of a meta-analysis of the data from 2018 patients including all histological types, showed that cetuximab improves efficacy when added to standard first-line chemotherapy over chemotherapy alone, regardless of the regimen implemented: 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 (odds ratio=1.463; 95% CI: 1.201–1.783; $P < 0.001$). The administration of cetuximab with chemotherapy resulted in a 13% lower chance of patients dying within a 3-year follow-up period ($P = 0.10$). The absolute benefit at 1 year was 5% [119]. Unlike oral TK inhibitors, monoclonal antibodies do not depend on uptake across membranes. This may explain their efficacy in combination with chemotherapy [120]. New phase II studies have adopted a strategy of enrolling patients with high EGFR gene copy number evaluated by FISH. These patients were treated with standard chemotherapy or chemoradiation plus cetuximab, and presented higher response rates and outcomes, but the validity of these findings should be further examined [121,122]. Another issue is that of whether K-ras mutations are negative predictors of resistance to cetuximab in NSCLC as they are in colon cancer cases [123]. Retrospective data from the FLEX and BMS-009 reveal no difference in regard to PFS or OS between patients with wild-type and mutant K-ras tumors [118,124]. It seems that the K-ras status has no effect on response to cetuximab in NSCLC. Finally, cetuximab is effective in a wide spectrum of eligible patients, including those with squamous tumors. The subgroups that will really benefit from the drug are still unknown.

Panitumumab (Vectibix; Amgen, Thousand Oaks, California, USA) is a humanized IgG_2 monoclonal EGFR antibody approved in 2006 against refractory EGFR-expressing colorectal cancer, which can induce antibody-dependent cellular toxicity [125]. In a phase II NSCLC trial, 166 untreated patients with advanced disease were randomized to carboplatin versus paclitaxel every 3 weeks, with or without panitumumab, at a weekly dose of 2.5 mg/kg. Unfortunately, the addition of panitumumab to chemotherapy did not have any benefit. The TTP was 4.2 months for the panitumumab arm and 5.3 months for chemotherapy alone ($P = 0.55$). A grade III acne-like rash was observed in 4% of patients treated with the antibody. However, this negative result may be a result of nonselection of cohort patients [126,127].

Matuzumab (EMD 72000; Merck KGaA, Darmstadt, Germany) is another IgG_1-humanized monoclonal EGFR antibody involved in antineoplastic activity in preclinical models [128]. The inclusion of matuzumab, at a weekly dose of 800 mg, in systemic chemotherapy with paclitxel was well tolerated by 18 NSCLC patients with stage IIIB and IV disease. Of the total of 18 patients, 14 developed acneform rash [129]. Matuzumab is combined with pemetrexed as a second-line treatment regimen.

Targeted EGFR treatment in NSCLC should not be blindly provided, but rather aligned to the patient EGFR and K-ras mutational profile. Patients with EGFR mutations have a more responsive form of lung cancer to anti-EGFR treatment or even to chemotherapy. There are as yet no data available on the clinical differences among patients with various EGFR mutations. New available TK inhibitors and current developments in molecular oncology in conjunction with new prospective trials offer the opportunity of discovering the most promising EGFR-targeting therapy regime. Epidermal growth factor

blocking with either TK inhibitors or monoclonal antibodies, treatment timing, and appropriate patient selection remain challenges [130].

HER-2 monoclonal antibodies

Trastuzumab (Herceptin; Roche) is a humanized monoclonal antibody directed against the extracellular domain of the HER-2 receptor. The mechanisms of action of trastuzumab include degradation of the HER-2 receptor, G1 cell phase arrest, inhibition of the PI3K-AKT cascade, prevention of the production of p95 (an active HER-2 part), and angiogenesis suppression through changes to proangiogenic and antiangiogenic molecular status [131–133]. Trastuzumab has played a major role against HER-2-overexpressing (+3) breast cancer tumors since 2006. In regard to NSCLC, trastuzumab has been the subject of several phase II studies. Administered as monotherapy or in combination with chemotherapy, initially at a dose of 4 mg/kg and subsequently at 2 mg/kg weekly, trastuzumab showed zero or minimal clinical activity in tumors with HER-2 + 1 or + 2 expression, as evaluated by immunohistochemistry. Hematologic toxicity and ventricular ejection fraction decrease were observed [134–138]. There is a discrepancy of results in HER-2 + 3 tumors. Similarly, in a trial including 103 chemonaive HER-2-positive patients with advanced NSCLC, there was no difference in response rates and PFS owing to the addition of trastuzumab to cisplatin and gemcitabine. Generally speaking, the platinum doublet with trastuzumab showed a response rate of 36% and PFS of 6.1 months versus 41% and 7.0 months, respectively, for chemotherapy alone. Nevertheless, six patients with + 3 HER-2 tumors evaluated by FISH showed 83% response rates, showing an increased PFS of 8.5 months [139]. Other trials suggested no advantages for HER-2 + 3 cancers [135,136]. Very few patients with HER-2 + 3 tumors seem to benefit from trastuzumab [140]. It could also be speculated that patients with HER-2 amplification or HER-2 gene mutations, although rarely encountered, could gain maximum responses from trastuzumab [141]. Current trials with trastuzumab in NSCLC cases are pursuing this direction. On account of the fact that, as already mentioned, HER-2 dimerizes with EGFR members, a molecule targeting the HER-2 dimerization process and the EGFR TK domain would be a more attractive option than trastuzumab, which only works when the HER-2 receptor is overexpressed [142].

Pertuzumab (Omnitarg; Genentech/Roche) is a recombinant humanized HER-2 dimerization inhibitor, binding to a different HER-2 epitope; there is an increasing body of evidence of the efficacy of pertuzumab in breast and ovarian cancers [143,144]. In NSCLC, pertuzumab elicited no response among pretreated patients. As a single therapy, it achieved a PFS of 6.1 weeks, which is shorter than other monotherapies. Nevertheless, a small number of patients (six of 22) in a phase II study showed metabolic responses to pertuzumab expressed as a reduction in standardized uptake value, when assessed with positron emission computed tomography imaging. Extended PFS was noted for this patient group [145,146], although the activity of pertuzumab was not correlated with HER-2 expression levels. A probable role of pertuzumab in NSCLC treatment may emerge from combinations with cytotoxic or targeted agents. *In vitro*, its clinical activity seems to be enhanced when combined with an EGFR inhibitor [147].

Multikinase inhibitors

Simultaneous targeting of several kinases offers a theoretical advantage over single kinase inhibitors, as NSCLC and most cancers have complex signaling pathways and are not driven by a single protein aberration. Such a multi-front attack may also impede the activation of escape mechanisms for NSCLC cells. In contrast, toxicity is probably exacerbated, leading to more off-target effects. These agents, although often aiming at the same pathways, seem dissimilar in their activities, which may induce different outcomes in NSCLC [148] (Table 3).

Sunitinib (Sutent; Pfizer) is a small oral molecule approved in 2006 against renal cell carcinoma and imatinib-resistant gastrointestinal stromal tumors. The inhibitory activity of sunitinib includes the VEGFR 1-3, platelet-derived growth factor receptor, stem cell factor receptor (c-Kit), colony stimulated factor receptor-1, Fms-like TK-3 (FLT3) and glial cell-line-derived neurotrophic factor receptor (RET) [149]. In NSCLC, 63 patients with advanced disease received sunitinib at the standard schedule of a daily dose of 50 mg for 4 weeks followed by a 2-week of treatment in a 6-week cycle, in a phase II trial. All patients had had prior platinum-based chemotherapy. Their overall response rate was 11.1% (95% CI: 4.6–21.6%) with seven PR. The median PFS and OS were 12 and 23.4 weeks, respectively. Adverse events included grade III and IV hematological toxicity, fatigue, nausea, mucositis, diarrhea, and hypertension. Pulmonary bleeding occurred in squamous carcinomas [150]. In another phase II trial in 47 pretreated patients, a continuous daily dose of 37.5 mg led to one PR (overall response rate of 2.1%) and 11 SD [151]. Sunitinib is currently being studied in combination with cytotoxic agents such as gemcitabine, pemetrexed, and docetaxel.

Sorafenib (Nexavar; Bayer, Leverkusen, Germany) is another oral VEGFR 1-3, PDGFR, FLT-3, RET, c-Kit, and RAF (part of the mitogen-activated protein kinase/ERK pathway) inhibitor authorized for use against renal and hepatocellular carcinomas. Sorafenib was initially administered at a dose of 400 mg twice daily, continuously, in patients with relapsed or refractory NSCLC. After 8 weeks of treatment, the conditions of 30 of the 51 patients in a phase II study had stabilized. No PRs were

Table 3 New agents against NSCLC

Name	Targets	Development	Setting
VEGF Trap (Aflibercept)	VEGFR 1, -2	Phase II	2nd line
IMC-1121b	VEGFR-2	Phase II	1st line
Lapatinib (Tykerb)	EGFR/HER-2	Phase II	1st and 2nd line
HKI-272	EGFR/HER-2	Phase II	1st and 2nd line
CI-1033 (Carnetinib)	EGFR, HER-2, HER-3, HER-4	Phase II	2nd line
EKB-569	EGFR, HER-2	Phase II	2nd line
CL 387,785	EGFR	Preclinical	
BIBW 2992 (Tovok)	EGFR, HER-2	Phase III	Relapsed NSCLC
PF-0029804	EGFR, HER-2, HER-3, HER-4	Phase II	Relapsed NSCLC
Panitumumab (Vectibix)	EGFR mAb	Phase II	1st and 2nd line
Matuzumab	EGFR mAb	Phase II	2nd line
Trastuzumab (Herceptin)	HER-2 mAb	Phase II	1st and 2nd line
Pertuzumab (Omnitarg)	HER-2 dimerization inhibitor	Phase II	Relapsed NSCLC
Sunitinib (Sutent)	VEGFR 1-2, PDGFR, c-Kit, CSF-1R, FLT, RET	Phase II	1st and 2nd line
Sorafenib (Nexavar)	VEGFR 1-2, PDGFR, c-Kit, FLT, RET	Phase III	1st line
Vandetanib (Zactima)	VEGFR-2, EGFR, RET	Phase III	1st line and relapsed NSCLC
PTK-787 (Vatalanib)	VEGFR 1-3, PDGFR, c-Kit, FLT	Phase II	1st and 2nd line
AZD-2171 (Recentin)	VEGFR 1-3, PDGFR, c-Kit	Phase III	1st line
BIBF 1120 (Vargatef)	VEGFR, PDGFR, FGFR	Phase III	1st and 2nd line
XL-647	VEGFR-2, EGFR, HER-2	Phase II	1st and 2nd line
BMS-690514	VEGFR-2, EGFR, HER-2	Phase II	Relapsed NSCLC
Axitinib (AG013736)	VEGFR 1-3, PDGFR, c-Kit, CSF-1R	Phase II	1st line and relapsed NSCLC
Pazopanib (GW786034)	VEGFR 1-3, PDGFR, c-Kit	Phase II	Adjuvant, Neo-adjunvant, 1st line
Motesanib (AMG706)	VEGFR 1-3, PDGFR, c-Kit	Phase III	1st line

c-Kit, stem cell factor receptor; CSF-1R, colony stimulated factor receptor; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; FLT, Fms-like tyrosine kinase; HER, human epidermal growth factor receptor 2; mAb, monoclonal antibody; NSCLC, nonsmall cell lung cancer; PDGFR, platelet derived growth factor receptor; RET, glial cell-line derived neutrophic factor receptor; VEGFR, vascular endothelial growth factor receptor.

described. The PFS was 2.7 months and OS 6.7 months. Responsive patients achieved a PFS of 5.5 months [152]. Another study with 15 evaluable patients reported two patients with PR and seven with SD and a TTP of 5 months. The side effects in these trials included rash, diarrhea, hand-foot syndrome, hypertension, grade II and III hematological toxicities and electrolyte disorders, all of which were fully reversible after treatment postponement [153]. At third-line setting, sorafenib seemed superior to placebo. Data from 83 patients showed SD at a ratio of 47% for patients who had received sorafenid (24 out of 51) and 19% for patients (six out of 32) who had received placebo ($P = 0.01$). The median PFS was 3.6 and 2.0 months for sorafenib and placebo, respectively ($P = 0.009$) [154]. The few responders from these studies do not reflect a clear sign of sorafenib's activity as a single therapy for pretreated patients. The drug achieved significant tumor control in many cases.

The addition of sorafenib to chemotherapy with carboplatin and paclitaxel in a large ($n = 926$) first-line phase III Evaluation of Sorafenib, Carboplatin and Paclitaxel Efficacy in NSCLC trial has not proved beneficial compared with placebo. Interim analysis results have revealed a median OS of 10.6 months for patients treated with sorafenib and chemotherapy versus 10.7 months for patients on chemotherapy alone ($P = 0.93$). However, detrimental effects have been observed in patients with squamous cell histology assigned to the sorafenib arm. This subgroup presented greater mortality rates compared with other squamous tumors that had been treated with chemotherapy alone. Paradoxically, this phenomenon has not been reported in earlier trials and cannot be attributed to toxicity, though the main cause of death was disease progression. Fatal pulmonary hemorrhage incidents (1.4%) have been similar for all arms, even to a lesser extent than in the bevacizumab case (4%). The trial has stopped and has led to the exclusion of squamous tumors from another ongoing phase III trial, namely, the NexUS (NSCLC research Experience Utilizing Sorafenib). This awkward situation has set a new basis for patient selection [155].

Vandetanib (Zactima; Astra Zeneca, London, UK) is a VEGFR-2, EGFR, and RET inhibitor, known earlier as ZD6474, approved for the treatment of rare thyroid tumors with antineoplastic activity in lung tumor xenografts and also in a variety of other solid tumors [156]. Concurrent VEGFR-2 and EGFR inhibition is reported, which has synergistic antitumor effects [157]. Compared with other TKIs in pretreated NSCLC patients, vandetanib significantly prolonged PFS ($P = 0.13$) at a dose of 300 mg/m^2 once daily versus gefitinib in a phase II trial [158], but this was not so when compared with erlotinib. A phase III trial (ZEST-ZACTIMA Efficacy Study vs. Tarceva) including 1240 patients showed equivalent efficacy in PFS and OS for vandetanib and erlotinib. The adverse events rate for grade III was higher in the vandetanib arm (50 vs. 40%) [159]. After platinum-containing chemotherapy in patients with advanced NSCLC, vandetanib showed a longer PFS in combination with docetaxel than docetaxel with placebo in a phase II trial. Vandetanib was administered at doses of 100 and 300 mg/m^2. The 100 mg arm and docetaxel achieved a PFS of 18.7 weeks, compared with 17.0 weeks for the 300 mg arm with docetaxel and 12.0 weeks for docetaxel

alone. Interestingly, the 100 mg dose proved more effective and showed lesser toxicity. At higher doses, vandetanib's EGFR activity may prevail over its anti-angiogenic effects [160,161]. The 100 mg vandetanib dose every 21 days, as a second-line therapy/treatment, was evaluated in a phase III randomized placebo-controlled trial (ZODIAC-ZACTIMA in combination with docetaxel in NSCLC) with docetaxel ($n = 694$) versus docetaxel alone ($n = 697$) in pretreated patients. Vandetanib led to statistically significant improvement in PFS versus docetaxel (4.0 and 3.2 months, $P < 0.001$) and in overall response rate (17 vs. 10%, $P < 0.001$). OS did not reach statistical significance ($P = 0.196$). Diarrhea and neutropenia were the most common adverse events correlated with vandetanib. Qtc prolongation was described, but was asymptomatic and below 2% [162]. The combination of vandetanib and pemetrexed as a second-line treatment regime versus pemetrexed alone also showed positive trends for this combination for PFS ($P = 0.108$) and OS ($P = 0.219$), with a significant overall response rate of 19.1 versus 7.9% ($P < 0.001$) in a phase III trial (ZEAL-ZACTIMA Efficacy with Alimta in Lung cancer) with 534 enrolled patients. Rash (38 vs. 26%), diarrhea (26 vs. 18%), and hypertension (12 vs. 3%) appeared more frequently in patients treated with vandetanib and pemetrexed [163]. Remarkably, vandetanib is the first TK inhibitor that has some clinical benefit when combined with cytotoxic agents. An ongoing multicentre phase III study (ZEPHYR-ZACTIMA Efficacy trial for NSCLC patients with history of EGFR-TK inhibitor and chemo-resistance) investigates the efficacy of vandetanib versus the best supportive care in patients resistant to anti-EGFR treatment.

PTK787 (Vatalanib; Novartis, Basel, Switzerland) is an oral TK inhibitor against the VEGFR 1–3 the PDGF, c-Kit and FLT receptor kinases. A phase II trial of PTK787 was conducted to assess its clinical activity (SD > 12 weeks) at a dose of 1250 mg once or twice daily in patients ($n = 54$) with disease progression after first-line platinum chemotherapy. PFS and OS were 2.4 and 7.0 months for the once-daily group, respectively, and 3.7 and 6.8 months for the twice-daily group. PTK787 exhibited a response rate of 2% at the 1250 mg dose and 7% at the 1250×2 dose. There were two episodes of pulmonary hemorrhage and one episode of pulmonary embolism [164].

AZD2171 (Recentin; Astra Zeneca) inhibits VEGFR 1–3, PDGFR, and c-Kit. As a continuous oral daily dose, AZD-2171 has a maximum tolerated dose of 45 mg daily in combination with chemotherapy. This resulted from a phase I study of AZD2171 plus carboplatin and paclitaxel. There were 20 enrolled patients with no earlier anti-VEGF intervention who received AZD2171 at either 30 or 45 mg. The median TTP was 7.6 months with PR nine confirmed and a response rate of 45%. The 45 mg cohort was associated with more severe fatigue, headache, diarrhea, and thrombotic events. The AZD2171 combination with carboplatin and paclitaxel led to severe gastrointestinal toxicity and neutropenia. The 30 mg cohort was more easily tolerated and is indicated for long-term administration [165]. However, the AZD2171 extension into a phase III trial (BR.24) with carboplatin and paclitaxel in the first-line treatment was halted owing to unacceptable toxicity in the AZD2171 arm (Astra Zeneca, press release). The administration of AZD2171 at a dose of 30 mg daily and pemetrexed every 21 days is currently under investigation among recurrent NSCLC patients with or without prior bevacizumab treatment. Data from 31 evaluable patients of this phase II trial have confirmed a disease control rate of 71% for patients who had not received bevacizumab ($n = 19$) and 67% for those who had been treated with bevacizumab ($n = 12$). The grade III and IV toxicities included neutropenia, fatigue, and diarrhea. No major hemorrhage has been observed [166]. AZD2171 seems to be effective even among patients with earlier bevacizumab administration.

BIBF 1120 (Vargatef; Boehringer Ingelheim) is a molecule with triple anti-angiokinase activity, well tolerated at doses of 25–100 mg in xenograft models. The VEGFR, PDGFR, and the FGF receptor (FGFR) fall within its inhibition range [167]. In phase II development, BIBF 1120 was tested on 73 patients – including some with treated brain metastases – with advanced NSCLC as second-line or third-line therapy. Patients were randomized to receive BIBF 1120 at doses of either 250 ($n = 36$) or 150 mg ($n = 37$) twice daily. Both schedules were equally effective. The median PFS and OS were 1.7 and 5.5 months, respectively, with a disease control rate of 48%. Patients with performance status between 0 and 1 ($n = 57$) achieved a better median PFS of 2.9 and an OS of 9.5 months. Disease control was 59% for this subgroup. In general, the study proved safe, and diarrhea, nausea, and abdominal pain were the main adverse events [168]. Phase III placebo-controlled trials of BIBF 1120 in combination with docetaxel or pemetrexed are in progress.

XL647 (Exelexis) is a potent oral VEGFR-2, HER-2, and EGFR inhibitor with an additional effect on T790M-expressing cells [169]. Early results from two phase II studies are available at this stage. In the first study, 36 untreated patients with advanced lung adenocarcinoma and at least one of the following criteria (Asian origin, female sex, minimal or never smoker) were administered XL647 350 mg on days 1–5 in a 14-day cycle or XL647 300 mg daily continuously. Of the 36 patients in the 350 mg arm, 10 had PR, while seven had EGFR mutations. The median PFS was 9.1 months for patients with mutations and 3.8 months for those without mutations. SD for 3 months reached a ratio of 36% [170]. In the second study, XL647 was administered at a dose of 300 mg daily to NSCLC patients with acquired resistance

to erlotinib or gefitinib. Of the 23 participants, one developed PR and seven had SD. One of the responders had a T790M mutation [171]. XL647 seems promising for effective treatment in selected populations.

Bristol Myers-Squibb-690514 (BMS) is a selective EGFR, HER-2, and VEGFR-2 antagonist with antiproliferative and proapoptotic effects on NSCLC cell lines and in T790M mutants [172]. Interim analysis from BMS-690514 200 mg assessment in erlotinib-naive ($n = 28$) and erlotinib-resistant ($n = 32$) NSCLC tumors revealed some disease control for 11 of 28 (39%) and seven of 32 (22%) for erlotinib-naive and erlotinib-resistant patients, respectively. A high disease control rate was more frequent for patients with EGFR mutations (six of eight) than with wild-type EGFR (five of 18). Two patients from the resistant group, who were carriers of T790M mutations, had stable disease for more than 4 months. Toxicity was manageable [173]. A comparison study between BMS-690514 and erlotinib is currently in progress.

Axitinib (AG013736; Pfizer) is an oral agent with anti-VEGFR 1-3, PDGFR, c-Kit, and colony stimulated factor-1 activity already used in a variety of cancers. Axitinib was evaluated in a phase II study with 32 enrolled metastatic patients. Of these patients, nine patients had received no prior chemotherapy, while three patients saw their tumor size shrink (9%), with a median PFS of 5.8 months and OS of 12.8 months. The disease control rate reached a ratio of 41%. The 1-year survival rate for patients with or without earlier chemotherapy was 57 and 78%, respectively. Fatigue, hypertension, and hyponatremia were mostly observed [174].

Pazopanib (GW786034; GSK, London, UK) is another oral VEGFR 1–3, PDGFR, and c-Kit inhibitor with some proven effect on renal cell carcinoma and gynecological tumors [175]. An interesting trial for pazopanib has been presented. Patients with operable early NSCLC (stage I–IIA) were treated with pazopanib 800 mg daily for a median of 18 days, before surgery. Impressively, 20 of 23 patients (87%) achieved tumor reduction with three PRs. The grade III adverse events were hypertension and increased ALT. No grade IV toxicities were observed [176]. Pazopanib seems a highly effective drug; there are ongoing evaluation studies at preoperative and post-operative settings and scheduled trials investigating various combinations of pazopanib with cytotoxic agents.

Motesanib (AMG706; Amgen*)* is a small molecule that inhibits VEGFR 1-3, PDGFR, and c-Kit**.** MOtesanib NSCLC Efficacy and Tolerability-1 study is a placebo-controlled phase III trial of motesanib plus carboplatin and paclitaxel in a first-line setting treatment against advanced NSCLC, in which patients with squamous histology treated with motesanib presented higher mortality rates and an increased frequency of hemoptysis. This information was derived from the safety data review of 600 patients, and led to the immediate discontinuation of the trial in squamous NSCLC. However, nonsquamous patients are still under trial (Amgen, press release). As in the cases of sorafenib in the Evaluation of Sorafenib, Carboplatin and Paclitaxel Efficacy study and AZD2171 in the BR.24 study, the MOtesanib NSCLC Efficacy and Tolerability-1 trial indicates that squamous tumors face high risk from multi-target intervention. VEGF inhibition by either agent might be an explanation. Thus, one of our primary intentions when enrolling patients for clinical trials should also be to prevent the participation of patients who might be endangering their safety.

Combined targeted agents

The latest strategy in targeted NSCLC therapy is to combine agents with multiple, functionally linked, inhibitory properties. EGFR and VEGFR have common downstream signaling pathways. EGFR activation induces VEGF expression in cancer cells, and tumor-associated endothelial cells express EGFR [177]. The results on the efficacy of this treatment approach are promising, as shown by vandetanib and XL647. The question is whether the combination of selective protein kinase inhibitors that block EGFR and VEGFR could be more promising. To this end, trials with bevacizumab and erlotinib in concurrent administration are being conducted. An initial phase I/II study of these two agents in recurrent NSCLC was designed to assign 40 patients to bevacizumab 15 mg/kg every 21 days and erlotinib 150 mg daily. Of the 40 patients, eight achieved a PR while 26 had SD, with rash, diarrhea, and proteinuria being the main adverse events. The OS and PF were 12.6 and 6.2 months, respectively [178]. The same combination in untreated patients ($n = 38$) resulted in a TTP of 5.5 months. Hypertension (2.7%), thrombosis (2.7%), and hemorrhage (2.6%) were added to the already known side effects [179]. Another study randomized 120 patients – after failure of a platinum regiment – to bevacizumab and erlotinib (Arm A), bevacizumab and chemotherapy (Arm B), or chemotherapy alone (Arm C). The rate of 1-year survival was 57.4% for the first arm, 53.8% for the second arm and 33.1% for chemotherapy. The bevacizumab/erlotinib arm provided the highest disease control with an OS of 13.7 months. Bevacizumab and chemotherapy and chemotherapy alone prolonged survival by 12.6 and 8.6 months, respectively. Drug discontinuation was more frequent in the chemotherapy group (24%) than in the bevacizumab/erlotinib arm (13%). The rates of fatal pulmonary hemorrhage were consistent with earlier results from bevacizumab trials [180]. The BeTA Lung (Bevacizumab Tarceva) was a multicenter controlled double blind phase III trial or bevacizumab plus erlotinib versus erlotinib alone as second-line therapy. A total of 636 patients with no prior treatment with TK inhibitors were allocated to the bevacizumab/erlotinib combination

or to the erlotinib/placebo arm. Patients with treated brain metastases and low-risk bleeding squamous cell disease were also included. Trial data presented a double ratio of PFS (3.4 vs. 1.7 months, $P < 0.0001$, HR=0.62) and response rate (12.6 vs. 6.2 months, $P = 0.006$) for the bevacizumab/erlotinib arm compared with the erlotinib/placebo group. However, the trial did not achieve its primary objective, as no improvement in survival rates (9.3 vs. 9.2 months, $P = 0.75$, HR=0.97) was reported [181].

The evaluation of bevacizumab plus erlotinib as a first-line option for advanced NSCLC is currently being conducted for the ATLAS trial. After six cycles of a platinum doublet plus bevacizumab, patients with no signs of disease progression continued on erlotinib (150 mg daily) and bevacizumab (15 mg/m^2 every 3 weeks) or bevacizumab plus placebo. The trial included patients with treated brain metastases and with peripheral or extrathoracic squamous tumors. The results from 743 participants that were randomised showed that PFS was superior for bevacizumab and erlotinib (4.8 months) compared with bevacizumab and placebo (3.7 months): HR=0.722 (95% CI: 0.592–0.881), $P = 0.0012$. There are no available data for OS. The expected side effects from erlotinib and bevacizumab administration occurred, but at a manageable and safe level. The addition of erlotinib to bevacizumab after first-line chemotherapy led to significant PFS improvement. The results of the biomarker evaluation suggested that patients with EGFR(+), EGFR-mutated or K-ras wild-type tumors presented the greatest PFS improvement [182,183].

Still at the first-line setting, the combination of erlotinib and sorafenib achieved significant antitumor effect in 50 patients with stage IIIB and IV NSCLC. A TTP of 4.6 months was shown. In addition to the usual adverse events of rash, diarrhea, and fatigue, one patient died as a result of an episode of fatal hemoptysis [184]. There are available data from a phase II trial of cetuximab and bevacizumab administered concurrently with carboplatin and paclitaxel and then as maintenance therapy to treatment-naïve patients with advanced NSCLC and nonsquamous histology. Among 104 assessable patients, with a median follow-up of 15 months, the PFS and OS are 7 and 14 months, respectively, with four treatment-related deaths [185].

Conclusion

It is apparent that the term NSCLC is not appropriate for treatment selection. There are two main entities. There is the nonsquamous NSCLC in which VEGF inhibition, particularly in the face of bevacizumab, remains the hallmark of targeted therapy. In contrast, there is the squamous NSCLC, whereby cetuximab offers a clinical benefit with a safe profile of administration. Monoclonal antibodies, such as bevacizumab and cetuximab, are also the only agents that have achieved increased survival in conjunction with systemic chemotherapy. TKIs have thus far shown a low response rate in general populations and are effective in specific groups of patients that have not yet been fully recognized. This is a tailored treatment regime that will broaden our knowledge with prospective trials and careful screening. The novel irreversible and multi-targeted inhibitors may be more likely to succeed against NSCLC than their 'ancestors', but it is quite premature to make any predictions about their future. Some years ago, targeted therapy for NSCLC entailed a few molecules. Today, we are in a position to introduce many agents that offer a new impetus in this particular method of treatment. This attempt stems from the great ambition to remove chemotherapy from the treatment field. The magnitude of such an achievement will not only deliver hope to patients with NSCLC, but will also change the way they face their disease. Are we heading towards the end of chemotherapy in NSCLC? Can we turn NSCLC into a chronic condition? Are we on the right track? The most probable answer is that we do not know, as these are early days yet. However, we may be optimistic about long-term results.

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