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Review

Targeted therapies in the treatment of advanced/metastatic NSCLC

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ABSTRACT

The treatment of advanced non-small cell lung cancer (NSCLC) has evolved substantially during the last years. Chemotherapy remains the cornerstone of treatment and prolongs survival with a positive impact on quality of life. However, we seem to have reached a plateau of activity in the treatment of NSCLC. Recently, the addition of bevacizumab or cetuximab to chemotherapy doublets has improved the outcome in selected patients with advanced NSCLC. Furthermore, the use of erlotinib and gefitinib is an alternative for second line treatment. Advances in our understanding of molecular biology of cancer and mechanisms of tumourigenesis have further enabled the discovery of several potential molecular targets and development of novel 'targeted therapies'. The purpose of this study is to review current data on the role of targeted therapies in the treatment of advanced NSCLC.

1. Introduction

Non-Small Cell Lung Cancer (NSCLC) accounts for approximately 80% of the 170,000 new cases of lung carcinoma diagnosed each year in the United States and remains the leading cause of cancer-related death in both men and women in Western countries. The majority of these patients present with advanced, unresectable or metastatic disease, and approximately 15% of unselected patients are expected to be alive at five years.[1]

Chemotherapy remains the cornerstone of treatment in advanced/metastatic NSCLC. A recent meta-analysis of 16 randomised trials including 2714 patients of chemotherapy compared with best supportive care demonstrated an advantage of chemotherapy with an absolute improvement in survival of 9% at 12 months.[2] Further studies have proven that

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doublet chemotherapy regimens are superior to single agent treatment; doublets should contain at least one new agent (taxane, vinorelbine, gemcitabine) and that three-drug combinations do not offer any benefit compared to two-drug regimens. Docetaxel and pemetrexed are considered as standard second line therapy in patients with good performance status.[3–5] However, chemotherapy has not substantially improved the survival for most lung cancer patients and it is clear that chemotherapy has reached a plateau of activity in the treatment of NSCLC.[6]

Advances in our understanding of molecular biology of cancer and mechanisms of tumourigenesis have enabled the discovery of several potential molecular targets and development of novel 'targeted therapies'. These therapies inhibit signalling pathways involved in the development and progression of cancer. As these pathways are preferentially activated in cancer cells as contrasted to normal cells, targeted therapies are presumably better tolerated compared to classical cytotoxic agents.

The purpose of this paper is to review the status of targeted therapies in the treatment of NSCLC.

2. Epidermal growth factor receptor antagonists

Epidermal growth factor receptor (EGFR) is over-expressed in majority of NSCLC and plays a strong stimulatory effect on cell proliferation, survival, migration and angiogenesis.[7,8] Inhibition of EGFR signalling has therefore been identified as a key therapeutic target in NSCLC. Strategies to block EGFR include tyrosine kinase inhibitors, monoclonal antibodies, antisense approaches and ligand-linked toxins. Among these approaches only tyrosine kinase inhibitors and monoclonal antibodies have reached clinical development.

2.1. *Tyrosine kinase inhibitors*

Tyrosine kinase inhibitors (TKIs) are small molecules that bind the ATP pocket in the EGFR intracellular tyrosine kinase domain and thus inhibit the phosphorylation and activation of EGFR and the downstream signalling pathway.[9] The effects of single agent TKIs in the treatment of NSCLC have been tested in the context of two placebo-controlled phase III studies, as second or third line treatment in patients who were not eligible for further chemotherapy. Erlotinib, a reversible TKI, improved survival in a randomised multicentre phase III study (BR.21 trial) with 731 previously treated NSCLC patients.[10] Median overall survival (OS) was 6.7 months for erlotinib versus 4.7 months for placebo (p-value = 0.0001). After this study, erlotinib has been licensed in USA and Europe as second or third line treatment for NSCLC in unselected patients. However, a similarly designed trial which compared gefitinib with placebo (ISEL study), as second line treatment in a larger cohort of patients (n = 1692) failed to demonstrate a statistically significant benefit in terms of OS.[11] Pre-planned subgroup analyses showed statistically significant benefit for Asian and never-smoker patients. The different results from the above trials may be explained by different study populations (the ISEL trial involved more patients with early relapse after first line chemotherapy) or by the difference in study drugs or dosing. Gefitinib dose in the ISEL study was 250 mg and erlotinib dose in the BR.21 trial was 150 mg, the latter being close to maximal-tolerated dose and equivalent to 600–700 mg of gefitinib.[12] Furthermore, two recently reported non-inferiority phase III trials demonstrated that gefitinib is equivalent to docetaxel in patients with advanced NSCLC previously treated with platinum-based chemotherapy.[13,14] (Table 1) All these trials have confirmed relatively favourable toxicity profile of these agents, with diarrhoea and acne-like rash being the most frequent side-effects.[15]

A very interesting study was reported during the 2008 European Society of Medical Oncology conference. Gefitinib was compared to carboplatin/paclitaxel doublet as first line treatment in 1217 clinically selected patients (Asian ethnicity only, adenocarcinoma histology and never or light ex-smokers (<100 cigarettes lifetime) (IPASS trial). This study demonstrated superiority of gefitinib relative to carboplatin/paclitaxel doublet in terms of progression free survival (PFS) (median PFS gefitinib versus carboplatin/paclitaxel: 5.7 months versus 5.8 months, 12-month PFS: 25% versus 7%; $p < 0.001$), however, the effect was not constant over time, initially favouring the chemotherapy doublet and then favouring gefitinib at 12 months. Overall survival was similar for both arms.[16] Gefitinib had a more favourable toxicity profile and quality of life improvement was significantly greater with gefitinib. EGFR gene mutations were assessed in 437 (36%) of all randomised patients. PFS was significantly longer for gefitinib in patients with EGFR-mutated tumours, while it was significantly shorter in EGFR mutation-negative patients.

Four studies have evaluated the combination of EGFR TKIs with chemotherapy in first line setting, versus chemotherapy alone. All these studies failed to demonstrate superiority for the combination of TKI with chemotherapy (see Table 2).[17–20] The most probable reason for lack of additivity and a possible antagonism from EGFR TKIs and chemotherapy is cell cycle redistribution. EGFR TKIs cause cell cycle arrest in G1 phase, which makes the cells relatively insensitive to phase-specific cytotoxic agents used in the above trials.[21] The concept of 'pharmacodynamic separation' of treating the patient with EGFR TKI from day 2 to day 16 of chemotherapy cycle is currently being tested in several phase II trials. Another negative trial was SWOG 0023 which evaluated the role of gefitinib as maintenance treatment after therapy with concurrent chemo-radiotherapy for locally advanced NSCLC, followed by docetaxel consolidation.[22] The biological reasons for significant inferior survival with gefitinib in this study are unclear. However, a preliminary report from a phase III study (SATURN) showed that erlotinib as maintenance therapy after platinum-based chemotherapy results in a significant prolongation of PFS in advanced NSCLC patients.[23]

Several new TKIs are under development (Table 3). Data from phase I trials have proven the favourable toxicity profile of these agents, with diarrhoea being the most frequent side-effect.[24–31] Lapatinib has been tested in a randomised phase II trial comparing two schedules and doses in chemo-naïve NSCLC patients. There were no significant adverse events, while efficacy results from this study are pending.[32] XL647 has been tested in a phase II study for chemo-naïve adenocarcinoma patients with clinical characteristics favouring

Table 1 – Phase III trials of TKI single agent treatment in NSCLC patients.

Study	Treatment	n	ORR (%)	Median survival (mo)	*p*-value
BR.21[10]	Erlotinib	427	8.9	6.7	<0.001
	Placebo	211	<1	4.7	
ISEL[11]	Gefitinib	1129	8	5.6	0.11
	Placebo	563	<1	5.1	
INTEREST[14] (*non-inferiority*)	Gefitinib	733	9.1	7.6	–
	Docetaxel	733	7.6	7.6	
Niho et al.[13] (*non-inferiority*)	Gefitinib	245	22.5	11.5	0.33
	Docetaxel	244	12.8	14.0	

Table 2 – TKIs in combination with chemotherapy.

	ORR	Median survival (mo)
INTACT-1[20]		
CMT + Placebo	47.2	10.9
CMT + gefitinib (250 mg)	51.2	9.9
CMT + gefitinib (500 mg)	50.3	9.9
INTACT-2[19]		
CMT + placebo	28.7	9.9
CMT + gefitinib (250 mg)	30.4	9.8
CMT + gefitinib (500 mg)	30.0	8.7
TRIBUTE[17]		
CMT + placebo	19.3	10.6
CMT + erlotinib	21.5	10.5
TALENT[18]		
CMT + placebo	29.5	44.1 (wks)
CMT + erlotinib	31.5	43.0 (wks)
SWOG S0023 (post CMT-RT)[22]		
Maintenance gefitinib		23
Maintenance placebo		35

Gemcitabine 1250 mg/m^2, d1 and 8, / cisplatin 80 mg/m^2 d1 (INTACT I and TALENT).
Carboplatin (AUC 6) / paclitaxel 225 mg/m2 d1, q3w (INTACT II and TRIBUTE).

Table 3 – EGFR TKIs currently in development.

	Target	Type of binding	Development phase	
Canertinid[24] (*CI 1033*)	*Erb-1/2/4*	Irreversible	Phase I	DLT: diarrhoea, oedema; rash, nausea, stomatitis DLT: diarrhoea
HKI-272[25,31]	*Erb-1/2*	Irreversible	Phase I	Nausea, anorexia, asthenia; DLT: diarrhoea
EKB-569[27,28]	*Erb-1/2*	Irreversible	Phase I	Rash, asthenia, anorexia, nausea, stomatitis, vomiting
PF-00299804[128]	*Pan-Erb*	Irreversible	Phase I	Most common toxicities: diarrhoea, rash
BIBW2992[29,35]	*Erb-1/2*	Irreversible	Phase II	DLT: dyspnoea, rash
Lapatinib[26,32]	*Erb-1/2/3*	Reversible	Phase II	DLT: diarrhoea, rash
XL647[30,33,34]	Erb-1/2, VEGFR, Flt-4, EphB4	Reversible	Phase II	DLT: pneumonitis, QTc prolongation

response to TKIs (smoking history less than 15 pack-years, female sex, Asian ethnicity). The response rate was 29% (10/34 patients) with diarrhoea being the most frequent adverse event.[33] A second ongoing phase II trial in patients with acquired resistance to EGFR TKIs has reported adequate activity (1 patient had partial response and 7 stable disease), without any significant adverse events.[34] Preliminary results of a single-arm phase II trial of BIBW2992 in patients with adenocarcinoma and activating EGFR mutations reported rapid responses and a favourable toxicity profile.[35]

2.2. *Monoclonal antibodies*

Monoclonal antibodies (MAbs) are competing with ligands for the binding sites of the extracellular part of EGFR. When ligand binding is inhibited, the EGFR-dependent downstream

Table 4 – Phase II trials of anti-EGFR MAbs in the treatment of NSCLC.

Study	Line therapy	N	Treatment	ORR (%)	PFS	OS
Cetuximab[39]	1st	132	GMB (1000–1200 mg/m^2, d1 and 8), platinum,q3wks ± cetuximab	27.7 versus 18.2	5.09 versus 4.21	11.9 versus 9.2
Cetuximab[40]	1st	86	CDDP (80 mg/m^2, d1) VNB (25 mg/m^2, d1 and 8) ± cetuximab	35 versus 28	4.7 versus 4.2	8.3 versus 7.0
Cetuximab[129]	1st	81	TXT (75 mg/m^2, d1) + Carbo (AUC6, d1, q3wks) + cetuximab	14.5	4.6	11
Cetuximab[130]	1st	68	TXT (30 mg/m^2, d1 and 8) + GMB (1000 mg/m^2, d1 and 8) + cetuximab	18	4.5	8
Cetuximab[131]	⩾2nd	66	Cetuximab single agent	3.3	2.3	8.1
Cetuximab[132]	Neo-adjuvant	16	CDDP (80 mg/m^2, d1) GMB (1250 mg/m^2, d1 and 8) + cetuximab	37.5	NR	NR
Cetuximab[133]	2nd	23	Pemetrexed + cetuximab	8.7	TTP: 25wks	NR
Cetuximab[134]	1st	57	Carbo (AUC6) + cetuximab (cetuximab maintenance in responding pts)	5.3	3.0	8.2
Cetuximab[135]	1st	53	TXL (100 mg/m^2/wk) + Carbo (6 AUC) + cetuximab (cetuximab maintenance in responding pts)	57%	5.53	13.8
Panitumumab[42]	1st	166	Carbo (AUC6), paclitaxel (200 mg/m^2, q3wks) +/− panitumumab	15 versus 11	4.2 versus 5.3	8.5 versus 8.0
Matuzumab[136]	>2nd	–	Pemetrexed ± matuzumab	–	NR	NR
Pertuzumab[137]	>2nd	43	Pertuzumab single agent: 840-mg/m^2 induction dose; 420 mg/m^2, q3wks	0/20.9 SD	6.1 wks	NR

GMB, gemcitabine; TXT, docetaxel; CDDP, cisplatin; VNB, vinorelbine; and Carbo, carboplatin; ORR, overall response rate; OS, overall survival; SD, stable disease; TTP, time to tumour progression; PFS, progression free survival; NR, not reported.

pathway cannot be activated (in case it is not permanently activated by a mutation event). Several antibodies have been tested in the context of phase II trials in NSCLC patients, as single agent or in combination with chemotherapy (Table 4). All these studies support the favourable toxicity profile of these agents with rash and diarrhoea being the most frequent adverse events. Cetuximab has been tested in combination with radiotherapy for locally advanced NSCLC[36–38] and the combination was found to be feasible and tolerable. Furthermore, cetuximab has been evaluated in the context of two randomised phase II trials in combination with chemotherapy as first line treatment in NSCLC.[39,40] Both studies have demonstrated improvement in response rate and overall survival with the addition of cetuximab to chemotherapy, leading to cetuximab testing in a phase III setting. Panitumumab, another anti-EGFR MAb, failed to demonstrate any improvement in terms of response rate, time-to-progression and overall survival in randomised phase II studies in advanced NSCLC.[41,42]

Based on the encouraging results of phase II studies, two prospective, randomised phase III trials of the combination of cetuximab with cytotoxic drugs were planned. The first compared a cisplatin (80 mg/m^2, d1) –vinorelbine (25 mg/m^2, d1 and 8) doublet versus the same chemotherapy plus cetuximab in 1125 chemo-naïve NSCLC patients with EGFR immunohistochemistry (IHC) positive tumours. After six chemotherapy cycles cetuximab was continued as maintenance treatment in the arm allocated to the three drugs regimen. Although, PFS was identical between the two arms (4.8 versus 4.8 months), the cetuximab arm had significantly longer survival (11.3 versus 10.1 months; $p = 0.044$) (FLEX study).[43] Furthermore, a new analysis demonstrated that OS for patients receiving cetuximab, who experienced any grade of rash (acne-like rash) within 3 weeks of treatment initiation was 15.0 months compared to 8.8 months in those patients who developed no rash (HR = 0.63; $p < 0.001$).[44] The magnitude of benefit while statistically significant was modest (1.2-month improvement in median survival) and noteworthy, this trial has been criticised as being 'over-powered'.[45] Furthermore, the target selection (EGFR-positive tumours by immunohistochemistry) is equivocal, since there is no clear correlation between EGFR expression and response to anti-EGFR treatment.[46] A second phase III trial investigating carboplatin plus a taxane (either paclitaxel or docetaxel) with or without cetuximab as first line treatment for patients with metastatic NSCLC failed to show any improvement in progression free survival, the study's primary end-point (median PFS for carboplatin/taxane versus carboplatin/taxane/cetuximab: 4.2 versus 4.4 months, respectively; $p = 0.23$) (BMS099 study).[47] A possible explanation for the discrepancy between the FLEX study and the BMS099 study might be related to the relatively low number of patients participating in the latter trial. However, given that survival benefit observed in the FLEX study was modest and that BMS099 study failed to demonstrate an OS benefit (although not adequately powered for OS), further studies are needed to elucidate the role of cetuximab in the treatment of NSCLC.

2.3. *Clinical predictors of response*

Numerous clinical data suggest that clinical predictors of response include female sex, adenocarcinoma histology, Asian ethnicity and absence of smoking.[10,48–50] However, these factors are not absolute. Even for poor prognosis subsets (i.e. men, non-adenocarcinoma histology, non-Asian ethnicity and current or former smokers), there was a reduction in the hazard ratio for death among patients treated with erlotinib.[10]

Unfortunately the first randomised trial run in pretreated patients comparing two active treatments, gefitinib versus docetaxel [INTEREST trial[14]] and not a TKI versus placebo [ISEL[11] and BR.21 trials,[10]] showed their prognostic and not predictive role for survival.[51] On the other hand the IPASS study demonstrated that gefitinib was at least as active as carboplatin/paclitaxel doublet in terms of PFS in a highly selected population on the basis of these clinical characteristics.[16] Similarly, in the FLEX trial of chemotherapy ± cetuximab, Asian ethnicity had a significant prognostic value, but not predictive for cetuximab efficacy.[45] Another feature associated with clinical efficacy of TKIs and MAbs is the development of cutaneous toxicity, such as acne-like rash.[44,52] All agents targeting the EGFR pathway, including both small molecule TK inhibitors and monoclonal antibodies that bind EGFR, are associated with dermatologic toxicity (predominantly dry skin and an acneiform rash). This is thought to be due to high levels of EGFR expression in the basal layer of the epidermis.[53] The presence of rash strongly correlated with overall survival and this correlation increased with rash severity grade in patients with NSCLC.[52] Similarly, NSCLC patients treated with cetuximab who developed skin toxicity derived a survival benefit, compared to patients who did not experience skin toxicity.[54] This feature should not be interpreted as a clinical predictive marker, because it is assessable only after the treatment with EGFR inhibitor is started.

2.4. *Molecular predictors of response*

EGFR expression as determined by immunohistochemistry and its correlation with sensitivity to EGFR TKIs has been extensively studied in NSCLC. The published results are conflicting and both positive and negative correlations have been reported.[9] However, it should be underlined that these differences could be attributed to differences regarding the methodologies used in all these studies.[46]

EGFR gene copy number, as assessed by fluorescence in situ hybridisation (FISH), has also been tested as predictive factor for response with TKIs treatment. It should be noted that the *EGFR* gene is rarely amplified in NSCLC patients (gene copy number to chromosome 7 centromere number ratio of >2), and increased gene copy number most frequently results from chromosome 7 polysomy.[46] FISH positivity has been associated with significantly higher response rate and overall survival in randomised phase III trials of TKIs versus placebo.[55,56] Patients with high gene copy number treated with gefitinib or erlotinib had significantly longer survival than patients receiving placebo, while in patients with FISH-negative tumours there was no difference.[55,56] However, no predictive value of FISH EGFR analysis for survival was observed in randomised trials comparing TKI treatment with chemotherapy.[14,57] Increased EGFR gene copy number has also been correlated with clinical outcome in NSCLC patients treated with monoclonal antibodies against EGFR.[58]

Most of the somatic mutations of EGFR observed in NSCLC affect the tyrosine kinase coding domain (exons 18–21). Most common of these are in-frame deletions in exon 19 (codons 746–750) and a missense mutation leading to a substitution of arginine for leucine at codon 858 (L858R).[59] There is strong correlation between the presence of these mutations and EGFR TKIs sensitivity *in vitro* and *in vivo*.[8,9,48,59,60] Apoptosis is efficiently induced by TKIs in NSCLC cells harbouring activating EGFR mutations and this is mediated through the mitochondrial or intrinsic apoptotic pathway via the proapoptotic BH3 only protein, BIM.[61–64]

Several single-arm prospective phase II studies with TKI therapy in NSCLC bearing EGFR mutations have reported response rate in the range of 80% and a median progression free survival of 7.7–14 months.[65–70] These results must be confirmed in prospective randomised phase III trials versus standard chemotherapy. Such a study is currently active in the Spanish Lung Cancer Study Group with erlotinib. K-ras gene mutations have been reported as negative predictors of response to single agent TKIs in advanced NSCLC[71] and to anti-EGFR MAbs (alone or in combination with chemotherapy) in patients with metastatic colorectal cancer.[72,73] However, the INTEREST study did not report any difference in OS between gefitinib and docetaxel irrespective of EGFR gene-copy number, EGFR gene mutation and K-ras gene mutation status.[14] Similarly, K-ras mutation status was not predictive of cetuximab benefit in the BMS099 study.[74] On the other hand, the IPASS study demonstrated that gefitinib was at least as active as the carboplatin/paclitaxel doublet in terms of PFS and EGFR mutation status was a strong predictive biomarker for the effect of gefitinib. However, this study did not report an OS difference and is still reported only in abstract form.[16] The relationship between somatic mutations of EGFR and response to MAbs therapy remains unclear. Preclinical data on NSCLC cell lines *in vitro* demonstrated that there was no association between presence of EGFR somatic mutations and efficacy of cetuximab treatment.[75] A small retrospective study in 38 NSCLC patients treated with cetuximab monotherapy confirmed this observation.[76] Further preclinical and clinical studies are warranted to elucidate this issue. In conclusion as for clinical factors all biomarkers, including K-ras mutation, when studied in a phase III-randomised trial comparing two active treatments (INTEREST trial) failed to show any predictive role for survival while were strong prognostic factors.

2.5. *Resistance to EGFR TKIs treatment*

Ras plays an important role in the EGFR downstream signalling pathway, by activating the Raf-kinase, the mitogen-activated protein kinase (MAPK) and promoting cell proliferation.[77] K-ras mutations are observed in about 15–30% of NSCLC patients and result in EGFR-independent activation of MAPK. These mutations are mutually exclusive with EGFR mutations and have been proposed as a mechanism of primary resistance to TKIs in NSCLC.[59,71,78] In contrast to EGFR abnormalities, mutations in K-ras predict resistance to EGFR antagonists.[71] Although several data support that MAbs are not active against colorectal cancer patients with mutated K-ras, limited data are available about the role of MAbs in K-ras-mutated NSCLC patients.

Acquired resistance to TKIs treatment is associated with development of secondary EGFR mutations. The most extensively studied is the substitution of methionine for threonine in codon 790 (T790M), in exon 20.[59] The T790M mutation is

present in approximately 50% of patients who relapse after initial response to TKIs and although the exact mechanism through which T790M causes TKIs resistance is not quite clear, it is believed that it leads to steric hindrance of inhibitor binding to the tyrosine kinase of EGFR.[8,59,79] T790M mutations are usually present in small fraction of tumour cells before treatment with EGFR TKIs and resistance results from their clonal selection.[80] Novel 'second-generation' of EGFR TKIs HKI-272[31] and EKB-569[81] is able to overcome resistance owing to T790M mutation *in vitro*, although there are no results from studies in humans to support its clinical use.

A recent publication demonstrated the development of MET amplification in NSCLC cell lines after exposure to gefitinib.[82] MET amplification leads to EGFR-independent activation of the PI3K/Akt pathway, through an ERbB-3-dependent activation.[83] Concomitant inhibition of MET and EGFR signalling restored sensitivity to gefitinib in NSCLC cell lines.[82]

These two mechanisms are responsible for approximately 60–70% of all cases of acquired resistance to TKIs treatment. Further studies are needed to discover other potential mechanisms of resistance to anti-EGFR treatment.

3. Antiangiogenic therapies

Angiogenesis, the formation of new blood vessels, is a fundamental process for the development of solid tumours and for the growth of secondary metastatic lesions. Tumours greater than 2 mm^3 must recruit a new blood supply in order to remain metabolically active and expand in size.[84] A large number of endogenous proteins that act to promote normal and tumour angiogenesis have been described, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiopoietins 1 and 2, interleukin-8 and platelet-derived growth factor beta (PDGF-β).[85,86]

3.1. *Monoclonal antibodies*

Bevacizumab is a recombinant, humanised, monoclonal antibody against VEGF, and is the most extensively studied antiangiogenic agent. A pivotal phase III trial ECOG 4599 demonstrated survival superiority (prolongation of median survival by 2 months) for the combination of bevacizumab and paclitaxel/carboplatin followed by bevacizumab until disease progression. In the European AVAiL phase III trial, two doses of bevacizumab were compared to gemcitabine/cisplatin followed by bevacizumab. This study failed to show survival improvement, although time-to-progression was significantly longer for either dose of the drug[87,88] (Table 5). Bevacizumab was registered in the United States based on the results of ECOG 4599 study and the drug was registered in Europe based on the data from both trials. Squamous histology, metastases to central nervous system, history of haemoptysis and history of documented haemorrhagic diathesis were reported as exclusion criteria in both studies, limiting the potential use of bevacizumab to selected NSCLC patients. The ECOG 4599 study reported significantly higher toxicity for patients receiving bevacizumab. Indeed, the rates of hypertension, proteinuria, bleeding, neutropenia, febrile neutropenia, thrombocytopenia, hyponatraemia, rash and headache were significantly higher in the paclitaxel–carboplatin–bevacizumab group than in the paclitaxel–carboplatin group.[87] Importantly, there were only two treatment related deaths in the chemotherapy arm and 15 in the bevacizumab arm.[87] Similarly, in the AVAiL study, treatment-related deaths were higher in the 15 mg/kg bevacizumab arm (1% higher death rate due to serious adverse events) while it was similar in the 7.5 mg/kg bevacizumab arm and chemotherapy alone arm.[88] Incidence of neutropaenia, vomiting, hypertension and epistaxis was also higher with either dose of bevacizumab.[88] Subgroup analysis of the ECOG 4599 study reported that elderly ($\geqslant$70 years) patients did not derive any survival benefit from the addition of bevacizumab, while they experienced significantly higher toxicity, when compared to their younger counterparts.[89] In an unplanned subgroup analysis overall survival in women did not improve significantly with bevacizumab treatment, despite significant progression-free survival difference.[90] This finding has not been observed in the AVAiL study.[88]

There are several trials of bevacizumab in stage III, that allow for squamous histology, with radiation given before initiation of chemotherapy-bevacizumab combination. However, toxicity concerns, haemoptysis in particular, have lead to premature closure of these studies.[91] Other trials are evaluating the role of bevacizumab in patients with brain metastases and in patients with squamous histology. Although, no central nervous system haemorrhages have been observed, one patient developed grade III leukoencephalopathy.[92] A large phase III trial testing the combination of chemotherapy and bevacizumab in the adjuvant setting is ongoing in clinically unselected patients.

Table 5 – Phase III studies of bevacizumab in combination with chemotherapy.

Study	Treatment	N	ORR (%)	PFS (mo)	OS (mo)
ECOG 4599[87]	CMT	444	15	4.5	10.3
	CMT+Bev (15 mg/kg)	434	35	6.2	12.3
			$p < 0.001$	$p < 0.001$	$p = 0.003$
AVAiL[88]	CMT	347	20	6.1	NR
	CMT+Bev (7.5 mg/kg)	345	34	6.7 ($p = 0.002$)	
	CMt+Bev (15 mg/kg)	351	30	6.5 ($p = 0.03$)	

CMT ECOG 4599: paclitaxel (200 mg/m^2, d1), carboplatin (AUC6, d1; q3wks).
CMT AVAiL: cisplatin (80 mg/m^2, d1), gemcitabine (1250 mg/m^2, d1 and 8 ;q3wks).

3.2. AVE0005 (VEGF-trap)

AVE0005 is a chimeric, fusion molecule that comprises segments of the extracellular domains of VEGFR-1 and VEGFR-2, fused to the Fc domain of the IgG1 backbone. It binds circulating VEGF-A and prevents its binding to the cell membrane receptor. This agent has been tested as a single agent at a dose of 4.0 mg/kg every 2 weeks in platinum- and erlotinib-resistant NSCLC patients.[93] This study is ongoing but among the 33 first patients, two partial responses were reported and no significant (grade III or greater) haemoptysis was observed. Most frequent grade III-IV treatment emergent adverse events included dyspnoea, hypertension and non-cardiac chest pain, fatigue and anxiety. A phase III-randomised trial comparing docetaxel versus docetaxel + VEGF-trap in second line setting is ongoing.

3.3. VEGF-receptor tyrosine kinase inhibitors

Another approach for the inhibition of the VEGF downstream pathway is VEGFR small molecule tyrosine-kinase inhibitors (TKIs). A growing number of TKIs are being tested in NSCLC (Table 6).

3.3.1. Sorafenib

Sorafenib inhibits VEGFR-2, raf-kinases, platelet-derived growth factor beta (PDGFR-B) and c-kit.[94] As single-agent treatment was tested in chemo-naïve[95] or previously treated[96] patients with advanced/metastatic NSCLC and has shown no or moderate activity. Sorafenib 400 mg twice daily has been combined with paclitaxel/carboplatin doublet with encouraging results.[97] Response rate was 29%, and 50% of the patients had stable disease. The regimen was well tolerated, with diarrhoea and rash being the most common side-effects. On the basis of this trial three phase III studies are evaluating sorafenib in combination with chemotherapy (paclitaxel/gemcitabine in two trials, gemcitabine/cisplatin in one trial). However, the largest of these trials (ESCAPE trial, paclitaxel/gemcitabine ± sorafenib) has been prematurely closed, after interim analysis showed that the study would not meet its primary end-point of improved survival.[98] Furthermore, more treatment-related deaths were observed in patients with squamous histology in the sorafenib arm.[99]

3.3.2. Vandetanib

Vandetanib is a TKI of EGFR, VEGFR and RET receptors. It is well tolerated in daily oral doses of $\leqslant$300 mg, with rash, diarrhoea, hypertension and asymptomatic QTc prolongation as the most frequently observed adverse effects.[100] In a randomised phase II trial of 168 previously treated NSCLC patients vandetanib was compared to gefitinib. Patients were allowed to cross over treatment after progression. Improved PFS was observed in patients receiving vandetanib (11 weeks versus 8.1 weeks, $p = 0.025$).[101] On the basis of this study a phase III trial of vandetanib versus erlotinib as second line treatment is now accruing patients (ZEST trial), while another phase III trial is comparing vandetanib to placebo in patients who have progressed after both chemotherapy and an EGFR TKI (ZEPHYR trial).[102]

Furthermore, in a randomised phase II trial, vandetanib when added to docetaxel demonstrated prolongation of PFS (18.7 weeks versus 12 weeks; $p = 0.07$). In first line treatment the addition of vandetanib to front line carboplatin/paclitaxel doublet has demonstrated improvement in terms of PFS in the context of a randomised phase II trial.[103] Two phase III trials are evaluating chemotherapy (ZODIAK and ZEAL trials; docetaxel and pemetrexed, respectively) with or without vandetanib.

Table 6 – Antiangiogenesis tyrosine kinase inhibitors developed in NSCLC.

Agent	Target	Phase
Sorafenib[98,99]	*VEGFR2/3, C-RAF, B-RAF, PDGFR-β, c-kit*	Phase III (*1st line TXL/Carbo ± sorafenib* prematurely stopped *1st line GMB-CDDP ± sorafenib*)
Vandetanib(ZD6474)[101,103,138]	*Erb-1, VEGFR2*	Phase III (*2nd line: vandetanid versus erlotinib; vandetanib versus placebo 2nd line: docetaxel ± vandetanid; pemetrexed ± vandetanid*)
Sunitinib[104,105]	*VEGFR1/2/3, FLT PDGFR-β, c-kit*	Phase II (*2nd line single-agent, maintenance, after platinum-based CMT*)
Cediranib (AZD-2171)[139]	*VEGFR1/2/3 PDGFR-β, c-kit*	Phase II (*1st line TXL/Carbo ± cediranib*; stopped)
Vatalanib(PTK787)	*VEGFR1/2/3, C-Fms PDGFR-β, c-kit*	Phase II (*2nd line monotherapy*, stopped)
Axitinib[140]	*VEGFR1/2/3, PDGFR-α, c-kit*	Phase II (*single agent ORR: 9.4%; OS: 12.8 mo*)
Pazopanib	*VEGFR1/2/3, PDGFR-α/β, c-kit*	Phase II (*2nd line monotherapy, neo-adjuvant for stage I*)
Motesanib[141]	*VEGFR1/2/3, PDGF, c-kit*	Phase III (*TXL/Carbo ± motesanib*) Phase II (*TXL/Carbo + panitumumab versus TXL/Carbo + motesanib*)
CP-547,632[142]	*VEGFR-2*	Phase I/II (*1st line with TXL/Carbo, did not increase ORR*)
BIBF1120[143,144]	*VEGFR1/2/3,PDGFR, FGFR*	Phase I (*with TXL/Carbo*) Phase II ($\geqslant$*2nd line, single agent, ORR: 0%, SD:48%*)
XL647[145]	*EGFR, HER2, EphB4, VEGFR-2*	Phase II (*1st line, patients enriched for EGFR mutations*)
AEE788[146]	*EGFR, HER2, VEGFR-2*	Phase I (*DLT: diarrhoea*)
KRN951[147]	*VEGFR1/2, PDGF, c-kit*	Phase I (*DLT: asymptomatic proteinuria, ataxia, intracranial haemorrhage*)
ABT-869	*VEGFR1/2/3,PDGFR*	Phase II
OSI-930	*Kinase insert domain receptor, c-kit*	Phase I (*either as single-agent or in combination with erlotinib*)
BMS-690514	*VEGFR, pan-Erb*	Phase I (*either as single-agent or in combination with TXL/Carbo*)

CMT, chemotherapy; TXL, paclitaxel; Carbo, carboplatin; GMB, gemcitabine; CDDP, cisplatin; DLT, dose limiting toxicity; ORR, overall response rate; OS, overall survival.

3.3.3. *Sunitinib*

Sunitinib has been tested in the context of phase II trials, in previously treated NSCLC patients. The drug was administered either in a 4 weeks on and 2 weeks off schedule,[104] or continuously.[105] Response rate were 11.1% and 2% with the 4 weeks on, 2 weeks off schedule and continuous administration, respectively. Fatigue, dyspnoea and nausea were the most common adverse events. Two treatment-related deaths due to haemorrhage were observed with the 4 weeks on and 2 weeks off schedule[104] while one treatment-related death due to congestive heart failure was observed with the continuous administration.[105] Several phase I/II trials are evaluating sunitinib in combination with chemotherapy (paclitaxel/carboplatin, gemcitabine/cisplatin, pemetrexed/cisplatin, pemetrexed/carboplatin, docetaxel) while a phase III trial is investigating sunitinib versus placebo in NSCLC patients who have failed treatment with both chemotherapy and an EGFR inhibitor.[106] Another phase III trial is evaluating the erlotinib–sunitinib doublet versus single-agent erlotinib in the second line setting.

3.4. *Vascular disrupting agents*

A new treatment strategy under investigation involves targeting tumour vasculature with small molecule vascular disrupting agents (VDAs), such as the tubulin-depolymerising combretastatin A-4-phosphate (C4AP) and the microtubule-independent ASA404 [5,6-dimethylxanthenone-4-acetic acid (DMXAA)]. The tumour-VDA, ASA404, induces apoptosis of tumour vascular endothelial cells and cytokine production, leading to tumour vascular collapse.[107] Two randomised phase II trials have evaluated the combination of chemotherapy (paclitaxel/carboplatin doublet) plus or minus ASA404 as first line treatment of NSCLC at a dose of 1200 mg/m^2 and 1800 mg/m^2, respectively.[108,109] Both studies reported an increased response rate and improved TTP and OS in favour of the ASA404 arm. No significant toxicity was observed with the addition of ASA404.

4. Signal transduction

4.1. *IGF-1R inhibition*

The insulin-like growth factor (IGF) 1 receptor (IGF-1R) is activated and overexpressed in NSCLC. The receptor is activated by two ligands (insulin-like growth factors 1 and 2; IGF1, IGF2).

Several anti-IGF-1R monoclonal antibodies are being developed in phase I–III clinical trials that include NSCLC patients. CP-751871 was evaluated in a phase II trial in patients with advanced NSCLC, in which 150 patients were randomly assigned to paclitaxel plus carboplatin with or without CP-751871.[110] The response rate was higher with CP-751871 than with chemotherapy alone. The objective response rate was increased in patients with all histologies, but was particularly high in patients with squamous carcinoma. The most significant toxicity was severe hyperglycaemia, which was observed in 20 of 97 patients (21%). Phase III clinical trials are in progress to investigate the role of inhibition of the IGF pathway in the treatment of advanced NSCLC. These trials explore combined treatment with chemotherapy or with EGFR inhibitors.

4.2. *m-TOR inhibition*

The mammalian target of rapamycin (mTOR), a serine/threonine kinase, is a downstream mediator in the phosphatidylinositol 3-kinase/Akt signalling pathway, which plays a critical role in regulating basic cellular functions including cellular growth, proliferation and cell survival.[111] m-TOR inhibits autophagy, a suggested tumour suppressor mechanism. Temsirolimus was evaluated as single-agent front line treatment in NSCLC patients within a two-stage phase II trial, but the study did not meet its prespecified efficacy criteria.[112] Everolimus was tested in patients previously treated with two or fewer chemotherapy lines (arm 1) or patients previously treated with two or fewer chemotherapy lines and small molecule epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs). Reported partial responses were 4.8% (arm 1) and 2.3% (arm 2), respectively.[113]

5. Promoting apoptosis

The majority of NSCLC exhibit dysregulated antiapoptotic pathways involving the transcription factor NF-κB. In addition, in NSCLC both chemotherapy and radiation upregulate antiapoptotic and cell-cycle regulatory proteins through NF-κB-dependent signalling mechanisms. Preclinical and phase I clinical trials suggest that inhibition of NF-κB markedly attenuates the resistance of NSCLC to undergo apoptosis and sensitises these cells to chemotherapy. Modulation of the antiapoptotic cascade mediated by NF-κB, combined with either traditional or novel chemotherapeutic agents, is being investigated in patients with NSCLC.

5.1. *Proteasome inhibition*

Proteasome is a multicatalytic complex critical to the regulated degradation of intracellular proteins involved in proliferation and apoptosis. Several proteasome substrates have been identified and include p21 and p27 (cell cycle inhibitors), p53 (transcription factor), IkB (inhibitor of NF kappa-B) and Bcl-2 (regulation of apoptosis).[114] Bortezomib is a small molecule reversible proteasome inhibitor which mediates apoptosis through regulation of the Bcl-2 family of molecules.[115] Bortezomib has been tested in phase I and phase II studies in NSCLC and has shown only moderate activity (Table 7). Interestingly, the activity exhibited restriction to the histological subtype bronchioloalveolar carcinoma and two ongoing trials are evaluating its activity specifically in this histological subtype. Bortezomib has also been investigated in combination with chemoradiation for locally advanced NSCLC.

5.2. *Histone deacetylase inhibition*

Histone acetyltransferases and histone deacetylases (HDAC) are a family of enzymes that plays an important role in regulation of gene transcription. Acetylation acts to neutralise the

Table 7 – Bortezomib in the treatment of NSCLC.

Study	Phase	N	Treatment	Outcome
Ho et al.[148]	II	14	1st line, 1.3 mg/m^2/d (d1, 4, 9 and 11, q21d)	RR: 0% (prematurely stopped)
Lilenbaum et al.[149]	II	64	1st line in pts with PS 2 Arm A: TXT 30 mg/m^2 (d1,8,15 q28d) + Cetuximab (400 mg/m^2 week 1 then 250 mg/m^2 weekly), Arm B: TXT 30 mg/m^2 (d1,8,15 q28d) + bortezomib 1.6 mg/m^2 (d1,8,15; q28d)	A: RR: 10.5% PFS 3.1 OS 3.8 B: RR: 13.6% PFS 1.8 OS 3.3
Davies et al.[150]	I	114	1st line GMB 1000 mg/m^2 (d1 και 8), Carbo AUC 5 + bortezomib 1.0 mg/m^2 (d1,8,15); q21d	RR: 20% PFS: 5 months OS:11 months
Fanucchi et al.[151]	II	115	2nd line Arm A: Bortezomib 1.5 mg/m^2 (d1,4, 8,11; q21d) Arm B: TXT 75 mg/m^2 + bortezomib 1.3 mg/m^2 (d1,4, 8,11; q21d)	A:RR: 10.0% PFS 42.5d OS 3.8 B: RR: 15.6% PFS 86d OS 3.3
Lynch et al.[152]	II	50	2nd line Arm A: erlotinib 150 mg/d Arm B: erlotinib 150 mg/d + bortezomib 1.6 mg/m2 (d1,8; q28d)	A: RR: 4/24 PFS 2.7 m B: RR: 2/24 PFS 1.4 m (prematurely stopped)

TXT: docetaxel, GMB: gemcitabine, Carbo: carboplatin, RR: response rate, PFS: progression free survival, OS: overall survival

positive charge of histones relaxing chromatin and promoting gene transcription. Deacetylation restores the positive charge allowing chromatin to pack into a tightly coiled, transcriptionally silent, conformation.[116] Accordingly, inhibition by histone deacetylase inhibitors promotes tumour suppressor gene expression, effectively modulating the proteome. Consequently, HDAC inhibitors induce apoptosis and can achieve this selectively in malignant cells.[117,118] Several HDAC inhibitors are being evaluated in NSCLC. Vorinostat is the most developed and is tested in a placebo-controlled randomised phase II/III trial in combination with paclitaxel/carboplatin doublet that was unfortunately stopped early due to negative results. Preclinical data suggest that HDAC inhibition may increase E-cadherin expression and sensitise NSCLC cells to EGFR inhibitors.[119] Phase I/II study testing the combination of HDAC and EGFR inhibitors is completed in advanced pretreated NSCLC patients and the results are pending. CI-994 was tested in randomised phase II trials in combination with gemcitabine[120] or with paclitaxel/carboplatin doublet.[121]

6. Rational combinations of targeted agents

Several trials are currently evaluating the combination of different targeted agents in treatment of NSCLC (Table 8). The

Table 8 – Combination of targeted agents.

Regimen	Phase of development	
Erlotinib + bevacizumab[122]	Phase II	PFS:4.4mo; OS:13.7mo
Erlotinib + bortezomib[152]	Phase II	PFS:1.7mo; prematurely stooped
Cetuximab + erlotinib + bevacizumab[153]	Phase I	Cetuximab 250 mg/m^2 weekly; erlotinib 50 mg daily; bevacizumab 10 mg/kg q2wk DLT: rash
Cetuximab + gefitinib	Phase I	Cetuximab 250 mg/m^2 weekly; gefitinib 250 mg daily; ongoing
Erlotinib + sorafenib[154]	Phase I	Sorafenib 400 mg/bid; erlotinib 150 mg/d No DLT
Erlotinib + sunitinib	Phase I	Ongoing
Gefitinib + everolimus[155,156]	Phase I/II	Everolimus: 5 mg/d gefitinib 250 mg/d DLT: hypotension, stomatis ORR (phase II): 17%
Gefitinib + sorafenib[157]	Phase I	Sorafenib 400 mg/bid; gefitinib 250 mg/d DLT: elevated ALT
Gefitinib + cediranib[158]	Phase I	Cediranib (20–45 mg/d) gefitinib 250 mg/d No DLT
Sorafenib + bevacizumab[159]	Phase I	Sorafenib 400 mg/bid; bevacizumab 5 mg/kg q2wk DLT: proteinuria, thrombocytopaenia
Motesanib + panitumumab[141]	Phase Ib	Motesanib 125 mg qd; panitumumab 9 mg/kg No DLT

ORR, overall response rate; PFS, progression free survival; OS, overall survival; DLT, dose limiting toxicity; qd, once daily; bid, twice daily.

most mature results are for the combination of erlotinib with bevacizumab. In a randomised phase II trial of the combination of bevacizumab with either chemotherapy (docetaxel or pemetrexed) or erlotinib versus chemotherapy alone in 120 previously treated NSCLC patients,[122] erlotinib/bevacizumab regimen produced encouraging results with a median PFS of 4.4 months and median OS of 13.7 months. One-year survival rate was 57.4% for bevacizumab–erlotinib arm and 53.8% for bevacizumab-chemotherapy arm compared with 33.1% for chemotherapy alone.[122] There were no unexpected toxicities observed in the erlotinib/bevacizumab arm. Unfortunately a phase III trial comparing erlotinib versus erlotinib + bevacizumab (BETA-Lung trial) in second line setting was negative reducing the enthusiasm for this promising approach.[123] On the other hand, the ATLAS phase III trial demonstrated a significant PFS benefit with bevacizumab + erlotinib combination as first line maintenance therapy following first line treatment with chemotherapy + bevacizumab, compared to maintenance therapy with bevacizumab + placebo.[124] Everolimus, an m-TOR inhibitor, has been tested in combination with erlotinib as second line treatment in the context of phase I/II trial. The dose limiting toxicities observed were rash, diarrhoea, vomiting and neutropaenia. Preliminary data reported one complete and three partial responses, while 17 patients had stable disease.[125]

7. Discussion

Although several new cytotoxic agents have been introduced in the treatment of NSCLC during the last decade, only small improvements in the survival of patients with advanced/metastatic lung cancer have been observed. It is clear that chemotherapy has reached a plateau of activity in the treatment of NSCLC[6] and further improvement in treatment is likely to require integration of novel targeted therapies.

The introduction of targeted therapies has led to some progress in the treatment of NSCLC. Inclusion of bevacizumab in first line treatment in combination with cytotoxic agents demonstrated a survival prolongation beyond the historical benchmark of 12 months. Erlotinib significantly prolongs survival and improves quality of life in patients with one or two primary lines of treatment, while gefitinib has recently demonstrated similar efficacy to docetaxel as second line treatment.

However, despite this progress several important issues in the clinical development of these targeted agents remain challenging for the future. First of all we need to identify the way to most effectively integrate these targeted agents to conventional therapies. We need to find out the best way to combine or sequence cytotoxic agents, radiotherapy and targeted therapies, in order to achieve the maximum clinical benefit. Furthermore, the role of targeted agents must be evaluated also in other disease stages (adjuvant, neo-adjuvant and maintenance). A second challenge is to develop reliable predictive factors which will allow the selection of patients who are most likely to benefit from a particular agent and save others from toxicity of ineffective treatments. A number of molecular predictors are being used for the selection of patients who would be most likely to benefit from anti-EGFR treatment. However, all clinical data are retrospectively derived and there is a clear need to validate these predictors in prospectively designed clinical trials. Prospective testing of biomarkers predictive of benefit from targeted agents is feasible as demonstrated in several ongoing phase II and phase III clinical trials. In these studies, biomarker data may be used as entry criteria ('enriched population' strategy) or as stratification factors with adequate power for comparisons in subsets of patients according to biomarker status. With limited therapeutic benefit achieved so far with targeted therapies in NSCLC and large number of novel drugs, it is clear that the successful drug development will depend on early identification of patients subsets in whom these therapies are effective.

NSCLC development is a multistep process linked to several intracellular pathways and several genetic alterations. More detailed information about molecular alterations in different lung cancer histologies are needed for drug development and successful tailoring targeted therapies to individual tumour and patient characteristics. An example of such approach is a comprehensive molecular analysis of lung adenocarcinomas, revealing several novel mutations.[126] Thus, using a single targeted agent may not be the optimal strategy to substantially improve clinical outcome. Therefore, another significant challenge for future research is development of combinations of agents targeting several different pathways, or drugs targeting more than one molecule.

Separation of 'all' lung cancer into subsets on the basis of molecular characteristics of the patient and the tumour will lead to better understanding of the natural history of this disease and will allow the initiation of patient-tailored-targeted treatment programs.[127]

Many clinical trials are currently evaluating targeted therapies in NSCLC. It is hoped that one or more of these approaches will prove successful and lead to substantial progress in the treatment of this common and fatal disease.

Conflict of interest statement

Athanasios G. Pallis: no conflict of interest to declare; Lucile Serfass: no conflict of interest to declare; Rafal Dziadziusko: R.D. received fees as a consultant for Roche and GSK Bio; Jan van Meerbeeck: no conflict of interest to declare; Dean Fennel: no conflict of interest to declare; Denis Lacombe: no conflict of interest to declare; John Welch: no conflict of interest to declare; Cesare Gridelli: C.G. received honoraria as speaker bureau and advisory board member for Eli Lilly, Roche, Sanofi-Aventis, Merck-Serono.

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