1. Introduction

Non-small cell lung cancer (NSCLC) accounts for approximately 80–85% of all cases of lung cancer, and is the most common cause of death in men and second only to breast cancer in women [1]. Treatment of NSCLC is guided by disease stage. Surgery is the treatment of choice for early-stage localized disease, whereas multimodality therapy remains the norm for patients with locally advanced disease. Patients with advanced metastatic disease may derive a benefit from palliative chemotherapy. About 40% of patients with NSCLC present at an advanced stage, with metastatic or locally advanced disease, which underscores the importance of identifying therapeutic schemes that may benefit this large patient population.

Combination chemotherapy, usually platinum-based, is currently the first-line therapy of choice [2]. Based on various studies, doublet regimens containing cisplatin or carboplatin with paclitaxel, gemcitabine, docetaxel, vinorelbine or irinotecan are administered [3]. The choice of combination drugs, however, varies in different countries, but several studies have shown similar degrees of efficacy among different combinations in the treatment of advanced NSCLC [4]. According to the ASCO guidelines [3] first-line chemotherapy should be stopped at 4 cycles in patients who are not responding and administered for no more than 6 cycles. Data from a recently published meta-analysis suggest that extending chemotherapy (>6 cycles), particularly with a third-generation regimen, improved progression-free survival (PFS) substantially, but had only modest effects on overall survival (OS) [5].

The prognosis for patients with advanced NSCLC is poor. Recent, large, randomized phase III trials have demonstrated that platinum-based chemotherapy combinations yield a median survival time of 8–11 months, a 1-year survival rate of 30–45% and...
a 2-year survival rate of 10–20% [6,7]. A landmark meta-analysis has shown that the use of chemotherapy in patients with advanced NSCLC, when compared with best supportive care (BSC), produced an improvement of 10% in 1-year survival and 2 months in median survival [8]. Although these results were statistically significant, the magnitude of this benefit is modest, and the duration of chemotherapy that maximizes survival benefit and symptom palliation in advanced NSCLC is currently unclear. The treatment of NSCLC is therefore a major unmet need and new therapies focusing on the molecular mechanisms that mediate the growth of lung cancer cells are urgently needed.

2. Monoclonal antibodies

2.1. Cetuximab

Monoclonal antibodies that bind the extracellular domain of EGF-R prevent the receptor from interacting with its ligand, EGF, and thus prevent intracellular signal transduction. In addition, antibodies have the inherent ability to recruit effector cells such as macrophages and monocytes to the tumor through the binding of the antibody constant Fc domain to specific receptors on these cells [9]. Cetuximab (Erbitux®, Merck, Germany) is a chimeric monoclonal antibody (IgG1 subtype) that competitively binds the extracellular domain of EGF-R (Fig. 1). Cetuximab was initially introduced as treatment for metastatic colorectal cancer and is now well established in all lines of treatment [10]. Among all anti-EGF-R monoclonal antibodies, cetuximab has been extensively studied in the treatment of NSCLC and has recently been reviewed [11]. Cetuximab has also demonstrated activity in the first-line setting for NSCLC treatment. A randomized phase II trial (N = 86) compared the activity of chemotherapy with cisplatin and vinorelbine with and without cetuximab [12]. This trial demonstrated the safety of the combination, and the response rates, median PFS, and OS were numerically higher on the cetuximab-containing treatment arm. This led to the development of a phase III trial (known as the FLEX trial) with the primary endpoint of OS comparing cisplatin and vinorelbine with and without cetuximab [13]. Patients (N = 1125) were required to have EGF-R expression by immuno-histiochemistry (IHC), and 85% of the patients screened demonstrated EGF-R expression. There are currently multiple biomarkers being investigated for predicting clinical benefit of anti-EGF-R direct therapies including IHC, fluorescence in situ hybridization (FISH), and activating EGF-R mutations. The optimal biomarker or set of biomarkers for predicting clinical benefit or resistance has yet to be determined, and the technical methods for testing biomarkers has yet to be standardized. Patients with a performance status of 2 (18% of the patients enrolled) and squamous histology (33% of patients enrolled) were eligible, but patients with brain metastases were excluded. Patients randomized to the cetuximab-containing arm demonstrated a statistically significant higher response rate and improvement in OS (median 11.3 months vs. 10.1 months. There was no difference in PFS between the two treatment arms. Patients on the cetuximab-containing arm compared with the chemotherapy arm did experience a statistically significant higher rate of febrile neutropenia (22% vs. 15%), grade 3/4 acne-like rash (10% vs. <1%), diarrhea (5% vs. 2%), and infusion-related reactions (4% vs. <1%). The rate of treatment related deaths on the cetuximab and chemotherapy arms were similar (3% and 2%, respectively). A second phase III trial of carboplatin and taxane therapy (either paclitaxel or docetaxel at the discretion of the treating physician) with and without cetuximab with the primary endpoint of PFS by independent radiological review has been performed [14]. The patients were not selected on the basis of EGF-R expression by IHC, and this trial was substantially smaller than the FLEX trial (676 vs. 1125 patients, respectively). Similarly to the FLEX trial, no statistically significant difference in the PFS was observed. Recent results indicated no statistically significant differences in OS. The HR observed on this trial was similar to the HR observed on the FLEX trial, and this trial was not sufficiently powered to detect a difference in OS. A meta-analysis has been published most recently which included four randomized Phase II/III clinical trials with a total of 2,018 patients across all histologies [15]. The analysis showed the benefit of cetuximab when added to standard first-line platinum-based chemotherapy for OS, PFS and ORR (OS: p = 0.010; PFS: p = 0.036, ORR: p = 0.001) compared to chemotherapy alone. Due to lack of sufficient evidence cetuximab is currently not approved for the treatment of advanced NSCLC. To date, only NSCLC patients treated in clinical trials should receive cetuximab. Moreover, preliminary results from ongoing studies suggest that
cetuximab is less active in patients harboring a mutated EGF receptor ([16], Dr. Jeff Engelman, Boston, unpublished results), therefore it is strongly recommended to analyse EGF-R expression as well as the mutation of the receptor. Currently, cetuximab is extensively studied in many clinical trials for NSCLC and additional data will be expected in the next years. Data have also been published for panitumumab (Vectibix®, Amgen, USA), a fully human monoclonal anti-EGF-R antibody (IgG2 subtype). A randomized phase II trial in previously untreated advanced stage IIIB/IV NSCLC patients compared carboplatin (AUC 6 every 3 weeks) and paclitaxel (200 mg/m² every 3 weeks) with or without panitumumab (2.5 mg/kg weekly). In this trial there was no benefit appreciated with regard to time to disease progression (4.2 months vs. 5.3 months for chemotherapy alone, p = 0.55). In addition, there was no reported benefit in RR or median survival time [17].

2.2. Bevacizumab

Bevacizumab (Avastin®, Roche, Switzerland) is a monoclonal antibody with high affinity for VEGF (Fig. 2). The clinical activity of bevacizumab in inoperable locally advanced, metastatic or recurrent NSCLC was first shown by the 3-arm phase II trial AVF0757g, in which bevacizumab (7.5 or 15 mg/kg, every 3 weeks) was combined with PC chemotherapy in chemotherapy-naive patients [18]. Compared with platinum-based therapy alone, addition of the VEGF antibody in the higher dosage improved both response rate and median PFS. Patients with squamous cell cancer, however, had an increased risk of severe pulmonary bleeding. In the following phase III trials, therefore, only patients with non-squamous cell histology and without a history of relevant hemoptyses were recruited. The EU approval of bevacizumab for the first-line therapy of advanced or recurrent NSCLC without predominantly squamous cell histology is based on the results of the trials ECOG 4599 and AVAiL (Avastin in Lung). In both phase III trials with similar inclusion and exclusion criteria the patients received platinum-based doublet chemotherapy with or without bevacizumab [19,20]. In the US ECOG trial, chemo-naive patients were treated with up to 6 cycles of carboplatin (AUC 6) and paclitaxel (200 mg/m²) plus bevacizumab (15 mg/kg, q3w, until disease progression) (N = 434) or received PC alone (N = 444) [19]. In the 3-arm AVAiL study conducted mainly at European centres, up to 6 cycles of cisplatin (80 mg/m²) and gemcitabine (1250 mg/m²) were administered in the first-line setting in combination with bevacizumab (7.5 or 15 mg/kg, q3w, until disease progression) (N = 345 and N = 351) or placebo (N = 347) [20]. In the ECOG 4599 trial, addition of the VEGF antibody to PC significantly improved OS defined as the primary endpoint, PFS and RR [19]. Patients with adenocarcinoma (68.6% of the overall population) benefited from bevacizumab (N = 300) with an increase in OS by about 3.9 months to a median 14.2 months. The AVAiL trial confirmed the efficacy of the angiogenesis inhibitor in combination with the cisplatin/gemcitabine regimen commonly used in Europe. Bevacizumab significantly prolonged PFS defined as primary endpoint in this trial and improved the RR. OS analysed as secondary endpoint could not be significantly improved by the combination with bevacizumab; in all three study arms, however, unusually high median survival times of more than 13 months were observed. It should be considered, that more than 60% of all patients received follow-up therapies that were not specified in the study design and which may have variably influenced survival time [20]. In both phase III studies the possible influence of gender and age on the efficacy of bevacizumab was investigated. An unplanned subgroup analysis of the ECOG 4599 trial showed a significant benefit in median overall survival for men (B + CTX: 11.7 months vs. CTX: 8.7 months; p = 0.001), but not for women (B + CTX: 13.3 months vs. 13.1 months; p = 0.87) [21]. Median PFS was similar (6.3 and 6.2 months), although the response rate was lower in men than in women (23.6% and 38.5%). In an exploratory subgroup analysis of the AVAiL trial, bevacizumab in the 7.5 mg dosage reduced the risk of progression by about 25% both in men and women (HR 0.74 and 0.77), although only women benefited from the 15 mg dosage with a marked decrease in progression risk (women: HR 0.59; men: HR 0.99) [22]. For elderly patients (>70 years) the results of a retrospective subgroup analysis of the AVAiL trial showed a trend to improvement of PFS and response, but no prolongation of OS with an increase in severe adverse events [23]. On the other hand, a corresponding subgroup analysis of the AVAiL trial showed that...
patients ≥65 years derived a similar, statistically significant PFS benefit from bevacizumab (7.5 mg/kg) as younger patients. Considering the conflicting results of the subgroup analyses treatment with bevacizumab regardless of age and gender is feasible if the specific safety criteria and toxicities are carefully monitored.

In the ECOG 4599 trial the addition of bevacizumab led to a slight increase in bleeding, hypertension, proteinuria, neutropenia, febrile neutropenia, thrombocytopenia, hyponatremia, rash and headache compared with chemotherapy alone. The rate of severe bleeding, by which 9.1% of the patients in the phase II trial AVF0757g were affected [18], was markedly reduced by selecting patients with non-squamous NSCLC and was only 2.3% in the ECOG 4599 trial. In the AVAi1 trial 1.5% and 0.9% respectively developed severe bleeding in the two bevacizumab arms [20]. The safety and efficacy of bevacizumab-based first line therapy in daily practice are currently being evaluated in the international SAiL (Safety of Avastin in Lung) trial and the US American ARIES (Avastin Regimens: Investigation of Treatment Effects and Safety) trial [24,25]. Both trials are also enrolling elderly subjects, patients with poor performance status (ECOG 2) and therapeutic anticoagulation and are combining bevacizumab with different chemotherapy regimens. The interim analyses of the two phase IV trials (SAiL: cut-off: March 2009; N = 2166. ARIES: cut-off: February 2009; N = 1758) confirmed the safety profile known from the phase III trials; in the SAiL trial the already available effectiveness data with a median OS of 15.3 months simultaneously demonstrated the efficacy of bevacizumab. The incidence of grade ≥ 3 hemorrhage (pulmonary and cerebral) was not higher either in the SAiL trial or the ARIES trial, in which patients with cerebral metastases also participated, than in the randomized controlled studies. Since the ATLAS and PASS-PORT trials, in which 85 patients with treated cerebral metastases received bevacizumab, showed no increased risk of complications [26], the angiogenesis inhibitor is now also approved for patients with CNS metastases. In the phase III trials ECOG 4599 and AVAi1, after completion of the chemotherapy bevacizumab was given as monotherapy until disease progression. The results of the ATLAS trial, in which two different targeted therapies were combined, may possibly provide a further improvement in the treatment results. In the placebo-controlled phase III trial, patients who responded to bevacizumab-based first-line therapy with objective response or stable disease received bevacizumab in combination with the oral EGF-R inhibitor erlotinib in the maintenance therapy [27]. The trial met its primary endpoint with a significant 28% decrease in the risk of progression (HR 0.72; 95% CI 0.59–0.88; p = 0.006) and an increase in median PFS by 1.1 months (time of randomization: 4.8 months vs. 3.7 months). Data on OS have not yet been presented. The combination of bevacizumab with erlotinib was also effective in patients after failure of first-line chemotherapy in the placebo-controlled phase III trial BeTaLung. The combination of two targeted therapies improved median progression-free survival (3.4 months vs. 1.7 months; p < 0.0001) and response rate (12.6% vs. 6.2%; p = 0.006) compared with second-line therapy with erlotinib alone [28]. The median OS benefit (primary endpoint), however, was not significant (9.3 months vs. 9.2 months). The large number of follow-up therapies is also discussed as a possible cause. Ongoing trials are focusing on the efficacy of bevacizumab in the adjuvant setting (phase III trial ECOG 1505), maintenance therapy with bevacizumab/pemetrexed (AVAPERL trial) and the importance of biomarkers for therapeutic response (phase II trial ABIGAIL).

2.3. Figitumumab

The insulin-like growth factor type 1 receptor (IGF-1R) and its ligands play a key role in different cancers and are closely linked to EGF-R signalling (Fig. 1). Increased IGF-1 and decreased insulin-like growth factor binding protein-3 and -4 (IGFBP-3,4) are also associated with higher risk of lung cancer. In addition, IGF-1R is often overexpressed in lung tumors and can mediate the proliferation of lung cancer cells and resistance to therapy [reviewed by [29]]. Loss of IGF-BPs was reported to be involved in the resistance to EGF-R-targeted tyrosine kinase inhibitors [30]. Since IGF-1R has been shown to bypass the EGF-R pathway, small molecules (e.g. BMS-754807, Bristol-Myers Squibb, USA) targeting the intracellular domain and monoclonal antibodies (e.g. MK-0646 [Merck & Co., USA], IMC-012 [Imclone, USA], Figitumumab [Pfizer, USA]) that bind to the extracellular domain of this receptor have been developed [31]. In this regard it is interesting to note that small-molecule-type IGF-1R inhibitors have not been tested in clinical trials yet. This is mainly due to the observation that these compounds may have a “broader” target spectrum resulting in a more complete blockage of the IGF signalling system, and several in vivo studies have provided evidence that almost all tumor-bearing animals died due to treatment-refractory ketoacidosis/hyperglycemia following administration of the small molecule IGF-1R inhibitors.

Amongst all compounds under clinical development figitumumab is the most advanced drug. It is a fully human monoclonal antibody with a half-life of approximately 3 weeks that has been well tolerated in phase I studies [32,33]. Moreover, in combination with chemotherapy no dose-limiting toxicities were found for figitumumab. Based on these results a phase II study was conducted to determine the clinical efficacy of figitumumab in combination with paclitaxel and carboplatin in patients with previously untreated locally advanced or metastatic NSCLC [34]. Patients (all histologies) were randomly assigned (2:1) to paclitaxel (200 mg/m[2]), carboplatin (AUC 6), and figitumumab (10–20 mg/kg) (PCI) or paclitaxel and carboplatin (PC) alone every 3 weeks up to 6 cycles. Patients with chemotherapy alone experiencing disease progression were eligible to receive figitumumab at investigator’s discretion. A total of 156 patients were enrolled onto the randomized portion of the study. PCI was well tolerated with a low incidence of treatment-related hyperglycemia events. Although these events were not significantly different between the arms, a relationship to figitumumab dosing was likely. Hyperglycemia has been observed in other figitumumab studies and seems to be a characteristic of anti-IGF-1R class of drugs. 54% of the patients treated with PCI and 42% of patients treated with PC had objective responses. The overall clinical benefit for PC was approximately 50% which is in line with other studies. Treatment with PCI[0] translated into a PFS benefit of 4-6 weeks, according for censor-ship for cross-over, whereas no PFS benefit was seen for the PCI[1] group. Interestingly, the response rate in patients with squamous cell tumors treated with PCI reached 70%. Although these data should be interpreted with caution (small sample size, no independent confirmation of radiographic response) several lines of evidence have indicated that de-regulation of the IGF-1R pathway may be common in NSCLC of squamous histology [35]. Since these data suggested that PCI is safe and effective in patients with NSCLC 3 phase III clinical trials (ADVIGO 1016: first-line treatment: PC±figitumumab, primary endpoint: OS, N = 820; ADVIGO 1017: first-line treatment: gemcitabine/cisplatinum±figitumumab, primary endpoint: OS, N = 1100; ADVIGO 1018: refractory treatment: erlotinib±figitumumab, primary endpoint: OS, N = 600) have been started and are currently open for enrollment.

3. Small molecules

3.1. EGF-R inhibitors

The discovery of somatic mutations in the tyrosine kinase domain of EGF-R in NSCLC represents a dramatic step in elucidating genomic changes in lung cancer and their role in developing treatment strategies. These gain-of-function mutations enhance EGF-R
activation, markedly increase sensitivity to EGF-R RTK inhibitors and are transforming. Retrospective studies suggest particularly promising results with EGF-R RTK inhibitors therapy among patients harboring EGF-R mutations, with response rates higher than 65% and median survival of 20–30 months [36]. Characteristics associated with EGF-R mutations enable clinical profiling of patients to enrich for mutations among patients with NSCLC. Nearly 90% of these mutations occur as either multinucleotide in-frame deletions in exon 19 or as single missense mutations that result in substitution of arginine for leucine at position 858 (L858R). Both mutations are associated with increased sensitivity to the selective EGF-R kinase inhibitors gefitinib and erlotinib [37]. About 70% of the patients with EGF-R mutations respond to EGF-R tyrosine kinase inhibitors including gefitinib and erlotinib, whereas only 10% of those without the mutations do so [38]. While most patients with EGF-R mutations derive benefit from EGF-R RTK inhibitors, there is variability in the degree and duration of response. Some patients exhibit de novo resistance, and the remainder are highly likely to develop acquired resistance after a period of initial response. De novo resistance mechanisms among patients with EGF-R mutations have not been well studied, though some genomic mechanisms of acquired resistance are recognised, including a secondary point mutation in EGF-R (T790M) that blocks the capacity for gefitinib or erlotinib to inhibit EGF-R. Mutations that substitute methionine for threonine at position 790 in the EGF-R kinase domain (‘gatekeeper mutation’) have been found in approximately 50% of lung adenocarcinomas from patients with acquired resistance to the EGF-R inhibitors gefitinib and erlotinib [39]. Threonine 790 is the ‘gatekeeper’ residue, an important determinant of inhibitor specificity in the ATP binding pocket. The T790M mutation has been thought to cause resistance by sterically blocking binding of tyrosine kinase inhibitors such as gefitinib and erlotinib, but this explanation is

Fig. 3. Chemical structures of some EGF-R- and VEGF-R-blocking agents.
difficult to reconcile with the fact that it remains sensitive to structurally similar irreversible inhibitors. Thus far, gatekeeper mutants have proved particularly difficult to overcome in the clinic presumably because many kinase inhibitors are designed to interact with the adjacent hydrophobic (selectivity) pocket. This knowledge has led to the identification of alternative EGF-R inhibitors that can overcome T790M-mediated resistance in vitro and potentially in patients.

3.1.1. Gefitinib

Gefitinib (Iressa®, AstraZeneca, UK, Fig. 3) is targeted against tyrosine kinase activity on the EGF-R pathway. Gefitinib has an interesting development history and has contributed greatly to our understanding of the biology of NSCLC and the role of the EGF-R signalling pathways. During the phase II dose finding studies (IDEAL studies 1 and 2) gefitinib showed activity as monotherapy in patients with advanced NSCLC who had received prior chemother-therapy with overall response rates of 15% (IDEAL 1, Asian-European trial) and 10% (IDEAL 2, US trial) [40]. These phase II studies were instrumental to gaining marketing approvals in Japan and the USA. However, tumor analyses from these studies already raised some issues in identifying the patients who would be most likely to benefit. Although originally response to the EGF-R tyrosine kinase inhibitors had been thought to be most likely in patients expressing high levels of EGF-R, an analysis of the samples from the IDEAL studies showed that tumor EGF-R membrane staining was not clinically relevant for predicting response in patients receiving gefitinib monotherapy [41]. This led to looking for indicators inside the cell and one study by Natale [42] was able to show that gene expression could act as a predictor of response, a finding that has been subsequently built upon by other investigators. Patient who express mutated EGF-R appeared more likely to respond to gefitinib than those who did not and the type of mutations of the EGF-R kinase appeared to be in-frame deletions and missense mutation [43]. Further in vitro studies showed that the mutated EGF-R had increased and prolonged activation by EGF which was also more sensitive to inhibition by gefitinib. Interestingly the presence of these mutations is now known to be closely correlated to the sub-groups of patient who tended to show the best response to therapy from retro-pective studies of the clinical trials [44]. These groups include females, Asian ethnicity, never-smoking history and a histology of adenocarcinoma. Unfortunately, the original phase III studies did not have the benefit of this knowledge when designed; the ISEL study, testing against BSC, and the INTACT studies 1 and 2 (cisplatin and gemcitabine with or without gefitinib) failed to show a benefit in OS in the overall study population [45]. However, a pre-planned sub-analysis of the ISEL data demonstrated significantly longer survival in the gefitinib group compared with the placebo group for never smokers (HR 0.67; 95% CI 0.49–0.9; p = 0.012), as well as for patients of Asian ethnicity (HR 0.66; 95% CI 0.48–0.91; p = 0.01) [46]. In a subset analysis of all Asian patients from the ISEL trial, significant improvements in survival with gefitinib were seen for patients with adenocarcinoma, never smokers, and female sex [47]. Subsequently, the association of several factors (never-smoker, Asian origin, female sex, and adenocarcinoma histology) with response to gefitinib and their correlation with the presence of EGF-R mutations in tumor tissue has been shown by several studies (reviewed by [48]). With the insight gained from the previous phase III studies the development of gefitinib moved towards investigating its role as an alternative to chemotherapy with the potential of lower toxicity and a higher quality of life. One study to explore gefitinib as a ther-apeutic alternative was the international SIGN study of gefitinib vs. docetaxel in patients unresponsive to first-line chemotherapy. Although this was not powered to demonstrate an efficacy dif-ference between the groups, it did show that gefitinib was better tolerated with no clear disadvantage in efficacy [49]. Similar results were seen in a Japanese phase III V-15–32 trial [50]. To demonstrate non-inferiority of gefitinib vs. mono-chemotherapy as second line treatment required a much larger international study; the INTER-EST study. This open-label randomized phase III study was similar to the SIGN study in that it also was testing against docetaxel, although patients were allowed to have received previous taxanes. INTEREST recruited 1466 patients (79% non-Asian, 21% of Asian ethnic origin) and demonstrated that gefitinib was non-inferior in overall survival (593 vs. 576 events; HR 1.020, 95% CI 0.905–1.150) and similar in tumor response and PFS to docetaxel (median sur-vival 7.6 months vs. 8.0 months) [51]. Efficacy was irrespective of the patients’ EGF-R protein expression (N = 380), EGF-R gene muta-tion (N = 297), or K-RAS gene mutation status (N = 275). The result for gene copy number (N = 374) was unexpected; previous work had suggested that it may be predictive for EGF-R-TKI efficacy; yet there was no difference to the docetaxel group. Significantly more patients had sustained and clinically relevant improvement in quality of life with gefitinib than with docetaxel, as assessed by FACT-L total score (OR 1.99, 95% CI 1.42–2.79; p = 0.0001) and the FACT-L TOI (1.82, 1.23–2.69; p = 0.0026). Another unexpected finding was that the clinical factors linked to overall improved patient outcome from the ISEL study (never-smoker, Asian origin, female sex, and adenocarcinoma histology) were associated with observed longer survival in both treatment groups. Previously, it was generally believed that chemotherapy (docetaxel) produces similar survival in all patients. It is believed that the large number of patients refractory to chemotherapy in the ISEL study (90% vs. 58% in INTEREST) might partly explain why ISEL did not show a sig-nificant improvement in OS with gefitinib compared with placebo, since these patients represent a population who are difficult to treat and have a poor prognosis. In another study, Inoue et al. [52] in Japan showed that patients with poor performance status who were positive for EGF-R mutations and who would be unsuitable for “standard chemotherapy regimens” benefited from gefitinib. They demonstrated that the median PFS increased compared to histori-cal expectations [52]. In the IPASS study, gefitinib has been used as a first-line treatment compared to PC. The primary aim was to prove non-inferiority of the targeted treatment in PFS. Here, 1217 chemo-naive patients were randomized, being selected for pre-defined clinical factors (never-smoked or light smoker, predominantly ade-noacarcinoma, and performance status 0–2) and they were also assessed for their EGF-R mutation status. In the ITT population, gefi-tinib demonstrated superior efficacy with fewer events and a higher ORR (PFS: HR 0.74; 95% CI 0.65–0.85; p < 0.0001; ORR: HR 0.59; 95% CI 1.25–2.01; p = 0.0001), a better quality of life (FACT-L 48% vs. 41%, OR 1.34, 95% CI 1.06–1.69, p = 0.0148; TOI 46% vs. 33%, OR 1.78, 95% CI 1.40–2.26, p < 0.0001); and similar symptom improve-ment rates with a more favourable tolerability profile compared to a PC chemotherapy regimen. A subgroup analysis of the patients with evaluable samples for EGF-R mutations (N = 437; EGF-R mutation positive: N = 132) showed that PFS was significantly longer with gefitinib than PC in the EGF-R mutation-positive subgroup (HR 0.48; 95% CI 0.36–0.64; p = 0.0001), and significantly shorter with gefitinib than PC in the mutation-negative subgroup (HR 2.83; 95% CI 2.05–3.98; p < 0.0001). The ORR in the mutation-positive subgroup also favoured gefitinib (71.2% vs. 47.3% for gefitinib and PC, respectively, odds ratio 2.75; 95% CI 1.65–4.60; p = 0.0001). The initial superiority of PC was attributed to the EGF-R mutation-negative subgroup benefiting from chemotherapy but not from gefitinib, while prolonged PFS in the EGF-R mutation-positive subgroup explained subsequent improvement favouring gefitinib [53,54]. In another phase III study from Japan, patients harboring EGF-R mutation-positive tumors were specifically selected for inclusion and were randomized to receive either gefitinib or PC chemotherapy as first-line treatment for advanced NSCLC. After inclusion of 200 patients a pre-planned interim analysis showed that PFS was
significantly improved with gefitinib (N = 98) compared with the doublet chemotherapy (N = 96) (median PFS 10.4 months vs. 5.5 months, respectively; HR 0.357 [95% CI 0.25–0.51; p < 0.001]). Due to this early demonstration of efficacy at the interim analysis the study was closed early as it was considered unethical to continue [55].

Gefitinib is now approved by the CHMP in Europe and is indicated for the treatment of adult patients with locally advanced or metastatic NSCLC with activating mutations of EGF-R tyrosine kinase, independent of treatment line.

3.1.2. Erlotinib

Erlotinib (Tarceva®, Roche, Switzerland, Fig. 3) inhibits EGF-R signalling by binding to the intracellular TK domain. In a phase II trial 57 patients with advanced pretreated NSCLC in stage IIIB/IV received erlotinib (150 mg/day) [56]. Despite the strong previous treatment (median 2 previous treatments) two patients achieved a complete response, while 5 had a partial response (objective response 12.3%, 95% CI 5.1–23.7%). Median OS was 8.4 months (95% CI 4.8–13.9 months) and 1-year survival 40% (95% CI 28–54%). These data were confirmed by the results of the phase III trial BR.21. In this trial relevant for the approval of erlotinib as second/third-line therapy of advanced NSCLC, 731 patients with stage IIIB/IV NSCLC were randomized in a 2:1 ratio after failure of at least one chemotherapy and were treated with erlotinib (150 mg/day) or placebo with BSC [57]. The study met its primary endpoint with a significant improvement in median OS of 2 months (6.7 months vs. 4.7 months; HR BSC [57]. The study met its primary endpoint with a significant improvement in median OS of 2 months (6.7 months vs. 4.7 months; HR 0.70; p < 0.001). Erlotinib also improved the response rate (8.5% vs. <1%; p < 0.001) and median duration of response (7.9 months vs. 3.7 months) as well as median PFS (2.2 months vs. 1.8 months; HR 0.61; p < 0.001). Objective efficacy was associated with a significant prolongation of the time to deterioration of the core symptoms dyspnea (4.7 months vs. 2.9 months; p = 0.04), chest pain (2.8 months vs. 1.9 months; p = 0.03) and cough (4.9 months vs. 3.7 months; p = 0.04) [58]. Physical function (31% vs. 19%; p = 0.01) and global quality of life (35% vs. 26%; p < 0.0001) were also significantly better than with BSC alone. Erlotinib showed a favourable tolerability profile. The most commonly reported adverse events were rash (all grades: 76%; grade 3/4: 9%) and diarrhea (all grades 55%; grade 3/4: 6%) [57]. In 19% of the patients the erlotinib dose was reduced mainly due to cutaneous adverse events, and 5% discontinued the treatment due to adverse events. Since rash was associated with longer survival in the BR.21 trial and in two phase III trials in pancreatic cancer and is probably an important pharmacodynamic factor [59], various therapeutic strategies have now been developed for the treatment and control of this typical side effect to allow continuation of the therapy. The efficacy and tolerability of erlotinib (150 mg/day) are being evaluated under conditions of daily practice in the TRUST study. The interim analysis of February 2008 was based on an analysis of the data of 6809 patients who showed progression after chemotherapy/radiotherapy or were not suitable for these treatments [60]. With a median age of 63 years, 25% with poor performance status (ECOG 2/3) and 55% and 24% with adenocarcinoma or squamous cell carcinoma respectively, the population was typical of patients with advanced NSCLC. The disease control rate of 69%, with 12% complete or partial remissions, the median PFS (RECIST criteria) of 14.3 weeks and the 1-year overall survival of 38.6% were similar to those in the randomized trial BR.21. The incidence and intensity of the acne-like cutaneous events (all grades: 70%; grade 1/2: 58%) also confirmed the results of the phase III trial. Onset of rash in the first weeks of treatment was identified as the most important pharmacodynamic predictor of the efficacy of erlotinib in the phase III study. Although significantly increased response rates were also seen in women (p = 0.006), patients with adenocarcinoma (p = 0.001), non-smokers (p < 0.001) and Asians (p = 0.02) in the BR.21 study [57], the survival benefit conferred by erlotinib was independent of gender (HR 0.8 [women] vs. 0.78 [men]) and tumor histology (HR 0.71 [adenocarcinoma] vs. 0.67 [squamous cell carcinoma] [61]. Lifelong non-smokers, on the other hand, not only had a better prognosis but also a greater survival benefit from erlotinib (HR 0.42 vs. 0.87) compared with current/former smokers. The reduced efficacy in current smokers is probably attributed to the fact that tobacco smoking accelerates the elimination of erlotinib by inducing the enzymes CYP1A1 and CYP1A2, and the area under the plasma concentration time curve (AUC) is consequently smaller than in non-smokers [62]. To achieve similar AUC values in smokers as in non-smokers, the single dose had to be doubled from 150 to 300 mg in a dose escalation study. Since a phase II trial showed that the dosage can be escalated, depending on toxicity, to up to 300 mg/day in current/former smokers, conventionally dosed erlotinib (150 mg/day) is being compared with a dosage escalated up to 300 mg/day in a randomized phase III trial in current smokers (Protocol MO22162). The primary endpoint is the non-progression rate (NPR) after 6 weeks. The detection of EGF-R-mutations (exon 19 deletion and L858R) in the tyrosine kinase of the EGF receptor in the BR.21 study was associated with a significantly better response to erlotinib compared with the wild-type (27% vs. 7%; p = 0.03) [63]. However, the mutation status was not predictive of OS. The second-/third-line therapy with erlotinib reduced the mortality risk both in patients with exon 19 or 21 mutations (HR 0.55; p = 0.1217) and in patients with EGF-R wild-type (HR 0.74; p = 0.0924). Patients with a high EGF-R copy number (FISH positive) showed a significantly higher response rate in this trial compared with patients without EGF-R gene amplification (21% vs. 5%; p = 0.02) and had a statistically significant survival benefit from erlotinib (HR 0.43; p = 0.0042) in contrast to patients with FISH-negative NSCLC (HR 0.80; p = 0.3525). The efficacy of erlotinib in the first-line setting in patients with advanced NSCLC and activating EGF-R mutations was demonstrated in a phase II trial of the Spanish Lung Cancer Group (SLCG) [64]. Screening of 2507 patients detected activating EGF-R mutations in 358 patients, of whom 217 participated in the study. 70.6% achieved an objective response (12.2% with complete response), TTP was 14 months and median OS was 27 months. To determine the predictive value of activating EGF-R mutations in first-line therapy with erlotinib, the phase III trial EURTAC was initiated, in which patients with proven activating EGF-R mutations receive erlotinib or a platinum-based doublet chemotherapy in the first-line setting. The efficacy of erlotinib in patients with advanced NSCLC (N = 1949) who did not show progression after first-line therapy with doublet chemotherapy (4 cycles) (N = 889), was demonstrated by the placebo-controlled phase III trial SATURN (Sequential Tarceva in Unresectable NSCLC) [65]. Maintenance treatment with erlotinib (150 mg/day) until disease progression or unacceptable toxicity statistically significantly reduced the risk of progression (primary endpoint) by 29% (all patients: HR 0.71; p < 0.0001) and 31% (subgroup with EGF-R-IHC-positive tumors: HR 0.69; p < 0.0001). While the clinical factors gender, smoking status, tumor histology and ethnicity had no relevant influence on PFS, the detection of activating EGF-R mutations was associated with a much greater reduction in the risk of progression compared with the EGF-R wild-type (HR 0.10; p < 0.0001 [activating mutations] vs. HR 0.78; p = 0.0185 [wild-type]) [66]. The greater benefit in PFS, however, did not correlate with a correspondingly greater benefit in OS. In the overall population, erlotinib reduced the mortality risk by 19% (HR 0.81; p = 0.0088) with an increase in median OS in the overall population from 11.0 to 12.0 months. Patients with EGF-R wild-type (HR 0.77; p = 0.0243) experienced comparable benefit with erlotinib as patients with activating EGF-R mutations (HR 0.83; p = 0.6810) [67]. The results of the SATURN study, therefore, confirm the data of the BR.21 trial, in which erlotinib also reduced the mortality risk independently of the EGF-R mutation status. The question regard-
ing the optimal time to start erlotinib therapy, however, cannot be answered by the SATURN study, as comparability of the post-study therapies was not assured because of the study design. This issue should be addressed in future protocols, however it is already being clear that close follow-up of patients and timely start of second-
line therapy is of great importance. Because of its good efficacy in the advanced tumor stages, erlotinib is also being investigated for use in operable NSCLC. In the placebo-controlled phase III trial RADIANT, erlotinib (150 mg/day) is being evaluated in patients with completely resected NSCLC in stage Ib-IIIA and optional adjuvant chemotherapy.

It should be noted that other drugs targeting the EGF-R family have also been evaluated for NSCLC treatment [68]. Amongst them, lapatinib (Tykerb®, GlaxoSmithKline, UK), a small molecule targeting erbB-1/2, has been undergone phase II/III testing, however, data are not available yet. In addition, trastuzumab (Herceptin®, Roche, Switzerland), a monoclonal antibody targeting HER-2, pro-
duced disappointing results when combined with chemotherapy. As a result, very few studies are currently recruiting patients.

3.1.3. **BIBW-2992**

Multitargeted agents represent the next generation of targeted therapies in solid tumors. The benefits of individually targeting the EGF-R and VEGF-R signalling pathways have been clinically validated in recent years in a number of solid tumor types including NSCLC. Given the heterogeneity of this tumor type and potential cross-talk between these key signalling pathways, dual inhibition has the potential to offer additional clinical benefits in NSCLC.

The aniline–quinazoline derivative BIBW-2992 (Tovok®, Boehringer Ingelheim, Germany, Fig. 3) is an oral irreversible dual TKI of the EGF-R and HER-2/neu [69]. The IC50 is 0.5 nM for EGF-R kinase and 14 nM for HER2 kinase. Importantly, BIBW-2992 was found to be active against tumors overcoming EGF-R with the secondary T790M point mutation, which confers resistance to the first-generation EGF-R inhibitors gefitinib and erlotinib [69]. In phase I studies BIBW-2992 has shown a stable disease rate of approximately 40% in various tumor types. When given daily for 2 weeks every 4 weeks, the drug is well tolerated, with adverse events being rash, diarrhea, and transaminase elevations. These adverse events were found to be reversible, and the pharmacokinetic profile was predictable and compatible with oral dosing [70]. To date, 800 patients have been treated with BIBW-2992.

BIBW-2992 is currently in phase Ib/III clinical development in NSCLC (LUX-Lung Studies, Table 1). Preliminary data from the LUX-Lung 1 trial (BIBW-2992 vs. BSC in NSCLC patients failing 1–2 lines of chemotherapy and erlotinib or gefitinib) of the first 40 patients treated with BIBW-2992 revealed that 40% had an objective response, however, this trial is continuing recruitment [71]. New data presented from the ongoing phase II trials (LUX-Lung 2) showed that NSCLC patients with activating EGF-R mutations treated with BIBW-2992 experienced a high ORR (64%; 43/67 patients) and a high rate of disease control (96%; 64/67 patients) [72]. Due to these encouraging results BIBW-2992 was granted Fast-Track status by the FDA and a first-line study (LUX-Lung 3: BIBW-2992 vs. gefitinib vs. erlotinib) has started recruiting patients recently.

### Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Company</th>
<th>Target(s)</th>
<th>Development phase</th>
</tr>
</thead>
<tbody>
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<td>ArQule</td>
<td>c-MET</td>
<td>II</td>
</tr>
<tr>
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<td>Johnson &amp; Johnson</td>
<td>c-MET</td>
<td>I</td>
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<td>I</td>
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<td>Pfizer</td>
<td>c-MET, ALK</td>
<td>II/III</td>
</tr>
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<td>Exelixis</td>
<td>c-MET, VEGF-R1-3, Ret, Kit, Flt-3, Tie-2</td>
<td>III</td>
</tr>
<tr>
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<td>GlaxoSmithKline</td>
<td>c-MET, Ron, VEGF-R1-3, PDGF-R, Kit, Flt-3, Tie-2</td>
<td>II</td>
</tr>
<tr>
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<td>SuperGen</td>
<td>c-MET, Ret, Rad51, Kit, PDGF-R, Flt-3</td>
<td>I</td>
</tr>
<tr>
<td>MCGD265</td>
<td>Methylgene</td>
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</tr>
<tr>
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<td>Merck &amp; Co.</td>
<td>c-MET, KDR, FGF-R1-3, Flt-1,3,4</td>
<td>II</td>
</tr>
<tr>
<td>AMC 102</td>
<td>Amgen</td>
<td>c-MET</td>
<td>II</td>
</tr>
</tbody>
</table>

### Table 1

**Clinical trial programme for BIBW-2992 in NSCLC (BSC: best supportive care).**

<table>
<thead>
<tr>
<th>Study name</th>
<th>Phase</th>
<th>Design</th>
<th>Criteria</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>LUX-Lung 1</td>
<td>IIb/III (randomized)</td>
<td>BIBW-2992 plus BSC vs. placebo plus BSC</td>
<td>Progression after erlotinib or gefitinib</td>
<td>Currently recruiting (N = 560)</td>
</tr>
<tr>
<td>LUX-Lung 2</td>
<td>II (single arm)</td>
<td>BIBW-2992</td>
<td>Patients who have failed one line of chemotherapy or are chemotherapy-naive and have EGF-R activating mutations</td>
<td>Currently recruiting (N = 120)</td>
</tr>
<tr>
<td>LUX-Lung 3</td>
<td>III (randomized)</td>
<td>BIBW-2992 vs. erlotinib vs. gefitinib</td>
<td>First-line</td>
<td>Just started</td>
</tr>
</tbody>
</table>
encouraging. Several multitargeted therapies have also been under investigation in clinical settings and have demonstrated promise, particularly with regard to tyrosine kinase inhibition, and among them PF-02341066 (Pfizer, USA) is the most advanced compound. In a most recently published phase I study [77] with 37 patients PF-02341066 (an oral MET and ALK inhibitor) was found to be well tolerated, the most frequent adverse events were GI-related (nausea, vomiting, diarrhea) and fatigue. Treatment with PF-02341066 resulted in dramatic clinical activity against tumors carrying activating ALK gene fusions or MET alterations. Within the cohort of NSCLC patients an overall response rate of 53% (10/19 patients) was seen and the disease control rate at 8 weeks was found to be 79% (15/19 patients). Due to these encouraging results several phase II/III studies (recommended dose: 250 mg bid) in NSCLC patients with PF-02341066 either as monotherapy or in combination with EGF-R inhibitors are currently underway.

A putative treatment algorithm for EGF-R blocking agents is shown in Fig. 4.

### 3.3. VEGF-R Inhibitors

VEGF is the prototype of a large family of angiogenic and lymphangiogenic growth factors, which includes six structurally homologous, secreted glycoproteins called VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placenta growth factor [78]. VEGF-A (commonly referred to as VEGF) was the first such molecule to be identified by the virtue of its ability to induce vascular permeability. The VEGF ligands trigger biological effects on their interaction with specific cell-surface receptors. The diversity of these receptors also adds to the biological complexity of angiogenesis and lymphangiogenesis. Two receptors were originally identified on vascular endothelial cells: VEGF-R-1 (a 180-kD transmembrane protein, also called Flt-1) and VEGF-R-2 (a 200-kD transmembrane protein, also called KDR). A third structurally related tyrosine kinase receptor is the 180-kD VEGF-R-3 (also called Flt-4), which is expressed broadly on endothelial cells during early embryogenesis [79]. VEGF-R-2 is expressed in most, if not all, adult vascular endothelial cells as well as on circulating endothelial progenitor cells. Interestingly, both epithelial and mesenchymal tumor cells more typically express VEGF-R-1 than VEGF-R-2; however, in several experimental tumor models tumor cell-specific VEGF-R-2 expression has been shown to be the critical driver in the pathogenesis of tumors. VEGF binding induces conformational changes within VEGF-R-2 followed by receptor dimerization and autophosphorylation of tyrosine residues in the intracellular kinase domain [80].

#### 3.3.1. Cediranib

Cediranib (Recentin®, AstraZeneca, UK, Fig. 3) is an oral, highly potent inhibitor of VEGF signaling that inhibits all known VEGF-R tyrosine kinases (VEGFR-1, -2 [KDR] and -3); in preclinical studies it has been shown to inhibit VEGF-induced angiogenesis, neo-vascular survival, and growth of human tumor xenografts [81]. VEGF is overexpressed in NSCLC, so makes it a logical therapeutic target. In a phase I study in NSCLC cediranib showed that when combined with PC there was noticeable antitumor activity with only a manageable increase in side effects [82,83]. There have also been a number of phase II studies; however, the BR24 phase II/III study of cediranib 30 mg in first-line NSCLC did not continue into phase II following the planned end of phase II efficacy and tolerability analysis. Although there was evidence of clinical activity, there were a number of adverse events that were more frequent in the cediranib group of patients; namely diarrhea, dehydration, hand-foot syndrome, hypertension and neutropenia. At least one dose reduction to 20 mg was required in 37% of the patients receiving cediranib compared to only 6% in the control group. Consequently, a decision was taken to halt the trial, to allow a re-evaluation of dose and toxicity for this combination. As a result of this re-evaluation, research is still on-going with cediranib in NSCLC as some of the side effects were considered to have been dose related and may be avoided by a reduced dose. In 2009 the National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) has commenced a phase III trial of cediranib in advanced NSCLC comparing 20 mg cediranib plus PC chemotherapy vs. chemotherapy alone (BR29).

#### 3.3.2. Vandetanib

Vandetanib (ZD6474, AstraZeneca, UK, Fig. 3) is a multi-target TKI that selectively targets VEGF-R, EGF-R and RET tyrosine kinase activity. While RET kinase is of specific relevance in hereditary medullary thyroid cancer, where vandetanib has shown very promising results in phase II [84] and is currently undergoing phase III trials, vandetanib is also being developed in NSCLC. Preclinical data have shown that vandetanib inhibits VEGF-R and EGF-R in tumor models at doses equivalent to both monotherapy (300 mg) and combination (100 mg) doses. Results from phase II studies have led to the use of two doses when used as monotherapy and when in combination with chemotherapy; the MTD, 300 mg, when used as monotherapy and the 100 mg dose when used in combination with cytotoxic agents. A recent phase II study suggested that the higher dose when used with docetaxel might be less effective than the lower dose [85]. This phase II study led to the design of the ZODIAC phase III study of vandetanib with docetaxel. This was a randomized, double-blind, placebo-controlled study evaluating

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**Fig. 4.** Putative treatment algorithms for EGF-R blocking agents. Bevacizumab (a VEGF blocking agent) has been included since it is approved for first-line therapy. Patients with EGF-R mutations at progression to EGF-R TKIs should be treated within clinical trials based on the pre-dominant resistance mechanism (T790 M mutation? c-MET amplification? IGF-1R overexpression?). *Non-squamous histology.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Treatment Algorithm</th>
</tr>
</thead>
<tbody>
<tr>
<td>No EGF-R</td>
<td>Chemotherapy, or Gefitinib or Erlotinib</td>
</tr>
<tr>
<td>EGF-R</td>
<td>Resistance, ±Chemotherapy ±Chemotherapy ±IGF-1R Inhibitor ±Cetuximab ±Bevacizumab</td>
</tr>
<tr>
<td>Resistance</td>
<td>±Chemotherapy ±Chemotherapy ±IGF-1R Inhibitor ±Cetuximab ±Bevacizumab</td>
</tr>
</tbody>
</table>

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**Diagram:**

- **NSCLC Stage III B/IV**
  - **EGF-R Mutation**
    - Chemotherapy or Gefitinib or Erlotinib
    - Resistance
    - ±Chemotherapy ±IGF-1R Inhibitor ±Cetuximab ±Bevacizumab
  - ±Chemotherapy ±IGF-1R Inhibitor ±Cetuximab ±Bevacizumab
the combination of vandetanib 100 mg once daily plus docetaxel vs. docetaxel alone in patients with locally advanced or metastatic NSCLC, treated with one prior anti-cancer therapy. It enrolled 1391 patients at 250 centres throughout Europe, North America, South America and Asia Pacific. This study recently reported that the addition of vandetanib increased PFS by 26%, meeting its primary endpoint (4 months vs. 3.2 months, HR 0.79; 95% CI 0.70–0.90; p < 0.001). There was also evidence that the addition of vandetanib controlled lung cancer symptoms longer than chemotherapy alone thus improving the quality of life for these patients [86]. In the ZEAL study vandetanib (100 mg) was combined with pemetrexed. This study was similar in outcomes to the ZODIAC study, however study did not meet the primary endpoint of prolongation of PFS (median PFS 17.6 weeks vs. 11.9 weeks, HR 0.86, p = 0.108). The lack of statistical significance may be due to the smaller size of the ZEAL trial (N = 534) [87]. Both studies showed a trend to increased overall survival in the vandetanib-treated groups; however this too did not reach statistical significance. Vandetanib was well-tolerated with the side effects known from both EGF-R and VEGF-R kinase inhibitors; rash, diarrhoea and hypertension. Two large phase III trials of vandetanib 300 mg as a monotherapy were conducted; ZEST, which compared it to erlotinib, and ZEPHYR, where it was compared to BSC. The ZEST study showed that vandetanib did not significantly prolong PFS compared to erlotinib, however, a pre-planned non-inferiority analysis demonstrated similar efficacy to erlotinib in both, PFS and OS [88]. Thus it remains an open question as to where multi-kinase inhibitors fit into the armamentarium vs. the “single-action” kinase inhibitors. The ZEPHYR study might help answer this as it includes patients who have previously failed on EGF-R inhibitors. The ZEPHYR study meanwhile had completed recruitment but had yet to report. Another area where vandetanib could be of interest is in combination with radiotherapy. Preclinical models suggest that it may provide significant clinical efficacy as the anti-VEGF activity is thought to inhibit the efficacy of radiotherapy [89].

3.3.3. BMS-690514

BMS-690514 (Bristol-Myers Squibb, USA, Fig. 3) is an oral selective inhibitor of EGF-R, HER-2, VEGF-R1-3 and Flt-3. Since preclinical and clinical data (phase I/II trials) support combination inhibition of the VEGF-R and the EGF-R pathways in solid tumors (reviewed by [90]), BMS-690514 was also assessed in patients with advanced NSCLCs. Previous data from phase I studies have shown that BMS-690514 is generally well tolerated and demonstrated encouraging evidence of antitumor activity and disease control in NSCLC patients [91]. In a most recently published study [92], erlotinib-naïve and erlotinib-resistant patients with advanced or metastatic NSCLC were treated with BMS-690514 (200 mg/day, until disease progression or toxicity). For 60 patients treated, disease-control rates were 39% and 22% for erlotinib-naïve and erlotinib-resistant patients, respectively. The disease-control rate was significantly higher among patients harboring an EGF-R mutation than those with wild-type EGF-R. Most frequent treatment-related toxicities were diarrhea (90%), skin rash (31%), asthenia (29%), anorexia (27%), and hypertension (26%). Results from this study suggested that BMS-690514 is active in NSCLC patients, including erlotinib-resistant and those with wild-type EGF-R, EGF-R T790 M or K-ras mutations. A double-blind, randomized, parallel 2-arm phase II trial of BMS-690514 vs. erlotinib in previously treated NSCLC patients is currently recruiting patients, primary endpoint is PFS.

3.3.4. BIBF-1120

Although VEGF-(R) inhibition has made a significant impact on cancer treatment, preclinical findings suggest that long-term clinical outcome may improve with blockade of additional pro-angiogenic RTKs: platelet-derived growth factor receptors (PDGF-R) and fibroblast growth factor receptors (FGF-R). The indolone derivative BIBF-1120 (Vargatef®), Boehringer Ingelheim, Germany, Fig. 3) is an oral triple TKI of VEGF-R, PDGF-R, and FGF-R (IC50: 20–100 mmol/l) [93]. The ability of BIBF-1120 to inhibit FGF-R is of great interest since several lines of evidence have demonstrated that human tumors are able to develop resistance against VEGF signalling blockade by switching from VEGF secretion to alternative ligands, notably bFGF (reviewed by [29]). The FGF-R inhibition property of BIBF-1120 may therefore provide the opportunity to address these types of resistance suggesting that BIBF-1120 may be effective where agents inhibiting only VEGF-(R) are no longer effective. In all preclinical models tested so far BIBF-1120 has shown significant tumor growth inhibition either as monotherapy or in combination with different cytotoxic drugs such as pemetrexed or docetaxel [93]. In phase 1 studies, BIBF-1120 was well tolerated at a dose of 200 mg twice daily when given in combination with pemetrexed or PC in NSCLC patients [94]. Adverse events including nausea, vomiting and diarrhea were mostly mild to moderate (safety profile based on more than 500 cancer patients treated with BIF-1120) [72]. Results from a phase II study with 73 patients with locally advanced or metastatic NSCLC provided evidence that BIBF-1120 is active in this patient population [95]. Of particular note were results from a subset of 57 patients with good performance status (ECOG 1–2); these patients experienced longer OS (median OS was 9.5 months), longer PFS (2.9 months), and a higher stable disease rate of 59% compared with the overall study population. Based on these encouraging phase II results an extensive phase III trial programme for BIBF-1120 has been conducted (LUME-Lung Studies, Table 3) with the objective to assess BIBF-1120 in combination with standard chemotherapy (pemetrexed and docetaxel) in patients with advanced NSCLC.

3.3.5. Sunitinib

Sunitinib malate (Sutent®, Pfizer, USA, Fig. 3) is an oral, multitargeted TKI with antiangiogenic and antitumor activities. It inhibits VEGF-R-1–3, PDGF-R-α, PDGF-R-β, KIT, RET and Flt-3 and is approved for treatment of renal cancer and gastrointestinal stroma cell tumors. Its role for treatment of NSCLC patients has recently been reviewed in 3 excellent papers [96–98]. Briefly, in NSCLC sunitinib was evaluated in a phase II clinical trial where 63 patients with advanced NSCLC who failed platinum-based chemotherapy were treated with sunitinib (50 mg/day) for 4 weeks followed by 2 weeks of no treatment for each 6-week cycle. Seven patients achieved a
partial response, and 18 patients had stable disease. The median PFS was 12.0 weeks (95% CI, 10–16.1 weeks), and the median OS was 23.4 weeks (95% CI, 17–28.3 weeks). The 1-year survival rate was 20.2% [99]. Grade 3 or 4 adverse events included fatigue/asthenia (29%), pain/myalgia (17%), dyspnea (11%), and nausea/vomiting (10%). Three hemorrhage related deaths were reported among the 63 total participants. Two of the hemorrhage-related deaths were attributed to sunitinib, and both resulted in pulmonary hemorrhage [99]. A second phase II trial with the same inclusion criteria was designed to evaluate a continuous dosing schedule for sunitinib. In this trial sunitinib was given 37.5 mg/day orally. 47 patients were accrued and evaluated with a median duration of therapy of 92 days (range 12–336 days). A response rate of 2.1% (95% CI, 0.1–11.1) with a 19.1% rate of disease stabilization was reported. The median TTP was 12.3 weeks (95% CI, 8.9–16 weeks), and the median survival time was 38.1 weeks (95% CI, 31.1 to unavailable) [100]. Although these trials cannot be directly compared since they were performed in a sequential fashion, both dosing schedules showed activity of sunitinib in NSCLC. There are several ongoing phase I/II clinical studies in NSCLC incorporating sunitinib. One is a phase III trial of the Cancer and Leukemia Group B (CALGB) evaluating the use of maintenance sunitinib compared with placebo in patients with advanced stage IIIB or stage IV NSCLC who have non-progression disease after 4 cycles of platinum-based chemotherapy. The primary endpoint is PFS. This study is currently recruiting patients. Furthermore, the efficacy of sunitinib in NSCLC is currently investigated in the SUN study programme [101]. In 2007, a multicentre, randomized, double-blind, placebo-controlled phase III, efficacy and safety study of sunitinib in patients with advanced/metastatic NSCLC treated with erlotinib was conducted (N = 956, SUN 1087) to test whether treatment with erlotinib plus sunitinib is better than erlotinib alone in patients with advanced/metastatic NSCLC who have received previous treatment with a platinum-based regimen. The study is ongoing, recruitment is completed (primary endpoint: OS). Another study of this programme is SUN 1058, a randomized, double-blind, phase II study of erlotinib with or without sunitinib in the treatment of metastatic NSCLC (N = 155). This study is ongoing, but enrollment is closed (primary endpoint: radiographic progression of disease). In addition, in a large phase II programme sunitinib is currently being evaluated in combination with a number of standard regimens commonly used in NSCLC as well as a maintenance drug after first-line platinum-based treatment of advanced NSCLC. Results of these trials are eagerly awaited and will help to define the role of sunitinib in the therapeutic approach to NSCLC.

3.3.6. Sorafenib
Sorafenib (Nexavar®, Bayer, Germany, Fig. 3) is an oral multi-kinase inhibitor that targets RAF, VEGF-R-2, and VEGF-R-3. The drug is approved for treatment of renal cancer and hepatocellular carcinoma. In a phase II trial that evaluated 54 patients with relapsed or refractory NSCLC approximately 60% of patient achieved disease stabilization [102]. When sorafenib was combined with PC in 15 patients with advanced, progressive NSCLC the disease control rate (objective response plus stable disease) was 79%. The duration of response was 25 weeks, and the median PFS was 34 weeks [103]. One small phase II trial employed sorafenib alone in 25 patients with chemo-naive stage IIIB (wet) or stage IV patients. Three patients had a partial response and 7 patients had stable disease. PFS and medium survival was 2.9 and 8.8 months, respectively [104]. The phase III ESCAPE trial (Evaluation of Sorafenib, Carboplatin and Paclitaxel Efficacy) that evaluated sorafenib with PC in patients with NSCLC was stopped early when a planned interim analysis concluded that the study would not meet its primary endpoint of improved OS. A higher mortality was observed in the subset of patients with squamous cell carcinoma who received sorafenib and chemotherapy compared with those that only received chemotherapy. Currently, sorafenib treatment for NSCLC is being evaluated in several phase III studies. Two randomized controlled trials comparing safety and efficacy of carboplatin and paclitaxel plus or minus sorafenib in chemotherapy-naive patients with stage IIIB/IV NSCLC have just been completed (N = 1200, primary endpoint is OS). Results have not yet been published. A phase III trial comparing the efficacy of gemcitabine, cisplatin and sorafenib to gemcitabine, cisplatin and placebo in first-Line treatment of patients with stage IIIB/IV NSCLC (NCT00449033) is ongoing, but recruitment is completed (NexUS: NSCLC Research Experience Utilizing Sorafenib). Primary endpoint again is OS (N = 907). In addition, several phase II studies are open for enrollment, details are provided by www.clinicaltrial.gov/search.

3.3.7. Motesanib
Motesanib (Amgen, Takeda, Millenium, USA, Fig. 3) is a highly selective, oral agent that is being evaluated for its ability to inhibit angiogenesis by targeting VEGF-R-1/3 [105]. It is also under investigation for its potential direct antitumor activity by targeting PDGF-R and c-KIT. The efficacy and safety of motesanib is currently being explored in a phase III trial evaluating motesanib in combination with PC for first-line treatment of NSCLC (Motesanib NSCLC Efficacy and Tolerability Study; MONET 1). The primary endpoint of MONET 1 is OS, and secondary endpoints include PFS, objective response rate in patients with measurable disease, duration of response, and safety. The Independent Data Monitoring Committee (IDMC) for the MONET 1 trial has recently recommended the trial resume enrollment of patients with non-squamous NSCLC following a three-month enrollment suspension. Non-squamous NSCLC is a histological subtype of NSCLC representing approximately two-thirds of the study population.

4. Miscellaneous
4.1. mTOR inhibitors
The mammalian target of rapamycin (mTOR) is a serine-threonine kinase that functions as a central regulator of multiple signalling pathways that control cell growth, division, metabolism, and angiogenesis [106,107]. Clinical trials are ongoing with rapamycin and its analogs temsirolimus (torisel, Pfizer, formerly Wyeth, USA), everolimus (RAD001, Novartis, Switzerland) and deforolimus (Ariad Pharmaceuticals, USA) in various tumor types. Although mTOR plays a central role in many biologic processes, rapalogs have been generally well tolerated. Toxicities have included asthenia, mucositis, nausea, cutaneous toxicity, diarrhea, hypertriglyceridermia, thrombocytopenia, hypercholesterolemia, elevated transaminases, hyperglycemia, and pneumonitis [108]. Toxicity was more common with higher doses in some studies [109]. These adverse events were transient and reversible with interruption of dosing. Several phase I/II studies have shown that mTOR inhibitors induce tumor regressions and prolonged stable disease in a variety of malignancies including NSCLC [110]. In vitro and in vivo studies with everolimus (an orally available macrolide) has been done in NSCLC cell lines and murine lung cancer models that have helped assess their effect on cell signalling pathways and growth. In addition, the combination of erlotinib (or gefitinib) plus everolimus has also been studied in lung cancer xenografts. These agents can have additive or synergistic interactions. Recently, La Monica et al. [111] have provided evidence that everolimus could restore gefitinib sensitivity in resistant NSCLC cell lines in vitro suggesting that combination treatment might be of value in refractory NSCLC patients. Based on these data several phase I/II trials have
been conducted [112]. Clinical evidence for the efficacy of the combi-
nation came from a phase I study [113]. This study was conducted of the combination of everolimus and gefitinib to determine a daily
dose of everolimus with gefitinib in patients with advanced NSCLC.
Ten patients were enrolled and the MTD of everolimus was found to
be 5 mg when administered daily with gefitinib (250 mg). A similar
phase I study (23 patients) has been published for the combina-
tion of everolimus in combination with docetaxel in recurrent or
refractory NSCLC [114]. The recommended doses of docetaxel were
60 mg/m² and 10 mg everolimus. It is worth to note that promising
antitumor activity was found with clinical benefit in 55% of treated
patients. In a most recently published phase II study [115], 85 pre-
viously pretreated (platinum-based chemotherapy alone [N = 42]
or in combination with EGFR inhibitors [N = 43]) patients with
relapsed NSCLC received everolimus (10 mg/day) until progression or
unacceptable toxicity. Overall response rates (primary objective)
were 7.1% and 2.3%, respectively. Overall disease control rate was
47.1%, median PFS was 2.6 and 2.7 months, respectively. Based on
these data further studies of everolimus plus standard chemother-
apy for metastatic NSCLC are underway (e.g., phase II/I study with
everolimus plus docetaxel in relapsed NSCLC). In the meantime a
new generation of mTOR inhibitors is being developed. In contrast
to rapalogs, catalytic site inhibitors of mTOR inhibit both mTORC1
and mTORC2, and inhibition of mTORC2 will affect the activation
of Akt. Agents such as BEZ235 (Novartis, USA) and EX147 (Exelixis,
USA) are dual PI3K/mTOR inhibitors and thus may bypass feedback
loops, potentially increasing their efficacy compared with rapalogs.
The tolerability and efficacy of these agents are currently being
tested in clinical trials.

4.2. HDAC inhibitors

Histone deacetylases (HDACs) regulate the acetylation status of
histones at prominent amino-terminal lysine residues, and
their inhibitors have recently inspired great interest in the cancer
research community as a possible treatment option for solid and
hematologic tumors. Currently available HDAC inhibitors (HDIs)
fall into six structural classes, and suberoylanilide hydroxamic acid
(SAH A) is a prototype of the hydroxamate class that inhibits class
I and class II HDAC enzymes with similar potency [116]. The basic
concept is that inhibition of HDAC enzymes repress gene expres-
sion by inducing the hyperacetylation of core histone proteins,
and indeed, many studies have shown altered gene expression
upon treatment of tumor cells with HDIs. Nevertheless, over the
last few years, it has also become clear that HDIs not only cause
a change in the histone acetylation status, but are also able to
influence the acetylation status of a number of other proteins
important for tumor formation and proliferation, such as p53, α-
tubulin, nuclear receptors, HSP90, signal transducer and activator
of transcription family members, such as STAT3, and subunits of
nuclear factor-κB (NF-κB) [117]. Clinical experience has indicated
that these agents are generally well tolerated, and active in several
haematological and solid tumors [118]. The most common drug-
related adverse events were anemia (81%), fatigue (62%), nausea
(62%), diarrhea (56%), vomiting (50%) and thrombocytopenia (50%)
[119]. HDAC inhibitors under clinical evaluation in the treatment
of NSCLC patients are pivanex (Titan Pharmaceuticals, USA), CI-994
(Pfizer, USA), vorinostat (Zolinza®, Merck & Co, USA), and panobio-
nostat (LBH589) (Novartis, Switzerland). Amongst them vorinostat
is the most advanced HDAC inhibitor under clinical development
for NSCLC. Since the drug has demonstrated activity in patients
with advanced solid tumors in phase I trials, vorinostat was tested
as single agent in patients with relapsed NSCLC [120]. A total of
16 patients were treated with vorinostat (400 mg). No objective
antitumor activity of vorinostat was detected in this setting,
however, it yields TTP in relapsed NSCLC similar to that of other
targeted agents. In order to combine vorinostat with other anti-tu-
mor agents, a randomized, double-blind, placebo-controlled phase
II study of carobaplatin (AUC 6 and paclitaxel (200 mg/m²) with or
without vorinostat (400 mg QD on days 1–14 of each 3-week cycle)
for first-line treatment of advanced NSCLC patients has been con-
ducted [121]. A total of 94 patients (stage IIIb/IV) were enrolled (2:1
randomization). The confirmed response rate was superior with
vorinostat over placebo (34% vs. 12.5%, p = 0.02), and at the time of
analysis, the preliminary median PFS for vorinostat and placebo
were 5.75 and 4.1 months, respectively. From this study it was
concluded that vorinostat significantly enhances chemotherapy in
NSCLC patients, a finding which merits additional clinical evalua-
tion to further clarify the role of HDAC inhibitors in the treatment
landscape for NSCLC.

4.3. Integrin inhibitors

Integrins are heterodimer transmembrane receptors for the
extracellular matrix composed of α and β subunit. Natural
integrin ligands include laminin, fibronectin, and vitronectin,
but they also include fibrinogen and fibrin, thrombospondin,
MMP-2, and FGF-2. Integrins bind ligands by recognising
short amino acid stretches on exposed loops, particularly the
arginine–glycine–aspartic acid (RGD) sequence. On ligation, inte-
grins mediate complex signalling events, alone or in combination
with growth factor receptors, regulating cell adhesion, prolifer-
ation, survival, and migration by activating canonical pathways,
such as integrin-linked kinase (ILK), protein kinase B (PKB/Akt),
mitogen-activated protein kinase (MAPK), Rac or nuclear factor
kappa B (NF-κB). In resting vessels, integrins interact with the
basal membrane, thereby maintaining vascular quiescence. During
angiogenesis, they are essential for endothelial cell migration,
proliferation, and survival [122]. In preclinical studies, pharmacologic
inhibition of integrin function efficiently suppressed angiogene-
sis and inhibited tumor progression. Of the 24 known integrin
heterodimers, αvβ3 and αvβ5 were the first vascular integrins
targeted to suppress tumor angiogenesis. These encouraging pre-
clinical results stimulated researchers and industry to develop
pharmacologic inhibitors of integrin function for clinical testing.
Three classes of integrin inhibitors are currently in preclinical and
clinical development: monoclonal antibodies targeting the extra-
cellular domain of the heterodimer (e.g., vitaxin; MedImmune,
USA), synthetic peptides containing an RGD sequence (e.g., cilen-
gidite; Merck, Germany), and peptidomimetics (e.g., S247; Pfizer,
USA), which are orally bioavailable nonpeptidic molecules mim-
icking the RGD sequence [122]. Amongst them, cilengitide (EMD
121974) is the most advanced integrin inhibitor in clinical de-
velopment. It is a cyclic RGD-motif containing peptide binding with
high specificity to the αvβ3 and αvβ5 receptors [123]. As shown in
several phase I studies, cilengitide was well tolerated with
occasional patients experiencing joint and bone pain. Thrombosis,
thrombocytopenia, electrolyte imbalances, and anorexia were also
reported [124]. In a recently published randomized phase II study
140 patients with relapsed stage IV NSCLC received 1 of 3 cilen-
gidite doses (240 mg/m², N = 35; 400 mg/m², N = 35; 600 mg/m²,
N = 36) twice weekly or docetaxel (75 mg/m², N = 34) once every
3-week cycle for 6 months as second-line treatment. Respond-
ing patients could continue cilengitide for up to 1 year [125], PFS
(primary endpoint) in the docetaxel group was higher than that of
cilengitide at all doses. However, cilengitide monotherapy at a
dose of 600 mg/m² showed similar OS to docetaxel (181 days vs.
194 days, 1-year-survival rate 29% vs. 27%) and better tolerabil-
ity. These results are intriguing for several reasons. Despite a wide
range of dosages, no clear pattern of toxicity could be determined,
thus, the optimal dose of cilengitide to be carried forward for future
studies in NSCLC patients has not yet been established. Further-
more, it is far from being clear whether or not the target itself (αvβ3 and αvβ5) is a good one for NSCLC. Antiangiogenic drugs are unlikely to function as single agents, and preclinical evidence shows that integrin inhibitors may be most effective in combination with chemotherapy agents and in particular with radiotherapy [126]. The rationale for combining cilengitide with radiotherapy is further strengthened by the observation that αvβ3 expression in endothelial cells is upregulated with radiation [127]. To address these issues a phase I study (cilengitide in combination with whole brain radiotherapy in patients with brain metastases from NSCLC) is currently recruiting patients. In addition, another study aims to determine the MTD of cilengitide in combination with cetuximab, and platinum-based chemotherapy (cisplatin/vinorelbine or cisplatin/gemcitabine) in NSCLC patients. This study is open for recruitment.

4.4. RANKL inhibitors

Skeletal metastases affect as much as 65% of patients with advanced lung cancer and may lead to serious clinical complications such as severe bone pain, pathologic fractures, hypercalcaemia, cytopenias, and neurological impairment due to spinal cord compression. The pathogenesis of skeletal metastases (i.e., colonisation and expansion of tumor cells into bone) has been predicated on the ‘seed and soil’ hypothesis, which posits a reciprocal interaction between tumor cells and the bone microenvironment, in particular the osteoclastic and osteoblastic lineage cells [128]. This interaction manifests itself in excessive and uncoupled bone remodelling at the affected site characterized by increased osteoclast and osteoblast activity, which subsequently leads to the development of metastatic bone lesions with varying degree of bone resorption and/or bone formation. Recent research has identified the essential cytokine system for the regulation of bone remodelling in health and in disease [129]. This system consists of a ligand, a member of the tumor necrosis factor (TNF) ligand superfamily member called RANK ligand (RANKL) mainly produced by osteoblastic lineage cells and some tumor cells [130], a cellular receptor called RANK that is expressed on the surfaces of osteoclasts and osteoclast precursor cells, and a soluble decoy receptor, osteoprotegerin (OPG) predominantly produced by osteoblastic lineage cells. RANKL is essential for osteoclast formation, function, and survival, thus enhances bone resorption. OPG blocks RANKL, and prevents bone resorption. Preclinical research using animal models confirmed the essential roles of RANK, RANKL, and OPG in regulating bone remodelling through osteoclast-mediated bone resorption. Deletion of the RANK or RANKL gene in mice resulted in severe osteopetrosis and lack of osteoclast formation [131]. In contrast, deletion of the OPG gene in mice resulted in severe osteopenia and increased osteoclastogenesis [132]. Conversely, OPG overexpression in mice caused increased bone mass and reduced osteoclast count. Bone remodelling is thought to be governed by the relative balance between RANKL and OPG under physiologic conditions. Under pathologic settings, however, such as osteoporosis, rheumatoid arthritis, multiple myeloma, and cancer-induced bone metastases, this delicate balance is perturbed in favour of RANKL, resulting in enhanced osteoclastogenesis, osteoclast hyperactivity and subsequent osteolytic bone disease. In turn, increased osteoclast-mediated bone resorption releases growth factors (e.g., TGF-β, IGF-1, bone morphogenetic proteins [BMPs]) that further attract tumor cells and stimulate their growth, thereby propagating a ‘vicious cycle’ of bone resorption and tumor expansion. Recent evidence also suggests that soluble RANKL may act as a chemottractant that promotes migration of RANK-expressing tumor cells to bone. Furthermore, RANKL-induced osteoclastogenesis not only mediates osteolytic bone disease, but also contributes to the pathogenesis of osteoblastic bone disease resulting from tumors (Fig. 5).

Given the central role of RANKL in the development of bone metastases in several solid tumors including breast, prostate, and lung cancer and in the pathophysiology of cancer cell growth and survival, targeted inhibition of the RANKL/RANK/OPG pathway may prove to be a novel and promising therapeutic approach. Lung cancer metastases to bone lead to mixed osteolytic/osteoblastic lesions. In a study by Feeley et al. [133], RANKL inhibition by RANK-Fc in mice that underwent subcutaneous injection of an NSCLC cell line, which produces mixed osteolytic/osteoblastic bone metastases, resulted in a reduction in tumor volume in bone and inhibition of osteoclastogenesis. In addition, early studies in animal models have shown that RANKL inhibition resulted in marked suppression of bone resorption and increases in bone mass and bone strength. In another study by Tometsko et al. [134], expression of RANK mRNA was significantly higher in human lung tumors than in normal lung tissue. RANK mRNA was slightly elevated in lung tumors compared to normal tissue. Furthermore, a human lung cancer cell line that had the highest level of RANK mRNA also expressed RANK on the cell surface. Treatment of these cells with RANKL led to activation of downstream signalling pathways and upregulation of IL-8, a factor associated with cell migration and tumor metastasis to bone. These results suggest that RANK is expressed in human lung tumors and is functional on human lung cancer cells [135]. In another animal model of metastatic bone disease caused by non-small-cell lung cancer, RANKL inhibition by OPG Fc completely blocked osteoclastogenesis, osteolytic lesions, and skeletal tumor burden induced by either RANK-expressing or RANK-negative lung cancer cell lines. These results demonstrate that, irrespective of RANK expression on tumor cells, RANKL is an essential regulator of tumor-induced osteolytic lesions caused by lung cancer. RANKL inhibition with OPG Fc was tested in a
phase I trial in patients with multiple myeloma or breast cancer who exhibited radiographically confirmed bone lesions. The study found that OPG Fc was effective in reducing bone resorption similar to that observed after intravenous pamidronate treatment [136]. However, the long-term use of OPG Fc in humans may not be feasible due to the potential risk of developing neutralizing antibodies against endogenous OPG and the relatively short half-life of OPG Fc in cancer patients. Given the limitations of OPG Fc, denosumab, a novel RANKL inhibitor was developed as a potential treatment for cancer-induced bone disease. Denosumab (Amgen, USA), a fully human monoclonal anti-RANKL antibody with high affinity and specificity for human RANKL, has been shown to bind to RANKL in a manner similar to that of native OPG, and inhibited the interaction of RANKL and RANK. This in turn may reduce the differentiation, activity, and survival of osteoclasts and may inhibit osteoclast-mediated bone resorption. Denosumab is currently being investigated in a large clinical programme involving more than 10,000 patients with lung cancer, prostate cancer, breast cancer, multiple myeloma, and giant cell tumor of the bone. Preliminary data from these clinical trials have confirmed that denosumab treatment significantly suppressed bone turnover markers, increased bone mineral density, inhibited bone loss, and reduced the risk of skeletal related events [137]. These results are very promising and have the potential to translate into improved treatment options in the management of skeletal metastases in patients suffering from a wide variety of tumors including NSCLC.

4.5. Proteasome inhibitors

Bortezomib (Velcade®, Millennium Pharmaceuticals, USA) is a dipeptidyl boronic acid that functions as a specific and selective reversible inhibitor of the 26S proteasome. Inhibition of the proteasome results in disruption of protein homeostasis that adversely affects cell signalling cascades [138]. Although bortezomib has shown its greatest benefit in the treatment of refractory multiple myeloma, it targets many key cell cycle regulators that are relevant to tumor progression and therapy resistance in lung cancer. In a phase II trial of bortezomib alone and in combination with docetaxel in 155 previously treated patients with advanced NSCLC the 1-year OS was modestly improved in the combined therapy arm, 39% vs. 33% [139]. The most common adverse effects of bortezomib include peripheral neuropathy, transient thrombocytopenia, and gastrointestinal disorders (nausea, diarrhea, and constipation). A SWOG phase II study (S0339) evaluated 114 patients with chemotherapy-naive stage IIIB and stage IV disease. Patients received gemcitabine/carboplatin with bortezomib. One-year and 3-year survival rates were 47% and 19%, respectively. The overall disease control rate was 68%. PFS and median OS were 5 and 11 months, respectively [140]. Based on this trial, a phase III trial is planned. Inhibition of the proteasome with bortezomib is a novel approach to the treatment of lung cancer. Single-agent responses are modest, yet at 8% are in keeping with other approved second-line therapies. Although bortezomib has shown some antitumor activity alone in lung cancer, it is likely to have its greatest clinical benefit when given in combination with other therapeutics. In support of this, a most recently published phase II study demonstrated that bortezomib as first-line monotherapy in metastatic NSCLC patients is not active. Although well tolerated, the study was terminated after the first 14 patients have been enrolled [141].

The phase II SWOG study of bortezomib in combination with gemcitabine/carboplatin yielded promising PFS and OS rates; however, a phase III trial is required to confirm these findings. Although the preclinical rationale to proceed with the development of bortezomib was strong, with the plethora of new agents being investigated in lung cancer, it is hard to predict where bortezomib will fit into the therapeutic landscape. Results from ongoing studies of erlotinib/bortezomib in combination and bortezomib in NSCLC will shed some light on the potential therapeutic roles for bortezomib.

4.6. Polo-like kinase inhibitors

Targeting mitosis is an established approach for cancer therapy, and taxanes as well as Vinca alkaloids are currently used to treat a broad variety of cancer types including NSCLC. However, these drugs have significant limitations as they are ineffective for many cancer types and patients suffer from toxicities related to the broad requirement for microtubule function in critical cellular processes unrelated to mitosis. New mitosis-specific targets for cancer therapy have emerged in recent years, including the mitotic kinesins and the Polo-like kinases (PLKs) and Aurora families of serine/threonine kinases, and a first wave of inhibitors are currently under evaluation in early clinical trials [142]. PLKs are a group of highly conserved serine/threonine protein kinases that play a key role in processes such as cell division and checkpoint regulation of mitosis. In mammals, four members of the family have been identified (i.e., PLK1, PLK2, PLK3, and PLK4) [143]. Of the mammalian PLK family members, PLK1 is the most extensively characterized. PLK1 controls critical steps in the passage of cells through the M phase of the cell cycle, including initiation of entry into mitosis, centrosome maturation and separation necessary for the formation of a bipolar mitotic spindle, metaphase to anaphase transition and mitotic exit, and onset of cell division. About 80% of human tumors, of various origins, express high levels of PLK transcripts. However, PLK mRNA is mostly absent in surrounding healthy tissues making them an attractive, selective target for cancer drug development [144]. Several PLK inhibitors are in preclinical development and some of them (HMN-214, Nippon Shinyaku, Japan; ON 019190.Na, Onconova Therapeutics, USA; GSK461364, GlaxoSmithKline, UK; BI 2536, BI 6727, Boehringer Ingelheim, Germany) are already undergoing phase I/II evaluations as potential cancer treatments (reviewed by [145]). Amongst them, BI 2536 is the most advanced PLK inhibitor in clinical development. BI 2536, a dihydropteridinone derivative (Fig. 3), is an ATP-competitive kinase inhibitor, and causes perturbation of the spindle assembly, leading to mitotic arrest and subsequent apoptosis. BI 2536 is a highly selective inhibitor of the PLK1 enzyme (IC50: 0.83 nM). Furthermore, PLK1 mRNA was mostly absent in surrounding healthy tissues of erlotinib/bortezomib in combination and bortezomib in NSCLC.

A phase II study in patients with NSCLC has also been completed with BI 2536 as a single agent using different 3-week schedules [147]. In this study patients with relapsed advanced or metastatic NSCLC, the efficacy, safety, and PK of two dosing schedules of BI 2536 were investigated. Patients were randomized to receive BI 2536 either on day 1 (200 mg) or on days 1–3 (3 × 50 mg/3 × 60 mg) of 21-day treatment courses. Clinical benefit (partial response and stable disease) was observed in 54% of the patients. The PFS and OS times were 58 days (95% CI, 48–85) and 189 days (95% CI, 175–304; 47 patients censored), respectively [147]. Grade 4 neutropenia occurred in 36% of patients and two patients died due to sepsis and pulmonary hemorrhage. Other common adverse events, which were generally mild, included fatigue and anorexia. Due to these encouraging results a phase III development programme is underway. The small-molecular inhibitors of PLKs are new and promising agents in the treatment of NSCLCs and their future development in comparative phase III trials will be followed with interest.
4.7. Pro-apoptotic receptor agonists

Apoptosis is a highly regulated physiological process for the removal of damaged or dysfunctional cells and its regulation is commonly disrupted in tumors. Apoptosis is mediated by intracellular cysteine proteases (caspases). Activation of caspases and hence apoptosis occurs via two main pathways: the extrinsic and intrinsic pathways. The extrinsic pathway is activated through cell-surface death receptors (DRs), and the intrinsic pathway is tightly controlled by p53. Inactivation of p53 is one of the most common mutations in cancer and renders tumor cells resistant to standard chemotherapy. The extrinsic pathway triggers apoptosis independently of p53. Therefore, novel pro-apoptotic therapies that directly activate the extrinsic apoptosis pathway have the potential to induce apoptosis, both in cancers that are responsive and in those that have become resistant to conventional therapy.

Apol2/TRA1L (TNF-related apoptosis-inducing ligand) is a member of the tumor necrosis superfamily of cytokines that selectively induces apoptosis in cancer cells by binding to DR4 and DR5. The Apol2/TRA1L pathway directly participates caspases and induces p53-independent cell death through the extrinsic pathway of apoptosis [148]. DR4 and DR5 are expressed in multiple tumor types suggesting that DR4 and DR5 agonists may be broad spectrum anticancer therapies. Several of these agonists (rApol2/TRA1L and conatumumab [Amgen, USA], mapatumumab and lexatumumab [HGS, USA] are currently undergoing clinical evaluation, and one of these, conatumumab, a fully human monoclonal antibody that binds to the extracellular domain of human DR5 and is thought to activate caspases thereby inducing apoptosis selectively in cancer cells, is the most advanced compound for NSCLC. Conatumumab indirectly activates the intrinsic pathway in some cancer cells. Because of its unique mechanism of action, conatumumab has the potential to function either as a single agent or to cooperate with existing anticancer therapies. Conatumumab is currently being studied for its potential as an anticancer agent in a variety of tumors. First results from a first-in-human study of conatumumab in 16 adult patients with advanced solid tumors showed antitumor efficacy including one observed partial response in a patient with NSCLC who experienced a 46% reduction in tumor volume by RECIST [149]. In another study of conatumumab in combination with PC chemotherapy in patients with advanced NSCLC, among the 12 patients evaluated, 1 complete remission, 3 partial responses, 3 stable disease were seen [150]. Phase II/III studies in NSCLC are ongoing.

5. Conclusion

Despite recent advances in NSCLC treatment clinical outcome of these patients still remains a challenge. The search for innovative therapeutic agents in NSCLC that are more effective and have fewer side effects than older chemotherapeutic drugs has spurred the development of more than 500 molecularly targeted agents and thereby has introduced the concept of individualized therapy. In the process of identifying targets for therapy, our understanding of the molecular pathways involved in malignancy has also increased, and this knowledge of mechanisms of tumor cell growth and survival has translated into clinical trials of drugs that have clearly changed the treatment landscape. With a median age at diagnosis of 70 years, many NSCLC patients previously considered ineligible for anti-cancer therapy because of comorbidities and frailty may now receive treatment. Yet despite these apparent advances, however, for most patients with NSCLC targeted therapies have not dramatically changed clinical outcome. The molecular complexity of lung cancer underlies these disappointments and stresses the need for optimizing treatment by seeking a more personalized approach to care. Therefore clinical trials that investigate the activity of novel agents, and incorporate patient selection based on clinical and molecular factors, are required.

Conflict of interest statement

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