The Role of Pemetrexed Combined with Targeted Agents for Non-Small Cell Lung Cancer

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Abstract: Pemetrexed is a novel third-generation multitargeted antifolate agent used in the first- and second-line treatment of unresectable pleural mesothelioma and advanced non-small cell lung cancer (NSCLC). Owing to its mild toxicity, this compound is a preferred partner in the multidrug regimens. In the last few decades, better understanding of molecular oncology and genetics has allowed for the development of an array of molecular targeted agents, many of which have been found active in NSCLC. It has been hoped that these compounds will disrupt tumor signaling pathways complementary to those targeted by chemotherapy. This review outlines the current preclinical and clinical studies using pemetrexed in combination with targeted agents in advanced NSCLC. Clinical experience with the use of these combinations is still limited and mostly includes phase I and II trials. These investigations have mainly focused on compounds previously shown to be active in NSCLC: anti-angiogenic agents (bevacizumab and small molecule tyrosine kinase inhibitors) and inhibitors of epidermal growth factor receptor (cetuximab and erlotinib). Preliminary results have shown the feasibility of these combinations and their promising activity but large phase III studies are warranted to verify the real value of this strategy. Combinations of pemetrexed with other targeted agents, such as mTOR inhibitors and compounds targeting proteasome are still at early stages of development.

Keywords: Non-small cell lung cancer, pemetrexed, targeted therapy, angiogenesis inhibitors, EGFR inhibitors, mTOR inhibitors, agents targeting proteasome.

INTRODUCTION

Lung cancer is one of the most common malignancies and is the leading cause of cancer-related deaths [1, 2]. Non-small cell lung cancer (NSCLC), which includes the histological sub-types of squamous, adeno- and large-cell carcinomas, accounts for over 80% of all lung tumors [3]. Around 65% of NSCLC patients present with locoregionally advanced or metastatic disease [4, 5]. In addition, many patients who undergo surgical resection or definitive radiotherapy will develop distant metastases [6, 7]. Treatment outcome in patients with disseminated NSCLC is particularly grim; median survival rate with best available therapies is merely a few months, and most patients die of tumor within two years of diagnosis [6]. Thus, the management of this group has remained essentially palliative and primarily directed at relieving disease-related symptoms. A series of metaanalyses performed in the 1990s showed that in advanced NSCLC chemotherapy produces longer survival and provides a better quality of life compared with best supportive care [8-11]. In consequence, platinum-based chemotherapy is currently recommended as the first-line treatment of advanced NSCLC patients with good performance status [12, 13]. However, a benefit from chemotherapy is typically transient, almost all patients develop progression within a median of 3–6 months of treatment commencement, and the median survival is in the range of 8-10 months [14, 15]. Currently, a proportion of patients who failed primary chemotherapy are offered second-, or even third-line treatment, however the benefit achieved with these attempts is uncommon and modest. In the general opinion, efficacy of chemotherapy in advanced NSCLC appears to have reached a plateau and finding better therapies is critical to improve survival.

One of the few new active cytotoxic agents that demonstrated activity in this disease is pemetrexed (Alimta, LY231514, Eli Lilly and Company, Indianapolis, IN). Pemetrexed is a third-generation multitargeted antifolate agent that inhibits three enzymes involved in folate metabolism: thymidylate synthase and, to a lesser extent, dihydrofolate reductase and glycinamide ribonucleotide formyltransferase [16]. Pemetrexed is well tolerated and its main toxicities (myelosuppression and mucositis) are alleviated by the vitamin B12 and folate supplementation. This compound has shown its anticancer activity in the first- and second-line treatment of malignant pleural mesothelioma [17, 18]. In advanced NSCLC pemetrexed was initially approved as a second-line chemotherapy, based on a randomized, open-label randomized study that showed similar activity and slightly lower toxicity of this drug compared to single agent docetaxel [17]. In the first-line treatment of patients with locally advanced or metastatic NSCLC, a phase III study showed similar efficacy of pemetrexed-cisplatin and gemcitabine-cisplatin doublets, with slightly better toxicity profile of the former [19]. Additionally, in patients with non-squamous cell histology overall survival (OS) was significantly better in the pemetrexed-cisplatin arm, whereas the squamous cell subtype favored gemcitabine combination. This differential clinical activity of pemetrexed in the various subtypes of NSCLC is most likely due to higher mRNA and protein expression of thymidylate synthase in squamous-cell lung cancer compared with adenocarcinoma. Another phase III study comparing carboplatin and pemetrexed versus carboplatin and gemcitabine in the first-line setting showed similar effi-

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cacy outcomes between both regimens, however patients in the pemetrexed-carboplatin arm experienced fewer cases of myelosuppression [20]. Most recently, maintenance therapy following four cycles of standard platinum doublet has been shown to extend progression-free survival (PFS) and OS in advanced NSCLC [21].

In the last few decades, a better understanding of molecular oncology and genetics has allowed the development of an array of molecular targeted agents, many of which have been found active in NSCLC. It has been hoped that these compounds will disrupt tumor signaling pathways complementary to those targeted by chemotherapy [22]. This review will outline the current clinical experience with pemetrexed combined with targeted agents in advanced NSCLC.

ANGIOGENESIS INHIBITORS

Angiogenesis is a critical feature in the pathogenesis of tumor growth and dissemination. The growth of tumors over a few millimeters in diameter necessitates the establishment of a new blood supply [23]. In tumors, neovascularisation is stimulated through the secretion of pro-angiogenic growth factors, in particular basic fibroblast growth factor and vascular endothelial growth factor (VEGF) [24]. VEGF is expressed in many tumors and plays a key role in *de novo* blood vessel formation [25]. The family of VEGF pro-angiogenic and pro-lymphangiogenic cytokines includes six members: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor. These molecules bind to one of three transmembrane VEGF receptors: VEGFR-1, VEGFR-2 and VEGFR-3 [26]. In NSCLC VEGF is expressed in 42 to 75% of cases and correlates with poor clinical outcome [27-30]. Approaches used to disrupt tumor-induced angiogenesis include monoclonal antibodies against VEGF ligands or their receptors, small-molecule inhibitors of VEGFR tyrosine kinase activity and other strategies. Whereas anti-VEGF or VEGFR therapies alone have only modest activity, their combining with standard cytotoxic therapy that directly targets rapidly proliferating neoplastic cells seems to be promising [31].

BEVACIZUMAB

Bevacizumab (AvastinTM; Genentech [South San Francisco, CA]/Roche [Nutley, NJ]) is a recombinant humanized monoclonal antibody that targets VEGF. Preclinical studies demonstrated synergistic activity of this compound in combination with various cytotoxic agents [32]. A randomized phase II study (AVF0757g) compared bevacizumab, 7.5 mg/kg or 15 mg/kg once every 3 weeks, in combination with carboplatin–paclitaxel (CP) with CP alone [33] in advanced NSCLC. The median time to progression (7.4 *versus* 4.2 months) and response rates (31.5% versus 18.8%) favored the 15-mg/kg bevacizumab cohort in combination with chemotherapy, however this was achieved at the expense of increased incidence of pulmonary hemorrhage, particularly in patients with squamous cell cancer located close to major blood vessels. Based on these results, a subsequent pivotal ECOG 4599 phase III study of bevacizumab, 15 mg/kg every 3 weeks, in combination with 6 cycles of CP as first-line therapy in advanced NSCLC excluded patients with squamous cell histology and brain metastases [34]. Patients who did not develop progression continued with bevacizumab therapy until progression. Addition of bevacizumab resulted in the improvement of response rate (35% *versus* 15%, P< 0.001), median OS (12.3 *versus* 10.3 months, P = 0.003) and PFS (6.2 *versus* 4.5 months, P< 0.001). The most common toxicities of bevacizumab included hypertension, proteinuria and minor bleeding. In another phase III trial (AVAiL) patients with nonsquamous advanced NSCLC without brain metastasis were randomized to 6 cycles of cisplatin and gemcitabine (CG) chemotherapy with or without bevacizumab applied at two doses (7.5 and 15 mg/kg) [35]. PFS, the primary endpoint of this trial, favored both bevacizumab arms (a median of 6.7 months [P=0.002] for the 7.5 mg/kg cohort and 6.5 months [P=0.03] for the 15 mg/kg cohort compared with 6.1 months in the control group. Response rates were also higher at both doses of bevacizumab in combination with chemotherapy (34% and 30% in the 7.5 mg/kg and 15 mg/kg cohorts, respectively, compared with 20% in the chemotherapy alone arm). In this study, the OS benefit did not reach statistical significance and all study endpoints were similar at both doses of bevacizumab. Although these two trials demonstrated the benefit of bevacizumab in addition to first-line chemotherapy for NSCLC, about half of all patients were not eligible because they had either squamous histology, brain metastases or other exclusion factors. Most recently, another phase III study (ATLAS) addressed the safety and efficacy of maintaining bevacizumab (15 mg/kg) with or without erlotinib following first-line CP–bevacizumab [36]. In another ongoing phase III study (POINTBREAK) patients with advanced nonsquamous NSCLC are randomized to pemetrexed/carboplatin/bevacizumab induction chemotherapy followed by pemetrexed/bevacizumab maintenance or an induction with paclitaxel/carboplatin/bevacizumab followed by bevacizumab maintenance. Treatment consists of up to 4 cycles of induction therapy followed by maintenance therapy until disease progression or treatment discontinuation. As opposed to two previous trials, these two studies allowed inclusion of patients with brain metastasis.

A combination of pemetrexed with bevacizumab has been the subject of several phase II studies (Table **1**) [37-44]. A combination of these two agents in the second-line setting resulted in response rate of 10%, PFS of 4.1 months and OS of 8.6 months [37]. The study of Herbst *et al.* [38] randomly assigned 120 patients with nonsquamous NSCLC that had progressed during or after one platinum-based regimen to bevacizumab in combination with chemotherapy, bevacizumab in combination with erlotinib, or chemotherapy plus placebo. Patients assigned to receive chemotherapy were applied either docetaxel or pemetrexed at the investigators' discretion, however no separate analysis was performed for either drug. The dose of bevacizumab in this study was 15 mg/kg and pemetrexed 500 mg/m^2, both administered by intravenous infusion on the first day of each 3-week cycle. No unexpected adverse events were noted. Fewer patients (13%) in the bevacizumab-erlotinib arm discontinued treatment as a result of toxicity than in the chemotherapy alone (24%) or bevacizumab-chemotherapy (28%) arms. The incidence of grade 5 hemorrhage in patients receiving bevacizumab was 5.1%. Although not statistically significant, the risk of disease progression or death was reduced as compared to chemotherapy alone (HR 0.66; 95% CI, 0.38-

Table 1. Phase II Studies of Bevacizumab-Pemetrexed Combinations in Advanced NSCLC

Authors (ref.)	No. of patients	Setting	Treatment combination	Response rate (%)	PFS (m)	OS (m)	Major toxicity (≥G3)
Adjei *et al.* [37]	48	Second-line	Pemetrexed + bevacizumab	10	4.1	8.6	Neutropenia 19% fatigue 13% dyspnea 10%
Herbst *et al.* [38]	120 (40 arm 2)	Second-line	Arm 1: (pemetrexed or docetaxel) + placebo Arm 2 : (pemetrexed or docetaxel) + bevacizumab Arm 3: bevacizumab + erlotinib	12.5 (arm 2)	4.8 (arm 2)	12.6 (arm 2)	Leukopenia 6% neutropenia 8% fatigue 5%
Waples *et al.* [39]	69	First-line	Pemetrexed + oxaliplatin + bevacizumab (with maintenance bevacizumab)	26	7.8	16.7	Neutropenia 8% fatigue 9% hyperglycemia 6%
Heist *et al.* [40]	36	Second-line	Pemetrexed + oxaliplatin + bevacizumab	27	5.8	12.5	Hemoptysis 1% (G5) hypertension 17% fatigue 9%
Wozniak *et al.* [41]	20 (18 evaluable)	First-line	Pemetrexed + gemcitabine + bevacizumab	61	NR	NR	Neutropenia 20% thrombocytopenia 10%
Patel *et al.* [42]	50	First-line	Pemetrexed + carboplatin + bevciuzimab (with maintenance Pemetrexed + bevaciuzimab for responders)	55	7.8	14.1	Anemia 6% trombocytopenia 8% fatigue 8% infection 10%
Skaff *et al.* [43]	27	First-line	Pemetrexed + carboplatin + bevacizumab	36	7.2	NR	Anorexia 33% hypertension 9%
Casey *et al.* [44]	40 (20 each arm)	First-line	Pemetrexed + carboplatin + bevciuzimab with enzastaurin or placebo	20 25	4.3 4.2	NR	GI perforation 5% (G5)

1.16 among patients treated with bevacizumab and chemotherapy and 0.72; 95% CI, 0.42-1.23 in patients treated with bevacizumab and erlotinib). One-year survival rate was 57% for bevacizumab-erlotinib, 54% for bevacizumab-chemotherapy and 33% for chemotherapy alone. Other studies evaluated the addition of bevacizumab to pemetrexed-based multidrug combinations. The study of Waples *et al.* [39] evaluated the efficacy and safety of first-line bevacizumab (15 mg/kg) combined with oxaliplatin (120 mg/m^2) and pemetrexed (500 mg/m^2), all administered on day 1 of each 21-day cycle. The median OS was encouraging (16.7 months) and the response rate was 26%. The combination of oxaliplatin and pemetrexed was selected owing to its good efficacy and acceptable toxicity in advanced NSCLC [45]. The same regimen in the second-line setting was associated with a median PFS of 5.8 months and median OS of 12.5 months [40]. Preliminary data suggested a high efficacy (61% response rate) of a combination of pemetrexed with bevacizumab and gemcitabine [41]. A study of Patel *et al*. [42] evaluated the efficacy and safety of first-line pemetrexed (500 mg/m^2), carboplatin (area under the concentration-time curve of 6) and bevacizumab (15 mg/kg) every 3 weeks for six cycles. In patients with response or stable disease, pemetrexed and bevacizumab were continued until disease progression or unacceptable toxicity. The objective response rate was 55% and median PFS and OS were 7.8 months and 14.1 months, respectively. Treatment toxicity during the initial six cycles and during the maintenance phase was moderate, with no hemorrhagic or hypertension events of grade 3 or greater. Another study using this combination achieved response rate of 36% and median PFS of 7.2 months [43]. Based on these results, a phase III trial has been designed to compare four cycles of carboplatin, pemetrexed, and bevacizumab followed by maintenance pemetrexed and bevacizumab *versus* four cycles of carboplatin, paclitaxel and bevacizumab with maintenance bevacizumab alone [46].

Several ongoing phase II trials are investigating bevacizumab with different chemotherapy combinations regimens, including those containing pemetrexed [47].

SMALL MOLECULE TYROSINE KINASE INHIBITORS (TKIS) TO VEGFR

Within the last years, several small molecule TKIs with the VEGFR-blocking activity have been developed. Although small molecule TKIs were sought to selectively inhibit VEGFR-2, they are also active against other VEGFRs

and other tyrosine kinase receptors including bFGFR, EGFR family members, PDGF receptor-α (PDGFR-α), c-kit, and Flt3. These agents have shown apparent activity in several solid tumors including renal cell carcinoma, hepatocellular carcinoma and gastrointestinal stromal tumors, whereas their role in NSCLC is still a matter of clinical investigations.

Sorafenib

Sorafenib (Nexavar, BAY43-9006; Bayer Pharmaceuticals Corporation, West Haven, CT) is an orally available multitargeted TKI that targets raf-kinase, VEGFR-1, VEGFR-2, VEGFR-3, PDGFR- β, Flt-3, c-kit, and p38-α [48]. In advanced NSCLC, two phase II trials showed activity and good toxicity profile of single-agent sorafenib in second-line chemotherapy [49]. Three phase III first-line trials have been launched to compare standard chemotherapy (CP or GC) with sorafenib or placebo. However, one of these trials (ESCAPE) was prematurely stopped after an interim analysis concluded that it would not meet its primary end point of improved OS. Additionally, in the subset of patients with squamous cell histology, higher mortality was observed in the sorafenib arm [50]. A few ongoing studies are investigating the combinations of sorafenib with pemetrexed but no results are available so far.

Sunitinib

Sunitinib (Sutent, SU11248; Pfizer, New York, NY) is an oral multitargeted TKI that targets VEGFR-1,-2, and -3, PDGFR-α, c-kit, and Flt3 [51]. In NSCLC single-agent sunitinib was investigated in two phase II studies including previously treated patients [52, 53]. Clinical responses were modest (2% and 9.5%, respectively), whereas stable disease was achieved in 17% and 41% of patients, respectively. An ongoing phase III trial is comparing sunitinib versus placebo in NSCLC patients who have progressed after both chemotherapy and an EGFR inhibitor. Clinical experience with the combination of pemetrexed and sunitinib is very limited. A recent phase I study demonstrated good tolerance and encouraging antitumor activity of this regimen in 12 patients with solid tumors refractory to standard therapy, most of which included NSCLC cases [54].

Vandetanib

Vandetanib (Zactima, ZD6474; Astra-Zeneca, Macclesfield, UK) is a selective small-molecule TKI inhibiting VEGFR-2, VEGFR-3, RET and EGFR. Several phase II randomized studies have demonstrated a promising activity of this agent in advanced NSCLC,used as a single agent or in combination with chemotherapy. In a study including 168 previously treated advanced NSCLC patients, vandetinib was compared with gefitinib with crossover upon progression [55]. The median PFS in the vandetanib and gefitinib arms was 11 and 8.1 weeks, respectively (P=0.025). As opposed to these results, a recent phase III trial comparing vandetinib and erlotinib in the same setting failed to show a superiority of the former [56]. In another phase II trial of 127 patients after failure of first-line platinum-based chemotherapy, the addition of vandetanib to docetaxel resulted in prolongation of PFS over docetaxel alone (18.7 *versus* 12 weeks, P=0.07) [57]. In the first-line setting, 181 patients were randomly assigned 2:1:1 to receive vandetanib, vandetinib, carboplatin and paclitaxel or carboplatin and paclitaxel [58]. The vandetinib arm was stopped early due to its inferior activity. Median PFS for vandetinib plus chemotherapy *versus* chemotherapy alone was 24 weeks and 23 weeks, respectively (HR 0.76; p=0.098).

The feasibility of a combination of pemetrexed plus vandetanib was established in a study of Hanna *et al.* [59]. This combination has also been investigated in a recent phase III study (ZEAL) [60]. A total of 537 patients who progressed after previous chemotherapy were randomly assigned to pemetrexed plus vandetanib or pemetrexed plus placebo. This study has not reached its primary endpoint: despite higher response rate in the pemetrexed-vandetinib arm (19.1% *versus* 7.9%, P<0.001), PFS did not differ significantly. The adverse event profile was consistent with previous studies of vandetanib: rash (38% *versus* 26%), diarrhea (26% *versus* 18%) and hypertension (12% *versus* 3%) were more frequent in the pemetrexed-vandetanib arm.

Pazopanib

Pazopanib (GW786034, GlaxoSmithKline, Middlesex, UK) is an oral angiogenesis inhibitor targeting VEGFR-1, VEGFR-2, and VEGFR-3, PDGFR and c-kit [61]. Two phase II studies, both using single-agent pazopanib in the preoperative setting showed activity of this agent in NSCLC [62, 63]. The studies testing pazopanib in the second-line or third-line setting in advanced disease are ongoing. No published studies have so far reported the combination of pazopanib and pemetrexed.

Cediranib

Cediranib (Recentin; AstraZeneca, Macclesfield, UK) is an oral small molecule TKI targeting VEGFR-2. The combination of cediranib with standard chemotherapy regimens (CG and CP) has been tested in phase I studies [64, 65]. In the subsequent phase II/III trial patients with advanced NSCLC were randomly assigned to CP combination with either cediranib or placebo. Although evidence of clinical activity was seen, there was increased toxicity in the cediranib arm and the study was considered not to have met the predefined criteria for automatic continuation into phase III [66]. A recent phase I study of cediranib and pemetrexed in previously treated NSCLC patients showed acceptable toxicity and promising activity of this combination [67]. A phase II trial investigating this regimen is ongoing.

Axitinib

Axitinib (AG-013736, Pfizer) is an oral TKI inhibiting VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-β, and c-Kit. In the phase II study, this agent has shown clinical activity and manageable toxicity in advanced NSCLC [68]. There are no published studies investigating the combination of pemetrexed and axitinib. Phase I and II studies are ongoing.

Vatalanib

Vatalanib (PTK787/ZK222584, Novartis International AG, Basel, Switzerland) is another small molecule TKI that inhibits VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-β and c-Kit. This agent given as monotherapy demonstrated modest

efficacy in the second-line treatment of advanced NSCLC [69]. There are no published studies on the combination of pemetrexed and vatalanib, and one phase I study is ongoing.

Enzastaurin

Enzastaurin is a selective inhibitor of the beta isoform of protein kinase C (PKCβ) that acts by interacting competitively at its ATP-binding site [70]. This drug applied in second- and third-line treatment of advanced NSCLC showed modest activity and favorable toxicity profile [71]. A preclinical study showed that enzastaurin-pemetrexed combination was highly synergistic and significantly increased apoptosis [72]. A recent phase I study of this combination showed its good tolerance and suggested a clinical activity in advanced NSCLC [73]. However, a randomized phase II study of pemetrexed, carboplatin and bevacizumab with enzastaurin or placebo was prematurely closed due to the apparent lack of enzastaurin efficacy (median PFS of 4.3 and 4.2 months, respectively) and toxicity concerns [44].

OTHER ANTIANGIOGENESIS INHIBITORS

Thalidomide

Thalidomide (Celgene; Summit, NJ) exerts antiangiogenesis by inhibiting basic fibroblast growth factor (bFGF) and VEGF. In first-line chemotherapy of advanced NSCLC, this compound showed limited efficacy in combination with carboplatin/irinotecan [74]. A phase III study did not demonstrate improved PFS and OS by the addition of thalidomide to gemcitabine [75]. Additionally, patients in the thalidomide arm experienced increased rate of the thrombotic events. No published data are available on the combination of thalidomide and pemetrexed.

Lenalidomide

Lenalidomide (Revlimid, Celgene Co., Summit, NJ, USA) is a thalidomide analog that is registered for the treatment of multiple myeloma and myelodysplastic syndrome. The experience with this compound in NSCLC is very limited. One phase II trial (NCT00179686) has completed accrual but the results are not yet available. There are no published data on its combination with pemetrexed.

Aflibercept

Aflibercept (VEGF-trap/AVE0005, Regeneron Pharmaceuticals, Tarrytown, NY) is a fully human soluble fusion protein comprised of segments of the extracellular domains of VEGFR-1 and VEGFR-2 fused to the constant region (Fc) of human IgG1, with potent antiangiogenic activity. It exerts antiangiogenic activity by binding circulating VEGF-A and placental growth factor, thus preventing their binding to the cell membrane receptor. In a phase II study including patients with advanced NSCLC that failed after previous chemotherapy with at least two regimens, two partial responses were observed in 33 evaluable patients and the drug was well tolerated [76]. There are no published data on the combination of aflibercept with pemetrexed, and one phase I study is ongoing.

INHIBITORS OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)

EGFR is an important pathway in promoting tumor growth, proliferation, invasion and angiogenesis. Binding EGFR with its ligands increases the activity of EGFR tyrosine kinase, and EGFR is autophosphorylated. This activates signaling cascade promoting cell proliferation and inhibiting apoptosis. EGFR is frequently expressed in NSCLC [77], therefore it is an attractive target for anticancer therapy. EGFR activity may be inhibited by monoclonal antibodies such as cetuximab and panitumumab, that bind to EGFRs' extracellular domain, or by small molecules (e.g. erlotinib and gefitinib) that specifically inhibit the EGFR tyrosine kinase. Within the last years, several clinical trials have been performed to examine the activity of EGFR inhibitors in NSCLC.

Cetuximab

Cetuximab (ErbituxTM; Bristol-Myers Squibb [New York]/ Merck KGaA [Darmstadt, Germany]/Imclone) [New York]) is a recombinant, chimeric murine-human monoclonal antibody directed at the ligand-binding site of EGFR. It competes with ligands for receptor binding with a higher affinity than the endogenous ligands, EGF and TGF-α. Single-agent cetuximab has been evaluated in a phase II study including patients with advanced NSCLC that have failed at least one prior chemotherapy regimen. The response rate was merely 4.5%, but 30% of patients retained stable disease and the median survival was 8.9 months [49]. The typical toxicities accompanying cetuximab therapy include diarrhea and acneiform rash. Most of the studies using cetuximab in advanced NSCLC included its combination with chemotherapy. In contrast to EGFR TKIs, the strategy of combining anti-EGFR antibodies and chemotherapy has been proved more successful. Two large phase III trials investigated the efficacy of cetuximab in addition to standard first-line chemotherapy. In the BMS 099 trial cetuximab was used in combination with carboplatin and a taxane [78]. The preliminary results of the study including 676 patients did not show the difference in PFS, the primary endpoint of this study, whereas the OS data were not reported. The second study (FLEX) randomized 1125 patients with advanced NSCLC showing immunohistochemical expression of EGFR to 6 cycles of vinorelbine plus cisplatin with or without cetuximab [79]. OS (the primary endpoint) favored the cetuximab arm, although the absolute difference was modest (median 11.3 *versus* 10.1 months; HR 0.871 [95% CI 0.762–0.996]; p=0.044). The combination of chemotherapy and cetuximab showed higher response rates (36% *versus* 29%; p=0.010), whereas PFS was not different. The main cetuximab-related adverse event was acne-like rash (10% of grade 3). Unlike in colorectal cancer, *K-RAS* mutation status was not predictive for cetuximab efficacy [80].

The combination of pemetrexed and cetuximab is justified by their single agent activity in NSCLC, non-overlapping toxicities and different mechanisms of action. In a phase I-II study including 23 stage IIIB/IV NSCLC patients previously treated with one platinum containing regimen, pemetrexed at 750 mg/m^2 every 21 days was combined with cetuximab at 400 mg/m^2 week 1, and 250 mg/m^2 weekly thereafter [81]. This combination is a subject

of two ongoing phase II studies and a phase III study in the second-line treatment. Based on the activity of cetuximab in addition to definitive radiotherapy in head and neck cancer patients, the combination of cetuximab and pemetrexed, together with cisplatin has also been tested as the addition to thoracic irradiation in patients with locally advanced NSCLC [82]. In this phase II study patients were randomly allocated to radiation (70 Gy) along with carboplatin (AUC 5) and pemetrexed 500 mg/m^2 on day 1 administered intravenously every 21 days for 4 cycles, or the same chemotherapy regimen with weekly cetuximab for 6 weeks concurrent with radiation (arm B). All patients received four additional cycles of pemetrexed (500 mg/m^2 every 21 days) as consolidation therapy. The primary endpoint in this study, the percentage of patients who lived longer than 18 months after starting initial treatment was met, however the addition of cetuximab did not confer an apparent benefit.

EGFR KINASE INHIBITORS

Erlotinib

Erlotinib (Tarceva; Genentech [South San Francisco, CA]/OSI Oncology [Boulder, CO]) is a small molecule reversible TKI that blocks the EGFR pathway, inhibiting proliferation, differentiation and angiogenesis. In a phase II study including patients with EGFR-expressing advanced NSCLC that failed previous platinum-based chemotherapy, the use of erlotinib resulted in a response rate of 12%, stable disease rate of 39%, and median survival of 8.4 months [83]. Despite its clinical efficacy as a single agent and preclinical data suggesting additive to synergistic effects, erlotinib added to first-line platinum-based chemotherapy did not increase its efficacy in terms of response rate, PFS or OS [84, 85]. However, a phase III study (BR.21) comparing erlotinib to placebo in stage IIIB or IV NSCLC patients who had received one to two prior combination chemotherapy regimens demonstrated significant, albeit moderate clinical benefit of erlotinib (median OS of 6.7 and 4.7 months for erlotinib and placebo, respectively, HR 0.7, P<0.001) [86]. Additionally, more patients treated with erlotinib had improvements in pain, cough and dyspnea. The most common side effect of erlotinib were acneiform rash and diarrhea (75% and 55%, respectively) although grades 3 or 4 toxicity occurred in less than 10% of patients. Currently erlotinib is considered a standard option in second- or third-line therapy of advanced NSCLC. Favorable predictive factors for the efficacy of erlotinib include female gender, adenocarcinoma histology, never smoking status, East Asian race and the occurrence of *EGFR*-activating mutations or high *EGFR* gene copy number.

There is a strong biological rationale for combining pemetrexed and erlotinib as both compounds have no overlapping toxicities and are active in the second-line setting of NSCLC. Importantly, in the *in vivo* model pemetrexed and erlotinib showed a strong synergism in NSCLC cells; pemetrexed increased EGFR phosphorylation and reduced Akt phosphorylation, whereas erlotinib significantly reduced thymidylate synthase expression and activity [87]. The synergistic effect of both compounds was demonstrated with their concurrent and sequential application (pemetrexed followed by erlotinib), whereas the exposure of cells to erlotinib followed by pemetrexed was mostly antagonistic in erlotinib-sensitive cells and additive at best in erlotinib-resistant cells [88]. In the clinical setting, a phase I study investigated safety and tolerability of two different schedules of intermittent erlotinib and pemetrexed [89]. The pharmacodynamic separation of chemotherapy and erlotinib was designed to overcome a potential antagonism noted in previous studies using erlotynib and multidrug chemotherapy concurrently [84, 85]. A total of 42 patients with advanced solid tumors, including 16 NSCLC, were included. Two dose-escalating schedules of erlotinib were tested: weekly on days 2, 9, and 16 (at 800–1400 mg) or on days 2 to 16 (150–250 mg), both combined with pemetrexed given every 21 days at a dose of 500 mg/m^2. A maximum tolerated dose was not reached. Dose-limiting toxicities included infection and neutropenic fever. Rash occurred in 55% and 90% of the patients applied either regimen, respectively. Notably, with the first schedule, among the 16 patients assessable for response, 5 patients with NSCLC had a partial response, with durations ranging from 3 to 16 months (no response was seen in patients with other malignancies). The second regimen seemed to be less effective, with just one response in a breast cancer patient. Currently, at least five phase II studies of pemetrexed with erlotinib in various combinations are ongoing and their results are awaited.

Gefitinib

Gefitinib (Iressa; AstraZeneca) is another orally active TKI, with the mechanism of action similar to that of erlotinib. In initial phase II studies this drug has shown an apparent clinical activity with response rates between 10% to 20% and benefits in symptom improvement in the second- or third-line setting [90, 91]. However, a phase III trial (ISEL) evaluating the survival benefit of gefitinib *versus* placebo in advanced NSCLC patients who failed one or two prior chemotherapy regimens did not show significant OS benefit [92]. A recent large phase III study (INTEREST) showed similar efficacy of gefitinib and docetaxel in patients with previously treated NSCLC [93]. Similarly to erlotinib, activity of gefitinib seems to be related to never smoking status, adenocarcinoma histology, East Asian ethnicity and *EGFR* gene alterations.

There are no published studies addressing a combination of pemetrexed and gefitinib. One phase II trial of pemetrexed and cisplatin followed sequentially by gefitinib versus pemetrexed and cisplatin in Asian "never smoker" patients with advanced NSCLC is currently ongoing.

mTOR INHIBITORS

The mTOR pathway plays an important role in transducing proliferative signals mediated through the PI3K and Akt signaling pathways [94]. mTOR is a serine/threonine kinase which is among the most important intracellular signaling enzymes regulating cell growth, survival and motility in cancer cells. It is as a downstream target of both the PI3K and Ras signaling pathways. Agents targeting the mTOR pathway have the potential for application in cancer treatment. Currently tested mTOR inhibitors include rapamycin (sirolimus) and its derivatives CCI-779 (temsirolimus), RAD001 (everolimus), and AP23573. Preliminary

clinical data suggest modest activity of single agent rapamycin derivatives in NSCLC [95]. Several phase I and II studies, mainly testing these compounds in combination with cytotoxic targeted therapies are ongoing. Clinical data on the combination of pemetrexed with mTOR inhibitors are very limited.

AGENTS TARGETING PROTEASOME

Bortezomib

The proteasome is a multicatalytic intracellular complex responsible for the degradation of misfolded or damaged proteins that are targeted by ubiquitination. Proteasome inhibitors affect multiple pathways within cells by blocking the activity of the 26S proteasome. Bortezomib (VelcadeTM; Millennium [Cambridge, MA]) is the first agent in this class to be evaluated in clinical studies and approved for treatment of multiple myeloma and mantle cell lymphoma. In NSCLC preclinical studies have shown that bortezomib used as a single agent exerts growth inhibition and apoptosis in numerous cell lines and has antitumor activity *in vivo* [96], whereas the clinical data on the combination of pemetrexed with bortezomib are very limited. One study using cell lines demonstrated an antagonistic effect between both compounds [97]. Phase I studies suggested feasibility and acceptable toxicity of this combination [98, 99].

CONCLUSIONS

The introduction of targeted agents has opened a new era in the treatment of advanced NSCLC. The most intensively investigated molecular targets in this tumor include EGFR and VEGFR. The addition of both bevacizumab and cetuximab to platinum-based combination chemotherapy improves survival in the first-line setting, although the benefit is modest. EGFR TKI, erlotinib prolongs survival in patients following progression after first-line or second-line therapy, whereas another compound of this group - gefitinib constitutes a valuable alternative to standard second-line chemotherapy. Clinical trials addressing the combination of EGFR and VEGFR inhibition have shown clinical responses [100], yet this strategy needs to be developed cautiously, as recent reports on patients with advanced colorectal cancer suggested potential antagonistic effect of the combination [101]. A number of other targeted agents have been intensively investigated in NSCLC and the early results are encouraging. NSCLC is characterized by large molecular heterogeneity, therefore better selection of patients using clinical and molecular predictive assays will hopefully improve the efficacy of this class of compounds.

The combination of targeted agents with chemotherapy has been addressed in several clinical studies, however optimal drug selection and sequencing still needs to be developed. Owing to its mild and typically not overlapping toxicity, the multitargeted antifolate agent pemetrexed is considered a preferred cytotoxic partner to targeted therapies. Additionally, preclinical studies suggested potential synergistic effect for some of these combinations. Clinical experience with the use of these combinations is still limited and mostly includes phase I and II trials. Not surprisingly, these investigations have mainly focused on compounds previously shown to be active in NSCLC: anti-angiogenic agents (bevacizumab and small molecule TKIs) and inhibitors of EGFR (cetuximab and erlotinib). Preliminary results have shown feasibility of these approaches and their promising activity but large phase III studies are warranted to verify the real value of this strategy. Combinations of pemetrexed with other targeted agents, such as mTOR inhibitors and compounds targeting proteasome, are still at early stages of development.

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