Novel agents in development for advanced non-small cell lung cancer

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***Abstract*:** The identification of *EGFR* mutations and *ALK* rearrangements in nonsmall cell lung cancer (NSCLC) has led to the rapid development of targeted therapies and significant changes in the treatment paradigm. Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) and crizotinib are now standard therapies for patients with the appropriate molecular alteration. Current investigations are determining the mechanisms of resistance to targeted therapies and developing novel agents to combat resistance. For patients with *KRAS* mutant NSCLC, a phase III trial of the MEK inhibitor, selumetinib, has been initiated. For patients without a defined mutation or a mutation without a known targeted therapy, immunotherapy, ganetespib, nintedanib and MET inhibitors in combination with EGFR TKIs are in development. Preliminary results of phase III trials raise doubts about the future development of dacomitinib as a second-line agent.

Keywords: anaplastic lymphoma kinase, dacomitinib, epidermal growth factor receptor, ganetespib, nintedanib, onartuzumab, targeted therapy, tivantinib

Introduction

Lung cancer remains the leading cause of cancer-related mortality in the United States and in the world [Jemal *et al.* 2011; Siegel *et al.* 2013]. The majority of the patients with lung cancer have the nonsmall cell (NSCLC) subtype and the majority of patients have advanced disease, defined as stage IIIB or IV, at the time of diagnosis [Govindan *et al.* 2006]. Under the previous staging system, American Joint Committee on Cancer (AJCC) TNM 6th edition, patients with malignant pleural and pericardial effusions were considered stage IIIB, often referred to as 'wet IIIB,' and were included in advanced stage trials [Greene *et al.* 2002]. Under the current staging system, AJCC TNM 7th edition, patients with malignant pleural or pericardial effusions are considered metastatic lesions (M1a) and patients with these conditions are considered as stage IV disease [Goldstraw *et al.* 2007]. In first-line cooperative group trials in the United States, the most common histology was adenocarcinoma (approximately 45–55% of the cases), followed by squamous histology (approximately 20–30% of the cases) and large cell histology (approximately 10–15% of cases) [Wakelee *et al.* 2006; Kelly *et al.* 2013]. Squamous histology is closely associated with tobacco use and the prevalence of squamous histology may vary depending on the prevalence of tobacco use [Kenfield *et al.* 2008].

The goals of treatment for patients with advanced stage disease are to improve overall survival (OS) and health-related quality of life (HRQOL), and to reduce disease-related symptoms. Historically, patients with advanced NSCLC were treated with a platinum-based doublet therapy without regard to histology. However, in a phase II trial of bevacizumab, a monoclonal antibody against the vascular endothelial growth factor (VEGF) A, a prohibitive rate of severe pulmonary hemorrhage was observed in patients with squamous histology [Johnson *et al.* 2004]. Consequently, patients with squamous histology were excluded from subsequent trials of bevacizumab. After the approval by the US Food and Drug Administration (FDA) of pemetrexed, analyses from phase III trials revealed the activity of pemetrexed is limited to patients with nonsquamous histology [Scagliotti *et al.* 2009]. Thus, patients with NSCLC are frequently divided into squamous and nonsquamous cohorts for treatment selection and drug development. An overview of the commonly used treatments for patients with nonsquamous and squamous stage

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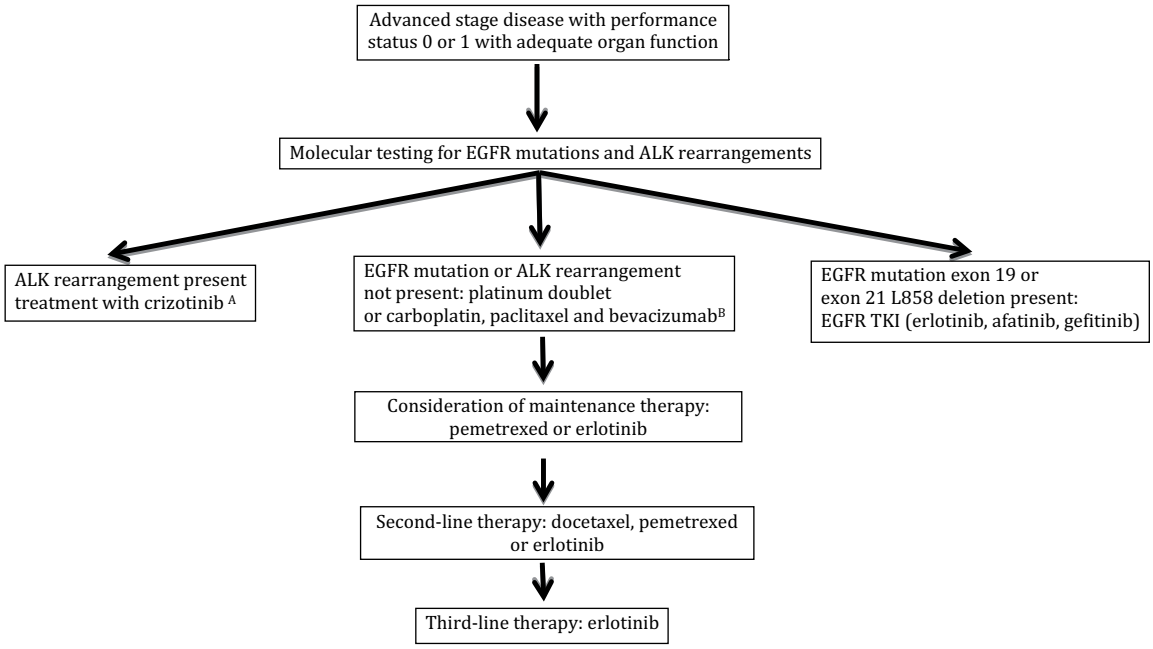

Figure 1. Commonly used treatment paradigms for advanced stage non-small cell lung cancer for non-squamous histology.
A: Crizotinib is approved by the US Food and Drug Administration without regard to line of therapy.
B: Bevacizumab is a treatment option for patients without contraindication (e.g. hemoptysis, uncontrolled hypertension).
ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; TKI: tyrosine kinase inhibitor.

IV disease with a good performance status is presented in Figures 1 and 2.

The identification of *EGFR* mutations and *ALK* rearrangements in NSCLC has further subdivided patients with advanced NSCLC [Lynch *et al.* 2004; Paez *et al.* 2004; Soda *et al.* 2007]. In the United States, patients with a known *EGFR* mutation can be treated with an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) in the first-line setting, and crizotinib is approved by the US FDA for patients with an *ALK* rearrangement without regard to the line of therapy. It is estimated that 10–15% of all NSCLC harbor an *EGFR* mutation and that 3–5% harbor an *ALK* rearrangement [Soda *et al.* 2007; Sequist *et al.* 2008].

A frequent clinical question is which NSCLC tumors should be tested for these uncommon but clinically important molecular alterations. These alterations are more prevalent in younger patients, patients with adenocarcinoma histology, or a history of never or light smoking [Rosell *et al.* 2009; Shaw *et al.* 2009] In NSCLC with adenocarcinoma histology it is estimated that 5-10% of tumors have an *ALK* rearrangement and 10–20% have an *EGFR* mutation [Kris *et al.* 2011]. *EGFR* mutations have been detected in tumors from patients with a significant history of tobacco use, suggesting that the history of tobacco use is not sufficient to exclude patients from molecular testing [D'Angelo *et al.* 2011; Lindeman *et al.* 2013]. The current diagnostic standard is to test for *EGFR* and *ALK* molecular alterations in all non-squamous tumors regardless of clinical characteristics [Lindeman *et al.* 2013].

The need for routine testing for *EGFR* mutations and *ALK* rearrangements for patients with squamous histology is debated, in part due to the low prevalence of these molecular alterations. The rate of *EGFR* mutations in patients with squamous histology is reported to be 1–15% [Chou *et al.* 2005; Kim *et al.* 2005; Pallis *et al.* 2007; Park *et al.* 2009; Miyamae *et al.* 2011]. One issue with basing the decision to perform molecular testing on histology is that there can be significant inter-observer variability among pathologists in the classification of squamous and nonsquamous histology when hematoxylin–eosin slides are used [Grilley-Olson *et al.* 2013]. Given the clinical implications of the classification between squamous and nonsquamous histology, pathologists

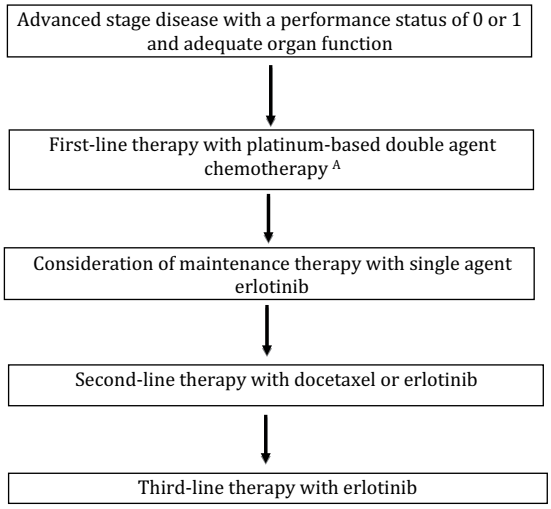

Figure 2. Commonly used therapies for advanced non-small cell lung cancer with squamous histology. A: Pemetrexed and bevacizumab are not approved by the US Food and Drug Administration for use in patients with squamous histology non=small cell lung cancer.

frequently use immunohistochemistry (IHC) to assist in the classification. A single IHC test is not sufficient to distinguish between squamous and nonsquamous histology. The expression of p63 and the *N*-terminal truncated p40 (ΔNp63) and the absence of thyroid transcription factor (TTF-1) expression are consistent with squamous histology [Bishop *et al.* 2012; Pelosi *et al.* 2012]. Poorly differentiated adenocarcinomas may express p63 and rarely squamous histology may have focal expression of TTF-1. Thus, the presence of p63 or the absence of TTF-1 is not sufficient to determine histology. The use of IHC improves the classification of NSCLC subtypes [Steinfort *et al.* 2012].

A retrospective study found the rate of *EGFR* and *KRAS* mutations in squamous cell carcinoma when IHC classification was used to identify histology was 0% [95% confidence interval (CI): 0–3.8%] [Rekhtman *et al.* 2012]. A review of previous cases of *EGFR* and *KRAS* mutations reported in patients with squamous histology revealed that the histology in the vast majority of the cases would be reclassified as poorly differentiated adenocarcinoma or adenosquamous with the addition of IHC staining to morphological assessment histology [Rekhtman *et al.* 2012]. These data suggest that, with better histological classification, the rate of *EGFR* and *KRAS* mutations observed in squamous histology tumors will be lower than the rate observed in previous series.

A reasonable approach is to test patients with squamous histology for *EGFR* mutations and *ALK* rearrangements if the histological diagnosis is uncertain, if the histology is adenosquamous, or in a patient with squamous histology with a limited history of tobacco use.

Unfortunately, acquired resistance to targeted therapy generally develops within 1 to 2 years. In the general NSCLC patient population, patients who receive first-line chemotherapy experience disease progression within 6 months and patients receiving second-line therapy generally experience disease progression within 3 or 4 months. Thus, there is a significant need for novel agents, and increasingly novel agents are being developed in molecularly or histologically defined patient populations.

EGFR mutant NSCLC

For patients with a known *EGFR* activating mutation (exon 19 deletion and exon 21 L858R point mutation) treatment with an EGFR TKI (e.g. erlotinib, gefitinib or afatinib) is a standard first-line therapy [Keedy *et al.* 2011]. Multiple phase III trials have compared EGFR TKI therapy with platinum-based doublet chemotherapy as first-line therapy for patients with *EGFR* mutant NSCLC. These trials have consistently demonstrated an improvement in objective response rate (ORR), progression-free survival (PFS) and HRQOL with EGFR TKI compared with chemotherapy [Mok *et al.* 2009; Maemondo *et al.* 2010; Mitsudomi *et al.* 2010; Zhou *et al.* 2011; Rosell *et al.* 2012; Sequist *et al.* 2013; Yang *et al.* 2013a]. The ORR and PFS observed in EGFR TKI arms were approximately 60–80% and 10–15 months, respectively. A median OS of approximately 20–30 months was observed in both treatment arms. A high crossover rate from chemotherapy to EGFR TKI therapy is thought to be responsible for the similar OS. Once a patient experiences disease progression on EGFR TKI, the available treatment options are local radiation if the patient has an isolated site of progression and continuation of the EGFR TKI, continuation of the EGFR TKI in combination with chemotherapy, discontinuation of EGFR TKI and initiation of chemotherapy alone [Weickhardt *et al.* 2012; Goldberg *et al.* 2013; Ohashi *et al.* 2013; Yu *et al.* 2013a]. The optimal treatment paradigm is unclear and there are limited data available to base treatment decisions. Most physicians select treatment based on the individual patient since there is no defined standard therapy.

In several studies, a biopsy at the time of disease progression has been performed to characterize the molecular mechanisms of acquired resistance [Sequist *et al.* 2011b; Yu *et al.* 2013a]. One study of 37 paired pre- and post-EGFR TKI samples revealed that five patients (14%) had transformed to small cell lung cancer (SCLC), and the most commonly identified mechanisms of resistance were an acquired *EGFR* exon 20 T790M mutation in 18 patients (49%), *MET* amplification in 2 patients (5%), and *PIK3CA* mutations in 2 patients (5%) [Sequist *et al.* 2011b]. A larger study of 155 patients with acquired resistance on EGFR TKI therapy revealed that 98 patients had an acquired *EGFR* exon 20 T790M (63%), 4 had SCLC (3%), and *MET* amplification (5%); *HER2* amplification was observed in 3 of 24 patients (13%) [Yu *et al.* 2013]. The development of *BRAF* mutations as a mechanism of acquired resistance has been observed as well [Ohashi *et al.* 2012]. Patients with the *EGFR* exon 20 T790M are resistant to EGFR TKI, but appear to have a more favorable prognosis and indolent disease course [Oxnard *et al.* 2011]. Collectively, these data suggest that multiple mechanisms are responsible for EGFR TKI resistance and there is value in performing a repeat biopsy at the time of disease progression, especially if conversion to SCLC is suspected.

In a retrospective analysis of patients receiving afatinib in 3 clinical trials, 14 patients were identified as having a *de novo* T790M mutation alone (n = 3) or combination with other mutations (n = 11) [Yang *et al.* 2013b]. The ORR, median PFS and median OS observed with afatinib in this patient population were 14.3% (n = 2), 2.9 months (95% CI: 0.3–13.8) and 14.9 months (95% CI: 1.5–30.5). These data suggest the single-agent activity of afatinib in NSCLC with a T790M mutation is low. A single-arm phase Ib trial investigated afatinib and cetuximab, a monoclonal antibody against the extracellular domain of EGFR, in patients who had experienced disease progression on erlotinib or gefitinib [Janjigian *et al.* 2012]. Patients were required to have an *EGFR* mutation or a response or stable disease for ≥6 months on prior EGFR TKI. Of the 100 patients enrolled, an EGFR T790M was detected in 53 tumor samples and not detected in 42 tumor samples. The primary grade ≥3 toxicities were rash (18%), diarrhea (7%) and fatigue (9%). The ORR was 40%, 94% of patients experienced disease control (defined as response or stable disease), and the median PFS was 4.7 months. The ORR in the patients with a T790M and without a T790M was 38% and 47%, respectively. A similar phase I/II trial of erlotinib and cetuximab did not demonstrate efficacy [Janjigian *et al.* 2011]. The combination of afatinib and cetuximab appears to have greater activity than single-agent afatinib in patients who develop progressive disease after an EGFR TKI therapy and warrants further investigation.

Another method of combating acquired resistance is the development of an EGFR TKI that is active against both the T790M mutation as well as the baseline activating EGFR mutations (Table 1). CO-1686 is active against the activating *EGFR* mutations and the T790M mutation, and has limited inhibition of EGFR wildtype which may reduce the rate of rash and diarrhea. CO-1686 is being investigated in an ongoing phase I/II trial in patients with EGFR mutant NSCLC who have experienced disease progression on an EGFR TKI [ClinicalTrials.gov identifier: NCT01526928]. The phase I portion of the trial revealed six of nine patients with an acquired T790M mutation experienced an objective response [Soria *et al.* 2013]. Patients have not experienced rash, and the most common toxicities (all grades) observed in 56 patients were nausea (20%), diarrhea (20%), fatigue (20%), vomiting (15%), and decreased appetite (10%). This trial is ongoing to determine the recommended dose for phase II trials and to determine the optimal formulation of the agent for further investigation. AZD9291 is an irreversible EGFR TKI with activity against activating EGFR mutations and the T790M mutation. AZD9291 is being investigated in a phase I trial [ClinicalTrials.gov identifier: NCT01802632]. Initial efficacy data are available from the dose escalation cohort and the expansion cohort for patients T790M mutations [Ranson *et al.* 2013]. The ORR in all patients was 46% and the response rate in patients T790M mutation-positive NSCLC was 58%. Only grade 1 or 2 rash and diarrhea have been observed in the multiple dose cohorts. Dose escalation continues to further define toxicity and to determine the recommended dose for phase II trials. Both these agents require further investigation but have demonstrated promise for patients who have acquired resistance to EGFR TKI.

NSCLC with an *ALK* rearrangement

Crizotinib is currently approved by the US FDA for patients whose tumors demonstrate an *ALK* rearrangement without regard to line of therapy. A phase III trial compared crizotinib with standard

Table 1. Select ongoing phase II or III trials for patients with *EGFR* mutant NSCLC or *ALK* rearranged NSCLC.[44]

Agent	Patient population	Phase	NCT number	Primary endpoint (s)
CO-1686	EGFR mutation positive previously treated with EGFR TKI	I/II	01526928	Grade 3 or 4 adverse events, ORR
AZD9291	EGFR mutation positive previously treated with EGFR TKI	I with expansion cohort	01802632	Safety and tolerability
LDK378	ALK positive, crizotinib naïve	II	01685138	ORR
LDK378	ALK positive, previously untreated	III (LDK378 *versus* platinum/ pemetrexed)	01828099	PFS
CH5424802	ALK positive, previously treated with crizotinib	II	01871805	ORR
Ganetespib	ALK positive, and have failed up to three therapies	II	01562015	ORR

ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; NCT, National Clinical Trial; NSCLC, non-small cell lung cancer; ORR, objective response rate; PFS, progression-free survival; TKI, tyrosine kinase inhibitor.

second-line chemotherapy (pemetrexed or docetaxel) in patients with NSCLC with an *ALK* rearrangement. Patients assigned to the crizotinib compared with the chemotherapy arm experienced a higher ORR (65% *versus* 20%, $p < 0.001$), longer PFS [hazard ratio (HR): 0.49, 95% CI: 0.37–0.64; $p = 0.001$; median 7.7 and 3.0 months, respectively), and a better HRQOL [Shaw *et al.* 2013]. The OS was similar in the crizotinib and chemotherapy arms (HR: 1.02, 95% CI: 0.68–1.54; p=0.54; median 20.3 and 22.8 months, respectively). The ORR and median PFS in the pemetrexed and docetaxel treated patients were 29% (95% CI: 21–39%), and 7% (95% CI: 2–16%), respectively, and 4.2 and 2.6 months, respectively. These data suggest a higher ORR in NSCLC with *ALK* rearrangements with pemetrexed than in unselected nonsquamous NSCLC. A phase III trial comparing crizotinib with carboplatin or cisplatin and pemetrexed in patients with NSCLC with an *ALK* rearrangement and nonsquamous histology has completed accrual [ClinicalTrials.gov identifier: NCT01154140]. This trial will provide data on the efficacy of crizotinib compared with standard double agent platinum-based therapy in the first-line setting. For patients who have isolated progression in the brain or oligometastatic progression, local therapy with radiation and continuing crizotinib may be a treatment option, and patients who have more indolent and asymptomatic disease progression continuing crizotinib beyond disease progression are reasonable options [Weickhardt *et al.* 2012; Ou *et al.* 2014].

In a case series of 18 patients who underwent biopsy after experiencing disease progression on crizotinib, 4 patients had a mutation within the *ALK* tyrosine kinase domain and an additional patient had amplification to *ALK* fusion gene [Katayama *et al.* 2012]. Other mechanisms of resistance identified include amplification of *KIT* and increased autophosphorylation of the EGFR, suggesting activation of the EGFR pathway as a mechanism of resistance. A similar case series of 11 patients identified secondary mutations in the tyrosine kinase domain in 4 patients, *ALK* copy number gain in 2 patients (1 patient also demonstrated an *ALK* resistance mutation), *KRAS* mutation in 2 patients (1 without the evidence of the original *ALK* rearrangement), 1 patient developed *EGFR* mutant NSCLC without evidence of a persistent *ALK* rearrangement, and 1 patient developed an *ALK* rearrangement negative NSCLC [Doebele *et al.* 2012]. The limited data available suggest multiple mechanisms are responsible for crizotinib resistance including 'gate keeper mutations,' copy number gain, and gain or loss of oncogenic driver mutations.

One concern with crizotinib is a relatively low penetration of the blood–brain barrier. In one study, 5 hours after taking crizotinib the crizotinib concentration in the cerebrospinal fluid (CSF) was 0.0014 μmol, and the CSF to plasma ratio was 0.0026 [Costa *et al.* 2011]. The CSF levels were significantly below the 50% growth inhibition of *ALK* rearranged cell lines. The pharmacokinetic data and clinical data raise concerns that the brain may be a 'pharmacokinetic sanctuary' and patients may experience intracranial disease progression while having extracranial disease control [Weickhardt *et al.* 2012; Camidge, 2013].

This pattern of disease progression is relevant as novel ALK inhibitors enter clinical trials.

Several 'second generation' ALK inhibitors are currently in development that may have potential therapeutic advantages compared with crizotinib (Table 1). LDK378 (now known as ceritinib) is a more potent and selective ALK inhibitor than crizotinib and was investigated in phase I trial of patients with malignancies that harbored *ALK* rearrangement. A total of 26 patients with *ALK* rearranged NSCLC who had experienced disease progression on crizotinib were treated with LDK378 at a dose of ≥400 mg daily and 21 patients responded (86%) including brain metastases responses [Mehra *et al.* 2012]. A second ALK inhibitor, CH5424802 (now known as alectinib), has activity against *ALK* rearranged cell lines with the gatekeeper mutation L1196M in cell lines and xenograft models [Sakamoto *et al.* 2011]. This agent was investigated in phase I/II trial and patients enrolled could not have received previous therapy with an ALK inhibitor. In the phase II portion, 43 of 46 patients (93.5%, 95% CI: 82.1–98.6%) experienced an ORR; the PFS was unavailable at the time of analysis [Seto *et al.* 2013]. In addition, AP26113 is a dual ALK/EGFR inhibitor with activity in cell lines with *ALK* rearrangement with gate keeper mutations [Camidge *et al.* 2013]. In a phase I study among patients with *ALK* rearranged NSCLC with previous therapy with crizotinib, 12 of the 16 (75%) patients responded and responses were observed in 4 of 5 (80%) patients with untreated or progressing brain metastases. All of these agents are early in development, but the preliminary evidence indicates activity in patients who have progressed on crizotinib, increased ALK selectivity and greater potency, and the potential for intracranial disease responses. Several agents that inhibit the heat-shock protein (HSP) 90 have also revealed single activity in patients with *ALK* rearrangement. Trials are ongoing with these agents (Table 1) [Sequist *et al.* 2010; Socinski *et al.* 2013].

KRAS mutant NSCLC

KRAS mutations are the most common mutation detected in NSCLC and are associated with a history of tobacco use and adenocarcinoma histology. The rate of *KRAS* mutations observed in patients with adenocarcinoma and squamous histology reported in a recent analysis were 34% and 6%, respectively [Shepherd *et al.* 2013]. The rate of *KRAS* mutations observed among former/current smokers and never smokers in a recent meta-analysis were 25% and 6%, respectively [Mao *et al.* 2010]. Unfortunately, a targeted therapy is not available for this patient population and the utility of routine clinical testing is debated [Roberts and Stinchcombe, 2013]. MEK1/MEK2 are two downstream kinases in the RAS-RAF-MEK-ERK pathway and inhibition of MEK is one strategy to block signaling [Janne *et al.* 2013]. Selumetinib is a MEK1/MEK2 inhibitor, and preclinical evidence revealed activity in *KRAS* mutant xenograft models and synergy with docetaxel. A randomized phase II trial investigated docetaxel (75 mg/m^2 every 3 weeks) with and without selumetinib in patients with *KRAS* mutant NSCLC who had progressed after first-line therapy; the primary end-point was OS. Patients assigned to the docetaxel and selumetinib (n = 44) compared with the docetaxel arm (n = 43) experienced a statistically significant improvement in ORR (37% *versus* 0%, $p < 0.0001$) and PFS (HR: 0.58, 80% CI: 0.42–0.79; $p = 0.014$), and a numerically superior OS (HR: 0.80, 80% CI: 0.56–1.14; $p = 0.21$). The rate of grade 3 or 4 neutropenia observed in the docetaxel and selumetinib and docetaxel arms was 82% and 67%, respectively, and the rate of febrile neutropenia was 18% and 0%, respectively. The rate of any adverse event leading to hospitalization in the docetaxel and selumetinib and docetaxel alone arms was 48% and 19%, respectively. While the efficacy results of this phase II study are promising, the toxicity observed in the combination arm is concerning. A phase III trial of docetaxel with selumetinib or placebo as second-line therapy for patients with KRAS mutant NSCLC has been initiated [ClinicalTrials.gov identifier: NCT01933932]. The primary endpoint is PFS and patients in both treatment arms receive prophylactic pegylated granulocyte colony stimulating factor.

Immunotherapy

Historically NSCLC was not thought to be susceptible to immunotherapy, but several recent trials have challenged this perception. In order for tumors to develop, grow and metastasize, the malignant cells must evade the immune system. Malignant cells are able to avoid immune detection and destruction by modifying several immune 'check points.' Programmed death 1 (PD-1) is an immune check point receptor which is expressed on activated T cells and is part of the process of immunosuppression [Topalian *et al.* 2012]. PD-ligand-1 (PD-L1) and PD ligand-2 (PD-L2)

are ligands which bind to the PD-1 receptor, and are expressed on some tumor cells and stromal cells [Topalian *et al.* 2012]. Blockade of the PD-1 receptor and PD-L1 ligand interaction leads to T-cell stimulation and can overcome tumor immune resistance.

A monoclonal antibody against PD-1, BMS-936558 (now known as nivolumab), was investigated in a phase I trial in patients with advanced cancer and multiple tumor types. The ORR observed in patients with advanced NSCLC was 18% (14 of 76 patients). To assess the role of intratumoral PD-L1 expression, IHC analysis was performed on pretreatment specimens from 42 patients. Among the 17 patients with PD-L1 negative tumors, no responses were observed; in contrast among the 25 patients with PD-L1 positive tumors, nine responses were observed ($p = 0.006$). Long-term follow up of the phase I trial revealed an ORR of 16% and a median OS of 9.6 months (95% CI: 7.4–13.7) [Brahmer *et al.* 2013]. Among patients with nonsquamous histology the ORR and median OS were 15% and 9.6 months (95% CI: 5.3–13.7), respectively, and among patients with squamous histology the ORR and median OS were 19% and 9.2 months (95% CI: 7.6 to not reached), respectively. The initial impression was that nivolumab was more active in squamous NSCLC, but with longer follow up and larger numbers it appears to have similar activity in both squamous and nonsquamous NSCLC. On long-term follow up, the most common grade 3 or 4 adverse events were fatigue, pneumonitis and elevated aspartate aminotransferase (2% each); two-drug related death due to pneumonitis were observed.

A similar trial of an anti-PD-L1 monoclonal antibody, BMS-936559, enrolled patients with advanced cancer including 75 patients with NSCLC [Brahmer *et al.* 2012]. Of the 49 patients with NSCLC evaluable for response, 5 patients experienced an objective response and 6 additional patients experienced stable disease for at least 24 weeks [Brahmer *et al.* 2012]. MPDL3280A, a monoclonal antibody that binds PD-L1, was investigated in a phase I study advanced NSCLC [Spigel *et al.* 2013]. At the time of analysis, 53 patients were enrolled and 37 patients were evaluable for response. The ORR was 24% (9 of 37 patients) and responses were observed in both squamous and nonsquamous histology. A preliminary analysis revealed that the ORR among patients with PD-L1 expression was 100% (4 of 4 patients) and the ORR among patients without PD-L1 expression was 15% (4 of 26 patients).

At this time, numerous trials investigating a variety of immunotherapy agents are ongoing in advanced NSCLC. The role of PD-L1 expression as a biomarker and the optimal method of testing for PD-L1 expression are areas of investigation. The data from available clinical trials are too limited and immature to determine if targeting PD-1 or PD-L1 will have greater efficacy and/or a lower toxicity.

Multi-targeted tyrosine kinases

Several phase II and III trials have investigated agents that target multiple tyrosine kinases; the majority of these agents inhibit angiogenesis through inhibition of the VEGF receptors [Scagliotti *et al.* 2010, 2012a, 2012b]. Nintedanib (BIBF1120) is a multi-targeted tyrosine kinase that inhibits VEGF receptors 1–3, fibroblast growth factor receptor 1–3 and platelet derived growth factor receptor. A phase III trial investigated docetaxel with nintedanib or placebo in patients who have experienced disease progression after first-line chemotherapy [Reck *et al.* 2013]. The primary endpoint was improvement in PFS by independent radiological review (IRC) and OS was the secondary endpoint. Patients assigned to the nintedanib arm compared with the placebo arm experienced a statistically significant improvement in PFS (HR: 0.79, 95% CI: 0.68–0.92; p=0.0019; median PFS of 3.4 and 2.7 months, respectively). The absolute difference in median PFS was modest. In the intent-to-treat patient (ITT) population, a significant difference in OS was not observed (HR: 0.94, 95% CI: 0.83–1.05; $p = 0.2720$). In a subset analysis of patients with adenocarcinoma histology ($n = 568$), patients assigned to the nintedanib compared with the placebo arm experienced a significantly longer OS (HR: 0.83, 95% CI: 0.70–0.99; $p = 0.0359$). In the squamous histology subset ($n = 555$), a statistically significant difference in OS was not observed (HR: 1.01, 95% CI: 0.85–1.21; $p = 0.8907$). The rate of drug-related grade ≥3 adverse events was higher in the nintedanib than the placebo arm (50.8% and 42%, respectively); the most common adverse events that were observed at a higher rate in the nintedanib arm were diarrhea, nausea and elevated liver function tests. The rate of grade ≥3 hemorrhage and hypertension were similar in the two treatment arms.

HSP 90 inhibitor

HSPs are part of a protein complex that forms a chaperone complex which regulates protein folding, stability and function [Shimamura and Shapiro, 2008]. Many of the client proteins are involved oncogenesis and inhibiting the HSP complex may successfully inhibit multiple oncogenic pathways [Socinski *et al.* 2013]. This class of agents has demonstrated activity in patients with NSCLC with an *ALK* rearrangement as discussed previously. Ganetespib, a HSP-90 inhibitor, has demonstrated single-agent activity in NSCLC, and preclinical data indicates synergy between chemotherapy and ganetespib [Socinski *et al.* 2013]. This agent was investigated in a randomized phase IIb trial in patients who had experienced disease progression after first-line therapy. The coprimary endpoints were PFS in patients with *KRAS* mutant NSCLC and patients with an elevated lactate dehydrogenase (LDH), and secondary endpoints were PFS and OS in patients with adenocarcinoma. After the trial was initiated, enrollment was restricted to adenocarcinoma histology due to concerns about pulmonary hemorrhage and a lack of efficacy in the nonadenocarcinoma histology cohort. In patients with adenocarcinoma histology (n = 252), patients assigned to the ganetespib containing compared with the docetaxel alone arm experienced a nonstatistically significant difference in PFS (HR: 0.83, 90% CI: 0.64–1.06; p = 0.108) and a statistically significant difference in OS (HR: 0.73, 90% CI: 0.55–0.98; p = 0.041). Patients assigned to the ganetespib compared with docetaxel alone experienced a numerically higher rate of diarrhea (48% *versus* 16%), fatigue (34% *versus* 24%), and grade 3 or 4 febrile neutropenia (11% *versus* 2%). When time since diagnosis of advanced disease was analyzed (>6 months *versus* ≤6 months from time of diagnosis), patients with a diagnosis of advanced disease >6 months appeared to benefit from ganetespib and patients with a diagnosis of advanced disease ≤6 months appeared to benefit from standard therapy (p-value for interaction = 0.0064). It is unclear if this observation is due to the multiple comparisons, a difference in the biology of NSCLC, or a difference in treatment effect; therefore, it should be interpreted with caution. A phase III trial is enrolling patients with adenocarcinoma with >6 months from diagnosis is comparing docetaxel/ganetespib with docetaxel [ClinicalTrials.gov identifier: NCT10798485].

Dacomitinib

Dacomitinib is an irreversible inhibitor of EGFR, HER2 and HER4. It was compared to erlotinib in a randomized phase II trial in patients with advanced NSCLC who had progressed on one or two lines of chemotherapy (n = 188) [Ramalingam *et al.* 2012]. Patients were not selected based on *EGFR* mutation status and 30 patients (16%) enrolled had *EGFR* mutant NSCLC. The primary endpoint was PFS and patients assigned to the dacomitinib compared with the erlotinib arm experienced a statistically significant improvement in PFS in the ITT population (HR: 0.66, 95% CI: 0.47–0.91; p = 0.012). Several subset analyses were performed for PFS based on *EGFR* and *KRAS* mutational status. Among patients with *KRAS* wildtype tumors/*EGFR* wildtype (n = 100), a statistically significant improvement in PFS was observed with dacomitinib compared with erlotinib (HR: 0.61, 95% CI: 0.37–0.99; p = 0.043) and among patients with *KRAS* wildtype tumors (HR: 0.55, 95% CI: 0.35–0.85; p = 0.006). Among patients with *EGFR* mutant NSCLC, a numerically longer PFS was observed in the patients assigned to dacomitinib compared with erlotinib (HR: 0.46, 95% CI: 0.18–1.18; p = 0.098). A phase III trial compared dacomitinib with erlotinib in the second-line setting; the coprimary endpoints were PFS by IRC in the ITT patients and in the *KRAS* wildtype patient populations [ClinicalTrials.gov identifier: NCT01360554] [Pfizer, 2012]. A phase III trial, BR.26, compared dacomitinib with placebo in patients who have progressed after first-line chemotherapy and erlotinib or gefitinib; the primary endpoint was OS [ClinicalTrials.gov identifier: NCT01000025]. Neither of these trials met the primary endpoints based on the preliminary press release and additional data be will presented in the future [Pfizer, 2014]. An ongoing phase III trial is comparing dacomitinib with gefitinib as first-line therapy for patients with *EGFR* mutant NSCLC; the primary endpoint is PFS by IRC [ClinicalTrials.gov identifier: NCT01774721].

MET inhibitors in combination with EGFR TKI

MET is a tyrosine kinase receptor that is directly involved in cell proliferation, survival and invasion, and is commonly dysregulated in malignant cells [Trusolino *et al.* 2010]. MET is activated by binding of hepatocyte growth factor (HGF) and onartuzumab is a monoclonal antibody that binds to the extracellular domain of MET and prevents HGF binding [Spigel *et al.* 2013a]. A randomized

phase II trial investigated erlotinib with onartuzumab or placebo in patients who had progressed after one or two standard therapies (n = 137). A preplanned subset analysis was performed to assess the efficacy of onartuzumab in patients with tumors that demonstrated MET overexpression as assessed by IHC. In the MET-negative patients squamous histology was more common (42% *versus* 15%) and never-smokers were less common (5% *versus* 20%). Patients were not selected based on EGFR mutational status, and 13 patients with EGFR mutations were enrolled in the trial; 6 patients in the placebo arm and 7 patients in the onartuzumab arm

In the ITT patient population, there was no significant difference in PFS and OS. In the subset analysis patients with MET-positive tumors by IHC (n = 66), a statistically significant longer PFS (HR: 0.53, 95% CI: 0.283–0.99; p = 0.04) and OS (HR: 0.37, 95% CI: 0.19–0.72; p = 0.002) was observed with the addition of onartuzumab. Conversely among patients MET-negative tumors (n = 62) patients, a worse PFS (HR: 1.82, 95% CI: 0.99–3.32; p = 0.05) and OS (HR: 1.78, 95% CI: 0.79–3.99; p = 0.16) was observed with the addition of onartuzumab. The rates of rash, diarrhea, fatigue and nausea were similar in the two treatment arms. The rate of peripheral edema (all grades) was numerically higher in the onartuzumab than the placebo arm (23.2% and 7.5%, respectively). The ongoing phase III trial is comparing erlotinib with onartuzumab or placebo in MET-positive patients by IHC who have progressed on one or two lines of therapy [ClinicalTrials.gov identifier: NCT01456325].

Tivantinib is an oral MET inhibitor and a randomized phase II trial revealed an improvement in OS among patients with nonsquamous who were assigned to erlotinib and tivantinib compared with erlotinib and placebo [Sequist *et al.* 2011a]. Patients were not selected based on *EGFR* mutation status for the phase II trial and the majority of patients enrolled were *EGFR* wildtype (85%). A phase III trial of erlotinib and tivantinib or placebo in patients with nonsquamous histology was initiated. The trial was stopped after a planned interim analysis revealed that the trial would not meet the primary endpoint of improvement in OS (HR: 0.98, 95%CI: 0.84–1.15; p = 0.81) [Scagliotti *et al.* 2013]. The rates of rash, diarrhea and asthenia/fatigue were similar in the two treatment arms. The rate of grade 3 or 4 neutropenia was numerically higher in the erlotinib and tivantinib than the erlotinib and placebo arm (10% and 1.0%, respectively). Patients were not selected based on *EGFR* mutation status for the phase III trial and 109 patients with *EGFR* mutant NSCLC were enrolled. The OS in the *EGFR* mutant subgroup in the erlotinib and tivantinib and erlotinib and placebo arms was similar (HR: 0.72, 95% CI: 0.35–1.48).

A retrospective subset analysis of patients with high MET expression by IHC (n = 211) revealed patients assigned to the erlotinib and tivantinib compared with the erlotinib and placebo experienced statistically significant improvement in OS (HR: 0.70; p = 0.03). Patients with low MET expression by IHC (n = 234) assigned to the erlotinib and tivantinib and erlotinib placebo experienced similar OS (HR: 0.90; p = 0.53). The clinical characteristics (e.g. gender, histology, performance status, smoking history, rate of brain metastases) were similar of the high and low MET expression subgroups. Subsequent to the completion of the trial, cell-line data indicated that the cytotoxic activity of tivantinib was not based on MET inhibition alone but inhibition of microtubule assembly as well [Basilico *et al.* 2013; Katayama *et al.* 2013]. The future development of tivantinib in combination with EGFR TKI in NSCLC is unclear, and any potential development will most likely require selection of patients by MET expression.

Conclusion

The identification of *EGFR* mutations and *ALK* rearrangements has led to the rapid development of targeted therapies and changes in the treatment paradigms for these patient populations. Current investigations are focused on determining the mechanisms of resistance to targeted therapy and developing novel agents to combat mechanisms of resistance. *KRAS* mutations are the most common mutations in NSCLC, and a phase III trial investigating selumetinib, a MEK1/MEK2 inhibitor, in patients with *KRAS* mutant NSCLC has been initiated. For patients without a defined mutation or mutation without a known targeted therapy immunotherapy, ganetespib, nintedanib and MET inhibitors in combination with EGFR TKI therapy are second-line agents in development. The future development of dacomitinib in the second-line setting is in doubt based on the preliminary results of phase III trials.

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Conflict of interest statement

The authors declare no conflict of interest in preparing this article.

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