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Overcoming resistance to targeted therapies in NSCLC: current approaches and clinical application

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***Abstract*:** The discovery that a number of aberrant tumorigenic processes and signal transduction pathways are mediated by druggable protein kinases has led to a revolutionary change in nonsmall cell lung cancer (NSCLC) treatment. Epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) are the targets of several tyrosine kinase inhibitors (TKIs), some of them approved for treatment and others currently in clinical development. First-generation agents offer, in target populations, a substantial improvement of outcomes compared with standard chemotherapy in the treatment of advanced NSCLC. Unfortunately, drug resistance develops after initial benefit through a variety of mechanisms. Novel generation EGFR and ALK inhibitors are currently in advanced clinical development and are producing encouraging results in patients with acquired resistance to previous generation agents. The search for new drugs or strategies to overcome the TKI resistance in patients with *EGFR* mutations or *ALK* rearrangements is to be considered a priority for the improvement of outcomes in the treatment of advanced NSCLC.

Keywords: alectinib, ALK rearrangements, AZD9291, ceritinib, CO-1686, EGFR mutations, selective EGFR inhibitors, T790M

Introduction

It is now recognized that genetic alterations in epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*) define two unique subtypes of nonsmall cell lung cancer (NSCLC) that are highly responsive to tyrosine kinase inhibitors (TKIs) specifically active against these mutated forms. Somatic mutations in *EGFR* are identified in 10–30% of patients with NSCLC [Lynch *et al.* 2004; Paez *et al.* 2004; Pao *et al.* 2004]. Common *EGFR* alterations include the L858R point mutation in exon 21 and exon 19 deletions [Sharma *et al.* 2007]. These mutations result in enhanced EGFR signaling and confer sensitivity to the EGFR TKIs [Lynch *et al.* 2004; Paez *et al.* 2004; Pao *et al.* 2004]. ALK can be aberrantly activated by mutation, gene amplification or chromosomal rearrangement, leading to the expression of a potent oncogenic driver. In NSCLC, *ALK* rearrangement occurs in approximately 5% of cases [Shaw *et al.* 2014a].

In first-line treatment, EGFR inhibitors and the ALK inhibitor crizotinib produce objective response rates (ORRs) nearing 75% in patients with typical *EGFR* mutations or *ALK* rearrangements. Randomized trials have also demonstrated improved progression-free survival (PFS) for *EGFR*-mutant patients receiving gefitinib, erlotinib or afatinib, and for *ALK*-rearranged patients receiving crizotinib compared with chemotherapy [Mok *et al.* 2009; Maemondo *et al.* 2010; Rosell *et al.* 2012; Sequist *et al.* 2013; Solomon *et al.* 2014]. Despite this initial sensitivity, however, the long-term effectiveness of such therapies is universally limited by the development of resistance. Indeed, the median PFS after treatment with EGFR or ALK inhibitors in target populations is generally less than 1 year [Mok *et al.* 2009; Maemondo *et al.* 2010; Rosell *et al.* 2012; Kwak *et al.* 2010; Camidge *et al.* 2012; Kim *et al.* 2012]. Identifying the mechanisms underlying this resistance is an area of intense, ongoing investigation.

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The search for new drugs or strategies to overcome the TKI resistance in patients with *EGFR* mutations or *ALK* rearrangements is to be considered a priority for the improvement of outcomes in the treatment of advanced NSCLC.

Resistance to targeted therapies

Resistance to targeted therapies is generally classified as either primary (i.e. intrinsic) or secondary (i.e. acquired) [Gainor and Shaw, 2013]. Primary resistance describes a *de novo* lack of treatment response, whereas acquired resistance denotes disease progression after an initial response. Criteria for acquired resistance were recently proposed for *EGFR*-mutant NSCLC [Jackman *et al.* 2010]. These include that the patient must have received prior therapy with an EGFR TKI and that tumor genotyping confirms the presence of a typical *EGFR* mutation that is associated with sensitivity to EGFR TKIs. Alternatively, the patient must have achieved either a documented partial or complete response or prolonged stable disease (⩾6 months) based on Response Evaluation Criteria In Solid Tumors (RECIST) or World Health Organization (WHO) criteria. Moreover, disease progression must have occurred despite uninterrupted exposure to an EGFR TKI within 30 days and the patient must have not received additional systemic therapy since discontinuation of EGFR TKIs. Similar criteria have not been established for *ALK*-positive NSCLC but, in clinical practice, the approach can be considered similar.

Although diverse TKI resistance mechanisms have been identified within *EGFR*-mutant and *ALK*-positive patients, some common principles of resistance are shared between these groups [Zhang *et al.* 2011]. Mechanisms of acquired resistance in oncogene-driven malignancies are broadly divided into two categories. The first involves development of additional genetic alterations in the primary oncogene, which facilitates continued downstream signaling. This commonly arises through secondary mutations in the kinase target or through gene amplification of the kinase itself [Engelman and Janne 2008]. Alternatively, resistance can develop independently of genetic changes in the target. This occurs through upregulation of bypass signaling pathways, changes in tumor histology or alterations in drug metabolism [Pao and Chmielecki, 2010; Ellis and Hicklin, 2009].

Resistance to EGFR TKIs

Several clinical trials clearly demonstrated that PFS of patients treated with EGFR TKI therapy is significantly longer than that of those treated by conventional platinum doublet chemotherapy [Mok *et al.* 2009; Maemondo *et al.* 2010; Rosell *et al.* 2012; Sequist *et al.* 2013]. EGFR TKI therapy has dramatically changed the paradigm of lung cancer treatment.

Primary resistance

Although ORRs to EGFR TKIs are high among *EGFR*-mutant patients, some patients exhibit intrinsic resistance. Primary resistance may be due in part to differential TKI sensitivities across various *EGFR* mutations. 'Classic' *EGFR* mutations, namely exon 19 deletions and L858R, are associated with marked sensitivity to TKIs [Pao and Chmielecki, 2010]. Conversely, exon 20 insertions or duplications (about 4% of *EGFR* mutations) seem to be resistant to EGFR inhibitors [Yasuda *et al.* 2012]. Intrinsic resistance to EGFR inhibitors may also be due to secondary genetic alterations that co-occur with sensitizing *EGFR* mutations. *MET* amplification and T790M are common mechanisms of acquired resistance. When present *de novo*, it has been suggested that these genetic alterations may also promote intrinsic resistance if present at sufficiently high allelic frequencies. Yu and colleagues recently reported a very low incidence of *de novo* EGFR T790M mutation in 2774 sequentially tested patients with lung cancer (0.5%) and a limited benefit with erlotinib treatment [response rate (RR) = 8%] [Yu *et al.* 2014]. Alternatively, selective pressure from TKIs may permit cells containing T790M or *MET* amplification to emerge as dominant clones early during therapy. For instance, a T790M resistance mutation within *EGFR* has been occasionally identified as a minor clone within treatment-naïve tumor specimens containing classic activating *EGFR* mutations [Inukai, 2006]. Similarly, *MET* amplification has been reported in *EGFR*-mutant tumors before TKI exposure [Turke *et al.* 2010].

Acquired resistance

Secondary mutations. In *EGFR*-mutant NSCLC, the earliest reports of TKI resistance identified an analogous secondary mutation in exon 20 of *EGFR*, leading to a threonine-to-methionine substitution within the gatekeeper residue at position 790 (T790M) [Pao *et al.* 2005]. Secondary

T790M mutations have since been found in approximately 50% of TKI-resistant, *EGFR*-mutant patients, establishing this alteration as the dominant resistance mechanism in the clinic [Sequist *et al.* 2011]. Although other gatekeeper mutations sterically impede TKI binding, T790M causes resistance predominantly through changes in adenosine triphosphate (ATP) affinity [Yun *et al.* 2008]. *EGFR*-mutant tumors are generally sensitive to competitive inhibitors because such mutations reduce the receptor's affinity for ATP. The addition of T790M, however, restores the ATP affinity of the kinase back to wildtype levels, re-establishing ATP as the favored substrate rather than the TKI.

Bypass signaling. TKI resistance can also develop through reactivation of downstream signaling pathways *via* bypass pathways. There are multiple mechanisms of resistance *via* bypass pathways as MET amplification, HER 2 amplification, PIK3CA mutations and BRAF mutation [Gainor and Shaw, 2013]. *MET* amplification, the main bypass signaling resistance, identified in only 5% of resistant tumors, confers resistance through ERBB3-mediated activation of downstream PI3K/AKT signaling, effectively bypassing the inhibited EGFR [Engelman *et al.* 2007].

Phenotypic alterations. Changes in tumor histology on development of resistance to EGFR TKIs have been reported. The transformation from NSCLC to small cell lung cancer (SCLC) is well known [Sequist *et al.* 2011]. Another histologic change observed in EGFR TKI-resistant specimens is an epithelial to mesenchymal transition (EMT). EMT is characterized by loss of epithelial markers (e.g. E-cadherin) and gain of mesenchymal features, including surface expression of vimentin [Chung *et al.* 2011].

Treatment approaches to resistance to EGFR TKIs

Treatment options at EGFR TKI resistance include chemotherapy (standard treatment), EGFR TKI beyond progression, third generation TKIs, and chemotherapy plus EGFR TKI.

TKI continuation beyond progression

In routine practice, oncologists typically discontinue a given therapy at the time of disease progression. It remains unclear, however, whether similar approaches should apply to TKIs in *EGFR*-mutant patients because resistance may be heterogeneous and TKI discontinuation may precipitate a disease flare [Chaft *et al.* 2011]. In cases of isolated progression [e.g. central nervous system (CNS)], local therapies followed by continuation of the relevant targeted therapy may be a viable approach in select patients [Weickhardt *et al.* 2012; Gan *et al.* 2014].

Recently, a phase II open-label single-arm trial, named ASPIRATION, assessed the efficacy of first-line erlotinib until RECIST progressive disease (PD), efficacy beyond PD if erlotinib was continued by the investigator, and safety in Asian patients with EGFR mutation positive NSCLC [Park *et al.* 2014]. Patients received erlotinib 150 mg/day orally and the primary endpoint was PFS 1 (time to RECIST PD/death); secondary endpoints included PFS 2 (time to off-erlotinib PD if erlotinib was extended beyond RECIST PD). The intent-to-treat (ITT) population included 207 patients; 150 patients had a RECIST PD event at data cutoff and 81 patients continued post-PD erlotinib. Median PFS 1 was 10.8 months, while median PFS 2 was 13 months. In patients receiving post-PD erlotinib, the difference between PFS 1 and PFS 2 was 3.7 months. Post-PD erlotinib patients had deeper response, longer PFS, longer time from best overall response to progression, less new lung lesions and better Performance Status (PS) at progression. The authors concluded that the approach of post-PD erlotinib is feasible, with a difference between PFS 1 and PFS 2 of 3.7 months in post-PD erlotinib patients. However, the validation of the optimal patient subset needs to be further clarified. Moreover, it underlines the limitations of the ASPIRATION trial, that is, it is a single arm noncomparative phase II trial just suggesting the feasibility of the beyond-progression approach, without any demonstration of efficacy. In fact, considering that ASPIRATION was not a randomized trial, patients continuing beyond progression may have been selected because they had better TKI response or because they had a disease with a more favorable prognosis.

Chemotherapy plus EGFR TKI

For patients with acquired resistance, an option is continuing EGFR TKI therapy in combination with platinum-based doublet chemotherapy. This option is suggested to be beneficial because of the potential tumor heterogeneity at the time of EGFR TKI resistance, and it is also supported by

retrospective clinical studies. The phase III, double-blind IRESSA Mutation Positive Multicentre Treatment Beyond ProgrRESsion Study (IMPRESS) evaluated the efficacy and safety of continuing gefitinib plus pemetrexed/cisplatin *versus* placebo plus pemetrexed/cisplatin in patients with acquired resistance to first-line gefitinib [Mok *et al.* 2014]. The primary endpoint was PFS and secondary endpoints included overall survival (OS), objective response rate, disease control rate, safety/tolerability and health-related quality of life. In the 265 patients randomized, there were more patients ⩾65 years old in the gefitinib arm and more patients with baseline brain metastases in the chemotherapy arm. However, no statistically significant improvement in PFS for gefitinib *versus* placebo was reported. Median PFS was 5.4 months in each arm [hazard ratio (HR) = 0.86; $p = 0.273$]. The OS data was immature (33% of patients had died), with better OS for placebo *versus* gefitinib (HR = 1.62; $p = 0.029$). *Ad hoc* PFS and OS analyses included the addition of brain metastases at baseline as a covariate (brain metastases *versus* no brain metastases), but there was no difference in term of PFS. No treatment differences were found in RR and disease control rate (DCR), and the safety profile for gefitinib plus pemetrexed/cisplatin was in line with what is already known. Postdiscontinuation therapy in the ITT population was higher in the placebo arm, where 17% of patients received platinum-based regimens in comparison with 5% in the gefitinib arm, and 44% received EGFR TKI therapy *versus* 30% of patients in the gefitinib arm.

In conclusion, the IMPRESS study showed no statistically significant improvement in PFS with continuation of gefitinib in addition to chemotherapy beyond RECIST progression to first-line EGFR TKI for patients with *EGFR* mutation-positive NSCLC. The IMPRESS study confirms that doublet chemotherapy should continue to be the standard of care for patients who develop resistance to first-line EGFR TKIs.

Third-generation TKIs

Second-generation EGFR TKIs, such as neratinib, dacomitinib and afatinib, differ from gefitinib and erlotinib in that they form irreversible covalent bonds with EGFR [Ou, 2012]. In preclinical models, irreversible EGFR TKIs demonstrated promising activity against T790M [Kwak *et al.* 2005]. Unfortunately, clinical trials of these agents in patients with acquired resistance have been largely disappointing, likely as a result of dose limitations from toxicity caused by inhibiting wildtype EGFR [Miller *et al.* 2012]. Recently, third-generation EGFR inhibitors, such as AZD9291, WZ4002 and CO-1686, have been developed. In preclinical studies, these compounds are active against cell lines and murine models harboring T790M mutations and spare wildtype EGFR *in vitro* and *in vivo* [Zhou *et al.* 2009]. As a result, these mutant-selective inhibitors may be able to overcome T790M-mediated resistance while producing less toxicity. AZD9291 is a potent, selective, irreversible EGFR TKI, effective against the EGFR TKI sensitizing and resistance T790M mutations. Recently, updated and very promising results of safety and efficacy from an ongoing phase I study on AZD9291 have been reported [Yang *et al.* 2014]. Patients with EGFR mutation positive NSCLC and acquired resistance to EGFR TKIs were enrolled into dose escalation and expansion cohorts. AZD was administered orally, at doses of 20–240 mg once daily. In 253 patients enrolled, 127 were T790M positive. Adverse events were mostly mild [Common Terminology Criteria for Adverse Events (CTCAE) grade 1/2], with diarrhea (39%), rash (36%) and nausea (18%) the most commonly reported. No dose limiting toxicities were reported in any of the dose escalation cohorts. At the recommended phase II dose of 80 mg every day (QD), rash and diarrhea occurred in 27% (grade 3, 0%) and 20% (grade 3, 1%) of patients, respectively. The confirmed overall RR in patients with centrally tested *EGFR* T790 M positive tumors was 61% (78/127) and the DCR was 95%. In the total population, RR was 51% and, in patients with *EGFR* T790M negative tumors, the confirmed RR was 17%. Among the 78 patients with centrally tested *EGFR* T790M positive and confirmed response, the longest duration of response to date is ongoing at more than 11 months. Preliminary duration of response at 80 mg is 8.2 months. It should be noted that duration of responses to AZD9291 were much shorter in T790M-negative patients, and responses in T790M-negative were more likely to be seen in patients who had not been on another EGFR TKI immediately prior to AZD9291, suggesting that these responses may be a nonspecific 'EGFR TKI retreatment effect'.

Also CO-1686 is an oral, covalent TKI that targets common activating *EGFR* mutations and T790M, while sparing wildtype EGFR. In a

recent dose finding study, 88 patients with *EGFR* mutated advanced NSCLC, and previously treated with EGFR TKIs, were treated [Sequist *et al.* 2014]. Main adverse events were nausea (25%), fatigue (21%) and hyperglycemia (21%). Hyperglycemia was well managed with oral hypoglycemics and/or dose reduction. A recommended phase II dose of 750 mg twice daily (BID) has been selected. Promising activity against T790M+ *EGFR* mutant NSCLC was observed with a reported ORR of 58% to date.

Third-generation EGFR inhibitors are to be considered the most promising strategy to overcome resistance to first- and second-generation EGFR inhibitors. Two very relevant phase III randomized trials comparing cisplatin-based chemotherapy with AZD9291 (AURA 3 trial) [ClinicalTrials.gov identifier: NCT 02151981] or with CO-1686 (TIGER 3 trial) in second-line patients with T790M positive advanced NSCLC who have progressed following prior therapy with an EGFR TKI are ongoing and may establish a new standard treatment in this clinical setting.

Resistance to ALK inhibitors

The first-generation ALK inhibitor crizotinib, in a phase III study named PROFILE 1014, was recently established as the standard of care for patients with previously untreated advanced ALK-positive nonsquamous NSCLC [Mok *et al.* 2014]. In this randomized trial, crizotinib was compared in terms of efficacy and safety with pemetrexed–platinum chemotherapy. The study met its primary objective demonstrating superiority of crizotinib over chemotherapy in prolonging PFS (median 10.9 *versus* 7.0 months; HR: 0.454). The most common adverse events with crizotinib were vision disorders and gastrointestinal symptoms. However, resistance usually occurs within the first year of treatment with crizotinib [Gainor and Shaw, 2013].

Primary resistance

Primary resistance to crizotinib may be due to differences in crizotinib sensitivity observed between different EML4–ALK fusion variants and ALK fusion partners *in vitro* [Heuckmann *et al.* 2012]. Furthermore, rare cases of 'primary resistance' to crizotinib may be due to technical factors rather than intrinsic biology, for example, the false-positive genotyping by ALK fluorescence *in situ* hybridization (FISH) testing.

Acquired resistance

Multiple different mechanisms appear to occur for acquired resistance to crizotinib and are divided into two categories: ALK dominant group, in which ALK remains the dominant driver (secondary mutations in the kinase domain and *ALK* gene amplification of the kinase itself) *versus* ALK nondominant, when separate or second oncogenic drivers occur [Doebele *et al.* 2012].

ALK dominant mechanisms: mutation or amplification. Secondary mutations in kinases are a common mechanism of drug resistance to kinase inhibitors. It is noteworthy that *ALK*-positive patients develop multiple secondary mutations at the time of TKI resistance. This is in contrast to *EGFR*-mutant patients, in whom T790M is essentially the sole secondary mutation observed clinically.

The first major 'gatekeeper' mutation identified in the TK domain of EML4–ALK is L1196M [Choi *et al.* 2010]. The substitution of leucine for a methionine at position 1196 (L1196M) created a mutant bulky amino acid side chain in the ATP binding pocket of the receptor, ultimately interfering with the binding of crizotinib to its receptor. Other 'not-gatekeeper' second-site *ALK* mutations are distributed throughout the kinase domain, including the solvent front (G1202R, S1206Y), ATP binding pocket (G1269A), and *N*-terminal to the C-helix (1151Tins, L1152R and C1156Y) [Doebele *et al.* 2012; Katayama *et al.* 2012].

A gain of *ALK* gene fusion copy number (defined as more than two-fold increase in the mean of the rearranged gene per cells in the post-treatment specimen compared with the pretreatment specimen) has recently been implicated as a cause of crizotinib resistance. This was initially suggested by cell line models in which amplification of wildtype EML4–ALK was sufficient to confer crizotinib resistance, and subsequently in resistant clinical specimens [Doebele *et al.* 2012; Katayama *et al.* 2012].

ALK non dominant mechanisms: bypass signaling. Activation of alternative downstream signaling pathways contributes to acquired resistance in up to 20% of *ALK*-positive NSCLCs [Boland *et al.* 2013]. These include activation of the EGFR, HSP90, PI3K/AKT/mTOR pathways, KRAS mutations and KIT amplifications.

Treatment approaches for crizotinib resistance

Beyond progression

In clinical practice where there is the presence of an 'oligoprogressive disease' to crizotinib (e.g. CNS), local ablative therapy with the continuation of crizotinib may be a viable approach in selected patients. However, an immediate change of therapy should be the better strategy in patients with a significant and symptomatic progression. A recent retrospective analysis conducted on 414 *ALK*-positive NSCLC patients enrolled in PROFILE 1001 (expansion cohort) or PROFILE 1005 showed that patients derived clinical benefit from continued ALK inhibition with crizotinib (for >3 weeks) after RECIST defined PD. These patients were more likely to have good PS at the time of PD, had responded to and exhibited extended time to progression from initial crizotinib treatment, and had a site of PD particularly amenable to local therapy (brain). Not surprisingly, these patients also had a better prognosis as demonstrated by their longer OS from the start of initial crizotinib treatment [Ou *et al.* 2014]. However, treatment with crizotinib beyond progression should be noted to be questionable compared with alternative of transition to second-generation ALK inhibitors with established high response rate and clinical benefit as described in the next paragraph. At this time, timing of transition off of crizotinib and to a second-generation ALK inhibitor must be considered as subject to clinical judgment.

Next generation ALK inhibitors

Second-generation ALK TKIs may be a promising treatment approach in crizotinib-resistant ALK-dominant *ALK*-positive NSCLCs.

Ceritinib (LDK378). Ceritinib (LDK378, Novartis) is an orally, small molecule, ATP-competitive, selective TKI targeting ALK. In enzymatic assays, ceritinib is 20 times as potent as crizotinib against ALK. In xenograft models of *ALK* rearranged NSCLC, ceritinib showed marked antitumoral activity against both crizotinib-sensitive and crizotinib-resistance tumors, suggesting its potential activity in crizotinib naïve patients as well as in patients progressing after crizotinib [Shaw *et al.* 2014a]. These promising preclinical data were confirmed in a dose escalation phase I study enrolling patients with advanced cancers harboring genetic alterations in ALK (ASCEND-1 trial). After determination of maximum tolerated dose (MTD) at the dose of 750 mg once daily (dose-limiting toxic events included diarrhea, vomiting, dehydration, elevated aminotransferase levels and hypophosphatemia), this phase was followed by an expansion phase, for a total of 246 patients overall (163 patients pretreated with crizotinib and 83 patients crizotinib-naive) [Shaw *et al.* 2014a; Felip *et al.* 2014]. RR in the overall population was 58.5%; in 163 crizotinib-pretreated patients it was 54.6% and in 83 crizotinib-naïve patients it was 66.3%. Median PFS in the three groups was 8.2 months, 6.9 months and not estimable, respectively. Discontinuation of treatment because of adverse events occurred in 10% of patients. The most common adverse events of any grade were diarrhea (86%), nausea (80%), vomiting (80%), abdominal pain (54%) and fatigue (52%). Most common laboratory abnormalities of any grade were decreased hemoglobin (84%), increased alanine aminotransferase (ALT) (80%), increased aspartate transaminase (AST) (75%) and increased creatinine (58%). Most common grade 3/4 laboratory abnormalities were increased ALT (27%), increased AST (13%) and increased glucose (13%). One treatment-related death (interstitial lung disease) was reported. In conclusion, ceritinib was demonstrated to have a worse safety profile than crizotinib, in particular with a higher incidence of grade 3 or 4 drug-related diarrhea (7% *versus* 0%) and nausea (5% *versus* 1%) and often requiring dose reduction. Thus, ceritinib was active in the majority of patients with *ALK*-rearranged NSCLC who received crizotinib previously, reporting an ORR and PFS similar to those seen after initial crizotinib treatment. Responses were observed in patients with various resistance mutations in *ALK* and in patients without detectable mutations. These findings suggest that the large majority of crizotinib-resistant tumors may remain ALK-dependent and that an important factor contributing to crizotinib resistance may be subtherapeutic inhibition of the target, which may be overcome by more potent and structurally distinct ALK inhibitors such as ceritinib. Thus, it should be underlined that since crizotinib is substantially less potent *versus* ALK than second-generation ALK inhibitors like ceritinib, it is understandable that patients with acquired resistance to crizotinib can still respond to another (far more potent) ALK inhibitor.

Moreover, Shaw and colleagues recently reported the efficacy and safety of ceritinib therapy in the

subset of ALK+NSCLC patients with brain metastases treated in the phase I study ASCEND-1. Ceritinib 750 mg/day resulted effective in patients with brain metastases, whether ALK inhibitor pretreated or ALK inhibitor naïve. In fact, the RR in 98 ALK inhibitor pretreated patients was 50% and 69.2% in 28 ALK inhibitor naïve patients [Shaw *et al.* 2014b].

Two phase III trials are ongoing and comparing ceritinib with chemotherapy in patients previously treated with platinum-based chemotherapy and crizotinib [ClinicalTrials.gov identifier: NCT01828112] and as first-line setting in chemo- and crizotinib-naïve patients [ClinicalTrials.gov identifier: NCT01828099]. In the NCT01828112 trial, ceritinib is compared with pemetrexed or docetaxel, and in NCT01828099 trial with pemetrexed plus cisplatin or carboplatin [Shaw *et al.* 2014b].

Alectinib (CH5424802/RO5424802). Alectinib (Chugai and Roche Pharmaceuticals) is a highly potent selective ALK inhibitor (10-fold greater potency than crizotinib) and with activity against L1196M gatekeeper mutation as well as other secondary mutations such as F1174L and R1275Q. Results from a recent phase I/II study with alectinib in a Japanese population have been published [Seto *et al.* 2014]. In the phase II part of the study, a total of 46 patients (crizotinib pretreated and naïve) were treated with recommended dose of 300 mg BID with an ORR of 93.5% (43/46). Grade 3 treatment-related adverse events were reported in 12 (26%) of patients; serious side effects occurred in 5 patients (11%), including decreased neutrophils and increased blood creatine phosphokinase (CPK).

Alectinib showed a significant clinical activity also in 47 *ALK*-positive NSCLC patients who were refractory to crizotinib in a phase I/II trial [Gadgeel *et al.* 2013]. In the phase I part of the trial, alectinib at oral dose of 600 mg BID was chosen as recommended phase II dose. Most common adverse events (⩾15%) were fatigue, myalgia, peripheral edema, increased blood CPK and nausea; grade 3–4 adverse events included γ-glutamyltransferase increase (4%), neutropenia (4%), hypophosphatemia (4%), hyperglycemia, syncope, acute renal failure and pericardial effusion (2% each). The ORR was 54.5%. In both trials, alectinib demonstrated consistent and rapid clinical activity against CNS metastasis in *ALK*-positive NSCLC patients who progressed on crizotinib. This interesting result is probably due to higher penetration into the CNS of alectinib compared with crizotinib.

Recently, further efficacy and safety data were reported for alectinib in 28 crizotinib-pretreated NSCLC patients [Seto *et al.* 2014]. Confirmed RR was 58.3% and DCR was 83.3%. A total of 19 of 28 patients had brain metastases at baseline and 6 patients had no prior brain irradiation. A total of 13 patients with brain metastases, including 4 patients without prior irradiation, were still on study treatment without progressive disease. The safety profile was favorable, continued the same trend previously reported and no patients discontinued treatment for a safety reason. Gastrointestinal and visual disorders, characteristic of crizotinib treatment, were mild and not so frequent with alectinib. Three phase II/III trials with alectinib are in progress in crizotinib-naïve (ALEXA trial) [ClinicalTrials.gov identifier: NCT02075840] as well as in crizotinib-resistant patients [ClinicalTrials.gov identifier: NCT01871805, NCT01801111].

AP26113 and other novel ALK inhibitors. In addition to ceritinib and alectinib, other second-generation ALK inhibitors (AP26113, ASP3026, TSR-011, PF-06463922, RXDX-101, X-376, X-396, CEP-28122, CEP-37440) are being developed [Awad and Shaw, 2014]. These new agents are expected to exhibit efficacy in the CNS and might help overcome drug resistance. AP 26113 (Ariad Pharmaceuticals) is a novel inhibitor of ALK with activity against L1196M gatekeeper mutation as well as against ROS1 and EGFR (including mutant form with the T790M gatekeeper mutation). AP26113 has showed promising antitumor activity in patients with crizotinib-resistant *ALK*-positive NSCLC, including those with brain metastases, in a phase I/II trial [Gettinger *et al.* 2014]. X-376 and X-396, compared with crizotinib, inhibit ALK with approximately 10-fold greater potency in biochemical assays and 3–10 fold greater potency in cell-based assays. In contrast, crizotinib is a slightly more potent MET inhibitor. In addition, X-396 is an approximately 10-fold more potent inhibitor than crizotinib of the ALK mutants L1196M and C1156Y. Initial results of a phase I study of X-396 showed responses in both crizotinib-naïve and crizotinib-resistant *ALK*-positive NSCLC patients [Horn *et al.* 2014]. These new agents are to be considered further potential options that may or not play a role in treating

Table 1. Novel generation EGFR and ALK inhibitors: some ongoing phase III clinical trials.

Drug	Setting	Comparator regimen
AZD9291	Second-line patients with T790M-positive advanced NSCLC who have progressed following prior therapy with an EGFR TKI (AURA 3 trial)	Platinum-based chemotherapy
CO-1686	Second-line patients with T790M-positive advanced NSCLC who have progressed following prior therapy with an EGFR TKI (TIGER 3 trial)	Platinum-based chemotherapy
Ceritinib	ALK+NSCLC failed to platinum-based chemotherapy and crizotinib [ClinicalTrials.gov identifier: NCT01828112]	Pemetrexed or docetaxel
Ceritinib	ALK+NSCLC as first line [ClinicalTrials.gov identifier: NCT01828099]	Cisplatin or carboplatin plus pemetrexed
Alectinib	Advanced ALK+NSCLC as first line [ClinicalTrials.gov identifier: NCT02075840]	Crizotinib

ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; NSCLC, nonsmall cell lung cancer; TKI, tyrosine kinase inhibitor.

specific acquired resistance mutations or perhaps CNS disease.

Conclusion

The experience with first- and second-generation EGFR TKIs (gefitinib, erlotinib and afatinib) and with first-generation ALK TKI (crizotinib) in NSCLC exemplifies the successes and challenges of personalized cancer medicine. The finding that these agents were effective and changed substantially the prognosis in a relevant proportion of NSCLC patients with *EGFR* mutations or *ALK* rearrangements was a major advance; however, resistance to these agents sets in after approximately 1 year of treatment and remains the main challenge in this clinical setting. Third-generation EGFR TKIs such as AZD9291 and CO-1686 are being developed as part of the strategy to overcome treatment resistance to first- and second-generation EGFR TKIs. Phase III trials are ongoing (Table 1) and these agents represent the most promising approach to the issue. In addition, novel second-generation ALK inhibitors like ceritinib and alectinib are currently in clinical development (phase III trials) and are producing encouraging results in *ALK*-positive NSCLC, even in patients with acquired resistance to crizotinib. In particular, ceritinib is already approved by the US Food and Drug Administration (FDA) in the treatment of advanced crizotinib-resistant *ALK*-positive NSCLC.

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