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Overcoming resistance to first/second generation epidermal growth factor receptor tyrosine kinase inhibitors and ALK inhibitors in oncogene-addicted advanced non-small cell lung cancer

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***Abstract*:** Epidermal growth factor receptor (EGFR) activating mutations and anaplastic lymphoma kinase (ALK) gene rearrangement in advanced non-small cell lung cancer (NSCLC) represent the two oncogenic events with an impact on current clinical practice. EGFR tyrosine kinase inhibitors (TKIs) and crizotinib are the standard of care for the treatment of EGFR mutant and ALK gene rearranged advanced NSCLC patients. Unfortunately, despite initial clinical benefit, acquired resistance to EGFR-TKIs or crizotinib usually develops after an average of 10–12 months of treatment. The aim of this review is to describe the mechanisms of resistance to first/second generation EGFR-TKIs and crizotinib. In particular, we focus on strategies to overcome resistance due to secondary EGFR T790M mutation and mutations of the ALK domain.

Keywords: AZD9291, ceritinib, crizotinib, EGFR-TKIs, EML-ALK gene rearrangement, epidermal growth factor receptor mutations, resistance, rociletinib

Introduction

Lung cancer treatment has evolved significantly during the past decade but this disease remains the leading cause of cancer-associated mortality worldwide [Siegel *et al.* 2015]. Non-small cell lung cancer (NSCLC) accounts for almost 85% of all lung cancer cases [Ettinger *et al.* 2012; Janssen-Heijnen and Coeburgh, 2003] and can be divided in squamous cell lung cancer and non-squamous cell lung cancer (mainly adenocarcinoma and large cell carcinoma) [Kumar *et al.* 2013]. It is now well established that NSCLC can be further more classified to different subtypes according to the oncogenic events that drive carcinogenesis at a molecular level [Chan and Hughes, 2015]. The two oncogenic events that currently have an impact in clinical practice are activating mutations of the epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) gene rearrangement.

EGFR activating mutations are found in approximately 10–30% of patients with nonsquamous NSCLC [Lynch *et al.* 2004; Paez *et al.* 2004; Pao *et al.* 2004]. The most frequent mutations are small in frame deletions in exon 19 and a point mutation in exon 21 that leads to the substitution of leucine with arginine (L858R) and constitute 90% of all EGFR mutations [Sharma *et al.* 2007; Pao and Miller 2005]. The presence of these mutations is predictive for response to EGFR tyrosine kinase inhibitors (TKIs) such as gefitinib, erlotinib and afatinib. Several randomized phase III trials have shown that, in patients with advanced EGFR mutant NSCLC, first-line treatment with EGFR-TKIs is superior to chemotherapy in terms of overall response rate (ORR), progression-free survival (PFS), toxicity and quality of life (QoL) [Mok *et al.* 2009; Maemondo *et al.* 2010; Mitsudomi *et al.* 2010; Han *et al.* 2012; Zhou *et al.* 2011; Rosell *et al.* 2012; Sequist

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et al. 2013; Wu *et al.* 2014] and therefore treatment with either gefitinib, erlotinib or afatinib is now recommended as first-line treatment for advanced EGFR mutant NSCLC [Masters *et al.* 2015; Reck *et al.* 2014].

In 2007, Soda and colleagues discovered that rearrangements between the anaplastic lymphoma kinase (ALK) gene and the echinoderm microtubule-associated protein-like 4 (EML4) gene serve as oncogenes in a small subset of patients with NSCLC [Soda *et al.* 2008]. ALK gene rearrangements are found in 2–7% of all NSCLC patients and are more frequently described in younger patients (<50 years old), light/never smokers and adenocarcinoma histology [Shaw *et al.* 2009].

Two randomized trials have shown that crizotinib, an inhibitor of ALK, MET and ROS1, is superior to standard chemotherapy in patients with advanced/metastatic NSCLC that harbor ALK rearrangements in terms of ORR, PFS and QoL [Shaw *et al.* 2013; Solomon *et al.* 2014]. On the basis of these studies, crizotinib is approved for chemotherapy-naive and pretreated patients by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Unfortunately, despite initial clinical benefit, acquired resistance to EGFR-TKIs or crizotinib usually develops after an average of 10–12 months of treatment [Maione *et al.* 2015].

This review describes the mechanisms of resistance to first/second generation EGFR-TKIs and crizotinib. In particular, we focus on strategies to overcome resistance due to secondary EGFR T790M mutation and mutations of the ALK kinase domain.

Overcoming resistance to EGFR-TKIs

Acquired resistance to EGFR-TKIs has been defined as disease progression after treatment with a single agent EGFR-TKI in patients harboring an activating EGFR mutation [Jackman *et al.* 2010]. The molecular events that lead to this resistance can be divided in two main categories. The first is a secondary mutation in the primary driver oncogene (EGFR gene) that makes EGFR TKIs ineffective in inhibiting downstream signaling. The second is mutations on other genes that allow bypass signaling and continuous cell proliferation despite the inhibition of the mutant EGFR gene [Gainor and Shaw 2013].

The most common mechanism of resistance (50–60% of patients) is the acquisition of a secondary T790M mutation on exon 20 [Sequist *et al.* 2011; Kobayashi *et al.* 2005; Pao *et al.* 2005]. This mutation leads to the substitution of threonine by methionine at position 790 that encodes part of the kinase domain of the receptor and results in increased affinity for adenosine triphosphate (ATP), causing resistance to competitive inhibition by first/second generation EGFR-TKIs [Pao *et al.* 2005; Yun *et al.* 2008]. In a small subset of patients (<5%), other secondary mutations (D761Y, T854A, L747S) have been described but their role is still unclear [Nguyen *et al.* 2009].

Bypass signaling and activation of pathways that drive carcinogenesis independently from EGFR activation is another mechanism of resistance. Examples of bypass pathways are MET activation either by amplification or activation through its ligand, hepatocyte growth factor (HGF) (5%), human epidermal growth factor receptor type 2 (HER2) amplification (8–13%), PIK3CA mutation (2%) and BRAF mutation (1%). Phenotypic changes have been described in a small subset of patients: either transformation to small cell lung cancer (SCLC) (6%) or epithelial-to-mesenchymal transformation (EMT) (1–2%) [Camidge *et al.* 2014]. The complexity of resistance mechanisms highlights the importance of rebiopsying the tumor at the time of disease progression as this information can guide enrollment in relevant clinical trials and appropriate management.

Two prospective trials have addressed the feasibility of rebiopsying at progression. The GFPC 12-01 study enrolled 100 NSCLC patients from 18 centers and rebiopsy at progression was feasible in 82%, with sufficient material for histological examination in 96% of the cases. Rebiopsy had a clinical impact in almost 30% of patients, tailoring treatment accordingly [Chouaid *et al.* 2014]. A study by Yu and colleagues evaluated 175 EGFR mutant patients who underwent rebiopsy at the time of acquired resistance to first-line EGFR-TKI treatment. Rebiopsy was feasible in 92% of patients, with sufficient material for histology in 95% of the cases. EGFR T790M was the most frequent mechanism of resistance (63%) followed by MET amplification (5%), HER2 amplification (13%) and SCLC transformation (3%) [Yu *et al.* 2013].

Currently, there is no consensus regarding standard treatment after resistance develops and the following strategies have been described.

Continuing EGFR-TKI beyond progression

Prior to the increasing knowledge regarding mechanisms of resistance, clinicians had observed that discontinuation of first-line EGFR-TKI may lead to a rapid disease progression (disease flare) in up to 23% of patients [Chaft *et al*. 2011]. This led to the suggestion that continuing with the same treatment beyond progression may be beneficial for a subset of patients, especially in patients who are asymptomatic or mildly symptomatic at time of progression. This approach has shown to be viable especially in patients with oligometastatic disease in whom local therapy at the site of progressive disease (PD) (surgery, radiotherapy, radiofrequency ablation) and continuation of the same TKI has proven in small series to be beneficial [Oxnard *et al*. 2012; Weickhardt *et al*. 2012].

ASPIRATION, a recent single arm phase II trial, assessed the efficacy of continuing erlotinib in the first-line setting after progression in EGFR mutant Asian patients. The study enrolled 207 patients and 81 out of 150 who had confirmed progressive disease at data cutoff continued on erlotinib (150 mg/day) resulting in longer PFS (9.3 *versus* 7.2 months) [Park *et al*. 2014]. These results suggests that patients with asymptomatic or oligometastatic progression may benefit from continuing first-line EGFR beyond radiological PD.

Combination of first-line EGFR-TKI with chemotherapy

The phase III trial IMPRESS has recently addressed the question of potential benefit of continuing first-line EGFR-TKI with chemotherapy after PD. This study randomized 265 EGFR mutant patients treated with first-line gefitinib to receive standard chemotherapy with cisplatin/pemetrexed alone or combined with gefitinib. There was no difference in PFS between the two groups (5.4 months) and the authors concluded that platinum-based chemotherapy should be the standard approach [Soria *et al*. 2015].

Second generation EGFR-TKIs

Soon after acquired resistance to first generation EGFR-TKIs was found and gradually understood, researchers started to develop second generation EGFR-TKIs that bind irreversibly to EGFR, and inhibit HER2 and HER4 as well, in an effort to overcome resistance. Preclinical studies with these agents showed they can be effective in post first-line EGFR treatment setting with some activity against tumors harboring T790M mutation [Li *et al*. 2008; Engelman *et al*. 2007; Yang *et al*. 2012]. Following these findings afatinib, dacomitinib and neratinib, three second generation EGFR-TKIs, were evaluated in clinical trials,

Afatinib has been extensively studied in EGFR mutant patients in the first and subsequent lines of treatment. A large phase II trial (LUX Lung 2) evaluated afatinib in EGFR mutant patients with advanced/metastatic disease who had received no previous EGFR-TKI treatment and no more than one line of cytotoxic chemotherapy. Afatinib was found to be effective with an ORR of 61% [Yang *et al*. 2012]. Following this trial, afatinib was tested in the first-line setting in patients with EGFR mutant advanced NSCLC against cytotoxic chemotherapy in two large phase III trials (LUX-Lung 3 and LUX-Lung 6) and was found to be superior in terms of ORR, PFS and QoL [Sequist *et al*. 2013; Wu *et al*. 2014]. Based on these trials, afatinib has been approved as first-line for EGFR mutated patients with advanced/metastatic NSCLC and is now recommended by international guidelines [Masters *et al*. 2015; Reck *et al*. 2014].

Unfortunately, afatinib has showed only modest activity in the post first generation EGFR-TKI treatment with a PFS of 3.3 months and no overall survival (OS) benefit as shown in a phase III trial that evaluated afatinib *versus* placebo in this setting [Miller *et al*. 2012]. The combination of afatinib with cetuximab, a monoclonal antibody targeting EGFR, was tested in a phase Ib clinical trial [Janjigian *et al*. 2014]. The study cohort included EGFR mutant patients who had progressed after treatment with first generation EGFR-TKIs. The ORR (overall 29%) was comparable in T790M-positive and T790M-negative tumors (32% *versus* 25%; $p = 0.341$) and the median PFS was 4.7 months (95% CI 4.3–6.4). Although this trial showed that the combination can be effective, its clinical significance is limited by the increased toxicity observed with the treatment-related adverse events observed in 99% of patients, 46% of which was ⩾grade 3.

Dacomitinib has been evaluated in two large phase III trials. ARCHER 1009 evaluated dacomitinib *versus* erlotinib in unselected pretreated patients and the median PFS was similar in the two groups (2.6 months, 95% CI 1.9–2.8) [Ramalingam *et al*.

2014]. Dacomitinib was also tested *versus* placebo in pretreated patients who had received up to three previous lines of chemotherapy and either gefitinib or erlotinib. The study did not show any difference in OS between the two groups (6.83 *versus* 6.31 months, $p = 0.506$) for dacomitinib and placebo, respectively [Ellis *et al.* 2014]. The phase III trial ARCHER 1050 [ClinicalTrials.gov identifier: NCT01774721] is ongoing to evaluate dacomitinib *versus* gefitinib in the first-line setting in EGFR mutant patients.

Neratinib has been evaluated in a phase II trial in pretreated patients with advanced NSCLC. The study allocated 167 patients to 3 arms: arm A, EGFR mutant patients or patients with previous TKI exposure ⩾12 weeks; arm B, EGFR wildtype (WT) patients; and arm C, adenocarcinoma histology and light smoking history (⩽20 pack-years). Neritinib had low activity with ORR 3% in arm A; no responses were seen in arms B and C. The most common toxicity was diarrhea (grade 3: 50%) and improved with dose reduction [Sequist *et al.* 2010].

Tackling T790M mutation

Third generation EGFR-TKIs are designed to inhibit both T790M and EGFR activating mutations while sparing WT EGFR. So far, three agents of this class have been tested in a clinical setting: mereletinib (AZD9291, Astra Zeneca), rociletinib (Clovis Oncology) and HM61713 (Hanmi Pharmaceutical Company Ltd).

Mereletinib was initially tested in a multicohort phase I clinical trial (AURA) in EGFR mutant patients who progressed after treatment with first-line EGFR-TKI and produced an ORR for the entire cohort of 51% (95% CI 45–58) and a disease control rate (DCR) [complete response (CR) plus partial response (PR) plus stable disease (SD)] of 84% (95% CI 79–88). In patients harboring the T790M mutation, the ORR was 61% (95% CI 52–70) and the DCR was 95% (95% CI 90–98). Interestingly, there was activity in the T790M negative patients as well, with an ORR of 21% (95% CI 12–34) and DCR of 61% (95% CI 47–73). The median PFS was 9.6 months (95% CI 8.3 to not reached) in EGFR T790M-positive patients and 2.8 months (95% CI 2.1–4.3) in EGFR T790M-negative patients. Mereletinib was overall well tolerated, no dose limiting effects were observed and any grade ⩾3 adverse events were reported in 32% of patients. The main side effects were diarrhea and skin toxicity, which were mild [Jänne *et al.* 2015]. Recently, updated data were presented at the World Conference on Lung Cancer 2015 from the phase II extension cohort of this trial assessing the efficacy, tolerability and safety of mereletinib at the dose of 80 mg once daily in the T790M mutated patients progressing after EGFR-TKI treatment. At the time of presentation, 199 patients were included and the median time of follow up was 8.2 months. Similarly to the results from the phase I trial, the ORR in the T790M positive population was 61% (95% CI 54–68) with a DCR of 91% (95% CI 85–94). Mereletinib was overall well tolerated with drug-related grade ⩾3 adverse events reported in 12% of the patients and a discontinuation rate of 4% [Yang *et al.* 2015].

At the same conference, these encouraging results were further more supported by preliminary results from AURA 2, a phase II single arm study, assessing the efficacy of mereletinib 80 mg dose in T790M positive patients after progression on standard EGFR-TKI treatment. The ORR was 71% (95% CI 64–77) with a DCR of 92% (95% CI 87–95) and a median PFS of 6.8 months (with low maturity, 38% of events). Again, the drug was associated with low incidence of grade ⩾3 adverse events (11%) and a good safety profile [Mitsudomi *et al.* 2015]. Preliminary results of the cohort of patients treated as first-line were presented at the ASCO Annual Meeting 2015 and showed that 81% (95% CI 68–89) of patients were progression-free at 9 months with an ORR of 73% (95% CI 60–84) [Ramalingam *et al.* 2015].

Following these encouraging results, mereletinib is currently being tested in two randomized phase III trials: FLAURA [ClinicalTrials.gov identifier: NCT02296125] evaluates mereletinib as a first-line treatment in EGFR mutant patients *versus* standard EGFR-TKI treatment and AURA-3 [ClinicalTrials.gov identifier: NCT02151981] randomizes patients with T790M mutation at progression after first-line standard EGFR-TKIs to receive mereletinib or platinum/pemetrexed.

Rociletinib was initially tested in a phase I/II trial (TIGER X) in EGFR mutant patients who progressed after at least one line of EGFR-TKI treatment. Patients underwent rebiopsy with central screening for the T790M mutation. The ORR in the T790M positive cohort was 59% (95% CI 45–73) with a PFS of 13.1 months (95% CI

5.4–13.1). In the T790M negative population, ORR and PFS were 29% (95% CI 8–51) and 5.6 months (95% CI 1.3 to not reached), respectively. Rociletinib had a good toxicity profile with main side effects (any grade) being hyperglycemia (47%), nausea (35%), fatigue (24%) and diarrhea (20%). The most common grade 3 adverse event was hyperglycemia (22%) that was managed with dose reduction, oral hypoglycemic treatment or both. No treatment discontinuation was reported due to hyperglycemia. Based on preclinical studies, hyperglycemia is caused by a rociletinib metabolite, M502, that inhibits reversibly insulin growth factor type 1 receptor (IGF1R) and insulin receptor [Sequist *et al.* 2015]. Inhibition of IGF1R may prove beneficial as mediates resistance to EGFR inhibitors in NSCLC models [Cortot *et al.* 2013]. Rociletinib is currently being evaluated in phase II and III clinical trials.

TIGER 1 [ClinicalTrials.gov identifier: NCT02186301] is a phase II/III trial testing rociletinib *versus* erlotinib in the first-line setting in EGFR mutated patients, TIGER 2 [ClinicalTrials.gov identifier: NCT02147990] is a phase II trial evaluating rociletinib in second-line post standard EGFR-TKI treatment and TIGER 3 [ClinicalTrials.gov identifier: NCT02322281] is a phase III trial evaluating rociletinib *versus* standard chemotherapy in patients who have progressed after standard EGFR-TKI and platinum-based doublet chemotherapy. On the basis of available data, it is expected that mereletinib and rociletinib will be approved for use in clinical practice in 2016.

HM61713 is being evaluated in a phase I/II trial [ClinicalTrials.gov identifier: NCT01588145] in EGFR mutated patients who have progressed after standard first-line EGFR-TKI. Preliminary data showed that, in T790M positive patients, the ORR and the DCR were 58.8% and 97.1%, respectively. Treatment-related adverse events were reported in 87.3% of patients and included diarrhea, rash, skin exfoliation, nausea, pruritus, decreased appetite and dry skin [Park *et al.* 2015]. A phase II trial is currently ongoing [ClinicalTrials.gov identifier: NCT02444819] evaluating HM61713 in the first-line setting in EGFR mutated patients.

These promising results highlight the importance of understanding the underlying mechanisms of resistance prior to planning the next therapeutic step and thus the importance of rebiopsying at the time of acquired resistance.

Overcoming resistance to crizotinib

Mechanisms of resistance

As for EGFR-TKIs, patients with ALK positive (ALK+) NSCLC treated with crizotinib inevitably also develop acquired resistance to the drug within the first year of therapy. So far, several mechanisms underlying acquired resistance to crizotinib have been elucidated and they conventionally belong to two categories [Doebele *et al.* 2012; Camidge *et al.* 2014; Katayama *et al.* 2012]. The first group of mechanisms can be considered as target-dependent, as they preserve the dominance of ALK signaling. ALK-dominant mechanisms can occur through mutations in the kinase domain of ALK or ALK fusion gene amplification, the latter alone or in combination with resistance mutations [Doebele *et al.* 2012; Camidge *et al.* 2014]. ALK mutations account for approximately 30% of failures to crizotinib, have comparable frequencies and seem to be associated with different sensitivity to crizotinib and other ALK inhibitors [Camidge *et al.* 2014]. A broad spectrum of ALK mutations has been identified in preclinical and clinical models [Katayama *et al.* 2012]. The first and most well characterized is the L1196M mutation, also called a 'gatekeeper' mutation for its ability to interfere with the ligand site of crizotinib [Choi *et al.* 2010]. Other mutations are G1202R, S1206Y, G1269A, 1151ins, F1174L and D1203N [Choi *et al.* 2010; Sasaki *et al.* 2010]. Notably, different resistant mutant clones may exist in the same patient [Choi *et al.* 2010]. Another mechanism is the increase or amplification of the number of rearranged EML4-ALK genes per cell, relative to nonresistant cells [Katayama *et al.* 2012]. In this scenario, it is possible that not all EML4-ALK fusion proteins in a tumor are inhibited by standard doses of crizotinib, thus allowing sufficient downstream signaling for tumor cell survival.

The second group includes ALK nondominant mechanisms, as they determine the activation of other pathways, such as EGFR or KIT through c-KIT gene amplification [Doebele *et al.* 2012; Camidge *et al.* 2014; Sasaki *et al.* 2011; Tanizaki *et al.* 2012]. Acquired mutations in EGFR and KRAS genes after treatment with crizotinib in ALK+ tumors have also been described but their contribution to acquired resistance is unclear

[Katayama *et al.* 2012]. However, reports of the outgrowth of KRAS and EGFR mutated, ALK-negative tumors from patients with ALK translocated NSCLC previously treated with crizotinib might demonstrate the emergence of a separate oncogenic driver as a resistance mechanism [Doebele *et al.* 2012]. Heat shock protein 90 (Hsp90) is a molecular chaperone that regulates the correct folding, stability, and function of numerous client proteins [Sang *et al.* 2013]. The EML4-ALK fusion protein is one of these client proteins and inhibition of Hsp90 causes regression of EML4-ALK driven xenografts and murine lung adenocarcinomas [Chen *et al.* 2010]. This blockade might also overcome drug resistance mechanisms, therefore providing a rationale for the use of Hsp90 inhibitors in crizotinib-resistant ALK+ NSCLC [Sang *et al.* 2013]. There is a consistent fraction of patients for which disease progression occurs only in the central nervous system (CNS), suggesting inadequate CNS drug penetration [Gainor *et al.* 2013; Chun *et al.* 2012; Costa *et al.* 2011]. However, it is not possible to exclude that, in ALK+ NSCLC, the CNS could be simply a preferential location of metastatic spread. Indeed, approximately half of patients develop brain metastases independent of whether they receive crizotinib or not, suggesting that the drug might not affect the brain affinity (or organotropism) of the disease [Shaw *et al.* 2011; Costa *et al.* 2015; Preusser *et al.* 2013]. In addition, it is important to point out that mechanisms of acquired resistance have been exclusively studied from biopsies of progressive extracranial sites, thus precluding the possibility to describe correctly how the occurrence of secondary molecular events might explain intracranial failure [Katayama *et al.* 2012; Shaw *et al.* 2014a]. Unfortunately, the mechanism of resistance remains unknown in about 15% of the patients. There is an urgent need for novel and more potent compounds, able to overcome or possibly delay resistance and to inhibit tumor growth in sanctuary site of metastases such as the CNS.

Continuing crizotinib beyond progression

From a clinical point of view, radiological disease progression is most often evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST). NSCLC tumors that increase in size or in number of lesions while on crizotinib therapy are considered resistant to the drug [Eisenhauer *et al.* 2009]. Furthermore, not all RECIST-defined progressions necessarily require an immediate therapeutic change. Some patients with ALK+ NSCLC often progress slowly, in limited pre-existing sites (oligoprogression) or in a single new site, and without worsening of their symptoms. In such scenario, probably sustained by an ALK-dominant mechanism of resistance, premature discontinuation of ALK inhibition should not be the preferred therapeutic choice due to the risk of disease flare, as recently described [Pop *et al.* 2012; Kuriyama *et al.* 2013]. Although no randomized prospective trials have been design to specifically address this question, continuation of crizotinib beyond progression in association with local therapies, including radiotherapy, local ablation or surgery, could represent a suitable option for optimizing the duration of crizotinib therapy [Camidge *et al.* 2012; Weickhardt *et al.* 2012; Ou *et al.* 2014]. A retrospective series reported that the addition of local treatments directed against only the sites of disease progression may extend disease control by more than 6 months [Weickhardt *et al.* 2012]. Furthermore, continuation of crizotinib beyond progression was permitted in the PROFILE trial, which evaluated the efficacy and safety of crizotinib in patients with advanced ALK+ NSCLC [Ou *et al.* 2014]. In particular, in 120 patients enrolled in PROFILE 1001 and 1005 who continued crizotinib for >3 weeks post-RECIST progression, the median duration of crizotinib treatment beyond progression was 19.4 weeks (95% CI 16.7–28.9) and median OS from the time of first progression was significantly longer for patients continuing crizotinib compared with patients who stopped [16.4 *versus* 3.9 months; hazard ratio (HR 0.27), $p < 0.0001$). Notably, patients who benefited from continuing crizotinib were more likely to have a good performance status, had achieved an objective response to crizotinib, and had a site of progressive disease that was amenable to local therapy, such as the brain, highlighting the importance of appropriate patient selection.

In contrast, for patients experiencing rapid radiological and clinical progression, progressing clones have become completely refractory to crizotinib or addicted to another driver and treatment with crizotinib should therefore be replaced by conventional chemotherapy or a next generation ALK inhibitor.

Second generation ALK inhibitors

Several novel second generation ALK inhibitors are currently being investigated in clinical trials,

both in crizotinib-refractory and in crizotinib-naïve settings [Shaw *et al.* 2014a; Mok *et al.* 2015; Seto *et al.* 2013; Ou *et al.* 2013; Camidge *et al.* 2015]. Among them, ceritinib (Zykadia™, LDK378), alectinib (Alecensa™, CH/RO5424802) and brigatinib (AP26113) have produced interesting results and are currently in advanced phase of clinical development.

Ceritinib

Ceritinib (Zykadia™, LDK 378) is a novel second generation ALK inhibitor with a greater preclinical antitumor potency than crizotinib which has demonstrated efficacy in patients with acquired resistance to crizotinib [Shaw *et al.* 2014a]. The phase I study, ASCEND-1, enrolled 130 patients with ALK+ solid tumors, the majority of whom (94%) had advanced NSCLC; 68% of the patients with NSCLC had received crizotinib previously. In the crizotinib-resistant population, the ORR was 56% and responses were also durable [median duration of response (DOR) of 8.2 months, 95% CI 6.9–11.4]. The median PFS was 7.0 months (95% CI 5.6–9.5). Interestingly, 19 of the crizotinib-refractory patients underwent repeated biopsy at the time of study entry and in a small fraction was possible to detect a secondary ALK mutation (n = 5) or ALK gene amplification (n = 2), whereas the majority (n = 12) retained the original ALK translocation. Overall, the safety profile of ceritinib was similar to that of crizotinib, although there was a higher incidence of certain adverse events (e.g. grade 3 or 4 nausea). The most common adverse events (all grades) included nausea (82%), diarrhea (75%), vomiting (65%) and fatigue (47%). Based on these findings, in April 2014 ceritinib was granted FDA accelerated approval for the treatment of patients with ALK-positive crizotinib-refractory NSCLC. Updated results of the ASCEND-1 trial, after additional accrual in the expansion cohort (n = 246, 163 crizotinib-pretreated, 83 crizotinib-naive), have been recently presented [Felip *et al.* 2014]. In the overall population, ORR was 61.8%, with a median DOR exceeding 9 months and a median PFS of 9.0 months. In addition, these results provided additional information on the efficacy of ceritinib in patients with brain metastases. Data from 124 patients with CNS disease at baseline were collected and separately analyzed [Shaw *et al.* 2014b]. Systemic response, DOR and PFS were consistent with results observed in general population. Among the 74 evaluable patients, 10 (34%) had measurable disease and achieved a PR, whereas 5 patients with nonmeasurable disease obtained CR, with an intracranial disease control rate (IDCR) of 67.5%.

The ASCEND-2 [ClinicalTrials.gov identifier: NCT01685060] study enrolled 140 ALK-positive NSCLC patients pretreated with at least one prior chemotherapy regimen and who progressed <30 days from last treatment with crizotinib [Mok *et al.* 2015]. The vast majority of patients was Caucasian (60%) and presented with asymptomatic brain metastases (71.4%), of which approximately one third had not received palliative radiotherapy. In the overall population, whole body ORR was approximately 40% with an overall DCR of 77%, whereas median DOR and PFS were 9.7 months and 5.7 months, respectively. Evaluating these results according to the presence of brain metastases, efficacy measures numerically favored the group of patients without CNS involvement (ORR: 52% *versus* 33%; PFS: 11.3 *versus* 5.4 months). However, in the small group of patients with intracranial measurable disease, the IDCR reached 80% with CR or PR observed in 5 out of the 6 patients not previously treated with radiotherapy, thus supporting the potential role of ceritinib in controlling intracranial disease. Two large phase III trials comparing ceritinib *versus* chemotherapy are currently ongoing and have PFS as the primary endpoint. ASCEND-4 [ClinicalTrials.gov identifier: NCT01828099] compares ceritinib *versus* standard platinum-pemetrexed as first-line treatment, whereas ASCEND-5 [ClinicalTrials.gov identifier: NCT01828112] compares ceritinib *versus* pemetrexed or docetaxel in subjects previously exposed to platinum-doublet chemotherapy and crizotinib.

Alectinib

Another promising second generation ALK inhibitor is alectinib, which gained FDA breakthrough therapy designation for ALK+ NSCLC due to the encouraging results from an ongoing phase I/II trial [Ou *et al.* 2013]. This study enrolled 58 patients with ALK+ NSCLC and no prior ALK inhibitor therapy; the ORR for alectinib in the 46 patients enrolled on the phase II part of the study was 93.5% [Seto *et al.* 2013]. Alectinib has been shown to have activity post crizotinib as well. In a phase I study of alectinib in 37 patients with ALK-rearranged NSCLC who progressed after crizotinib and chemotherapy, the ORR was 48% in the overall population and 59.5% in patients receiving doses ⩾460 mg twice a day. Of these ALK+

NSCLC patients, 16 had CNS metastases and alectinib demonstrated rapid benefit in brain metastases in a number of patients, including those resistant to crizotinib. The most common adverse events were fatigue, myalgia, cough, liver enzyme elevation, peripheral edema and rash [Ou *et al.* 2013]. The activity of alectinib in crizotinib-refractory ALK+ NSCLC has been further investigated in the global phase II trial NP28673. A total of 138 patients were enrolled, with 84 having brain metastases at baseline. In the overall population, the ORR and DCR were 50% and 77%, respectively (median DOR 11.2 months), whereas the PFS was approximately 9 months. Alectinib also produced regression of brain metastases in more than 40% of cases, confirming the strong activity of the drug even in presence of intracranial disease [Ou *et al.* 2015]. The ALEX trial [ClinicalTrials.gov identifier: NCT 02075840], a phase III trial comparing head-to-head alectinib *versus* crizotinib, has just completed accrual and its results will shed some light on the impact of this second generation ALK inhibitor as frontline treatment in ALK+ NSCLC.

Brigatinib

Brigatinib (AP26113) is a novel, orally active kinase inhibitor that potently inhibits mutant activated forms of ALK and EGFR in cell culture models. Preclinical data showed that this agent had 100-fold selectivity for ALK-positive *versus* ALK-negative cell lines. In addition, brigatinib was active against several ALK mutations, including the L1196M gatekeeper mutation [Camidge *et al.* 2013]. In a recent phase I/II study including 79 ALK+ NSCLC patients pretreated with crizotinib, brigatinib showed an impressive ORR of 72% with a median PFS of 56 weeks. Of note, 6 out of 12 patients with measurable brain lesions at baseline obtained a PR, demonstrating good CNS penetration of this agent. The most common adverse events were fatigue (36%), nausea (45%) and diarrhea (36%), which were generally grade 1/2 in severity. Early onset (<7 days) of pulmonary events such as dyspnea, cough, hypoxia and pulmonary opacities represented a rare but clinical significant type of adverse event observed with higher dose of brigatinib [Camidge *et al.* 2015]. The phase II ALTA (ALK in Lung cancer Trial of AP26113) [ClinicalTrials.gov identifier: NCT02094573] study, designed to evaluate the safety and efficacy of brigatinib in crizotinib-refractory patients, has recently completed accrual and its results are eagerly awaited. In addition, a phase III trial comparing brigatinib to crizotinib as frontline treatment in ALK positive NSCLC is expected to start in early 2016. The efficacy of ceritinib, alectinib or brigatinib may differ according to the type of secondary ALK mutation present, reinforcing the importance to repeat a tumor biopsy at the time of progression.

Conclusion

Occurrence of drug resistance is one of the most relevant limitations in lung cancer therapy. At the beginning of the targeted therapy era, oncologists believed that novel targeted agents could possibly cure patients with advanced lung cancer. Unfortunately, even if targeted therapies are extending survival and improving QoL, all patients inevitably progress after an initial response and some patients are resistant even in the presence of the drug target. Identification of mechanisms of resistance is important due to the potential consequences to patient therapy, QoL and survival. For this reason, many investigators are currently evaluating the changes that occur in the tumor under therapy pressure and the role of new techniques for biomarker assessment. In lung cancer, tumor tissue available for analyses is often limited and repeating a tumor biopsy is not always feasible in a large percentage of patients. Optimizing procedures for biomarker assessment and the possibility of performing analyses in the blood, the so-called liquid biopsy, will be crucial for implementing our knowledge on the mechanisms underlying failure of biomarker-driven targeted therapy.

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Conflict of interest statement

R.C. declares a consultancy/advisory role for AstraZeneca, Roche, Clovis Oncology, Novartis and Pfizer. F.C. declares a consultancy/advisory role for AstraZeneca, Clovis, Novartis and Pfizer. L.L. declares an advisory role for Clovis. O.R. declares no conflicts of interest in preparing this article

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