# *ALK* inhibitors in non-small cell lung cancer: the latest evidence and developments

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***Abstract*:** The treatment of patients with advanced non-small cell lung cancer (NSCLC) harbouring chromosomal rearrangements of *ALK* (anaplastic lymphoma kinase) was revolutionized by crizotinib, a small molecule inhibitor of *ALK, ROS1* and *MET*. Unfortunately, the disease progressed within the first 12 months in most of the patients because of the development of crizotinib resistance in the majority of patients and the emergence of acquired resistance mutations in most of them. Many of them had been reported even before its approval leading to the rapid development of second-generation *ALK* inhibitors for crizotinib-resistant NSCLC. In the last few years, novel potent *ALK* inhibitors with promising results and a good toxicity profile have become available: ceritinib (LDK378), alectinib (RG7853/AF-802/RO5424802/CH5424802), brigatinib (AP26113), entrectinib (RXDX-101, NMS-E628), PF-06463922, ASP3026, TSR-011, X-376/X-396 and CEP-28122/CEP-37440. Moreover, HSP90 (90 kDa heat shock protein) inhibitors have demonstrated clinical activity in patients with *ALK*+ NSCLC. This review focuses on the molecular and clinical properties of this new generation of *ALK* inhibitors under development in the clinic.

***Keywords:*** alectinib, *ALK* rearrangement, ceritinib, crizotinib, NSCLC, resistance

## Introduction

Lung cancer is the most common cause of death from cancer worldwide. It is estimated to be responsible for nearly one in five deaths (1.59 million deaths, 19.4% of total cancer deaths) in 2012 [Ferlay *et al*. 2015]. Most lung cancers (~90%) are non-small cell lung cancers (NSCLCs), which comprise a number of subtypes driven by various activated oncogenes [Ettinger *et al*. 2010; Larsen *et al*. 2011]. Recent advances in molecular profiling technologies have significantly enhanced the development of personalised medicine, i.e. molecularly targeted therapies based on individual genetic or protein profiles [Arnedos *et al*. 2014; Meric-Bernstam *et al*. 2013]. Consequently, molecularly targeted agents for NSCLC patients have become one of the successful personalized cancer therapies [Cardarella and Johnson, 2013; Li *et al*. 2013; Moreira and Thornton, 2012].

Anaplastic lymphoma kinase (*ALK*), a member of the insulin receptor tyrosine kinase family (RTK) [Ullrich and Schlessinger, 1990], is encoded by the *ALK* gene on chromosome 2p23. *ALK* was first identified as part of the NPM-ALK oncogenic fusion protein, resulting from the translocation between chromosomes 2 and 5 (t[2;5][p23;q35]) associated with anaplastic large cell lymphoma [Morris *et al*. 1994]. The same translocation has also been described in Hodgkin lymphoma [Orscheschek *et al*. 1995]. Subsequently, a small inversion within chromosome 2p results in the formation of a fusion gene comprising portions of the echinoderm microtubule-associated protein-like 4 (*EML4*) gene and the *ALK* gene which was identified in a resected adenocarcinoma specimen from a 62-year-old male smoker [Soda *et al*. 2007]. *ALK* rearrangements occur in 3–7% of patients with NSCLC and are more common among patients with a never/light smoking history, adenocarcinoma histology, a younger age, female gender and in tumours wild type for *EGFR* and *KRAS* [Koivunen *et al*. 2008; Shaw *et al*. 2009; Wong *et al*. 2009; Takahashi *et al*. 2010; Camidge *et al*. 2010]. These factors may

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help clinicians to identify high-risk populations for *ALK* testing. However, according to the International Association for the Study of Lung Cancer (IASLC) and the European Society for Medical Oncology (ESMO) guidelines all patients with advanced-stage lung adenocarcinoma or tumours with an adenocarcinoma component, irrespective of clinical characteristics should be tested for *ALK* (see http://www.iaslc.org and http://www.esmo.org). Furthermore, at least 27 fusion variants have been identified according to the specific chromosomal location of the gene fusion [Sasaki *et al.* 2010].

The principles and practices of personalised cancer therapy significantly influenced the accelerated approval of the first-generation *ALK* inhibitor, crizotinib (Xalkori; PF-02341066; Pfizer), by the US Food and Drug Administration (FDA) in 2011 [Gerber and Minna, 2010; Ou, 2012] (see http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/202570s000lbl.pdf), and by the European Medicines Agency (EMA) in 2012 (see http://www.ema.europa.eu/docs/en_GB/document_library/EPAR__Summary_for_the_public/human/002489/WC500134762.pdf). Crizotinib is an oral, small molecule inhibitor targeting ALK, ROS1 and MET tyrosine kinases [Bergethon *et al.* 2012; Kwak *et al.* 2010; Ou *et al.* 2011] which showed significant (~60%) overall response rates (ORRs) in single-arm phase I [Kwak *et al.* 2010] and phase II trials [Crinò *et al.* 2011]. The results of the PROFILE 1007 phase III trial confirmed significantly higher response rates and longer progression-free survival (PFS) with crizotinib (65% and 7.7 months, respectively) compared with chemotherapy (20% and 3.0 months, respectively) as second-line treatment for *ALK*+ NSCLC [Shaw *et al.* 2013a]. Moreover, crizotinib has proved superior to standard first-line platinum/pemetrexed chemotherapy in untreated advanced *ALK*+ NSCLC (PROFILE 1014). The ORR was 74% in the crizotinib arm and 45% in the chemotherapy arm. The PFS was significantly longer in the crizotinib arm: median 10.9 months *versus* 7.0 months [Solomon *et al.* 2014]. No significant differences in OS were seen in both trials, potentially due to the confounding effects of crossover.

Unfortunately, as seen with other targeted therapies, such as the first-generation epidermal growth factor receptor (*EGFR*) inhibitors in *EGFR*-mutated NSCLC, despite initial major responses to crizotinib, the majority of patients develop crizotinib resistance within the first 12 months [Camidge *et al.* 2012].

## Limitations of crizotinib in *ALK*+ NSCLC

### *Primary or intrinsic resistance*

As mentioned before, initial response rates to crizotinib are approximately 60% which suggests primary resistance in a significant proportion of cases. Some preclinical data suggest that differences in specific *ALK* fusion gene products may partially account for heterogeneous treatment responses or probably false-positive genotyping due to the various techniques used to detect *ALK* rearrangements [Heuckmann *et al.* 2012]. Also, some *ALK* translocations may not generate functional rearrangements in all patients.

### *Secondary or acquired resistance*

To date, the principal mechanisms of acquired crizotinib resistance include secondary resistance mutations in the kinase domain of *ALK*, for example L1196M, the 'gate-keeper' mutation and the C1156Y mutation [Choi *et al.* 2010]. Many other resistance mutations have been described [Sasaki *et al.* 2011; Katayama *et al.* 2012; Doebele *et al.* 2012; Ignatius Ou *et al.* 2014]. Moreover, copy number gains of the *ALK* fusion gene [Katayama *et al.* 2011] and bypass track activation involving NSCLC drivers such as *EGFR, c-KIT* and *KRAS* [Sasaki *et al.* 2011; Doebele *et al.* 2012; Katayama *et al.* 2012] have been reported.

Central nervous system (CNS) penetration of crizotinib and measurements of cerebrospinal fluid (CSF) concentrations of the drug have not been fully investigated. Most small molecule TKIs, including crizotinib [Costa *et al.* 2011; Metro *et al.* 2015], imatinib [Motl *et al.* 2006], gefitinib [Jackman *et al.* 2006] and erlotinib [Clarke *et al.* 2010] have been shown to exhibit low CSF to plasma ratios. Consequently, the CNS is a sanctuary site where *ALK*+ NSCLC can disseminate [Gainor *et al.* 2013]. Despite the potential control of CNS disease with crizotinib, ~50% of patients develop CNS metastases during treatment with this agent [Costa *et al.* 2015]. Many strategies had been reported for the treatment of CNS disease: high-dose crizotinib [Kim *et al.* 2013] and high-dose pemetrexed combined with high-dose crizotinib [Gandhi *et al.* 2013]. Some experts suggested that isolated CNS progression could be treated with radiotherapy while continuing crizotinib [Takeda *et al.* 2013].

**Table 1.** Molecular characteristics of second-generation *ALK* inhibitors.

| Drugs | Targets other than *ALK* | Activity against L1196M resistance mutation | Activity against C1156Y resistance mutation | Activity against G1202R resistance mutation | Activity against other crizotinib-resistant mutations | Lack of activity against resistance mutations |
|---|---|---|---|---|---|---|
| Ceritinib | IGF-R1, InsR, ROS1 | Yes | No | No | G1269A, I1171T, S1206Y, L1152R, F1174L, V1180L | G1202R, F1174C |
| Alectinib | LTK, GAK | Yes | Yes | No | G1269A, S1206Y, L1152R, F1174L, 1151T-ins | G1202R, V1180L, I1171T |
| Brigatinib | ROS1, EGFR | Yes | Yes | Yes | G1269A, S1206Y, 1151T-ins, F1174C, I1171T, D1203N, E1210K, F1245C | NA |
| Entrectinib | TrkA, TrkB, TrkC, ROS1 | Yes | Yes | NA | NA | NA |
| PF-06463922 | ROS1 | Yes | NA | Yes | G1269A | NA |
| TSR-011 | TrkA, TrkB, TrkC | Yes | NA | NA | NA | NA |
| ASP3026 | ROS1, ACK | Yes | NA | NA | F1174L | NA |
| X-396 | MET | Yes | Yes | NA | NA | NA |
| CEP-37440 | FAK | NA | NA | NA | NA | NA |

ALK, anaplastic lymphoma kinase; NA, not available.

## Next-generation *ALK* inhibitors

Second-generation *ALK* inhibitors were developed to enhance anti-*ALK* activity, to overcome crizotinib-resistant mutations and to improve activity in CNS disease. The molecular characteristics of these drugs are listed in Table 1. The second-generation *ALK* inhibitors in clinical use and in the advanced phase of development are listed in Table 2, and the novel *ALK* inhibitors in the early phase of development are listed in Table 3.

### *ALK inhibitors in the clinic*

*Ceritinib (LDK378; Zykadia; Novartis).* Ceritinib is an oral, ATP-competitive, small molecule tyrosine kinase inhibitor of *ALK*, 20-fold more potent than crizotinib in terms of *ALK* selectivity [Friboulet *et al.* 2014; Marsilje *et al.* 2013]. In contrast to crizotinib, ceritinib does not inhibit MET kinase activity but it does inhibit the insulin-like growth factor 1 receptor (IGF-R1), the insulin receptor (InsR) and ROS1 [Shaw *et al.* 2013b]. In cell-based assays, ceritinib had an IC50 of 27–35 nM against the EML4-ALK and NPM-ALK fusion kinases. The IC50s for IGF-1R, InsR and ROS1 were approximately 5-11-fold higher. *ALK*+ cell line models of acquired resistance to crizotinib, including cell lines derived from biopsy samples from patients with crizotinib-resistant NSCLC, revealed that ceritinib potently inhibits resistant mutations, and especially L1196M, G1269A, I1171T and S1206Y mutations. However, ceritinib was not effective against G1202R and F1174C crizotinib-resistant mutations [Friboulet *et al.* 2014]. The primary source of clinical data was the first-in-human, multicentre, single-arm ASCEND-1 trial [ClinicalTrials.gov identifier: NCT01283516] of ceritinib in patients with *ALK*+ advanced tumours. A total of 59 patients were enrolled in the dose escalation phase. The maximum tolerated dose (MTD) was 750 mg once daily and dose-limiting toxicity (DLT) events occurred in six patients during cycle 1, at doses of 400 mg or more daily. These events included grade 3 diarrhoea (at a daily dose of ⩾600 mg), grade 3 vomiting (at 750 mg daily), grade 3 dehydration (at 600 mg daily), grade 3 elevated transaminases, grade 2 elevated alanine aminotransferase (ALT) levels (at 400 mg daily) and grade 3 hypophosphatemia (at 400 mg daily). These toxicities were resolved after treatment discontinuation. The trial was followed by an expansion phase and 71 additional patients were treated with the MTD. The majority of patients (122/130 patients) had advanced NSCLC and had previously received cytotoxic chemotherapy. A total of

**Table 2.** Characteristics of *ALK* inhibitors in clinical use and in advanced phase of development.

| Drug name | Study name or ClinicalTrial.gov ID | Phase | Population (number of patients) | Comparator | ORR | PFS | OS | Most common grade 1–2 AEs |
|---|---|---|---|---|---|---|---|---|
| Crizotinib | PROFILE 1007 [Shaw *et al.* 2013a] | III | Platinum-based chemotherapy pretreated ($n=347$) | Pemetrexed or docetaxel | 65% (95% CI 58–72%) *versus* 20% (95% CI 14–26%; $p<0.001$) | 7.7 *versus* 3.0 months (HR, 0.49; 95% CI 0.37–0.64; $p<0.001$) | 20.3 (95% CI 18.1–not reached) *versus* 22.8 months (95% CI 18.6–not reached) (HR, 1.02; 95% CI 0.68–1.54; $p=0.54$) | Visual disturbance (60%), diarrhoea (60%), nausea (55%), vomiting (47%), constipation (42%), aminotransferase elevation (38%), oedema (31%), fatigue (27%) |
| | PROFILE 1014 [Solomon *et al.* 2014] | III | Previously untreated ($n=343$) | Platinum plus pemetrexed | 74% (95% CI 67–81%) *versus* 45% (95% CI 37–53%; $p<0.001$) | 10.9 *versus* 7.0 months (HR, 0.45; 95% CI 0.35–0.60; $p<0.001$) | Median OS was not reached in either group (HR for death with crizotinib, 0.82; 95% CI 0.54–1.26; $p=0.36$) | Visual disturbance (71%), diarrhoea (61%), oedema (49%), vomiting (46%), constipation (43%), aminotransferase elevation (36%) |
| Ceritinib | ASCEND-1 [Shaw *et al.* 2014a] | I | *ALK*+ advanced tumours ($n=130$)* | No | 58% (95% CI 48–67%) | 7.0 months (95% CI 5.6–9.5 months) | NA | Nausea (82%), diarrhoea (75%), vomiting (65%), fatigue (47%), increased ALT level (35%) |
| | ASCEND-2 [Mok *et al.* 2015] | II | Chemotherapy and crizotinib pretreated ($n=140$) | No | 38.6% (95% CI 30.5–47.2%) | NA | NA | Nausea (81.4%), diarrhoea (80%), vomiting (62.9%) |
| | ASCEND-3 [Felip *et al.* 2015] | II | Crizotinib-naive ($n=124$) | No | 63.7% (95% CI 54.6–72.2%) | NA | NA | Diarrhoea (82.3%), nausea (74.2%), vomiting (66.9%) |
| | ASCEND-4 | III | Previously untreated | Platinum plus pemetrexed | Ongoing trial [ClinicalTrials.gov identifier: NCT01828099] | | | |
| | ASCEND-5 | III | Platinum-based chemotherapy and crizotinib pretreated | Pemetrexed or docetaxel | Ongoing trial [ClinicalTrials.gov identifier: NCT01828112] | | | |
| | ASCEND-7 | II | Five arms study to confirm the efficacy in *ALK*+ CNS disease | | Ongoing trial [ClinicalTrials.gov identifier: NCT02336451] | | | |

*(Continued)*

**Table 2.** (Continued)

| Drug name | Study name or ClinicalTrial.gov ID | Phase | Population (number of patients) | Comparator | ORR | PFS | OS | Most common grade 1–2 AEs |
|---|---|---|---|---|---|---|---|---|
| Alectinib | AF-001JP [Seto *et al.* 2013] [Tamura *et al.* 2014] | I/II¶ | *ALK* inhibitor naïve ($n=46$) | No | 93.5% (95% CI 82.1–98.6) | 2-year PFS: 76% (95% CI 60–86%) | 2-year OS: 79% (95% CI 63–89%) | Dysgeusia (30%), increased AST (28%), increased blood bilirubin (28%), increased blood creatinine (26%), rash (26%), constipation (24%), increased ALT (22%) |
| | AF-002JG [Gadgeel *et al.* 2014] | I/II | Crizotinib pretreated ($n=47$) | No | 55% | NA | NA | Fatigue (30%), myalgia (17%), peripheral oedema (15%) |
| | NP28673 [Ignatius Ou *et al.* 2015] | II | Chemotherapy and crizotinib pretreated ($n=138$) | No | 49.2% (95% CI 40.0–58.4%) | 8.9 months (immature) | NA | Myalgia (17%), constipation (15%), fatigue (14%), asthenia (11%), increased AST (10%) |
| | NP28761 [Gandhi *et al.* 2015] | II | Chemotherapy and crizotinib pretreated ($n=87$) | No | 47.8% (95% CI 35.6–60.2%) | 6.3 months (immature) | NA | Constipation (36%), fatigue (30%), peripheral oedema (22%), myalgia (22%), increased AST (21%), blood CPK increased (21%), nausea (20%), diarrhoea (18%), increased ALT (18%) |
| | ALEX | III | Previously untreated | Crizotinib | Ongoing trial [ClinicalTrials.gov identifier: NCT02075840] | | | |
| Brigatinib | NCT01449461 [Camidge *et al.* 2015] | I/II | *ALK*+ advanced tumours ($n=137$; 79 *ALK*+ NSCLC)§ | No | 71% in crizotinib-pretreated and 100% in crizotinib-naive group | 13.4 months in crizotinib-pretreated group | NA | Nausea (52%), fatigue (42%), diarrhoea (40%) |
| | ALTA | II | Crizotinib pretreated | No | Ongoing trial [ClinicalTrials.gov identifier: NCT02094573] | | | |

*Results from 114 NSCLC patients treated with at least 400 mg of ceritinib daily.
¶Study conducted in Japan.
§ORR and PFS data from 79 *ALK*+ NSCLC patients.
AEs, adverse events; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confident interval; CPK, creatinine phosphokinase; HR, hazard ratio; ID, identifier; ORR, overall response rate; OS, overall survival; PFS, progression-free survival.

**Table 3.** *ALK* inhibitors in early phase of development.

| Drug name | Study name or ClinicalTrials.gov identifier | Phase | Most common grade 1–2 AEs |
|---|---|---|---|
| Entrectinib | NCT02097810 | I/II | Paraesthesia (42%) nausea (37%), myalgia (34%), asthenia (27%), dysgeusia (27%), vomiting (21%), arthralgia (19%), diarrhoea (19%) |
| PF-06463922 | NCT01970865 | I/II | Hypercholesterolemia (48%), peripheral oedema (23%) and peripheral neuropathy (21%) |
| TSR-011 | NCT02048488 | I/II | Fatigue (17.4%), constipation (15.9%), QTc prolongation (15.9%), diarrhoea (14.5%) and headache (13%) |
| ASP3026 | NCT01401504 | I | Fatigue (44%), vomiting (39%), nausea (37%), and constipation (24%) |
| X-396 | NCT01625234 | I/II | Rash (31%), nausea (31%), vomiting (29%), fatigue (26%), oedema (17%) and pruritus (11%) |
| CEP-37440 | NCT01922752 | I | NA |
| CEP-28122 | Preclinical | | NA |

AEs, adverse events; ID, identifier; NA, not available.

83/122 NSCLC patients (68%) were pretreated with crizotinib. Among the 78 NSCLC patients treated with 750 mg of ceritinib daily, the overall response rate (ORR) was 59% (46/78 patients; 95% confidence interval [CI] 47–70%). In the subgroup of 80 NSCLC crizotinib-pretreated patients, the median PFS was 6.9 months (95% CI 5.3–8.8 months) and 10.4 months (95% CI 4.6 to could not be estimated) in the 34 NSCLC crizotinib-naive patients. The most common grade ⩾3 adverse events (AEs) were elevated ALT and aspartate aminotransferase levels (21% and 11%, respectively), diarrhoea (7%) and elevated lipase levels (7%), all of which were reversible after ceritinib discontinuation [Shaw *et al.* 2014a]. In April 2014, the FDA granted accelerated approval to ceritinib for the treatment of patients with *ALK*+ metastatic NSCLC with disease progression or who are intolerant to crizotinib (see http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/205755lbl.pdf). In February 2015, the EMA Committee for Medicinal Products for Human Use (CHMP) adopted a positive opinion, recommending the granting of a conditional marketing authorisation for ceritinib in patients with advanced *ALK*+ NSCLC previously treated with crizotinib.

The updated ASCEND-1 report on the evaluation of the efficacy of ceritinib at a daily dose of 750 mg was recently presented. A total of 246 patients with *ALK*+ NSCLC were enrolled, including 163 pretreated with an *ALK* inhibitor (crizotinib or alectinib) and 83 *ALK* inhibitor-naive patients. The ORRs were 56.4%, 72.3% and 61.8% and the median PFS was 18.4, 9.0 and 6.9 months in the group pretreated with an *ALK* inhibitor, *ALK* inhibitor-naive patients and the overall population, respectively. The most common grade ⩾3 AEs were elevated ALT/AST levels (29.8% and 9.8%, respectively), diarrhoea (5.9%), nausea (5.9%), fatigue (5.1%), anaemia (5.1%), vomiting (4.7%) and pneumonia (4.7%) [Felip *et al.* 2014]. Regarding its activity in CNS disease, 124 patients had brain metastases at baseline: 28 patients pretreated with an *ALK* inhibitor and 8 patients in the *ALK* inhibitor-naive group had measurable brain lesions (MBLs). The intracranial ORRs were 36% (95% CI 19–56%) in patients pretreated with an *ALK* inhibitor and 63% (95% CI 25–92%) in the *ALK* inhibitor-naive group [Shaw *et al.* 2014b].

The preliminary results of two phase II single-arm studies on ceritinib in patients with *ALK*+ NSCLC were recently presented: the ASCEND-2 trial [ClinicalTrials.gov identifier: NCT01685060] in patients who had received cytotoxic chemotherapy (one to three lines, including one platinum doublet) and had progressed on crizotinib as the last therapy, and the ASCEND-3 trial in crizotinib-naive patients [ClinicalTrials.gov identifier: NCT01685138]. The first trial enrolled 140 patients, 71.4% with brain metastases, 28% of whom had no prior brain radiation (BRT). The ORR was 38.6%

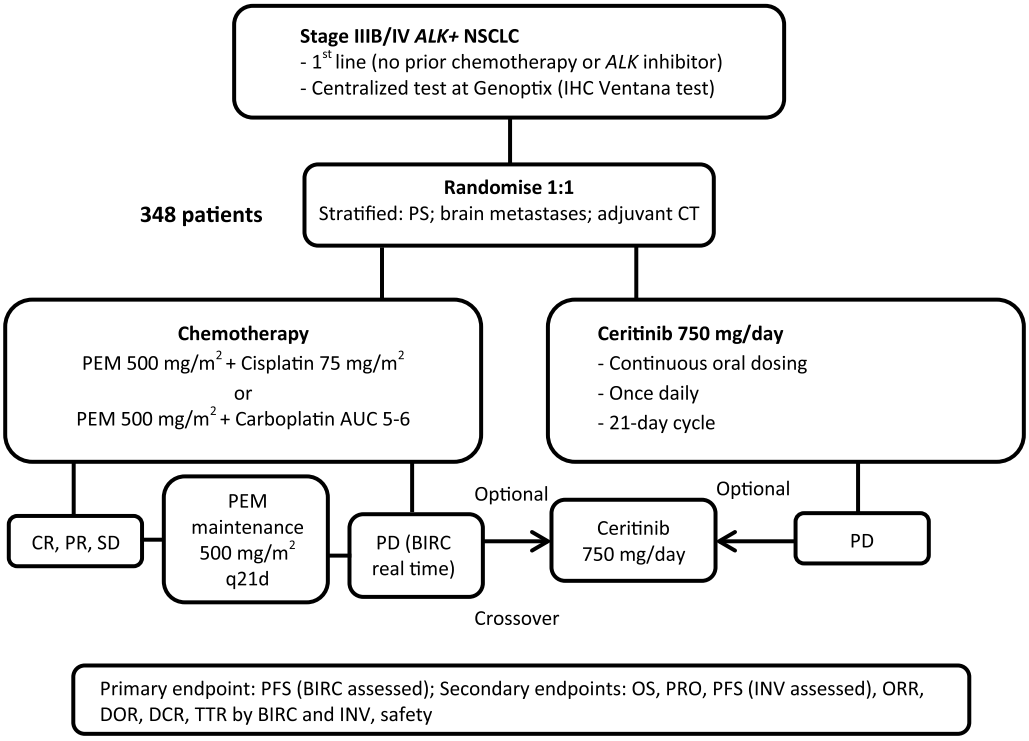


**Figure 1.** Ongoing phase III (ASCEND-4) study design.
AUC, area under the curve; BIRC, blinded independent review committee; CR, complete response; DCR, disease-control rate; DOR, duration of response; IHC, immunohistochemistry; INV, investigator; ORR, overall response rate; OS, overall survival; PD, progressive disease; PEM, pemetrexed; PFS, progression-free survival; PR, partial response; PRO, patient-reported outcomes; PS, performance status; SD, stable disease; TTR, time to response.

(95% CI 30.5–47.2%). Regarding its activity in CNS disease, 20 patients had investigator-assessed MBL at baseline. The intracranial ORR was 45% (95% CI 23.1–68.5%). The most common AEs (mostly grade 1–2) were nausea (81.4%), diarrhoea (80%) and vomiting (62.9%), and 7.9% patients discontinued treatment due to AEs, none of which were predominant [Mok *et al.* 2015]. The second trial enrolled 124 patients, 40.3% with brain metastases, 46% of whom had no prior BRT. The ORR was 63.7% (95% CI 54.6–72.2%). Regarding its activity in CNS disease, 10 patients had investigator-assessed MBL at baseline. The intracranial ORR was 20% (95% CI 2.5–55.6%). The most common AEs (mostly grade 1–2) were diarrhoea (82.3%), nausea (74.2%) and vomiting (66.9%), and 7.3% patients discontinued treatment due to AEs, none of which were predominant [Felip *et al.* 2015]. Another phase II study aimed at evaluating the efficacy and safety of ceritinib in patients with *ALK*+ NSCLC metastatic to the brain and/or to leptomeninges (ASCEND-7) is currently recruiting participants to confirm the efficacy of ceritinib in patients with *ALK*+ CNS disease [ClinicalTrials.gov identifier: NCT02336451]. Two randomized phase III trials are currently open to evaluate ceritinib *versus* first-line chemotherapy (ASCEND-4, Figure 1) and second-line chemotherapy (ASCEND-5). The primary endpoint of both studies is PFS [ClinicalTrials.gov identifiers: NCT01828099 and NCT01828112, respectively]. Given the positive results obtained from PROFILE 1014 where crizotinib has been compared with platinum/pemetrexed chemotherapy in the first-line setting, today, chemotherapy is probably not the best control arm, so further trials should be made with the choice of crizotinib as control arm.

*Alectinib (RG7853/AF-802/RO5424802/CH5424802, Chugai-Roche).* Alectinib is a highly selective, *ALK* inhibitor with a median inhibitory concentration of 1.9 nM for *ALK* activity. In addition, it exerts activity against LTK and GAK. Preclinical studies have shown that it is also active against crizotinib-resistant *ALK* mutations (L1196M, C1156Y and F1174L), but not against InsR, IGF-R1 and ROS1 [Kinoshita *et al.* 2012; Sakamoto *et al.* 2011]. Alectinib was granted

breakthrough therapy designation (BTD) by the FDA in June 2013 for patients with *ALK*+ NSCLC who progressed on crizotinib. Japan is the first country to approve alectinib in patients with advanced *ALK*-rearranged NSCLC, based on the results of the phase I/II AF-001JP trial targeting patients with *ALK*+ NSCLC who had not previously been treated with crizotinib or other *ALK* inhibitors. In the phase I study, 24 patients were treated with doses of 20–300 mg twice daily (BID). No DLTs or grade 4 AEs were observed up to the highest dose; thus 300 mg BID was selected as the recommended phase II dose. In the phase II trial, 46 patients were treated and 43 of them (93.5%) achieved an ORR (95% CI 82.1–98.6). The most frequent alectinib-related AEs were grade 1 or 2 dysgeusia (30%), followed by elevated AST (28%), blood bilirubin (28%) and blood creatinine (26%) levels, rash (26%), constipation (24%) and elevated ALT (22%). Grade 3 treatment-related AEs were recorded in 26% of the patients, and the most common were neutropenia and elevated creatine phosphokinase levels (4%). No grade 4 AEs or deaths were reported [Seto *et al.* 2013]. The 2-year PFS rate was 76% (95% CI 60–86%) and the 2-year OS was 79% (95% CI 63–89%) [Tamura *et al.* 2014]. Moreover, alectinib has demonstrated antitumour activity in *ALK*+ NSCLC patients resistant to crizotinib, including those with CNS metastases. A phase I/II trial conducted by Gadgeel and colleagues enrolled a total of 47 *ALK*+ NSCLC patients who progressed on or were intolerant to crizotinib. The ORR was assessable in 44/47 patients and was 55% in 24/44 patients, including one complete response (CR) and 23 partial responses (PRs). A total of 16 patients (36%) had stable disease. The subset analysis of 21 patients with CNS metastases at baseline showed a disease control rate approximating 90%. In contrast with the previous trial, the recommended dose for phase II studies was 600 mg BID [Gadgeel *et al.* 2014]. The results were recently confirmed by two clinical trials: the global NP28673 trial [ClinicalTrials.gov identifier: NCT01801111] conducted by Ignatius Ou and colleagues and the North American NP28761 trial [ClinicalTrials.gov identifier: NCT01871805] conducted by Gandhi and colleagues. These studies are phase II single-arm, open-label, multicentre trials evaluating the safety and efficacy of alectinib 600 mg BID in 138 (from 16 countries) and 87 (from US and Canada) *ALK*+ NSCLC patients respectively, whose disease progressed on crizotinib. In the response-evaluable population that was assessed (122 and 69 patients, respectively), treatment with alectinib resulted in ORRs of 49.2% (95% CI 40.0–58.4%) and 47.8% (95% CI 35.6–60.2%), respectively. Patients whose tumours shrank in response to alectinib continued to respond for a median of 11.2 and 7.5 months, respectively. For patients with baseline measurable CNS disease (34 and 16 patients, respectively), the ORR was 55.9% (95% CI 37.9–72.8%), and 68.8% (95% CI 41.3–89.0%), including five and two complete responses, respectively. The median PFS was 8.9 and 6.3 months (immature), respectively [Ignatius Ou *et al.* 2015; Gandhi *et al.* 2015]. In summary, alectinib has demonstrated a safety profile consistent with that observed in previous studies and yielded a robust treatment response, including excellent intracranial activity in patients who had progressed on crizotinib and had also failed on prior chemotherapy. A phase III trial (ALEX, Figure 2) to evaluate the efficacy and safety of alectinib 600 mg BID compared with critozinib 250 mg BID in treatment-naive *ALK*+ advanced NSCLC patients is currently recruiting patients [ClinicalTrials.gov identifier: NCT02075840].

### *Novel ALK inhibitors under clinical development*

*Brigatinib (AP26113, Ariad).* Brigantinib is a novel potent, orally available *ALK* inhibitor with an IC50 of 0.62 nM in a cell-free assay and a demonstrated ability to overcome crizotinib resistance mutations, including G1202R and activity against ROS1 (IC50 of 16–41 nM) [Zhang *et al.* 2010; Squillace *et al.* 2013]. This compound also inhibits mutant *EGFR*, including T790M [Rivera *et al.* 2012]. An update of a phase I/II ongoing trial in advanced malignancies, including *ALK*+ NSCLC, has been reported by Camidge and colleagues [ClinicalTrials.gov identifier: NCT01449461]. All patients received brigatinib at total once-daily doses of 30–300 mg. Patients were divided into three cohorts that received 90 mg, 90–180 mg (escalating after 7 days) or 180 mg. In the dose escalation phase, two DLTs were observed, grade 3 ALT elevation at 240 mg and grade 4 dyspnoea at 300 mg. The selected recommended phase II dose was 180 mg. Safety was evaluated in all 137 treated patients and efficacy in all 79 *ALK*+ NSCLC patients. Of 78 evaluable *ALK*+ NSCLC patients, 58 (74%) responded: 50/70 (71%) pretreated with crizotinib and 8/8 (100%) crizotinib-naive patients. The median PFS was 13.4 months in the

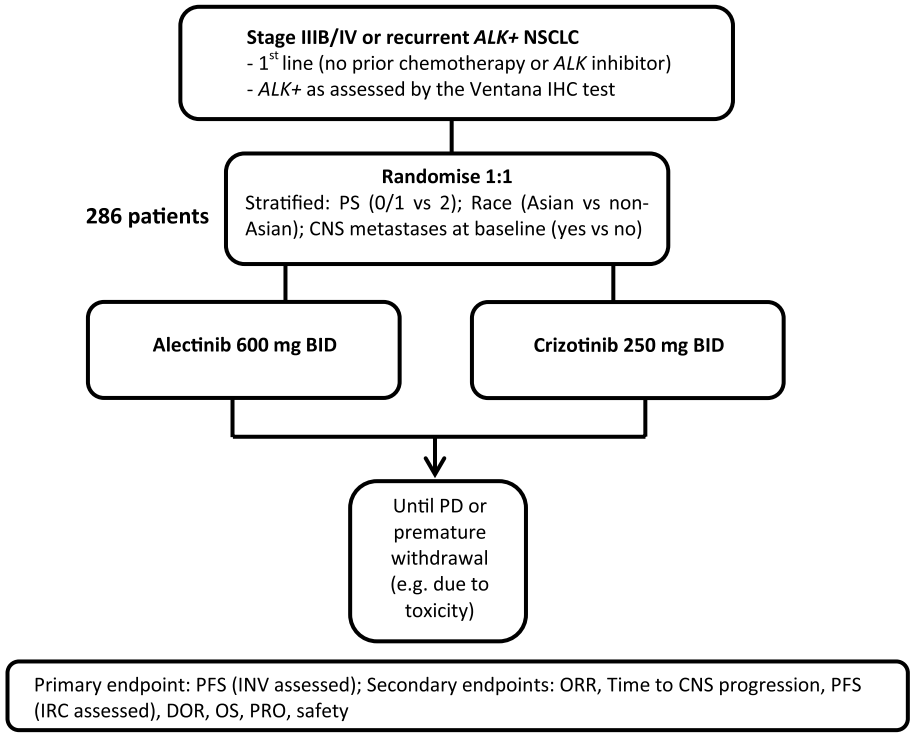


**Figure 2.** Ongoing phase III (ALEX) study design.
BID, twice a day; CNS, central nervous system; DOR, duration of response; IHC, immunohistochemistry; INV, investigator; IRC, independent review committee; ORR, overall response rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PRO, patient-reported outcomes; PS, performance status.

subgroup with prior crizotinib therapy, but not reached in crizotinib-naive patients. Of the 79 *ALK*+ NSCLC patients, 52 (66%) had CNS metastases identified at baseline: 17 had measurable disease and 35 had only unmeasurable disease. A total of 15/17 and 33/35 had at least one follow-up scan, respectively. A total of 8/15 (53%) with measurable CNS metastases achieved an intracranial response. The toxicity profile described in all 137 patients was nausea (52%), fatigue (42%) and diarrhoea (40%) which were the most common (all grades). Grade ⩾3 treatment-related AEs included elevated lipase (9%), dyspnoea (7%), hypertension (5%), hypoxia (5%), pneumonia (5%), elevated amylase (4%), fatigue (4%), pulmonary embolism (3%), elevated ALT (2%), hyponatraemia (2%) and hypophosphatemia (2%) [Camidge *et al.* 2015]. A pivotal phase II ALTA (*ALK* in Lung cancer Trial of AP26113) trial in patients with locally advanced or metastatic *ALK*+ NSCLC pretreated with crizotinib is currently enrolling new patients [ClinicalTrials.gov identifier: NCT02094573].

*Entrectinib (RXDX-101, NMS-E628, Ignyta).* Entrectinib is a novel, orally available, selective tyrosine kinase inhibitor of the Trk family of tyrosine kinases TrkA (coded by the *NTRK1* gene), TrkB (coded by the *NTRK2* gene) and TrkC (coded by the *NTRK3* gene), *ROS1* and *ALK*. Rearrangements in *NTRK1* were recently described in approximately 3% of NSCLC that were negative for other oncogenic driver mutations [Vaishnavi *et al.* 2013]. Entrectinib has demonstrated *in vitro* and *in vivo* antitumour activity against various TRK, ROS1 or *ALK*-driven xenograft models of different human cancers (NPM-ALK-driven lymphoma and EML4-ALK-driven NSCLC), and has demonstrated oral bioavailability and been observed to efficiently cross the blood–brain barrier in mice with intracranially injected NCI-H2228 EML4-ALK cells [Ardini *et al.* 2009, 2011]. Entrectinib is currently being evaluated in two phase I/II clinical trials, ALKA-372-001 and STARTRK-1 [ClinicalTrials.gov identifier: NCT02097810]. In the First-in-Human Study ALKA-372-001, entrectinib is administered in three different administration schedules. Schedule A (fasted, 4 days 'on' and 3 days 'off' for 3 weeks, 1 week rest) demonstrated significant antitumour activity [De Braud *et al.* 2014]. An update has recently been presented. They reported

on the completion of schedule A and the other two on-going schedules B (QD) and C (4 days 'on' and 3 days 'off'). A total of 38 patients have been enrolled, 65% (25 patients) of whom had NSCLC. Entrectinib was well tolerated. The majority of the patients reported grade 1–2 AEs, including paraesthesia (42%) nausea (37%), myalgia (34%), asthenia (27%), dysgeusia (27%), vomiting (21%), arthralgia (19%) and diarrhoea (19%). Asthenia and muscle weakness were the grade 3 AEs observed at doses of 1200 mg/m$^2$ (schedule A) and 400 mg/m$^2$ (schedule C), respectively, both reversible after dose reduction. No DLTs were observed [De Braud *et al*. 2015]. The phase I portion of the second study, STARTRK-1, designed to evaluate escalating doses of entrectinib administered in a continuous daily regimen (QD) was also recently presented. A total of 29 patients were enrolled at four different dose levels (100, 200, 400 and 800 mg/m2). Two DLTs occurred at a fixed dose of 800 mg/m2 (grade 3 cognitive impairment and grade 3 asthenia), then resolved upon study drug discontinuation [Patel *et al*. 2015]. Antitumour activity was reported in all 67 patients included in both studies. Among *ALK* inhibitor- or *ROS1* inhibitor-naive patients (n = 17), 10/11 (91%) patients treated at or above 400 mg/m2 achieved objective responses as early as cycle 1 with durable responses for up to 16 cycles. These data support further development of entrectinib and 400 mg/m2 was the selected recommended phase II dose for the trials [ClinicalTrials.gov identifier: NCT02097810].

*PF-06463922 (Pfizer).* PF-06463922 has been identified as an orally available ATP-competitive selective, brain penetrant inhibitor of *ALK* (including mutations) and the c-Ros oncogene 1 (*ROS1*). In *in vitro* and preclinical studies, PF06463922 is ~10-fold potent against wild-type EML4-ALK and ~40-fold potent against EML4-ALK L1196M compared with crizotinib [Johnson *et al*. 2014]. An ongoing phase I/II trial [ClinicalTrials.gov identifier: NCT01970865] is currently recruiting *ALK*+ or *ROS1*+ NSCLC patients with or without CNS metastases, TKI-naive or exhibiting disease progression after prior treatment with 1–2 TKIs. At ASCO 2015, Shaw and colleagues presented the preliminary results of portion I of this study. As of the cut-off date (20 April 2015), 33 *ALK*+ and 11 *ROS1*+ patients were enrolled across 7 once-daily (QD) dose levels and 3 BID dose levels. All 44 patients were evaluated for safety, 34 for overall tumour response and 25 for intracranial response. The ORR was 44% (95% CI 27–62%). In the 25 patients evaluable for intracranial response, 14 of whom had measurable disease, the ORR was 36% (95% CI 18–58%). The most common treatment-related AEs were grade 1–2 hypercholesterolemia (48%), peripheral oedema (23%) and peripheral neuropathy (21%). The only grade ⩾3 treatment-related AE was hypercholesterolemia (12%). One DLT occurred in a patient who received <16 of 21 planned 200 mg QD doses due to grade 1–2 cognitive effects [Shaw *et al*. 2015].

*TSR-011 (Tesaro).* TSR-011 is a potent, dual *ALK* and TrkA, TrkB and TrkC inhibitor. TSR-011 has high affinity for wild-type recombinant *ALK* kinase activity, with an IC50 value of 0.7 nM, and exerts sustained potent inhibition of *ALK*-dependent tumour growth in mouse models. Tesaro is evaluating TSR-011 in an ongoing phase I/IIa trial [ClinicalTrials.gov identifier: NCT02048488]. The results of the phase I were presented at the World Conference on Lung Cancer in 2013. A total of 19 patients were enrolled and the most frequent malignancies were NSCLC, ovarian and pancreatic cancer. The preliminary data demonstrated disease control (SD + PR) at 8 weeks attained in 65% of the 17 evaluable patients. TSR-011 exerted antitumour activity in at least 2/3 NSCLC *ALK*+ patients with dysaesthesia who had previously progressed on crizotinib. The reversible DLTs included and QTc prolongation [Weiss *et al*. 2013]. An update was recently presented. A total of 69 patients with advanced cancer, including 46 *ALK*+ NSCLC patients were treated at total daily doses of 30–480 mg, administered one, two or three times a day. Responses were observed at a total daily dose of 120 mg or more in 3/5 *ALK* inhibitor-naive patients (60%) and in 3/6 patients (50%) who had progressed after crizotinib as the only *ALK* inhibitor. Stable disease as the best response was observed in the 3 patients who progressed after ceritinib or alectinib. The most common grade 1–2 AEs were fatigue (17.4%), constipation (15.9%), QTc prolongation (15.9%), diarrhoea (14.5%) and headache (13%). Grade ⩾3 treatment-related AEs included fatigue (5.8%), anaemia (5.8%) and QTc prolongation (4.3%) [Arkenau *et al*. 2015].

*ASP3026 (Astellas Pharma).* ASP3026 is a selective, potent, ATP-competitive, small molecule oral inhibitor of *ALK* with an IC50 of 3.5 nM in enzymatic assays and an IC50 of 64.8 nM in H2228 cells. This agent also exerts activity against ROS1 (IC50 = 8.9 nM) and ACK, and against

L1196M, the crizotinib-resistant gatekeeper mutation. The compound was evaluated in an open-label, phase I, traditional 3+3 dose escalation design in patients with solid tumours and/or B-cell lymphoma (*ALK* positivity not required) [ClinicalTrials.gov identifier: NCT01401504]. A total of 30 patients were included. The most common AEs were fatigue (44%), vomiting (39%), nausea (37%) and constipation (24%). Grade 3 rash and elevation of AST and ALT were also observed. The MTD is 525 mg/daily but the clinical activity has not yet been reported [Patnaik *et al.* 2013]. The dose escalation phase finally enrolled 33 patients, including 3 *ALK*+ patients, and the expansion cohort enrolled another 13 *ALK*+ patients ($n=46$) to evaluate the activity of AP3026 in this sub-population. Of 15 *ALK*+ NSCLC patients who progressed on prior crizotinib, 7 (44%) achieved a PR and 8 (50%) showed stable disease as the best response. The median PFS in the *ALK*+ patients was 5.9 months (95% CI 3.8–9.4 months) [Maitland *et al.* 2014]. However, the company reported in February 2014 that it had discontinued the development of ASP3026 for strategic reasons.

*X-376 and X-396 (Xcovery).* X-376 and X-396 are more potent inhibitors of *ALK* but less potent inhibitors of *MET* compared with crizotinib, both in biochemical and cell-based assays. Moreover, X-396 could potently inhibit *ALK* kinases engineered with two point mutations (L1196M and C1156Y) associated with acquired resistance to crizotinib. Preclinical data also suggest that X-396 has the potential to overcome acquired resistance to crizotinib and exhibits CNS penetration [Lovly *et al.* 2011]. Preliminary results of the phase I/II trial of X-396 showed antitumour control in both crizotinib-naive ($n=5$) and crizotinib-resistant ($n=13$) *ALK*+ NSCLC patients. For the 11 evaluable *ALK*+ lung cancer patients, 6 achieved a PR (55%) and 2 had SD (18%) as the best response. Responses were observed in crizotinib-naive and in crizotinib-pretreated NSCLC patients. Responses have also been observed in 2 patients with brain metastases. The most common grade 1–2 drug-related AEs included rash (31%), nausea (31%), vomiting (29%), fatigue (26%), oedema (17%) and pruritus (11%) but grade 3–4 AEs were rare [Horn *et al.* 2014]. The expansion phase in patients with *ALK*+ NSCLC is ongoing [ClinicalTrials.gov identifier: NCT01625234].

*CEP-28122 and CEP-37440 (Teva).* CEP-28122 is a potent and selective *ALK* inhibitor (IC50 = 1.9 nM in enzymatic assays). In preclinical studies, CEP-28122 showed high selectivity against *ALK* among various types of tyrosine kinases, including InsR, IGF-R1 and c-MET [Cheng *et al.* 2012]. CEP-37440 is an inhibitor of *ALK* and of focal adhesion kinase (FAK). CEP-37440 is undergoing clinical development in phase I [ClinicalTrials.gov identifier: NCT01922752] in patients with advanced or metastatic solid tumours but no preliminary data are available.

## HSP90 inhibitors

HSP90 (90 kDa heat shock protein) is a molecular chaperone that plays a central role in regulating the correct folding, stability and function of numerous proteins [Taipale *et al.* 2010]. Inhibition of HSP90 activity results in aggregation or proteasomal degradation of these proteins, which in turn promotes the simultaneous disruption of numerous oncogenic signalling pathways critical for tumour cell proliferation and survival [Whitesell and Lindquist, 2005]. Many of these proteins are kinases that have been shown to be oncogenic drivers in subsets of lung adenocarcinoma, including EGFR, BRAF, HER2 and, notably, the EML4–ALK fusion protein. Targeting the chaperone function of HSP90 is therefore an alternative approach to direct kinase inhibition for therapeutic intervention in *ALK*-driven cancer. In early phase clinical trials, two HSP90 inhibitors, retaspimycin hydrochloride (IPI-504) and ganetespib (STA-9090) demonstrated clinical activity in *ALK*+ NSCLC patients [Sequist *et al.* 2010; Normant *et al.* 2011; Socinski *et al.* 2013]. Preliminary data from a phase II trial of AUY922 (a highly potent, nongeldanamycin HSP90 inhibitor) in 121 patients with advanced NSCLC, previously treated and stratified by molecular status, have been reported. Clinical activity was seen in patients with *ALK*+ and *EGFR*-mutated disease: a PR in 6/21 (29%) *ALK*+ NSCLC patients. Of these six responders, four were crizotinib-naive and two were crizotinib-pretreated patients. The estimated median PFS rate was 42% at 18 weeks in *ALK*+ patients. The most frequent AEs were grade 1–2 eye disorders (77%), diarrhoea (74%) and nausea (46%) [Felip *et al.* 2012].

Several clinical trials are on-going to evaluate HSP90 inhibitors combined with *ALK* inhibitors. Crizotinib is combined with ganetespib in crizotinib-naive patients [ClinicalTrials.gov identifier: NCT01579994], and two clinical trials are open

to patients with crizotinib-resistant disease. In one of them, onalespib (AT13387), a HSP90 inhibitor is administered alone or combined with crizotinib [ClinicalTrials.gov identifier: NCT01712217], and in the other trial, AUY922 is combined with ceritinib [ClinicalTrials.gov identifier: NCT01772797].

## Conclusion and future perspectives

Crizotinib has become a reference treatment for *ALK*+ NSCLC patients, and a promising treatment for tumours harbouring *MET* amplification and *ROS1* aberrations. Unfortunately, many patients develop acquired resistance during the first year of treatment and its efficacy is limited in CNS disease. Strategies are urgently needed to overcome inherent and acquired resistance to *ALK* inhibition. Today, several second-generation *ALK* inhibitors under various stages of clinical development have shown activity in crizotinib-resistant disease with promising activity in patients with CNS involvement, but resistance to these compounds has also been described.

One of the greatest uncertainties currently facing the research and oncology communities is: in what sequence should *ALK* TKIs be prescribed? Should crizotinib be prescribed in first-line targeted therapy and second-generation agents be reserved for subsequent lines of treatment, or, is it better to start with a more potent *ALK* inhibitor to achieve a more profound and prolonged response duration? To try to answer these questions, clinical trials with ceritinib [ClinicalTrials.gov identifier: NCT01828099] and alectinib [ClinicalTrials.gov identifier: NCT02075840] in crizotinib-naive patients are on-going but additional strategic studies on therapeutic sequences are needed to help us better select the best treatment sequences in *ALK*+ NSCLC in the near future.

In the last few years, immunotherapy has revolutionised cancer treatment. Hitherto considered a poorly immunogenic type of malignancy, lung cancer has recently emerged as an exciting new target for immune-based therapies [Brahmer and Pardoll, 2013]. Nivolumab (Opdivo®), a PD-1 inhibitor, was approved by the FDA in March 2015 for the treatment of squamous NSCLC having failed prior chemotherapy. Nivolumab has also demonstrated superior efficacy compared with standard of care (docetaxel) in previously treated nonsquamous NSCLC (CheckMate 057) [Paz-Ares *et al.* 2015] and the FDA simultaneously granted a breakthrough therapy designation in this setting. Ipilimumab (Yervoy®), which targets the CTLA-4 checkpoint on activated immune cells, was the first treatment ever shown to extend survival in patients with metastatic melanoma, and was approved for that indication in 2011. Based on the promising results of a phase II trial, it is now being tested in a phase III trial for NSCLC [ClinicalTrials.gov identifier: NCT01285609]. Combining immune checkpoint inhibition and *ALK* inhibitors may represent an opportunity to improve efficacy in crizotinib-resistant NSCLC patients. In this context, two early phase trials are ongoing: one to assess the safety and efficacy of ceritinib combined with nivolumab in patients with pretreated *ALK*+ NSCLC [ClinicalTrials.gov identifier: NCT02393625] and the other one is a modified phase I trial of ipilimumab combined with mutation-specific targeted therapy (crizotinib or erlotinib) stratified for the presence of *ALK* rearrangements or *EGFR* mutations [ClinicalTrials.gov identifier: NCT01998126).

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## Conflict of interest statement

The author(s) declare(s) that there is no conflict of interest.

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