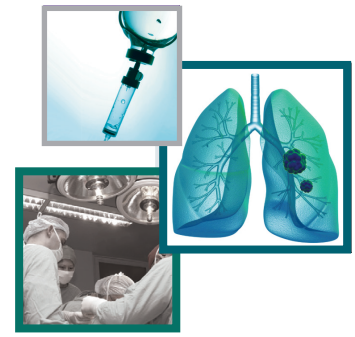
REVIEW

Lung Cancer Management



Targeting EGFR and ALK in NSCLC: current evidence and future perspective

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Practice points

- Despite recent improvements in diagnostic techniques and therapeutic approaches, the prognosis of advanced NSCLC remains dismal.
- Molecular analyses (at least for EGFR, KRAS, ALK, ROS1) are mandatory in every patient diagnosed with NSCLC.
- In oncogene-addicted NSCLCs, targeted therapies provide benefit in terms of both progression-free survival and overall survival.
- Resistance to treatment invariably occurs in virtually all patients who receive TKIs.
- Next-generation TKIs represent a valid options to overcome resistance to first-generation inhibitors.

The advent of molecular therapy targeting specific driver oncogenes has dramatically changed the prognosis of a subset of NSCLC, dilating survival and improving the quality of life of patients with advanced disease. Two of the major targets for treatment with receptor TKIs are the activated mutated forms of the *EGFR* and the *ALK* gene fusions. In advanced NSCLC patients harboring *EGFR* mutations or *ALK* rearrangements, the use of TKIs in the first-line setting, have provided unexpected large progression-free survival and overall survival benefits, compared with cytotoxic chemotherapy. However, despite initial responses and durable remissions, the development of resistance inevitably leads to treatment failure. The aim of this review is to discuss the treatment strategy currently used for tumors harboring these two genetic targets and to focus on what will be available in clinical practice in the near future.

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Lung cancer is the leading cause of cancer-related deaths worldwide in both men and women, accounting for roughly 27% of all cancer-related deaths in 2015 [1]. Unfortunately, the prognosis of advanced (IIIB/IV) NSCLC remains poor with a 5-year survival rate of less than 17% [1]. Nevertheless, recent advances over the understanding of molecular mechanisms underling the development and progression of NSCLC revealed the presence of different targetable oncogenic drivers, thus identifying specific subsets of patients with distinct pathological and clinical features who might benefit from targeted therapies. This evidence paved the way to the era of personalized therapy, with EGFR and ALK target therapies representing the forefront of treatment of advanced NSCLC. *EGFR* mutations more frequently occur in patients with specific clinical features, such as never smoker, female gender, Asian ethnicity (30% of advanced NSCLCs as opposed to 15% for the

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western population) and adenocarcinoma histology. However, it should be noticed that *EGFR* mutational status is not determinable only of the basis of the aforementioned clinical characteristics. On the other side, *ALK* rearrangements commonly occur in patients with certain clinicopathologic features including never smoking history, young age (median age of onset 52 years) and adenocarcinoma histology (solid or acinar growth patterns), accounting for 5% of all cases of NSCLC. In this paper, we will provide an overview of the two major subtypes of oncogenic drivers, *EGFR* and *ALK* in NSCLC as well as the development of target therapies available now and in the near future.

First- & second-generation EGFR–TKIs

Activating *EGFR* mutations (most commonly exon 19 deletion or exon 21 [L858R] point mutation) are associated with higher response rates (RRs) to EGFR–TKI therapy, than standard platinum-doublet chemotherapy. Such mutations are present in up to 17% of Caucasians and 50% of east Asian patients with lung adenocarcinoma [1].

Iressa Pan-Asia Study was the first Phase III randomized clinical trial to confirm *EGFR* mutation as a predictive biomarker for response to EGFR–TKIs [2].

Specifically comparing EGFR–TKI therapy with chemotherapy in an unselected population, with clinical features suggestive for activating *EGFR* mutations, patients with *EGFR*-mutated (m+) tumors achieved significantly longer progression-free survival (PFS) with gefitinib versus those receiving chemotherapy (hazard ratio [HR] for progression or death 0.48; 95% CI: $p < 0.001$). The dominant role of EGFR–TKIs as first-line treatment option over standard platinum-based doublet chemotherapy in patients with stage IIIb/IV NSCLC and suspected or known *EGFR* mutations, was further supported by multiple trials, showing significantly higher RRs and prolonged PFS occurred consistently across all of them **(Table 1)** [3-9].

As a result, erlotinib, gefitinib and most recently, the irreversible inhibitor afatinib have received approval for first-line treatment of *EGFR*m+ NSCLC, leading upfront molecular testing for *EGFR,* a mandatory analysis. Nevertheless, none of the above studies have documented a significant difference in overall survival (OS) between TKIs and chemotherapy, likely due to the high proportion of patients receiving different subsequent therapies and crossing over to the alternative treatments. In the pooled analysis of the Phase III randomized LuX-Lung 3 and LuX-Lung 6 trials [10], first-line afatinib was shown to improve OS versus chemotherapy in patients with advanced NSCLC with common mutations (median: 27.3 vs 24.3 months; HR: 0.81; 95% CI: 0.66–0.99; $p = 0.037$). In particular, subgroup analyses suggested that the OS benefit of afatinib was driven by patients harboring exon 19 deletions (31.7 vs 20.7 months; HR: 0.59; 95% CI: 0.45–0.77; $p = 0.0001$), while no difference was observed for the L858R cohort (HR: 1.25; 95% CI: 0.92–1.71; $p = 0.16$), noting that these two mutational patterns define different behavior and prognosis. With the aim to determine which of the currently available EGFR–TKIs is the treatment's choice for the advanced *EGFR*-positive NSCLC in the first-line setting, results from a Phase II randomized study, LuX-Lung 7 (comparing afatinib vs gefitinib), have been recently shown at the first ESMO Asia 2015 Congress in Singapore [11], while data from another Phase III randomized trial ARCHER 1050 (the irreversible TKI inhibitor, dacomitinib vs gefitinib) are pending [12]. In the first head-to-head LUX-Lung 7 trial, afatinib significantly reduced the risk of lung cancer progression by 27% versus gefitinib, with an higher proportion of patients alive and progression free at 18 months (27 vs 15%; $p = 0.018$) and 24 months (18 vs 8%; $p = 0.018$).

Speculating on the reason for the increased effectiveness of afatinib compared with gefitinib, we may argue that afatinib has some activity against T790M exon 20 mutation, present in up to 30% of TKIs naive. Although no difference between the two TKIs was found, in terms of discontinuation due to adverse events (6.3% for both arms), typical toxicity profiles have differently marked the two treatments, which might play a role to guide the selection of an *EGFR* inhibitor as a first-line choice. Despite remarkable initial responses, all patients with *EGFR*-mutant lung adenocarcinomas, eventually experience disease progression on EGFR– TKI therapy, resulting in median PFS ranging from 9.6 to 13.7 months.

Several acquired resistance mechanisms have been reported so far: EGFR exon 20 T790M mutation, uncommon EGFR secondary point mutations (e.g., D716Y, L747S, T854A), *MET* amplification, *PIK3CA* mutation, *BRAF*

Table 1. EGFR–TKI versus conventional chemotherapy in NSCLC harboring mutated *EGFR*.

Study	n (*EGFR*m+)	RR (%)	Median PFS (months)	Median OS (months)	Ref.
IPASS	261	71.2 vs 47.3	9.5 vs 6.3	21.6 vs 21.9	[2]
First-SIGNAL	42	84.6 vs 37.5	8.0 vs 6.3	27.2 vs 25.6	[3]
WJTOG 3405	172	62.1 vs 32.2	9.2 vs 6.3	34.8 vs 37.3	[4]
NEJGSG002	228	73.7 vs 30.7	10.8 vs 5.4	27.7 vs 26.6	[5]
OPTIMAL	154	83 vs 36	13.1 vs 4.6	28.8 vs 22.7	[6]
EURTAC	173	58 vs 15	9.7 vs 5.2	28.6 vs 22.1	[7]
LUX LUNG-3	345	56 vs 23	11.1 vs 6.9	28.2 vs 28.2	[8]
LUX LUNG-6	364	66.9 vs 23.0	11.0 vs 5.6	23.1 vs 23.5	[9]

m: mutation; OS: Overall survival; PFS: Progression-free survival; RR: Response rate.

mutation, *HER2* amplification, transformation to small cell lung cancer and epithelial to mesenchymal transition [13–16].

Third-generation EGFR–TKIs

Roughly 60% of cases of acquired resistance seem to be associated with the presence of a secondary missense mutation, namely T790M. This mutation consists into the replacement of a threonine with a methionine at codon 790 of exon 20 in the *EGFR* gene, affecting the catalytic adenosine 5′ triphosphate ATP binding pocket of the EGFR–TK domain [17]. As a consequence, T790M mutation enhances the binding affinity between the EGFR–TK domain and ATP. Importantly, in a subset of patients with *EGFR*m+ NSCLC who develop T790M-mediated acquired resistance, this mutation is detectable at baseline in a subgroup of clones through the use of highly sensitive techniques, becoming dominant following prolonged exposure to EGFR–TKIs [18]. In order to overcome such resistance a novel class of third-generation EGFR–TKIs has been designed to specifically target T790M mutation, providing a critical improvement in the treatment in T790M-positive NSCLC patients with acquired resistance to TKIs. Within this class, rociletinib (CO-1686), osimertinib (AZD9291), HM61713, ASP8273 and EGF816 are mutant-selective and EGFR wild type (WT) sparing TKIs, and are active against either sensitizing mutations and T790M. Interestingly, these molecules show a low affinity for WT form of EGFR, thus displaying a better toxicity profile compared with first- and second-generation EGFR–TKIs.

Osimertinib (AZD9291) is a monoanilinopyrimidine compound that irreversibly and selectively targets sensitizing as well as resistant T790M-mutant EGFR. In preclinical studies, osimertinib showed a significant efficacy in tumor xenograft and transgenic models [19]. In the Phase I/II AURA trial 253 patients with advanced *EGFR*-mutant NSCLC pretreated with EGFR–TKIs received osimertinib at disease progression. Of note, in 127 patients with documented T790M mutation was reported an impressive objective response rate (ORR) of 61%, with a disease control rate (DCR) of 95% and PFS and 9.6 months [20]. Conversely, in patients who tested negative for T790M, the ORR was 21%, DCR 61% and PFS only 2.8 months. Importantly, in first-line setting in EGFR–TKIs-naive patients harboring EGFR-sensitizing mutation, Ramalingam *et al.* reported an ORR of 70%, DCR of 97%, while PFS was not reached [21]. Besides, an ongoing Phase II clinical trial (AURA2) of osimertinib in *EGFR*-positive NSCLCs who progressed on EGFR–TKIs treatment and confirmed T790M showed encouraging results, achieving an ORR of 64%, a DCR of 90%, however, PFS was not reached [22]. Overall, osimertininb proved to be safe, with only 6–7% of adverse events leading to reduction or discontinuation of treatment. The most common side effects included: diarrhea (47%), rash (40%), nausea (22%) and interstitial lung disease (1.9%) [23]. The Phase III study (AURA3) is currently investigating osimertinib versus platinum-based therapy in advanced *EGFR*-mutant NSCLC patients with documented T790M mutation and progressive disease after treatment with upfront EGFR–TKIs [24]. Finally, in an effort to understand the most effective inhibitor among those of I and III generation, a randomized, Phase III study (FLAURA) comparing AZD9291, versus gefitinib or erlotinib in treatment-naive advanced NSCLCs harboring EGFR–TKI sensitizing mutations, has been recently opened to accrual, with the primary objective to compare PFS for

AZD9291 to standard of care EGFR–TKI, while PFS in T790M+ patients is a secondary objective [25].

Rociletinib (CO-1686) is another third-generation compound that selectively inhibit mutant *EGFR,* targeting either sensitizing and T790M mutations [26]. In a Phase I/II trial rociletinib allowed for an ORR of 59% and the DCR of 93% in patients harboring T790M, whereas in the T790M-negative patients experienced an ORR of 29% and DCR was 59%. Hyperglycemia nausea, rash, diarrhea and QTc prolongation were the most common side effects reported in this study [26]. EGF816 is a novel third-generation EGFR–TKI targeting T790M mutation with a 60-fold selectivity compared with WT EGFR [27]. Likewise osimertinib and rociletinib, *in vitro* studies confirmed that EGF816 potently inhibit also common *EGFR* mutations, including L858R, Ex19del and, remarkably, antitumor activity in exon 20 insertion mutant model. In the Phase I study EGF816X2101 42 EGF816 was administered to 42 patients, with 2% of them experiencing complete response, 24% partial response. However, an impressive DCR of 93% has been reported [27,28]. The most common AEs observed in the Phase I clinical trial were diarrhea (25%), stomatitis (22.5%) and rash (17.5%).

Along with the aforementioned compounds, novel third-generation EGFR–TKIs are currently under clinical investigation. Among them, ASP8273 and HM61713 already entered in human Phase I/II studies. With regard to ASP8273, in the Japanese Phase I/II dose escalation study, an ORR of 50% and 80% has been reported in all- and T790M-positive patients, respectively [29]. On the other hand in the Nord American trial an ORR of 28% and a DCR of 56% were observed, with 25% of patients harboring T790M mutation achieving a partial response [30]. Currently, ASP8273 is also being evaluated in a first-line Phase III clinical trial versus first-generation EGFR–TKIs, but data are still immature.

Lastly, HM61713 is another third-generation EGFR–TKI targeting either, sensitizing and T790M *EGFR* mutations, sparing the WT form of EGFR. Preliminary results from two expansion cohorts (300 and 800 mg) of the Phase I/II trial showed an ORR of 29.1 and 54.8% for 300 and 800 mg cohorts, respectively [31]. Again, data are still immature and need further investigation.

Future perspectives in *EGFR*m NSCLC

Given that the PD-1/PD-L1 pathway has been revealed as a promising target for treating NSCLC [32], its correlation with *EGFR* mutation needs to be confirmed by further studies. Indeed, data are still conflicting with some evidences stating that EGFR activation inhibits antitumor immunity through the PD-1/PD-L1 pathway, suppressing T-cell function and increasing levels of proinflammatory cytokines [33]. As opposite, recent studies concluded that *EGFR* or *KRAS* mutations did not correlate with RR to nivolumab for advanced NSCLC [34], neither a significant correlation between PD-L1 expression and EGFR, KRAS, BRAF or ALK status in limited disease was observed [35]. Recently, results from a multiarm Phase Ib trial, investigating osimertinib 80 mg in combination with durvalumab (anti-PD-L1 monoclonal antibody), savolitinib (MET inhibitor) or selumetinib (MEK 1/2 inhibitor) in patients with advanced EGFR-mutant lung cancer, have been released. The osimertinib and durvalumab combination represents one arm of the TATTON study, conceived with two parts; part A: a dose escalation phase in patients with advanced NSCLC that had received prior treatment with an EGFR–TKI. Part B: a dose expansion trial in EGFR–TKI treatment-naive advanced disease. Specifically, in patients with prior EGFR–TKI therapy, investigator-assessed ORR was 67 and 21% in T790M+ and T790M-, respectively, and 70% in *EGFR*-mutant treatment-naive patients. However, an increase in intersitial lung disease was reported with the combination of osimertinib and durvalumab compared with what would be expected with either drug alone (26% in part A, 64% in part B) [36]. If combining immunotherapy with TKI treatment, in oncogene-addicted disease, represents an exciting opportunity, and a potential answer to overcome the mechanisms of resistance, many gaps need to be fulfilled: the absence of available biomarkers with predictive capacity; the correct therapeutic strategy (combination vs a sequential approach); the unique toxicity profiles that these combinations may present.

First-generation ALK-inhibitor crizotinib

Crizotinib is an oral small molecule TKI of ALK, MET and ROS-1 kinases [37,38], which demonstrated an improvement in survival and RRs, over standard-of-care chemotherapy, for *ALK*-positive NSCLCs, regardless of the treatment

setting. Crizotinib development represents a paradigmatic example of a pretty fast approval process by the regulatory agencies, when an effective drug for a high unmet clinical need, is discovered. Indeed, based on the results of the trials listed below, crizotinib was granted accelerated approval by the US FDA in 2011 for the treatment of *ALK*-positive NSCLC, turned, 2 years later, into regular approval after the publications of confirmatory studies **(Table 2)** [39–46].

Mechanisms of acquired resistance to crizotinib

Unfortunately, as with EGFR–TKIs, mechanisms of intrinsic (30%) or acquired resistance to crizotinib, occur, after an average of 1 year since treatment start. Acquired resistance may be the result of either pharmacological or biological phenomena. In terms of pharmacological limitations, crizotinib appears to poorly penetrate the blood–brain barrier [47], resulting in inefficient CNS disease control [48–50]. In a retrospective analysis of pooled data from the profile 1005 and 1007, the intracranial ORR to crizotinib in patients with *ALK*-positive NSCLC was only 7%, despite a 12-weeks intracranial DCR of 60% [51]. When CNS represents the only site of recurrence, in the context of an extracranial disease control, brain local therapy (radiation or surgery) continuing crizotinib beyond progression, may be a reasonable option [52,53]. Biological resistance is expressed through ALK-dominant or nondominant mechanisms. The first involve an alteration in the drug target itself, the second the activation of alternative signaling pathways or 'bypass track.' As regards the target, *ALK* mutation and copy number gain account for 30–45% of crizotinib-resistant cases [54,55], where modifications of the 'gate-keeper' L1196M, represents the most common second site *ALK* mutation, to the left of broad range of others distributed throughout the ALK–TK domain, described so far (G1269A, G1202R, G1 123S/D, C1156Y, L1152R, S1206Y, 1151Tins, F1174C, D1203N) [56,57]. Among the bypass tracks mechanisms, the development of *EGFR* mutations/activation of WT EGFR, HER2 or KIT receptor, *K-RAS* mutations, has been described [58,59].

Second-generation ALK inhibitors

Two second-generation ALK inhibitors have been approved to date: ceritinib, received worldwide approval for *ALK*-positive NSCLC after critotinib failure; alectinib, approved in Japan for all patients with advanced *ALK*-positive NSCLC.

• Ceritinib

Ceritinib (LDK378; Zykadia; Novartis) is 20-times as potent as crizotinib against ALK and, in xenograft models of *ALK*-rearranged NSCLC, showed marked antitumor activity against both crizotinib-sensitive and crizotinib-resistant tumor [60,61]. Moreover, in *ALK*-positive cell line models, ceritinib was able to efficiently inhibit ALK harboring the crizotinib-resistant mutations L1196M, G1269A, I1171T and S1206Y but it was ineffective against the G1202R and F1174C [62]. Ceritinib inhibits also the IGF-1 receptor but not MET [63]. In a Phase I study (ASCEND, March 2014), ceritinib was shown to be highly effective in ALK-positive NSCLCs, both in the crizotinib-naive and crizotinib-treated settings, with 56% RR for 80 patients previously treated with crizotinib and 62% for the naive cohort [64]. Of note, responses were also seen in untreated CNS lesions in patients with crizotinib-resistant disease. Updated data were presented at ASCO 2014 (ASCEND-1): ORR 60% in the whole population (55.4% in ALK inhibitor pretreated, 69.5% in naive patients). Median PFS was 7 months in the overall population (6.9 months in patients previously exposed to ALK inhibitors) [65].

Two Phase II trails, both presented at American Society of Clinical Oncology (ASCO) 2015, have confirmed activity of ceritinib. ASCEND-2: ceritinib in patients who progressed after both chemotherapy and crizotinib, highlighted an ORR of 38.6% in the overall

Table 2. Summary of the Phase I–III studies on crizotinib for NSCLC with *ALK* rearrangements.

Study	n (*ALK*m+)	RR (%)	PFS (months)	OS (months)	Ref.
PROFILE 1001	82	57	9.7	1 year 76% 2 year 54%	[39]
PROFILE 1005	261	59.8	8.1	NA	[42]
PROFILE 1007	347	65	7.7	20.3	[44]
PROFILE 1014	343	74	10.9	Not reached	[46]

m: Mutation; NA: Not applicable; OS: Overall survival; PFS: Progression-free survival; RR: Response rate.

population and 33% in the brain metastases subgroup [66]. Median PFS (overall 5.7 months) significantly differed between patients with brain metastases (5.4 months) and those without (11.3 months). ASCEND-3: ceritinib in ALK inhibitor naive patients with *ALK*-rearranged NSCLC, showed an ORR of 63.7% (58% for those with CNS disease vs 67.6% in the cohort without), with median PFS 11.1 months (10.8 vs 11.1 months depending on the presence or not of brain metastases) [67].

With the aim to compare the activity of the drug with standard chemotherapy in untreated ALK-positive NSCLCs (ASCEND-4) and in previously treated with chemotherapy and crizotinib (ASCEND-5), two Phase III clinical trials with ceritinib have recently completed enrollment.

• Alectinib

In enzymatic assays, alectinib (RG7853/AF-802/RO5424802/CH5424802, Chugai-Roche) is five-times more potent than crizotinib against ALK, able to inhibit most of the clinically observed acquired ALK resistance mutation to crizotinib (L1196M, G1269A, C1165Y and F1174L) [68,69]. While it does not inhibit MET and ROS1, it showed activity against RET with a similar potency to ALK [70]. In a Japanese Phase I/II study, crizotinib-naive ALK-positive NSCLC patients exposed to alectinib, reached an RR of 93.5% [71]. With the goal to understand the activity of alectinib in crizotinib pretreated patients, the results of a Phase II global study (NP28673) have been reported, with an ORR of 50% in the whole population (57% for those with CNS involvement) and PFS 8.9 months (13 months for chemotherapy-naive patients) [72]. The molecule also has proven to meet the need to have a more active drug into the brain, which currently represents the 'Achilles heel' of all target therapies: an 83% intracranial DCR and a median CNS duration of response of 10.3 months [73].

Those results confirmed the ones showed in the previous Phase I/II study [74] with 60% of patients with brain metastases enrolled (ORR: 52%; median PFS: 8.1; CNS ORR: 75% and CNS DCR: 88.5%). Moreover, alectinib has shown to be effective in the setting of pretreated patients with leptomeningeal disease [75]. Weather alectinib should be used in first-line setting or in a sequential strategy is still an open issue. In order to address this question, two first-line studies comparing alectinib with crizotinib have recently completed enrollment: one conducted in Japan (J-ALEX; JapicCTI-132316) and a global one (ALEX; NCT02075840). However, the crucial point will be the magnitude of PFS benefit. Since sequential therapy with crizotinib followed by alectinib provides a combined median PFS of 18–20 months, we expect at least 6 months of amplitude of the PFS benefit to justify a switch from first-line crizotinib to first-line alectinib.

ALK inhibitors in clinical development

• Brigatinib

Brigatinib (AP26113, Ariad), is a dual ALK+/EGFRm inhibitor with preclinical activity against EML4–ALK (IC50 0.62 nM), included G1269S, G1202R, L1196M mutations and activity against ROS1 and T790M-mutant EGFR, without native EGFR inhibition [76]. The drug received breakthrough therapy designation by FDA for the treatment of ALK+ advanced NSCLCs resistant to crizotinib, based on results from a Phase I/II trial that showed antitumor activity in ALK+ NSCLC, including patients with active brain metastases. In the updated clinical data from this trial, objective responses were observed in ALK+ NSCLC patients, either TKI-naive or resistant to crizotinib. Of the 72 ALK+ NSCLC patients evaluable for response, 52 (72%) demonstrated an objective response with a median PFS of 13.4 months in the pretreated cohort. In a subgroup analysis, ten of 14 (71%) ALK+ NSCLC patients with active brain metastases had evidence of radiographic improvement [77].

• ASP3026 (Astellas Pharma)

ASP3026 is a potent inhibitor of ALK (IC50 3.5 nM) and activity against ROS1 (IC50 8.9 nM) ACK, L1196M, evaluated in 30 patients from an open-label, Phase I, escalation trial in patients with advanced tumors, excluding leukemia (ALK positivity not required; NCT01401504) [78]. At the 2014 ASCO annual report, 33 patients were enrolled in the dose escalation phase, including 3 ALK+ pts, plus another 13 ALK+ patients from the Phase Ib expansion cohort (n = 46). Out of the pretreated crizotinib cohort (15 ALK+), seven (44%) had a partial response and eight (50%) stable disease, with a median PFS of 5.9 months [79].

• PF-06463922 (Pfizer)

PF-06463922 is a dual inhibitor of ALK/ROS, with specific activity against ROS1 fusion variants including CD74–ROS1, SLC34A2–ROS1 and Fig–ROS1 (IC50 0.1–1 nM), and greater activity than crizotinib, especially against the gatekeeper L1196M [80]. A Phase I/II trial (NCT01970865) of ALK+ or ROS1+ NSCLC patients with or without CNS metastases, TKI-naive or 1–2 TKIs pretreated, has recently completed recruitment. Results from a Phase I portion of this study, presented at ASCO, out of 33 ALK+ and 11 ROS1+, 34 for overall tumor response and 25 for intracranial response, were evaluated: ORR was 44% in the whole population, 36% for those with brain measurable disease [81].

• Entrectinib

Entrectinib (RXDX-101, NMS-E628, Ignyta) is a multikinase inhibitor of TrkA/B/C, ROS1 and ALK kinases. Two Phase I/II clinical trials, ALKA-372-001 [82] and STARTRK-1 [83], have explored activity of this agent. The RR in the 11 patients across both studies was 91%, with nine of them patients reaching durable responses for up to 16 cycles. Specifically: 3/3 responses in patients with NTRK1/2/3 fusions, including patients with NSCLC, colorectal and pancreatic cancer; 5/6 responses, including one complete response, in patients with NSCLC ROS1+; 2/2 responses in patients with ALK fusions (one with NSCLC).

• TSR-011

TSR-011 (Tesaro) is dual ALK (IC50 value of 0.7 nM) and TrkA/B/C inhibitor (IC50 < 3 nM). Updated data from an ongoing Phase I/IIa trial [NCT02048488] have been recently presented: 46 points with advanced cancer, including 19 ALK+ and 11 TRK+ points, have been treated. Responses were achieved in: 3/5 ALK inhibitor-naive patients (60%); 3/6 patients (50%) progressed after crizotinib, stable disease in three patients after ceritinib or alectinib [84].

• X-376 & X-396

X-376 and X-396 (Xcovery) are potent inhibitors of ALK, less active for MET compared with crizotinib. X-396 showed activity against L1196M and C1156Y ALK mutants, and it demonstrated to penetrate blood–brain barrier [85]. In a Phase I/II trial of X-396, among 11 evaluable patients (both crizotinib-naive and resistant), six reached a PR and two SD, as well as two patients with brain disease. The expansion trail in ALK+ NSCLCs is ongoing (NCT01625234) [86].

• CEP-28122 & CEP-37440

CEP-28122 (Teva) is a selective ALK inhibitor (IC50 1.9 nM) with activity against InsR, IGF-R1 and c-MET [87]. CEP-37440 is a dual ALK/focal adhesion kinase inhibitor currently under investigation in a Phase I trial (NCT01922752). Focal adhesion kinase is implicated in cell adhesion and cell membrane–extracellular matrix interactions, thought to be involved in the carcinogenesis of colon cancer and other tumors of epithelial origin [88].

• Hsp90 inhibitors

Heat shock protein 90 (Hsp90) is a molecular chaperone essential for cellular survival preventing cellular proteins, included fusion proteins as EML4–ALK, from degradation by the ubiquitin–proteasome system in conditions of stress [89]. Main clinical trials evaluating Hsp90 activity in NSCLC are listed in **Table 3**.

Future perspectives in ALK-rearranged NSCLC

As per *EGFR* mutations, a potential effect of ALK translocation on PD-1/PD-L1 checkpoint expression cannot be excluded. Two recent studies had demonstrated that PD-L1 levels, were higher in patients with ALK translocations compared with the negative cohort; however, the association was not statistically significant and no evaluation about the clinical impact of different checkpoint expressions, emerged [99,100]. In mouse models, a vaccine against ALK induced a strong and specific immune response that both prophylactically and therapeutically impaired the growth of ALK-positive lung tumors. The vaccine used in combination with ALK TKI treatment, significantly delayed tumor relapses after TKI suspension. The study also confirmed that lung tumors containing ALK rearrangements induced an immunosuppressive microenvironment, regulating the expression of PD-L1 on the surface of lung tumor cells. High PD-L1 expression reduced ALK vaccine efficacy, which could be restored by administration of anti-PD-1 immunotherapy [101]. The hopes and concerns, about combination therapy with ALK-inhibitors and immunotherapy are pretty the same described with *EGFR* mutations, with a special interest in adverse events

Table 3. Hsp90 trials in oncogene-addicted NSCLC.

Drug name	Study name or ClinicalTrials.gov identifier	Phase	Patient population	Treatment arm(s)	Status	Ref.
IPI-504	NCT00431015	II	Advanced NSCLC	IPI-504	Completed	[90,91]
Ganetespib (STA-9090)	NCT02192541	I	Pretreated solid tumors (included advanced nonsquamous NSCLC)	Ganetespib and Ziv-aflibercept	Completed	[92–94]
	NCT01031225	II	Genotypically defined advanced NSCLC	Ganetespib	Completed	
	CHIARA (NCT01562015)	II	ALK+ advanced NSCLC	Ganetespib	Completed	
	NCT01579994	II	ALK+ crizotinib-naive advanced NSCLC	Ganetespib and crizotinib	Ongoing but not recruiting	
	GALAXY trial (NCT01348126)	II/ III	Advanced NSCLC	Ganetespib in combination with docetaxel versus docetaxel alone	Terminated for futility	
	GALAXY-2 (NCT01798485)	III				
AUY922	NCT01772797	Ib	ALK+ advanced NSCLC pretreated with crizotinib	Dose escalation study of LDK378 and AUY922	Ongoing but not recruiting	[95–97]
	NCT01752400	II	ALK+ advanced NSCLC pretreated with crizotinib	AUY922	Ongoing but not recruiting	
	NCT01124864	II	Advanced NSCLC progressed after two lines of prior chemotherapy	AUY922	Completed	
	NCT01922583	II	Advanced NSCLC with molecular alterations other than EGFR+, progressed after one line of systemic therapy	AUY922	Recruiting	
	NCT01854034	II	NSCLC with exon 20 insertion mutations in EGFR	AUY922	Recruiting	
	NCT01646125	II	Advanced EGFR+ NSCLC, progressed on prior EGFR-TKI treatment	AUY922 vs pemetrexed or docetaxel	Completed	
	NCT01259089	I/II	Advanced EGFR+ NSCLC progressed on prior eErlotinib	AUY922 and erlotinib	Completed	
AT13387	NCT01712217	I/II	Advanced ALK+ NSCLC pretreated with crizotinib	AUY922 alone (I) and with critotinib (II)	Ongoing but not recruiting	[98]

(i.e., pneumonitis), which are actually under focus in Phase I trials.

Conclusion

The identification of specific molecular targets in a significant fraction of NSCLC has led to the development of oncogene-directed therapies that have significantly changed the treatment of the advanced disease. TKI-sensitizing *EGFR* mutations and *ALK* rearrangements are the most important predictive biomarker for PFS and OS prolongation, as well as for a significant improvement in symptoms and quality of life, when TKIs are used for patients with advanced lung cancer. Therefore, the main challenge remains on how to overcome the inevitable acquired resistance to these therapies. Mechanisms of acquired resistances are roughly divided into two categories. The first involves onset of new genetic alterations in the native oncogene that guarantees the maintenance of the signal transmission. Resistance may also occur through several bypass signaling pathways, phenotypic transformation, chemical–physical barriers such as the blood–brain barrier.

This leads to two main implications: on the one hand the need of a new molecular characterization, via invasive tissue rebiopsy and/or liquid biopsies of circulating tumor DNA, when resistance occurs. On the other side, combination strategies (i.e., with immunotherapy) may represent a chance for more persistent remissions or to overcome mechanisms of relapse.

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