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Abstract

The therapeutic landscape of Non Small Lung Cancer (NSCLC) has been profoundly changed over the last decade with the clinical introduction of Epidermal Growth Factor Receptor (EGFR) tyrosine kinase inhibitors (TKIs) and the discovery of EGFR activating mutations as the major predictive factor to these agents. Despite impressive clinical activity against EGFR-mutated NSCLCs, the benefit seen with 1st and 2nd generation EGFR TKIs is usually transient and virtually all patients become resistant. Several different mechanisms of acquired resistance have been reported to date, but the vast majority of patients develop a secondary exon 20 mutation in the ATP-binding site of EGFR, namely T790M. The discovery of mutant-selective EGFR TKIs that selectively inhibit EGFR-mutants, including T790M- harboring NSCLCs, while sparing EGFR wild type, provide the opportunity for overcoming the major mechanism of acquired resistance to 1st and 2nd generation EGFR TKIs, with a relatively favorable toxicity profile. The development of this novel class of EGFR inhibitors poses novel challenges in the rapidly evolving therapeutic paradigm of EGFR- mutated NSCLCs and the next few years will witness the beginning of a new era for EGFR inhibition in lung cancer.
The aim of this paper is to provide a comprehensive overview of the increasing body of data emerging from the ongoing clinical trials with this promising novel therapeutic class of EGFR inhibitors.

Keywords	NSCLC; EGFR mutations; Osimertinib; Olmutinib; Rociletinib; Afatinib; EGFR TKI; resistance.
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INTRODUCTION

The therapeutic landscape of Non Small Lung Cancer (NSCLC) has been profoundly changed over the last decade with the clinical introduction of Epidermal Growth Factor Receptor (EGFR) tyrosine kinase inhibitors (TKIs) and the discovery of EGFR activating mutations as the major predictive factor to these agents [1]. Despite impressive clinical activity against EGFR-mutated NSCLCs and proved superiority over chemotherapy in the 1st line setting in molecularly-selected patients, the benefit seen with 1st and 2nd generation EGFR TKIs is usually transient and virtually all patients become resistant after approximately 9-13 months [2-6]. Several different mechanisms of acquired resistance have been reported to date, but the vast majority of patients (41-62%) develop a secondary exon 20 mutation in the ATP-binding site of EGFR, namely T790M [7-10]. Various strategies have been studied to overcome T790M mutation, but only few have reported clinical meaningful benefit. The discovery of mutant-selective EGFR TKIs that selectively inhibit EGFR- mutants, including T790M-harboring NSCLCs, while sparing EGFR wild type, provide the opportunity for overcoming the major mechanism of acquired resistance to 1st and 2nd generation EGFR TKIs, with a relatively favorable toxicity profile. Osimertinib is the first mutant-selective EGFR TKI approved by the FDA and EMA, but several other agents are in different development stages. The discover of this novel class of EGFR inhibitors poses novel challenges in the rapidly evolving therapeutic paradigm of EGFR-mutated NSCLCs and the next few years will witness the beginning of a new era for EGFR inhibition in lung cancer. However, the development path of a drug is a complex and difficult process, as recently observed with the unexpected disappointing results with the two 3rd generation EGFR TKIs Rociletinib and Olmutinib. The aim of this paper is to provide a comprehensive overview of the increasing body of data emerging from the ongoing clinical trials with this promising novel therapeutic class of EGFR inhibitors.

MECHANISMS OF ACQUIRED RESISTANCE TO 1st AND 2nd GENERATION EGFR INHIBITORS

After treatment with first or second generation EGFR TKIs, virtually all patients after approximately 12 months develop acquired resistance (AR). Different mechanisms of AR to EGFR TKIs have been reported to date and may broadly divided in two subgroups: pharmacological and biological mechanisms, including alterations in the drug target, bypass track mechanisms, phenotypic/histologic changes and downstream signaling pathways alterations [11, 12]. In 2005 two different groups first reported the development of a secondary mutation in the EGFR TK domain causing a substitution of a threonine with a methionine at the 790 site of the exon 20 (T790M

mutation), leading to resistance to first generation EGFR TKI Gefitinib after initial response [13, 14]. The T790M mutation is the major mechanism of AR, since it is detected *in vivo* in approximately 41-62% of the cases at re-biopsy after acquired resistance to 1st generation EGFR TKIs [7-10]. Following preclinical studies reporting the development of T790M mutation even after irreversible EGFR TKIs [15], a recent retrospective analysis confirmed T790M mutations as the major mechanism of AR, even in patients became resistant to the irreversible EGFR inhibitor Afatinib, with an overall frequency of ~50% [16]. The development of T790M is associated with AR to both 1st and 2nd generation EGFR TKIs by increasing the receptor affinity to the ATP and consequently limiting the potency of these ATP-competitive kinase inhibitors [17]. The emergence of T790M mutations in brain metastases is less frequent than extracranial sites (17% vs. 41% in a retrospective analysis), suggesting that other mechanisms of AR may be more relevant in the central nervous system (CNS), such as poor drug exposure [7].

The identification of T790M mutation after AR to EGFR TKIs raised the question whether these mutations were the results of a selection process of pre-existing clones T790M+ due to the selective pressure of EGFR TKIs or were acquired during treatment because of novel genetic and epigenetic alterations [18]. In favor of the "*selection model*" there are some preclinical and clinical evidences of the presence of pre-existing resistant clones before treatment, including T790M+, albeit their exact frequency and clinical significance is not yet fully understood [19-24]. Indeed, preclinical data indicate that tumors with AR likely harbor mixed populations of drug-sensitive and drug-resistant cells with differential growth rates, with the T790M+ cells showing slower growth than T790M- ones [25]. These data are further supported by clinical evidences indicating a more indolent natural history and a longer post-progression survival among T790M harboring patients [7, 26, 27].

In few cases the development of acquired resistance may involve the development of other rarer secondary mutations, including D761Y [28], L747S [29] and L854A [30].

Acquisition of a secondary mutation is not the solely mechanism of AR to EGFR TKIs, but different preclinical and clinical studies, but EGFR-mutated tumors may escape the EGFR blockage in several other ways, including: Small Cell Lung Cancer (SCLC) transformation (2-14%) [8-10, 31], Epithelial to Mesenchimal Transition (EMT) [9, 32], MET amplification (4-5%) [9, 10, 33], HER2 amplification (12-13%) [10, 33], PIK3CA mutations (5%) [9], AXL activation [34], BRAF mutations [35], and NFkB activation [36]. In some instances, multiple mechanisms may operate simultaneously, albeit in the majority of cases seems to be mutually exclusive [10].

The better knowledge of the inherited mechanisms of AR to EGFR TKIs contributed to the development of rationally strategies to overcome resistance.

Given its high frequency, it is not surprising that different strategies have been developed to restore the sensitivity of EGFR T790M-mutant cells to EGFR inhibition. To date, several different strategies have been evaluated in both preclinical and clinical models, but only few have been proved effective *in vivo*. Indeed, some promising *in vitro* strategies, such as the use of irreversible EGFR inhibitors ("second generation" EGFR TKIs) against T790M-mutants [37-39], failed *in vivo* due to a narrow therapeutic window [40-43]. Moreover, the use of a vertical blockage with an EGFR TKI and a monoclonal anti-EGFR antibody (i.e. Afatinib and Cetuximab), albeit proved effective against T790M-harboring tumors, is limited by the unfavorable toxicity profile [44].

The use of mutant-selective, EGFR wild type sparing, "*third generation*" inhibitors is an emerging therapeutic strategy in patients with AR to 1st and 2nd generation inhibitors. These newer agents differ from quinazoline-based reversible (Gefitinib and Erlotinib) and irreversible EGFR TKIs (Afatinib, Dacomitinib, Neratinib), because of their aminopyrimidine scaffold and have been specifically developed to target EGFR mutations, including T790M, with only minimal activity against wild type EGFR. The first-in-class third generation EGFR TKI reported was WZ4002, which did not progress to clinical trials [45], but now a few different 3rd generation EGFR TKIs are in active clinical development in NSCLC. In the following sections we will provide an overview of the latest preclinical and clinical data on the most promising agents of this novel class of EGFR TKIs.

OSIMERTINIB (AZD9291)

Osimertinib (AZD9291; AstraZeneca) is a novel irreversible, small molecule inhibitor, developed to target both sensitizing and resistant mutant forms of the EGFR while sparing the wild type form of the receptor [46].

This mono-anilino–pyrimidine compound is structurally and pharmacologically distinct from all other TKIs. Osimertinib binds irreversibly to the EGFR kinase by targeting the cysteine-797 residue in the ATP binding site via covalent bond formation [47, 48]. The drug exhibits nearly 200 times greater potency against L858R/T790M than *wild type* EGFR. Studies conducted *in vivo*, revealed that Osimertinib is metabolized to produce at least two circulating metabolite species, AZ5104 and AZ7550. AZ7550 had a comparable potency and selectivity profile, while AZ5104 is more potent against mutant and *wild type* EGFR forms [49, 50].

In vitro studies indicate that Osimertinib has activity against mutant EGFR, including T790M+, but selectivity margin against *wild type* EGFR. The drug has minimal *off-target* activity, with a limited number of non-HER kinases inhibited, but conserves activity against HER2/4. In contrast, Osimertinib is not effective against lines harboring non-T790M resistance, such NRAS mutations, MET amplification, and epithelial-to-mesenchymal transition (EMT) [49].

Studies conducted *in vivo* xenograft models demonstrated good bioavailability of Osimertinib and moderate clearance with half-life of 3 hours after oral dosing in the mouse. The circulating metabolites have a similar half-life and the total exposure level (AUC) were approximately 68% and 33% compared with parent compound for AZ7550 and AZ5104 respectively. Quantitative whole body autoradiography (QWBA) studies in rat brain indicate that Osimertinib has a brain-to-blood ratio of up to 2 over the first 24 hours, suggesting the potential of AZD9291 to penetrate the brain [49]. Studies in mouse models indicate that Osimertinib is distributed in the CNS at greater extent than Gefitinib, Rociletinib and Afatinib [51] and has potential activity also against leptomeningeal metastases (LMs) in both EGFR TKI-naïve and TKI-resistant tumors [52].

The AURA trial was a phase I/II study evaluating the safety, tolerability and activity of Osimertinib in patients with EGFR-mutated NSCLC progressing after a previous EGFR TKI. The phase I component of the study included a dose escalation part (Osimertinib 20 mg to 240 mg), using a rolling six design, and an expansion part with multiple biomarker-guided cohorts of patients. Before enrollment in the expansion cohort, the T790M status of the tumor was mandatory. The recommended phase II dose (RP2D) was 80 mg once daily. The phase II (extension part) component of the study enrolled T790M+ NSCLCs progressing on a previous EGFR TKI at the dose of 80 mg/d. A total of 253 patients (31 in the dose-escalation cohorts and 222 in the expansion cohort) were enrolled. EGFR T790M was detected in 62% of patients enrolled in the expansion cohort [53]. A maximum tolerated dose (MTD) was not found, however at the 160 mg and 240 mg dose levels there were an increase in the incidence and severity of adverse events (AEs) associated with EGFR wild type inhibition (namely, skin and gastrointestinal AEs), therefore the dose of 80 mg once daily was considered the RP2D dose. Of the 239 patients evaluated for response 51% had PR or CR, 33% SD and 14% PD. The disease control rate (DCR) was 84% (95% CI, 79 to 88). Among patients with EGFR T790M, the objective response rate (ORR) was 61% (78 of the 127 patients; 95% CI, 52 to 70), and the DCR was 95% (121 of the 127 patients; 95% CI, 90 to 98). Although the data published were immature, the median PFS was 8.2 months in the overall population, 9.6 months in the T790M+ group (95% CI, 8.3 to not reached) and 2.8 months (95% CI, 2.1 to 4.3) in patients with no detectable EGFR T790M [53]. These promising results were

recently confirmed in the phase II part of the AURA trial and in the open label phase II AURA 2 study.

In the phase II extension component of AURA trial, 201 EGFR-mutated and T790M-positive EGFR-TKI pretreated patients received Osimertinib 80 mg/d. The study confirmed the efficacy results of the phase I component of the trial, with an ORR by BICR of 62% (95% CI, 54 to 68) and a DCR of 90% (95% CI, 85 to 94), irrespective of EGFR mutation type (exon 19 deletions or L858R) and lines of treatment (2^{nd} vs. 3^{rd} or other). Median PFS by BICR was 12.3 months (95% CI, 9.5 to 13.8), with a non-significant trend in favor of longer PFS for patients with exon 19 deletions (median PFS 12.5 months) compared to those harboring L858R mutations (9.6 months) and for Asian versus non-Asian patients (12.6 months vs. 9.7 months). At a median follow-up of 13.8 months, median OS was not reached, with an estimated 1-year OS of 79% [54]. These results are in line with those of the open label phase II study AURA II, reporting an ORR of 70% with a DCR of 92% and a median PFS of 9.9 months in EGFR TKI-pretreated T790M-positive patients. PFS results were consistent irrespective of line of therapy, presence of CNS metastases, ethnicity (Asian vs. non-Asian), and mutation status (T790M co-occurring with exon 19 deletion vs. L858R) [55]. Osimertinib at the dose of 80mg/daily was associated in both studies with a favorable toxicity profile and an improvement in quality of life measures. ILD was observed in 2-4% of patients, irrespective of ethnicity [54, 55].

The phase III, open label, randomized study AURA3 (NCT02151981) evaluated the efficacy of Osimertinib 80 compared with platinum-based doublet chemotherapy in patients with locally advanced or metastatic NSCLC whose disease has progressed with previous EGFR TKIs and whose tumors harbored a T790M mutation [56]. Primary endpoint was PFS. Secondary outcome measurements were ORR, Duration of Response (DoR), DCR, and OS. 419 patients were randomized in a 2:1 ratio to receive either oral Osimertinib (at a dose of 80 mg once daily) or intravenous Pemetrexed 500 mg/m^2 q3wk plus either Carboplatin AUC5 or Cisplatin 75 mg/m^2 q3wk for up to six cycles; maintenance Pemetrexed was allowed. The trial met its primary endpoint, demonstrating a significant advantage in terms of PFS (10.1 months vs. 4.4 months; HR 0.30; 95% CI, 0.23 to 0.41; P<0.001). The advantage in PFS was consistent in all the subgroups analyzed, including patients with BMs (HR 0.32). Moreover, Osimertinib demonstrated also higher ORR (71%; 95% CI, 65 to 76) compared to platinum-Pemetrexed (31%; 95% CI, 24 to 40) (OR for objective response, 5.39; 95% CI, 3.47 to 8.48; P<0.001). The safety profile was in line with the results of previous phase I-II studies, with 23% of patients experiencing G3-4 AEs and a low discontinuation rate (7%) [56]. The outcome of patients treated with platinum-Pemerexed favorably correlated with previous observation in a similar population (median PFS of 4.4-5.4 months and

ORR of 31-34.1%) [57]. **Table 1** reassumes the efficacy data with Osimertinib in T790M-positive NSCLCs in clinical trials published to date.

The randomized double blind phase III FLAURA trial (NCT02296125) is evaluating Osimertinib in the first line setting versus Gefitinib or Erlotinib.

The intriguing preclinical intracranial activity of Osimertinib [51, 52] has been recently confirmed *in vivo* in patients with BMs. Osimertinib has a CSF concentration of 0.77-3.44 nmol/L [58] and is associated with a median PFS in patients with asymptomatic CNS metastases at baseline of 7.1-8.5 months [54, 56]. The intracranial activity of Osimertinib will be further explored in the phase I BLOOM trial, evaluating Osimertinib and AZD3759 in EGFR-mutated NSCLC patients with BMs and LMs. At the 2016 ASCO annual meeting, the preliminary results of the Cohort 1 of the study (EGFR-mutated NSCLCs with LMs) were presented, reporting a promising activity with Osimertinib 160 mg/d in this unfavorable prognostic subgroup of patients [59]. The Cohort 2 of the study is enrolling T790M+ NSCLC patients with LMs.

Osimertinib is being evaluated in multiple clinical trials across different settings and combinations, to understand its potential benefit for overcoming newly identified forms of resistance. The ongoing phase III trials are summarized in **Table 2**.

The ongoing TATTON study (NCT02143466), a multi-arm Phase Ib study, is designed to assess the safety, tolerability, pharmacokinetics and preliminary anti-tumor activity of Osimertinib in combination with ascending doses of novel therapeutics, including Durvalumab (anti-PDL1 inhibitor), Selumetinib (anti-MEK inhibitor) and Savolitinib (anti-MET inhibitor), in patients with EGFR-mutated advanced NSCLC who have progressed following therapy with an EGFR TKI. Finally, the phase III study CAURAL study (NCT02454933) will investigate Osimertinib in combination with Durvalumab as a potential second-line treatment for EGFR-mutated NSCLC patients carrying the T790M mutation to assess the efficacy and safety of Osimertinib in combination with Durvalumab versus Osimertinib monotherapy.

On 13 November 2015, Osimertinib received FDA approval for patients carrying a T790M mutation and whose disease has progressed after treatment with other EGFR TKI, followed by EMA approval on 2 February 2016, making Osimertinib the first new drug approved under the EMA expedited process [46]

ROCILETINIB (CO-1686)

Rociletinib (CO-1686; Clovis Oncology Inc.) is a novel, oral, mutant-selective, covalent EGFR inhibitor studied for the treatment of NSCLC.

Rociletinib is a potent 2,4-disubstituted pyrimidine molecule that covalently modify the Cys797 in the ATP-binding pocket of the EGFR kinase domain. Studies conduced *in vitro* demonstrated that CO-1686 is a potent inhibitor of L858R/T190M and approximately 22-fold more selective than *wild type* EGFR. Twenty-three targets inhibited more than 50% at 0.1 μmol/L; EGFR del19-, T790M-, L858R/T790M-, and L858Rmutant kinases have the highest degree of inhibition, however, other kinase targets were observed to be inhibited at lower potency, including focal adhesion kinase (FAK), CHK2, ERBB4, and Janus-activated kinase 3 (JAK3) [60, 61].

In vitro studies in NSCLC cell lines expressing EGFR mutants (T790M mutation, exon 19 deletion E746-A750, L858R/T790M double mutation), demonstrated that Rociletinib potently inhibits proliferation in the mutant EGFR NSCLC cells with Growth inhibition (GI_{50}) values ranging from 7 to 32 nmol/L. In comparison, the GI_{50} value for *wild type* EGFR cells was 547 nmol/L. CO-1686 also inhibits NRAS and KRAS mutations in WT EGFR cells at concentrations of 4275 and 1806 nmol/L, respectively. The efficacy of CO-1686 was also examined against other EGFR mutants including the exon 18 mutation G719S, an exon 19 insertion mutant (I744-K745insKIPVAI), an exon 20 insertion (H773-V774HVdup), and the exon 21 mutation L861Q. Rociletinib was active against these rare mutants also, with the exception of exon 20 insertions [60].

The TIGER program was an accelerated clinical development program for Rociletinib in patients with mutant EGFR NSCLC in different therapeutic settings (**Tab. 3**).

The phase I/II dose-finding TIGER-X evaluated Rociletinib in EGFR-mutated NSCLCs progressing after previous $1^{st}/2^{nd}$ generation EGFR TKIs. The study consisted of two parts, the phase I dose-escalation component followed by the phase 2 expansion part (requiring T790M status assessment before enrollment) to assess efficacy at 500 mg twice daily, 625 mg twice daily, and 750 mg twice daily. Two different formulations of Rociletinib were initially developed, the free-base form (entered the clinic in March 2013) and the hydrogen bromide salt (HBr), designed to improve the pharmacokinetic profile, introduced during dose escalation from August 2013 that replaced the free-base form. Primary objectives of the phase I were safety, toxicity profile, and pharmacokinetic characteristics of Rociletinib. Secondary end points included ORR, DoR, PFS, and QoL. Primary end points of the phase II part were ORR and DoR, whereas secondary and exploratory end points were the same as in the phase I component. A MTD was not defined in the phase I portion. Preliminary results, based on 130 patients enrolled, reported an ORR among 46 patients with centrally confirmed T790M-positive tumors 59%, with a DCR of 93%. Response rates were similar between patients with exon deletion 19 or L858R EGFR mutation. The estimated median PFS was

13.1 months. The ORR among 17 T790M-negative patients was 29%, with a DCR of 59% and a median PFS of 5.6 months. Among 20 patients whose tumors were not assessable for T790M the ORR was 15%. The predominant grade 3 AE was hyperglycemia (22%), which was generally manageable and did not result in treatment discontinuation [62]. Preclinical studies suggest that hyperglycemia is caused by a Rociletinib metabolite that inhibits the type I insulin-like growth factor receptor (IGF-IR) and induces activation of the IGFIR pathway, a proposed resistance mechanism for EGFR inhibition [63], although the contribution of the IGF-IR inhibitory effect of Rociletinib to its antitumor activity is currently unknown.

These preliminary results prompted the establishment of a rapid developmental program (**Table 2**) and granted, in May 2014, the U.S. FDA Breakthrough Therapy designation for the treatment of mutant NSCLCs with the T790M mutation after progression on EGFR-directed therapy.

Unfortunately, on May 2016 Clovis inc. announced the interruption of clinical development of Rociletinib and terminated enrollment in all ongoing sponsored clinical studies based on the unsatisfactory results of a pooled analysis of TIGER-X and TIGER-2 trials submitted to the US FDA, reporting a 32% ORR (95% CI 25, 40) with a median DoR of 8.8 months in T790M+ patients treated with Rociletinib at the doses of 625 mg BID and 23% ORR (95% CI 14, 34) with a median DoR of 9.1 months in patients who received 500 mg BID [64].

OLMUTINIB (BI 1482694/HM61713)

Olmutinib (Olita™, BI 1482694/HM61713; Hanmi Pharmaceuticals and Boehringer Ingelheim) is a 3rd generation EGFR TKI, developed to specifically inhibit EGFR mutants, including T790M, while sparing wild type EGFR.

The safety and pharmacokinetic profile of this agent was evaluated in the phase I/II HM-EMSI-101 study, a multicenter trial conducted in Korean patients previously treated with at least one EGFR TKI. At the recommended phase II dose (RP2D: 800mg qd), all eligible patients had to have confirmed T790M mutation in the tumor. The primary endpoint was ORR; secondary endpoints included DOR, DCR, PFS and safety. Data of patients treated at the RP2D of 800 mg qd were recently presented: 54% confirmed ORR by independent review and 90% DCR in T790M-positive TKI-pretreated NSCLCs with a favorable safety profile [65].

In July 2015, Hanmi Pharmaceuticals signed a licence and collaboration agreement with Boehringer Ingelheim for the development and commercialization of Olmutinib, launching the ELUXA 1 (HM-EMSI-202) pivotal Phase II global clinical trial, designed to further investigate the efficacy and

safety of Olmutinib in patients T790M-positive NSCLC with acquired resistance after first-line EGFR TKIs. Primary endpoint was ORR according to RECIST 1.1, while secondary endpoints were DCR, DoR, PFS, OS, TTP, tumor shrinkage, patients reported outcomes (PROs), and safety [66].

On December 2015, the US FDA granted breakthrough therapy designation to Olmutinib based on the promising results from the Phase I/II HM-EMSI-101 clinical trial, followed by accelerated approval in South Korea in May 2016 for the treatment of T790M-positive NSCLCs [67].

However, on September 2016, after a review of the available clinical data, with the report of two cases of toxic epidermal necrolysis, one of which was fatal, and a case of Stevens-Johnson Syndrome, in the HM-EMSI-10 trial, and the advances in the development of 3rd generation EGFR TKI Osimertinib, Boehringer-Ingelheim announced the termination of its deal with Hanmi Pharmaceuticals for the development of the drug, halting the ELUXA pivotal trial program (**Tab. 4**), which should have included the initiation of two Phase III studies comparing Olmutinib with chemotherapy in previously treated T790M+ NSCLCs (ELUXA 2) and in the first line setting versus Afatinib (ELUXA 3).

OTHER 3rd GENERATION EGFR TKIs

EGF816 (Novartis Pharmaceuticals) is a covalent, irreversible, EGFR TKI with high *in vitro* activity against EGFR mutants, including T790M. Preliminary results of the multi-arm phase I/II study (NCT02108964) in advanced NSCLC patients harboring T790M mutation showed a manageable safety profile, with the most common grade 3/4 AEs were maculo-papular rash (14%), anemia (6%), and diarrhea (6%), and an intriguing clinical activity with a confirmed 44% ORR and a 91% DCR. Median PFS was 9.2 months (95% CI 9.0-NE) [68].

ASP8273 (Astellas Pharma Inc) is another small molecule mutant-selective, irreversible EGFR inhibitor with higher *in vitro* affinity against EGFR-mutants than wild type that is being evaluated in phase II/III studies in EGFR-mutated NSCLCs. At the recommended phase II dose of 300 mg/d, ASP8273 showed a robust antitumor activity in both subjects with pre-treated EGFR mutation-positive NSCLC and T790M+. For the 45 subjects treated with ASP8273 300 mg with evaluable data, DCR was 62%, with 16 patients achieving a PR and 12 a SD. For the 40 T790M+ subjects with evaluable data, DCR was 65% with 15 PR and 11 SD. Preliminary median PFS was 6.7 months in both overall population and T790M+ patients [69]. The ongoing phase III trial SOLAR will compare the activity of ASP8273 in the first line setting versus 1st generation EGFR TKIs.

Brigatinib (ARIAD Pharmaceuticals), also known as AP26113, is dual ALK/EGFR with preclinical activity against the oncogenic ALK fusion protein and mutants resistant to Crizotinib, but also against activated and T790M-mutant EGFR [70]. Brigatinib received Breakthrough Therapy designation from the FDA in October 2014 for the treatment of patients with ALK-rearranged NSCLC with acquired resistance to Crizotinib and data from the phase I/II study (NCT01449461) in EGFR-mutated NSCLC are awaited.

Finally, PF-06747775 (Pfizer) and Avitinib (AC0010, Hangzhou ACEA Pharmaceutical Research) are other two novel 3rd generation EGFR TKIs in early development phase and results of phase the I/II studies are awaited.

More recently, Rho et al. through a high-throughput screen identified two novel 3-pyrazolypyrimidine compounds (GNS-1481 and GNS-1486) that selectively inhibit mutant EGFR, including T790M. Interestingly, GNS-1486 exhibit a superior selective action for mutant EGFR over WT form compared to Osimertinib, with an *in vitro* activity against also RET and a CNS-penetrant action in preclinical models [71]. Clinical trials testing GNS-1486 in EGFR-mutant NSCLC patients will be initiated soon.

EMERGING MECHANISMS OF ACQUIRED RESISTANCE TO 3rd GENERATION EGFR TKIs

Despite promising activity against EGFR mutant T790M+ NSCLCs, acquired resistance to 3rd generation EGFR TKIs ultimately occurs through different and, only partially, known mechanisms. Prior studies, evaluating the structural analogue tool compound WZ4002, reported activation of MAPK and IGF1R pathways as possible mechanisms of acquired resistance to irreversible mutant-selective EGFR TKIs [63, 72], but no additional mutations, a largely anticipated mechanism of resistance, were reported until recently.

This class of agents has been developed to inhibit EGFR T790M mutations, binding covalently to the cysteine residue 797 [45]. Therefore, it is not surprising that EGFR T790M+ cells acquiring resistance to mutant selective EGFR TKIs, due to the selective pressure of these agents, may develop a tertiary mutation that results in a cysteine to serine change at position 797 (C797S) in a region of the ATP-binding pocket of EGFR that is opposite to that of T790M. The EGFR C797S mutation resembles the acquired C481S mutation to the irreversible BTK (Bruton tyrosine kinase) inhibitor Ibrutinib in patients with chronic lymphocytic leukemia [73], suggesting that this mutation type may represent a common mechanism of AR to covalent kinase inhibitors.

Indeed, preclinical studies, using cultured patient-derived EGFR exon19del/T790M cell lines (MGH121) and through mutagenesis screen in EGFR mutant cells with or without T790M, reported the development of three tertiary mutations (C797S, L844V and L718Q) in the EGFR after acquiring resistance to the 3rd generation compound WZ4002 [74, 75]. The presence of these mutations and their allelic/genomic context has been reported to influence their sensitivity to the different EGFR TKIs available. Indeed, C797S conferred resistance to all the 3rd generation EGFR TKIs evaluated (WZ4002, Osimertinib and Rociletinib), but was sensitive to 1st and 2nd generation inhibitors (with reduced sensitivity in the context of L858R/C797S cells), L844V was associated with resistance to WZ4002 and Rociletinib, but not to Osimertinib (independently of the presence of T790M mutation), Gefitinib and Afatinib, while L718Q exhibited resistance to WZ4002 and Rociletinib, but not to Osimertinib, only in the context of exon19 del/L718Q [75]. A possible explanation may be found in the inherited differences in the molecular structure of Rociletinib and Osimertinib, since the first is structurally more similar to WZ4002 than the latter. Moreover, Niederst et al. reported that the allelic context of this acquired resistance may be essential for the therapeutic strategy of patients with EGFR mutation, since the development in the same alleles of T790M and C797S mutations (i.e. *in cis* mutations) confers resistance to all the EGFR TKIs known, but the development of these mutations in different alleles (i.e. *in trans*) may pave the way to a combinatory approach of both 1st and 3rd generation TKIs. Moreover, the front-line use of mutant-selective inhibitors and the subsequent development of C797S mutation may raise the opportunity of the use of 1st generation agents, since del19/C797S mutations are still sensitive to Gefitinib [74]. These findings suggest that different sequential and combinatory approaches may necessary in EGFR-mutated NSCLCs patients when treated with different classes of EGFR TKIs, since the mechanisms of AR may vary based on the molecular and therapeutic context.

The development of C797S mutation has been reported *in vivo* in a small cohort (15 subjects) of EGFR T790M positive patients progressing after Osimertinib: this tertiary mutation was found, using a droplet digital PCR (ddPCR) assay, in 40% of patients after AR to this agent, while 33% of the cases exhibited persistence of the secondary mutation T790M (without C797S) and 27% showed neither T790M nor C797S mutations [76]. The development of C797S has been recently confirmed *in vivo* in tumor samples obtained after re-biopsy in patients with acquired resistance to Osimertinib [77] and Olmutinib [78] and in plasma samples from patients with AR to Rociletinib, albeit with a less frequency than observed with Osimertinib (2% vs. 32%), suggesting a distinct pattern of resistance mechanisms [79].

Jia et al. recently identified an investigational compound, named EAI045, which targets selected drug-resistant EGFR mutants but spares the wild-type receptor. Unlike 3rd generation mutant-

selective EGFR TKIs, this compound is a non-ATP competitive inhibitor that binds to T790M-mutant EGFR in an allosteric site created by the displacement of the regulatory C-helix in an inactive conformation of the kinase. This different mechanism of action do not allow an efficient EGFR blockage as single agent, but a marked synergy was observed with the anti-EGFR antibody Cetuximab in mouse models of lung cancer driven by L858R/T790M EGFR and, interestingly, by L858R/T790M/C797S EGFR [80; 81]. Recently, the dual ALK/EGFR inhibitor Brigatinib demonstrated intriguing preclinical activity *in vitro* and *in vivo* in different EGFR triple-mutant models (C797S/T790M/activating-mutation) with AR to 3rd generation EGFR TKIs, with improved activity in combination the EGFR mAb Cetuximab [82].

In addition to C797S mutation, other tertiary mutations as possible mechanisms of AR to Osimertinib have been recently reported, including L792F/Y/H and L718Q [83, 84], albeit the relative frequency is still unclear.

Studying 32 cell lines with acquired resistance to several EGFR TKIs, including Gefitinib, Afatinib, WZ4002 and Osimertinib, Eberlein et al. reported that resistance to AZD9291 and other EGFR inhibitors *in vitro* is often associated with increased dependence on RAS signaling, through NRAS mutations (including the novel E63K NRAS mutation) and KRAS gain, and sensitivity to the MEK inhibitor Selumetinib [85]. These data are in line with previous reports indicating the RAS-MAPK signaling pathway as an alternative mechanisms of acquired resistance to EGFR inhibitors in both lung and colon cancer [86] in many different ways, including loss of NF1 [87], CRKL amplification [88], MAPK1 amplification [72]. Moreover, the combination of Osimertinib and Selumetinib delayed the emergence of resistance in EGFRm and EGFRm/T790M cells [85]. These data provide the rationale for concomitant use of Osimertinib and Selumetinib in the ongoing multi-arm phase Ib trial TATTON [NCT02143466].

Activation of alternative signaling pathways in an EGFR-independent way may also occur after acquired resistance to Osimertinib, including MET amplification, HER2 amplification and BRAF V600E mutation [89-91]. The emergence of by-pass track mechanisms of AR may be overcome with the use of effective combinatory strategies, such as the addition of MET inhibitors to the mutant-selective EGFR TKI [92].

In a small case series of 4 EGFR exon 19 del/T790M NSCLCs patients with acquired resistance to Osimertinib enrolled into the AURA trial, re-biopsy after AR revealed the presence of different mechanisms of resistance: none of the 4 patients developed C797S mutation, but loss of EGFR T790M mutation was observed in addition to alternative pathways activation, including FGFR1 amplification, PTEN deletion, MAPK1 and AKT3 overexpression, and histologic transformation (i.e. SCLC transition) [93], confirming previous *in vitro* findings [49, 72, 94, 95]. Similarly to a

previous report [31], SCLC transformation was associated with RB loss. This small case series conducted on patients progressing after both 1st and 3rd generation EGFR TKIs confirms the complexity and substantial overlay of mechanisms of acquired resistance, under the selective pressure of different classes EGFR TKIs.

The loss of secondary mutation T790M mutation after AR and SCLC transformation was also reported for Rociletinib. Piotrowska et al. using the MGH NGS platform recently reported in 13 biopsies among 12 EGFR T790M+ patients progressing after treatment with Rociletinib the loss of EGFR T790M mutation in 6 (with evidence of SCLC transformation plus RB1 loss in 2/6 cases) and persistence of T790M mutation in 7 samples (with EGFR amplification in 3/7 tissue biopsies), respectively. No C797S positive cases were reported, probably due to the small sample size and the population evaluated in the study: both primary and secondary resistant patients to Rociletinib were included and mostly with poor response to the treatment. Interestingly, using a patient-derived cell line from an Afatinib-resistant NSCLC, the authors also demonstrated the intratumor heterogeneity of T790M mutations within the same sample "*T790M positive*", providing a possible explanation for the apparent "*loss*" of this mutation at the AR to Rociletinib: it is likely expression of selection of pre-existing T790M-*wild type* clones rather than mutation/deletion of T790M alleles [96]. Moreover, a recent study using a CAPP-seq ctDNA, analysis, allowing simultaneously study of single-nucleotide variants (SNVs), insertions/deletions, rearrangements, and somatic copy-number alterations (SCNAs), revealed a largely underestimate heterogeneity of mechanisms of acquired resistance to 3rd generation EGFR TKIs, with co-existence of multiple mechanisms in the same patient at higher frequency than previously reported (46% in T790M-mutant vs. 5-15%) [79]. These findings have important clinical implications since intrapatient heterogeneity may negatively affect clinical response to 3rd generation EGFR TKis.

FUTURE PERSPECTIVES

The development of 3rd generation EGFR TKIs poses novel challenges in the therapeutic management of EGFR-mutant NSCLCs (**Fig. 1**). One of the possible future scenarios is the use of mutant-selective EGFR TKIs in the 1st line setting.

Front-line use of use of these agents might provide several advantages over the current approved EGFR TKIs: activity against EGFR mutants, including T790M, while sparing EGFR wild type and delay of acquired resistance, as demonstrated in *in vitro* models [97], with a better central nervous system (CNS) penetration. Indeed, Ballard et al. recently reported in a mouse model that

Osimertinib is distributed in the CNS at greater extent than Gefitinib, Rociletinib and Afatinib [51], providing the rationale for the use of Osimertinib in EGFR-mutant patients with brain metastases as first line option.

Several different clinical trials are evaluating the role of EGFR-mutant selective EGFR TKIs in TKI-naïve patients [**Tab. 5**].

Preliminary data from two cohorts of TKI-naïve patients enrolled into the phase I AURA trial treated with Osimertinib at 80 mg or 160 mg daily were recently reported, showing a promising activity in this subset of patients: 67% ORR in the 80 mg cohort and 77% in the 160 mg cohort, 97% DCR overall and a median PFS of 19.3 months in the overall population (median not reached in the 80 mg cohort and 19.3 months in the 160 mg cohort) [98].

The FLAURA trial (NCT02296125) is an ongoing double blind, randomized, phase III study comparing Osimertinib 80 mg/d with standard 1st generation EGFR TKIs, Gefitinib and Erlotinib. Rociletinib is being evaluating in both treatment-naïve and pretreated patients in the phase II part of TIGER1 trial and in combination with the anti-PDL1 inhibitor Atezolizumab in a phase Ib/II (NCT02630186). ASP8273 is also being compared with first generation EGFR TKIs in the 1st line setting in the open label phase III trial SOLAR (NCT02588261).

Osimertinib is also being studied in the adjuvant setting (stage IB-IIIA NSCLC with EGFR mutations after surgical resection with/without chemotherapy) in the phase III trial ADAURA (NCT02511106).

Another challenge posed by 3rd generation EGFR TKIs is the re-biopsy of patients with acquired resistance to 1st/2nd generation EGFR TKIs. Albeit tumor tissue genotyping is the gold standard, a re-biopsy is not always feasible in clinical practice because of scheduling problems, costs, risks of complications and issues related to tissue acquisition and preservation [99]. Moreover, tumor heterogeneity may represent an obstacle for tumor genotyping, since single region sampling may underestimate the genomic complexity of a tumor [100, 101]. Liquid biopsy may overcome some of the limits of traditional tissue biopsy and in particular plasma genotyping of circulating tumor DNA (ctDNA) has shown promising results. Accumulating data suggest that Osimertinib use in T790M-positive patients, as determined by liquid biopsy, achieve the same outcomes than those with a tissue rebiopsy [102, 103].

In the phase I AURA trial, patients with EGFR activating mutations and centrally confirmed tumor and/or plasma genotyping (BEAMing) T790M result were enrolled. Among 216 patients with both plasma and tissue genotyping results, the concordance rate was 82% for exon 19 deletions, 86% for L858R and 70% for T790M. Outcomes were similar for 179 patients T790M+ in tumor (62% ORR and 9.7 months PFS) and for 167 patients T790M+ in plasma (63% ORR, 9.7 months), but

unexpectedly differed for T790M- in plasma (46% ORR, 8.2 months PFS) and T790M- in tumor (26% ORR, 3.4 months PFS). These data suggest that patients with a T790M negative results in plasma genotyping may be divided in two subgroups with different outcome: T790M undetected (38% ORR, 4.4 months PFS) and T790M uninformative (64% ORR, 15.2 months PFS) [102]. Similar results were reported in a prospective study conducted at the Gustave Roussy in patients ineligible for a tissue rebiopsy, using the eTAmSeq assay on cfDNA and reporting an ORR 62.5%, with a DCR of 89%, and a 6-month PFS rate of 66.7% [103], which favorably correlate with data in patients with a tumor tissue genotyping in the AURA2 and AURA 3 trials [55, 56]. These data suggest that plasma and tissue genotyping can have complementary roles and that liquid biopsy should be offered early to identify T790M+ candidates for Osimertinib therapy, reserving traditional tissue biopsy to T790M- cases in plasma. The value of liquid biopsy for the decision-making in EGFR-mutated NSCLC treated with Gefitinib and Osimertinib will be evaluated in the randomized, open-label, phase II APPLE trial (EORTC 1613), with the aim to to explore whether liquid biopsies could become the new standard procedure for defining disease progression compared to RECIST criteria [104].

The favorable toxicity profile of 3rd generation EGFR TKIs pave the way to combinatory schedules with other agents in order to provide a more comprehensive anti-tumor activity and/or to prevent the emergence of resistant clones. Several clinical trials are ongoing evaluating different combinations in EGFR-mutated NSCLCs in various setting [**Tab. 6**].

Recently, safety concerns raised from the ongoing multi-arm phase Ib study TATTON evaluating different combinations and schedules of Osimertinib with other investigational agents, since 38% of patients treated with Osimertinib plus the anti-PDL1 inhibitor Durvalumab developed an interstitial lung disease (ILD) at an unexpectedly higher frequency compared with both single agents (2.9% and 2.0%, respectively) [105].

CONCLUSIONS

Preclinical and clinical data with mutant-selective EGFR TKIs are promising and are changing the therapeutic landscape of EGFR-mutated NSCLCs (**Fig. 1**). The rapid FDA approval of Osimertinib (only 2.8 months after first patient treated) and the fast development of Rociletinib and Olmutinib are profoundly influencing the therapeutic algorithm of EGFR mutant NSCLCs, with a new and effective option after acquired resistance to 1st and 2nd generation EGFR TKIs. Ongoing trials will produce the definitive evidences of the best in class EGFR TKI and the optimal therapeutic sequence of these agents.

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HIGHLIGHTS

- Treatment of EGFR mutated NSCLC is an evolving therapeutic paradigm;
- EGFR mutated patients treated with 1st/2nd generation EGFR TKIs develop AR after 9-13 months;
- T790M mutation is the major mechanism of AR to these agents;
- 3rd generation EGFR TKIs are emerging as a novel promising therapeutic strategy after AR;
- Osimertinib is the first mutant-selective EGFR TKI approved by FDA and EMA.

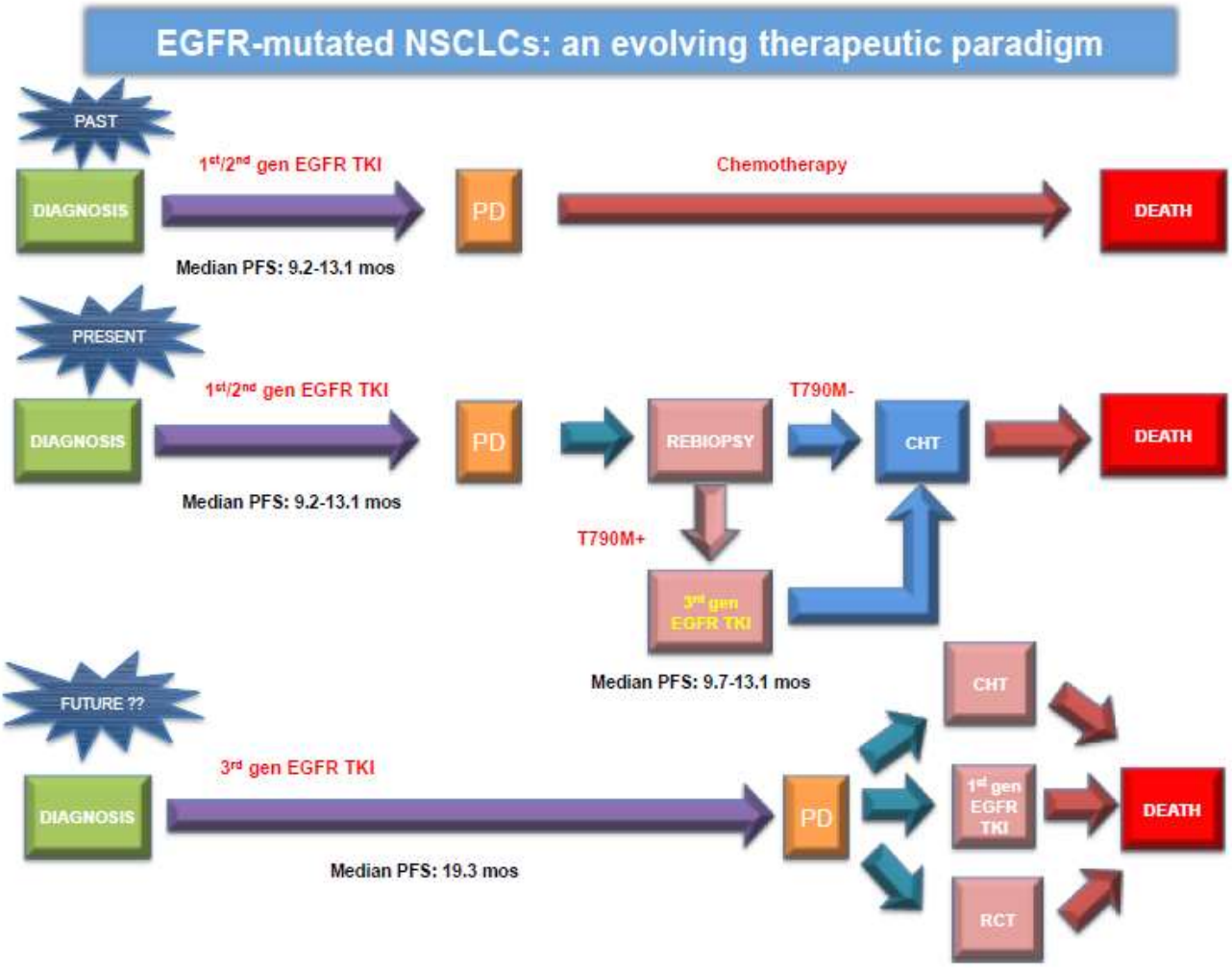

Fig. 1 Evolving therapeutic algorithm in EGFR mutated NSCLCs. Legend: CHT, chemotherapy; RCT, randomized clinical trial.

Table 1. Clinical data of efficacy with Osimertinib in T790M-positive NSCLC.

Study	Phase	n (T790M+ pts)	Osimertinib Dose	Line(s) of treatment	ORR	DCR	Median PFS
AURA [53]	1	138	20-240 mg	≥2	62%	95%	9.6 mos
AURA extension [54]	2	201	80 mg	≥2	62%	90%	12.3 mos
AURA 2 [55]	2	199	80 mg	≥2	70%	92%	9.9 mos
AURA 3 [56]	3	279	80 mg	2	71%	93	10.1 mos

Table 2. Ongoing phase III trials evaluating Osimertinib in NSCLC.

STUDY	PHASE	SETTING/POPULATION	TREATMENT(S)	PRIMARY ENDPOINT
FLAURA	III	EGFR-mut+ 1st line	Osimertinib vs. Gefitinib/Erlotinib	PFS
ADAURA	III	EGFR-mut+ Adjuvant setting	Osimertinib vs. Placebo	DFS
CAURAL	III	T790M+, EGFR-TKI-pretreated	Osimertinib + Durvalumab vs. Osimertinib	PFS
ASTRIS	III	T790M+, EGFR-TKI-pretreated	Osimertinib	Efficacy and safety in a real world setting

Table 3: Initial development program of Rociletinib.

STUDY	PHASE	SETTING POPULATION	TREATMENT(S)	PRIMARY ENDPOINT(S)
TIGER-1	II/III	EGFR-mut+ TKI-naive	Rociletinib vs. Erlotinib	PFS
TIGER-2	II	T790M+ prior TKI	Rociletinib	ORR
TIGER-3	III	T790M+ prior TKI and platinum doublet	Rociletinib vs. mono-chemotherapy	PFS
NCT02630186	Ib/II	EGFR-mut+ 1st line and pretreated	Rociletinib + Atezolizumab	Safety and activity
NCT02580708	I/II	EGFR-mut+ 1st line and pretreated	Rociletinib + Trametinib	Safety and activity

Table 4. Initial development program of Olmutinib.

Name	Phase	Population	Primary Objective (s)	Control Arm
HM-EMSI-101	I/II	T790M+ TKI-pretreated (expanded cohort)	Safety, Tolerability and Pharmacokinetic	-
ELUXA 1 (HM-EMSI-202)	II	T790M+ TKI-pretreated	ORR	-
ELUXA-2	III	T790M+ TKI-pretreated	PFS	Platinum doublet CHT
ELUXA-3	III	EGFR-mut+ 1st line	PFS	Afatinib
ELUXA-4	I/II	Japanese T790M+ TKI-pretreated	ORR	-
ELUXA-6	II	NSCLC for whom a needle biopsy may not be appropriate	Prospectively use blood-based biomarker testing to select patients with EGFR T790M+	-

Table 5. Selected studies with 3rd generation EGFR TKIs in the 1st line setting.

Treatment(s)	Phase	Population	Study Name
ASP8273 vs. Gefitinib/Erlotinib	III	EGFR-mut+	NCT02588261 (SOLAR)
Osimertinib vs. Gefitinib/Erlotinib	III	EGFR-mut+	NCT02296125 (FLAURA)
Osimertinib vs. Gefitinib	II	EGFR-mut+	APPLE/EORTC 1613
Osimertinib	II	EGFR-mut+	NCT02841579 (AZENT)
Osimertinib	II	EGFR-mut+ (liquid biopsy)	NCT02769286 (LiquidLung-O)
Osimertinib + Bevacizumab	I/II	EGFR-mut+	NCT02803203
EGF816	II	*de novo* T790M (Arm 3)	NCT02108964
ASP8273	II	EGFR-mut+	NCT02500927

Table 6. Ongoing trials combining 3rd generation EGFR TKIs with other agents in EGFR-mutated NSCLCs

DRUG	COMBINATION(S)	PHASE	STUDY
Osimertinib	Selumetinib (anti-MEK) Durvalumab (anti-PDL1) Savolitinib (anti-MET)	Ib	NCT02143466 (TATTON)
Osimertinib	Durvalumab (anti-PDL1)	III	NCT02454933 (CAURAL)
Osimertinib	Bevacizumab (anti-VEGF)	II	NCT03133546 (BOOSTER)
Osimertinib	Navitoclax (anti-Bcl-2)	I	NCT02520778
Osimertinib	Itacitanib (anti-JAK1)	I/II	NCT02917993
Osimertinib	INK-128 (anti-TORC1/2)	I	NCT02503722
Osimertinib	Necitumumab (anti-EGFR mAb)	I	NCT02496663
EGF-816	Nivolumab (anti-PD1)	II	NCT02323126
EGF-816	PDR001 (anti-PD1)	I	NCT02900664
EGF -816	Capmatinib (anti-MET)	I/II	NCT02335944

Conflicts of interest:
No potential conflicts of interest declared.