Review Article

Optimizing the clinical management of *EGFR*-mutant advanced non-small cell lung cancer: a literature review

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Background and Objective: Despite several steps forward in the treatment of epidermal growth factor receptor (*EGFR*)-mutant non-small cell lung cancer (NSCLC), however there are still pending issues and upcoming challenges requiring adequate addressing in order to optimize the clinical management of metastatic patients harboring molecular alterations within the *EGFR* gene. This review aims to summarize the most recent findings regarding the diagnostic testing and therapeutic strategies of *EGFR*-mutant advanced NSCLC.

Methods: Literature search was conducted using MEDLINE/PubMed, EMBASE, Scopus and Cochrane Library databases, up to December 2021. Relevant studies in English language published between 2004 and 2021 were selected.

Key Content and Findings: The increased detection of uncommon *EGFR* mutations in the real-word practice along with the clinical development of novel selective inhibitors, highlighted the issue of an adequate selection of the best EGFR-tyrosine-kinase inhibitor (TKI) to the right patient mutation. The advent of osimertinib in first-line has dramatically changed the spectrum of molecular mechanisms underlying both innate and acquired resistance to the EGFR-TKI therapy, accelerating the clinical investigation of novel genomic-driven sequential strategies as well as upfront targeted combinations. The recent approval of potent, selective inhibitors targeting the *EGFR* exon-20 insertions, renewed interest toward this patients' subset, questioning the diagnostic accuracy of old-standard genomic sequencing technologies and pushing the implementations of next-generation sequencing (NGS)-based molecular profiling in the real word practice scenario.

Conclusions: This review provides evidence-based answers to the aforementioned challenges aiming to optimize the clinical management of metastatic patients harboring molecular alterations within the *EGFR* gene.

Keywords: Epidermal growth factor receptor (EGFR); targeted therapy; resistance; combinations; next-generation sequencing (NGS); non-small cell lung cancer (NSCLC)

Introduction

Over the past few decades, the identification of oncogenic drivers predicting clinical response to targeted therapies produced a radical shift from histological to molecular subtyping of lung adenocarcinoma, establishing a new paradigm of precision medicine. Tumor molecular profiling is now considered as a crucial step of the diagnostic and therapeutic management of advanced non-small cell lung cancer (NSCLC), allowing to personalize therapeutic strategies and ultimately improve patients' survival (1). The epidermal growth factor receptor (*EGFR*) gene activating mutations represented the first molecular predictive biomarker discovered in lung cancer in 2004, underlying clinical responsiveness to the EGFR-tyrosine kinase inhibitors (TKIs) (2,3). Molecular alterations within the *EGFR* gene have been reported in about 40–60% and 12–15% of Asiatic and Caucasian adenocarcinoma patients, respectively, rarely occurring also in squamous cell carcinoma subtype (4). The most common targetable alterations include both exon 19 in frame deletion (Del19) and exon 21 L858R point mutation, accounting for about 85–90% of the overall *EGFR* mutations, predicting meaningful response to targeted agents. Conversely, uncommon alterations include a wide spectrum of mutations, deletions, as well as insertions, occurring among exons 18 to 21 of the *EGFR* gene (*Figure 1*), which are characterized by high heterogeneity in terms of response/resistance to the available EGFR-TKIs (4). Sometimes uncommon variants may occur at subclonal level, coexisting with either common or uncommon *EGFR* mutations, to define the "complex/compound" molecular subtypes. Co-occurring genomic alterations have been also reported in a significant subset of patients, highlighting the biological heterogeneity of the *EGFR*-mutant disease (5). Despite several steps forward in the treatment of *EGFR*-positive NSCLC, however there are still pending issues and upcoming challenges requiring adequate addressing. The increased detection of uncommon *EGFR* mutations following the advent of next-generation sequencing (NGS) in the real-word practice, along with the clinical development of novel selective inhibitors, highlighted the issue of adequate selection of the best EGFR-TKI to the right patient. The advent of osimertinib in first-line has dramatically changed the spectrum of both innate and acquired resistance mechanisms related to the EGFR-TKI therapy, accelerating the clinical investigation of novel treatment strategies as well as upfront combinations.

The recent approval of potent, selective *EGFR* exon-20 insertions inhibitors questioned the diagnostic accuracy of old-standard genomic sequencing technologies, pushing the implementations of NGS-based molecular profiling in the real word practice scenario.

In this review we summarize the most recent findings regarding diagnostic testing and therapeutic strategies of *EGFR*-mutant NSCLC, in order to provide evidence-based answers to the aforementioned challenges, aiming to optimize the clinical management of metastatic patients harboring molecular alterations within the *EGFR* gene. We present the following article in accordance with the Narrative Review reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-1/rc).

Methods

Literature search was conducted using MEDLINE/PubMed, EMBASE, Scopus and Cochrane Library databases, up to December 2021. The following keywords were used as literature search terms: lung cancer, non-small cell lung cancer, epidermal growth factor receptor, targeted therapy, resistance, next generation sequencing. Relevant studies in English language published between 2004 and 2021 were selected. The literature retrieval was supplemented by manual searches of abstracts meeting proceedings, including the American Society of Clinical Oncology (ASCO) and the European Society of Medical Oncology (ESMO) congress, as well as the World Conference on Lung Cancer (WCLC). Two authors (FP and PB) independently selected studies and disagreements were discussed and solved with a third author (GVS) (*Table 1*).

Selecting the best EGFR-TKI to the right patient

TKIs have shown their superiority over platinum-based chemotherapy in terms of progression-free survival (PFS) and overall response rate (ORR) in several phase 3 clinical trials conducted in patients with *EGFR*-mutation positive advanced NSCLC (6). Moreover, TKIs are better tolerated and improve patients' quality of life as compared to cytotoxic chemotherapy (6). The first generation of drugs comprises reversible inhibitors, such as gefitinib, erlotinib, and icotinib. Although characterized by some differences in terms of toxicity, these drugs have been considered the standard of care of *EGFR*+ advanced NSCLC for a long time. Afatinib is a second-generation, irreversible EGFR-TKI, which demonstrated longer time-to-treatment failure
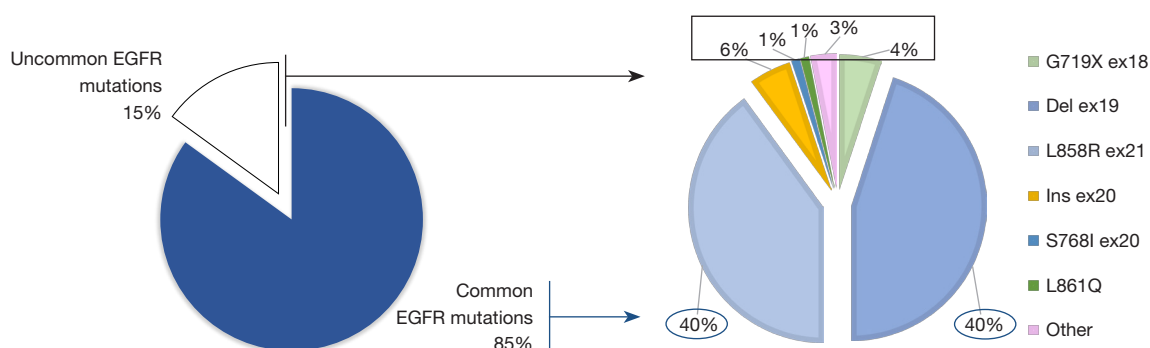
Figure 1 Targetable EGFR oncogenic alterations in advanced NSCLC. EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer.

Table 1 Search strategy summary

Items	Specification
Date of search (specified to date, month and year)	12th December, 2021
Databases and other sources searched	MEDLINE/PubMed, EMBASE and Cochrane Library Databases. ASCO, ESMO, WCLC abstracts meeting proceedings
Search terms used (including MeSH and free text search terms and filters)	Lung cancer, non-small cell lung cancer, epidermal growth factor receptor, targeted therapy, resistance, next generation sequencing
Timeframe	1st January 2004 to 12th December 2021
Inclusion and exclusion criteria (study type, language restrictions, etc.)	Relevant studies in English language were selected
Selection process (who conducted the selection, whether it was conducted independently, how consensus was obtained, etc.)	Two authors independently selected studies and disagreements were discussed and solved with a third author
Any additional considerations, if applicable	Not available

(TTF) and PFS as compared to gefitinib in a randomized phase IIB clinical trial (7). However, the study did not reach the co-primary end-point of OS, since afatinib failed to demonstrate a significant survival advantage in the overall study population [hazard ratio (HR) =0.86, 95% confidence interval (CI): 0.66–1.12]. Dacomitinib, another second-generation, irreversible EGFR-TKI, showed its superiority over gefitinib in a phase 3, randomized trial, in terms of both OS (HR =0.75, 95% CI: 0.59–0.94, P=0.0155) and PFS (HR =0.59, 95% CI: 0.47–0.74, P<0.0001), although with an increased toxicity (8,9). Finally, the randomized phase 3 FLAURA trial compared first-generation TKIs (gefitinib or erlotinib) with the third-generation inhibitor, osimertinib in patients with advanced NSCLC harbouring *EGFR* exon 19 deletions or exon 21 L858R mutation (i.e., "common" mutations) (10). Osimertinib is a potent, irreversible EGFR-TKI, highly selective for *EGFR* activating mutations

as well as T790M resistance mutation. Moreover, unlike first-generation drugs, osimertinib is characterized by great ability to penetrate the blood-brain barrier (11). The double-blind FLAURA trial reached its primary end-point, as osimertinib significantly increased PFS as compared to first-generation TKIs (HR 0.46, 95% CI: 0.37–0.57; P<0.001). However, the experimental treatment did not increase ORR nor disease control rate (DCR). An update OS analysis, with a median follow-up of 39 months and 58% data maturity, showed a median of survival of 38.6 months for osimertinib and 31.8 months for the control arm (HR 0.80, 95% CI: 0.64–1.00; P=0.046) (12). Thanks to all these positive data, clinicians are now spoiled for choice. Indeed, treatment decisions should be based on drugs efficacy, expected toxicity profile, patients and disease characteristics as well as molecular data. When dealing with "common" *EGFR* mutations, the strongest

data are coming from the phase 3 head-to-head FLAURA trial. Indeed, osimertinib significantly increase PFS with fewer adverse events (AEs) (grade 3: 34% *vs.* 45%) (10), although patients' reported outcomes did not differ between the two treatment arms (13). Moreover, the high blood-brain barrier penetration of osimertinib, witnessed by the superior brain PFS (HR 0.48; 95% CI: 0.26–0.86; P=0.014), intracranial response rate (66% *vs.* 43%, odds ratio 2.5, 95% CI: 1.2–5.2; P=0.011) as well as the reduced *de novo* central nervous system (CNS) progression rate (6% *vs.* 15%), make this drug the best treatment option for patients with CNS disease (14). While these compelling data apply to patients with *EGFR* exon 19 deletions and exon 21 L858R point mutations, evidences for less common mutation subtypes are more scarce. Since it is clear that patients harbouring *EGFR* exon 20 insertions should not be candidated to the current available EGFR-TKIs, and deserve, as of today, upfront platinum-chemotherapy, novel selective inhibitors, such as mobocertinib and amivantamab, have recently demonstrated promising activity in phase I-II clinical studies (15,16), and are rapidly coming in the clinical setting. As regards patients with other, non-exon 20 insertions, *EGFR* uncommon mutations, the selection of first-line treatment should be accurately evaluated based on scientific evidence and patients' characteristics. The largest clinical available datasets refer to afatinib, and suggest that this drug is somehow active against exon 18 G719X and E709X mutations, exon 19 insertions and missense mutations, exon 20 S786I and p.768I as well as exon 21 L861Q (17,18). More recently, small studies on osimertinib have been also reported, suggesting the role of this agent in most subgroups, with exception of exon 20 S786I and p.768I mutations (19,20). Moreover, in rare cases of *de novo* exon20 T790M mutations, osimertinib should be regarded as the treatment of choice. These data underline, once again, the need for a deep description of each specific *EGFR* molecular alteration to support clinicians in their treatment choices. As extended genomic profiling is becoming more common, the description of both compound mutations (i.e., concomitant *EGFR* mutations) and co-mutations (i.e., other genes mutations) are expected to increase in daily practice. While, at this point, we do not have strong data to support the use of one EGFR-TKI over another in patients harboring complex genotypes, ongoing studies will hopefully inform treatment choices in the next future. Indeed, although data about the negative predictive and prognostic role of *TP53* mutations are conflicting, results of ongoing studies (NCT04695925) aiming at treatment intensification in this

subgroup of patients are eagerly awaited (5).

Overcoming acquired resistance to osimertinib

The pre-existence of resistant clones and even more the onset of adaptive molecular aberrations influence the efficacy as well as the response duration to EGFR-TKI therapy. Over the past few years, we have witnessed radical changes in the biological landscape of EGFR-TKI related resistance, mostly due to the advent of osimertinib in first-line (21). A thorough understanding of both tumor heterogeneity and molecular background could help to build effective therapeutic strategies aiming to prevent as well as overcoming innate and acquired resistance to EGFR-TKIs.

The upfront administration of third generation TKI produced a dramatic decrease of on-target alterations, reported in about 10–15% of cases now as compared to 50–60% under both first- and second-generation EGFR-TKIs (22,23). The most common on-target resistance mechanism occurring during third-generation TKI therapy is the C797S mutation within the exon 20 of the *EGFR* gene, detected in about 7% of patients developing disease progression to Osimertinib in the FLAURA trial (23), while no T790M cases were detected in the same patients cohort (23). In absence of a coexisting T790M mutation, C797S mutation may potentially retain sensitivity to first/second-generation EGFR inhibitors, including gefitinib, erlotinib and afatinib. Conversely T790M and C797 coexisting tumor retain sensitivity to first or second-generations TKI only when such mutations occur *in trans* (on different alleles) (24). Fourth-generation EGFR inhibitors are currently under investigation, aiming to overcome EGFR-dependent mechanics of resistance (25). Different molecules (EAI001, EAI045 JBJ-04-125-02, DDC4002) have demonstrated *in vitro* and *in vivo* activity alone or in combination with third-generation TKI, not reaching yet the advanced stages of clinical development.

The analysis of paired pre- and post-osimertinib tumor samples by Schoenfeld *et al.*, demonstrated that off-target resistance is more frequently reported in the first line osimertinib cohort, suggesting to be a time-dependent mechanism resulting in less durable responses to the third-generation TKI (26).

Among the off-target molecular alterations, the amplification of MET gene was the most frequent bypass pathway conferring acquired resistance to osimertinib, reported in about 10–15% of patients included in the

FLAURA study (23). On this basis, early-phase clinical trials evaluated the efficacy of dual EGFR-MET inhibition strategy in this treatment context, showing preliminary promising results. The phase Ib TATTON study (NCT02143466) investigated the addition of the MET-TKI savolitinib to osimertinib in patients with both *EGFR*-mutant and MET-positive NSCLC patients failing prior EGFR-TKI. In the cohort B1, including 69 patients progressing to prior third-generation EGFR-TKIs, ORR and median PFS were 30% and 5.4 months, respectively (27). The phase 2 platform ORCHARD study is currently exploring the efficacy of molecularly-driven personalized treatments for *EGFR*-mutant NSCLC patients progressing to first-line osimertinib. Preliminary data from the small cohort of 17 *EGFR*-mutant patients harboring MET amplification showed a promising activity (ORR 40%) for the osimertinib and savolitinib combination (28), which is currently being investigated in the phase II SAVANNAH trial (NCT03778229). An alternative dual EGFR/MET targeting is provided by the use of bispecific antibodies. Particularly amivantamab (JNJ-61186372), a fully human EGFR-MET bispecific antibody with immune cell-directing activity, is currently being investigated in combination with the third-generations EGFR-TKI, lazertinib, within the CHRYSALIS phase I study. Preliminary data from the osimertinib-resistant cohort, showed an ORR of 36% and 41% in the molecularly unselected, platinum-naïve (n=45) and resistant (n=29) population, respectively. Higher rate of activity has been observed among those patients harboring either EGFR/MET-based resistance (ORR 47%, 8/17), as well as MET overexpression by immunohistochemistry (IHC) analysis (ORR 90%, 9/10) (29,30). A phase Ib CHRYSALIS-2 study is currently evaluating the safety of lazertinib as monotherapy or in combination with amivantamab (and platinum-based chemotherapy in a single cohort) in this treatment setting (NCT04077463). Although there is still lack of consensus regarding the definition of MET-positive disease as well as the optimal detection method, recent data coming from such studies suggested gene amplification as the more reliable biomarker to select best candidate for this treatment strategy.

HER3 is frequently overexpressed in the vast majority of lung cancer cells and is likely involved in the development of EGFR-TKI resistance occurrence. In a phase I trial including 57, heavily pretreated, TKI-resistant patients, a HER3 Directed Antibody Drug Conjugate (ADC), patritumab deruxtecan (U3-1402) showed promising activity, with an ORR of 39% and median PFS of 8.2 months in the overall unselected population (31). Clinical responses were observed across the spectrum of baseline HER3 protein expression as well as the different TKI resistance mechanisms, including *EGFR* C797S mutation, MET amplification, HER2 mutation, suggesting a potential efficacy regardless of tumor molecular landscape. A phase 2 prospective study is investigating single-agent patritumab deruxtecan after failure of EGFR TKIs and platinum-based chemotherapy therapy (NCT03260491). Other bypass pathways, included HER-2 amplification as well as molecular aberrations in the RAS-MAPK signaling pathway, reported in about 2% and 12–15% of NSCLC patients receiving upfront Osimertinib (23). Particularly, NRAS mutations were found in 1% of plasma samples from patients who received first-line osimertinib within the FLAURA study, while multiple KRAS mutations, including KRAS p.G12S and p.G12D have been reported in 3% of cases (23). Considering the RAS-MAPK signaling pathway, rare concomitant acquired BRAF V600E mutations and MET amplification have been described as a mechanism of resistance to first-line Osimertinib (32). Loss of PTEN seems to be a mechanism of primary resistance to EGFR TKIs, but has been also reported in cases of acquired resistance (33,34). Gene fusions involving driver oncogenes, such as ALK, RET, BRAF (23,35,36), as well as molecular alterations among cell cycle-related genes, such as amplification or mutations in cyclin D1, D2 and E1 genes, cyclin-dependent kinase (CDK) 4/6 and CDK inhibitor 2A genes, have been also detected in a significant subgroup of patients who progressed to first-line Osimertinib (23), and seem to be associated with negative outcomes and reduced EGFR-TKI activity (37), providing a rationale for combination therapies to overcome EGFR-TKIs resistance. Several other combinations with different drugs targeting different pathways (like CDK4/6 inhibitor, glutaminase inhibitor, JAK inhibitor, Bcl-2 inhibitor, aurora A kinase inhibitor, PARP inhibitor, AXL inhibitor, mTOR inhibitor) are currently under investigation in phase I/II clinical trial.

Tissue specimens' analyses under third-generation EGFR-TKI therapy disclosed histological transformation to small cell lung cancer (SCLC), as off-target resistance mechanism arising in a substantially higher proportion of cases than previously reported with first- or second-generation inhibitors (15% *vs.* 3–9%) (26). SCLC transition in *EGFR*-mutant patients dramatically affects survival outcomes, leading to rapid disease progression and transient response to SCLC-directed chemotherapies (38).

However, a comprehensive insight into the biological mechanisms underlying cancer cells' phenotype switching is still wanting. The intratumor heterogeneity, in terms of morphological, genetic, and epigenetic background remains a crucial issue to be adequately addressed in this research context. Offin *et al.* demonstrated that patients harbouring co-occurring *EGFR*/RB1/TP53 alterations (5% of all *EGFR*-mutant lung cancers) are uniquely at risk for SCLC transformation during their disease course (35). Although not all *EGFR*/RB1/TP53-mutant NSCLC patients will transform to SCLC during their disease course, both RB1 and TP53 loss-of-function mutations appear to be necessary, but not sufficient, for lineage plasticity. Taking together, these observations suggested that identifying such three-gene mutational signature (*EGFR*/RB1/TP53) at diagnosis could provide an opportunity for early intervention trials in those patients at higher risk of SCLC transformation. On this rationale, clinical trials have been initiated to test the combination of EGFR TKIs upfront with conventional SCLC therapy (NCT03567642).

Today, single-agent immunotherapy should be considered only once both targeted and chemotherapy options have been exhausted. Like in non-oncogene addicted NSCLC, modulation of the immune response through PD-1 inhibition may be enhanced by the potential immunogenic effects of both antiangiogenic agents and cytotoxic chemotherapy. In this regards, sub-group analysis of *EGFR* mutant patients progressing to prior EGFR-TKI, from the phase III trial IMPOWER-150, revealed significantly survival benefit with the addition of atezolizumab and bevacizumab to platinum-chemotherapy (39). Another phase II prospective, single arm study has recently shown great antitumor activity of pembrolizumab in combination with platinum-based chemotherapy in *EGFR*-mutant NSCLC patients failing prior EGFR-TKI, (ORR of 42%, PFS of 8.3 months and OS of 22.2 months) (40) but conclusive data are expected from ongoing prospective randomized studies (NCT03515837).

Developing effective and tolerable upfront combinations

Aiming to improve the efficacy of EGFR-TKI therapy, different upfront combination strategies have been recently explored, including cytotoxic chemotherapy, anti-angiogenic agents, immune-checkpoint inhibitors, as well as novel selective drugs targeting different signaling pathways.

Preclinical data showed that combining chemotherapy

with EGFR-TKIs may have a synergistic effect, resulting in the reduction of angiogenesis, along with the up-regulation of downstream EGFR signaling pathways, promoting the apoptosis of EGFR TKI-resistant cells (41,42).

The phase II NEJ005/TCOG0902 was the first randomized trial to investigate the efficacy of concurrent or sequential chemotherapy with gefitinib in 80 *EGFR*-positive NSCLC (43), showing a significantly longer OS, better PFS and similar ORR with the concurrent regimen compared to the sequential one (44). Han *et al.* evaluated platinum-based chemotherapy plus gefitinib versus either chemotherapy or gefitinib alone in 121 *EGFR*-positive NSCLC patients (45). The combination strategy provided longer PFS and OS compared to TKI alone. Noronha *et al.* evaluated platinum-chemotherapy plus gefitinib versus gefitinib alone in 350 untreated *EGFR*-positive NSCLC patients (46), revealing a clinical benefit in favour of the combination arm in terms of ORR and OS, with doubled PFS comparable to that observed within the FLAURA trial. The randomized phase III NEJ009 study evaluated gefitinib plus carboplatin-pemetrexed versus gefitinib alone (47), showing an improved RR, PFS and OS in favour of the experimental arm (*Table 2*). All the above mentioned studies showed that the addition of chemotherapy to the first-generation EGFR-TKI may be an effective strategy, increasing clinical responses and survival outcomes as compared to the EGFR-TKI alone. However the survival benefit associated to such combinations is similar to that obtained with single agent Osimertinib within the FLAURA trial. Therefore, considering the lack of CNS activity and the worse tolerability profile, characterized by an increased rate of severe AEs, especially the haematological toxicities, the use of such combinations is currently limited to specific countries where the clinical access to the third generation TKI is still denied. Limited data regarding the combination of osimertinib plus chemotherapy are currently available. Tanaka *et al.* tested osimertinib plus chemotherapy versus osimertinib alone in 62 T790M-positive NSCLC patients who failed prior EGFR-TKI (48). The study showed that the addition of chemotherapy to osimertinib was generally well tolerated without producing a significant impact on patients' survival. A retrospective analysis of 18 *EGFR*-positive advanced NSCLC patients treated with the combination of osimertinib plus different chemotherapy regimens, confirmed a tolerable profile, showing a median duration of treatment comparable to that observed with osimertinib alone within the AURA3 trial (49). Preliminary safety data from the first 30 patients enrolled within the FLAURA2

Table 2 Clinical trials of EGFR-TKIs plus chemotherapy in *EGFR*-mutant advanced NSCLC

Author	Phase	Treatment arms	Patient (n)	ORR (%)	PFS (months)	OS (months)
Sugawara *et al*., 2015	II	Concurrent or sequential alternating regimen with gefitinib and platinum-based chemotherapy	80	87.8 *vs*. 84.6	18.3 *vs*. 15.3; HR 0.71 (0.42–1.20; P=0.20)	41.9 *vs*. 30.7; HR 0.51 (0.26–0.99; P=0.042)
Oizumi *et al*., 2018	II	Concurrent or sequential alternating regimen with gefitinib and platinum-based chemotherapy	80	90.2 *vs*. 82.1	17.5 *vs*. 15.3; HR 0.68 (0.42–1.12; P=0.130)	41.9 *vs*. 30.7; HR 0.58 (0.34–0.97; P=0.036)
Han *et al*., 2017	III	Platinum-based chemotherapy + gefitinib *vs*. gefitinib *vs*. platinum-based chemotherapy	121	82.5 *vs*. 65.9 *vs*. 32.5	15.7 *vs*. 11.9 *vs*. 5.7; HR 0.48 (0.29–0.78; P=0.003); HR 0.16 (0.09–0.29; P<0.001)	32.6 *vs*. 24.3 *vs*. 25.8; HR 0.46 (0.24–0.87; P=0.016); HR 0.36 (0.20–0.67; P=0.001)
Noronha *et al*., 2020	III	Platinum-based chemotherapy + gefitinib *vs*. gefitinib	350	75 *vs*. 63	16 *vs*. 8; HR 0.51 (0.39–0.66; P<0.001)	NR *vs*. 17; HR 0.45 (0.31–0.65; P<0.001)
Hosomi *et al*., 2020	III	Platinum-based chemotherapy + gefitinib *vs*. gefitinib	345	84 *vs*. 67	20.9 *vs*. 11.9; (HR 0.490; P<0.001)	50.9 *vs*. 38.8; HR 0.722, P<0.01

EGFR, epidermal growth factor receptor; TKI, tyrosine-kinase inhibitor; NSCLC, non-small cell lung cancer; N, number; ORR, objective response rate; PFS, progression free survival; OS, overall survival; *vs*., versus; HR, hazard ratio.

Table 3 Ongoing clinical trials of osimertinib plus chemotherapy in *EGFR*-mutant advanced NSCLC

ID (trial name)	Phase	Treatment arms	Primary endpoint	Status
First line				
NCT04035486 (FLAURA2)	III	Platinum-based chemotherapy + osimertinib *vs*. osimertinib	PFS	Recruiting
NCT03567642	I	Osimertinib + platinum/etoposide	MDT	Recruiting
Second line				
NCT04765059 (COMPEL)	III	Platinum-based chemotherapy + osimertinib *vs*. platinum-based chemotherapy + placebo	PFS	Recruiting

EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; PFS, progression free survival; *vs*., versus; MDT, maximum dose tolerate.

study did not reveal new toxicity signals, supporting further investigation of osimertinib plus chemotherapy combinations in the upfront setting. Several studies are currently ongoing (*Table 3*) and the results will clarify soon the potential role of this treatment regimens at least in particular subgroups of *EGFR*-mutant NSCLC patients.

Preclinical studies found that up-regulated EGFR signaling increased VEGF expression through hypoxia-independent mechanisms, while elevated VEGF contributed to the EGFR-TKIs resistance occurrence, providing biological rational for combination strategies. Four prospective randomized trials have already established the benefit of dual EGFR/VEGF pathways inhibition, showing a consistent efficacy in terms of median PFS (50-53)

(*Table 4*). Both the NEJ026 and the RELAY trials demonstrated a significant survival benefit from the addition of bevacizumab and ramucirumab, respectively, to the first-generation EGFR-TKI erlotinib, leading to a median PFS that was comparable to that obtained with osimertinib within the FLAURA trial, and somewhat higher considering the subgroup of patients harboring the L858R mutation. However, three of these trials did not show any survival advantage while the final OS from the RELAY study is still pending. The randomized phase III BEVERLY trial has recently evaluated the addition of bevacizumab to erlotinib as first-line treatment for *EGFR*-mutated NSCLC (54), showing a significant PFS increase, without survival advantages as well as unexpected safety issues. In order to

Table 4 Clinical trials of EGFR TKIs plus anti-angiogenics in *EGFR*-mutant advanced NSCLC

Trial	Phase	Treatment arms	Patient (N)	ORR (%)	PFS (months)	OS (months)
NEJ026	III	Erlotinib + bevacizumab *vs.* erlotinib	228	72 *vs.* 66	16.9 *vs.* 13.3; HR 0.605 (0.417–0.877; P=0.016)	50.7 *vs.* 46.2; HR 1.00 (0.68–1.48)
ARTEMIS-CTONG1509	III	Erlotinib + bevacizumab *vs.* erlotinib	449	76 *vs.* 75	19.4 *vs.* 12.4; HR 0.59 (0.46–0.76; P<0.0001)	NR
RELAY	III	Erlotinib + ramucirumab *vs.* erlotinib	449	76 *vs.* 75	19.4 *vs.* 12.4; HR 0.59 (0.46–0.76; P<0.0001)	NR
ACTIVE	III	Gefitinib + apatinib *vs.* gefitinib	313	77.1 *vs.* 73.7	13.7 *vs.* 10.2; HR 0.71 (0.54–0.95; P=0.0189)	NR
BEVERLY	III	Erlotinib + bevacizumab *vs.* erlotinib	160	81.3 *vs.* 52.5	15.4 *vs.* 9.7; HR 0.60 (0.42–0.85; P=0.0039)	28.4 *vs.* 23.0; HR 0.70 (0.46–1.10, P=0.12)
NCT02803203	I/II	Osimertinib + bevacizumab	49	80 [67–91]	19 (95% CI: 15–24)	10.1 (6–NR, P=0.002)
WJOG9717L	II	Osimertinib + bevacizumab *vs.* osimertinib	122	86 *vs.* 82	22.1 *vs.* 20.2; HR 0.86 (0.53–1.39; P=0.21)	NR *vs.* 22.1; HR 1.02 (0.43–2.44; P=0.96)

EGFR, epidermal growth factor receptor; TKI, tyrosine-kinase inhibitor; NSCLC, non-small cell lung cancer; N, number; ORR, objective response rate; PFS, progression free survival; OS, overall survival; *vs.*, versus; HR, hazard ratio.

Table 5 Ongoing clinical trials of osimertinib plus anti-angiogenics in *EGFR*-mutant advanced NSCLC

ID (trial name)	Phase	Treatment arms	Primary endpoint	Status
First line				
NCT04988607 (FLAIR)	II	Osimertinib +/− bevacizumab	PFS	Not yet recruiting
NCT04425681 (OWBLM)	II	Osimertinib +/− bevacizumab	LM-PFS	Recruiting
NCT05104281	II	Osimertinib +/− bevacizumab	PFS	Recruiting
NCT04974879	II	Osimertinib +/− bevacizumab	PFS	Recruiting
NCT03909334 (RAMOSE)	II	Osimertinib +/− ramucirumab	PFS	Recruiting
NCT04181060	III	Osimertinib ± bevacizumab	PFS	Recruiting
Second line				
NCT02789345	I	Osimertinib + ramucirumab or necitumumab	DLT	Active, not recruiting
LY3009806-IIT-01	Ib	Osimertinib + ramucirumab	DLT	Active
NCT03133546 (BOOSTER)	II	Osimertinib +/− bevacizumab	PFS	Active, not recruiting

EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; PFS, progression free survival; LM, leptomeningeal metastasis; DLT, dose limiting toxicity.

enhance the efficacy of osimertinib and delay the onset of acquired resistance, a phase I/II trial tested osimertinib plus bevacizumab as first-line in 49 *EGFR*-positive NSCLC patients, showing a median PFS of 19 months which is comparable to that obtained with osimertinib in the front-line setting (55). Kenmotsu *et al.* compared osimertinib plus bevacizumab versus osimertinib alone in 122 untreated *EGFR*-positive NSCLC patients, showing no differences in terms of median PFS. In the subgroup analysis, former-smoker (HR 0.481) and patients with Ex19del (HR 0.622) showed a trend toward a not significant increase of PFS in favour of the combination arm, as previously observed in other similar studies (56). Further prospective clinical trials are currently ongoing (*Table 5*) to confirm the hypothesis that the addition of antiangiogenic agents to osimertinib may improve first-line clinical outcomes at least in

Table 6 Clinical trials of EGFR TKIs plus immunotherapy in *EGFR*-mutant advanced NSCLC

Author	Phase	Treatment arms	Patients (N)	AEs (%)	ORR (%)	PFS (months)	OS (months)
Oxnard *et al.*, 2020 (TATTON)	Ib	Osimertinib + durvalumab	23	ILD 38 (13/34)	67 (T790M+); 21 (T790M−)	NR	NR
Awad *et al.*, 2021	I/II	Erlotinib + pembrolizumab; Gefitinib + pembrolizumab	12; 7	Liver G3-4 70 (5/7)	42; 14	19.5 (3.0–9.5); 1.4 (0.2–13.0)	NR (19.5–NR); 13.0 (0.2–NR)
Creelan *et al.*, 2021	I	Gefitinib + durvalumab	56	20 (4/20)	63.3 (43.9–80.1)	10.1 (5.5–15.2)	NR
Gettinger *et al.*, 2018	I	Erlotinib + nivolumab	21	24 (G3)	15 [3–38]	5.1 (2.3–12.1)	18.7 (7.3–NR)
Rudin *et al.*, 2018	I	Erlotinib + atezolizumab	28	43 (G3)	75 [51–91]	15.4 (8.4–NR)	32.7 (32.7–NR)

EGFR, epidermal growth factor receptor; TKI, tyrosine-kinase inhibitor; NSCLC, non-small cell lung cancer; N, number; AEs, adverse events; ORR, objective response rate; PFS, progression free survival; OS, overall survival; NR, not reached; G, grade.

particular subsets of *EGFR*-mutant NSCLC.

Preclinical models reported a potential interaction between EGFR activation and PD-L1 expression in the development of EGFR-TKI resistance. Therefore the combination of anti-PD-1/PD-L1 and EGFR TKIs might have synergistic effects in NSCLC therapy (57). The phase Ib TATTON study investigated the safety and tolerability of osimertinib plus durvalumab combination in *EGFR*-positive patients with disease progression on prior EGFR-TKI (58). In terms of toxicity, interstitial lung disease (ILD) was reported in 38% of patients, being higher than expected with either drug alone, including five patients (15%) with grade 3–4 AEs. Despite the efficacy results, the primary end-point of safety was not met and enrolment was early interrupted. The phase I/II study KEYNOTE-021 evaluated the combination of pembrolizumab plus either erlotinib or gefitinib in untreated *EGFR*-positive NSCLC patients (59). The study showed that pembrolizumab plus gefitinib was not feasible due to grade 3/4 liver toxicity in five out of seven patients (71.4%), leading to permanent treatment discontinuation in four patients, while pembrolizumab plus erlotinib produced similar AEs than erlotinib monotherapy. Another open-label multicenter phase I trial evaluated the combination of gefitinib and durvalumab in TKI-naive patients with *EGFR*-positive NSCLC (60), showing higher rate of toxicity without significant ORR/PFS improvement compared to gefitinib alone, as previously reported in similar populations. In contrast to these data, other early phase clinical trials of concurrent immunotherapy and TKIs have shown acceptable safety profiles. Rudin *et al.* evaluated the combination of erlotinib plus atezolizumab in EGFR TKI-naïve and pre-treated NSCLC patients (61). Grade 3 AEs occurred in 43% of patients and the most common were pyrexia and increased ALT. The combination demonstrated a manageable safety profile with promising efficacy results. The phase I CheckMate 012 trial evaluated nivolumab in combinations with different other targeted agents, including erlotinib for the *EGFR*-mutant advanced NSCLC cohort (62). Study results showed that the combination was effective and tolerable, with Grade 3 toxicities occurring in 24% of patients, and no grade 4/5 AEs (*Table 6*). In summary, this data suggested that the optimal sequencing of TKIs and immunotherapy in *EGFR*-mutant patients remains to be defined in order to minimize the risk of AEs and increase the clinical benefit.

Recently, the phase I CHRISALYS study showed impressive results from the upfront combination of lazertinib and amivantamab (29), yielding a 100% ORR in the small cohort of treatment naïve, *EGFR* mutant NSCLC patients. Based on this encouraging evidence, the randomized phase III MARIPOSA study (NCT04487080) is currently randomizing *EGFR*-positive advanced NSCLC patients to first-line lazertinib plus amivantamab *versus* either osimertinib or lazertinib alone, aiming to further increase the survival plateau set by Osimertinib in this patients' subgroup. Other innovative combinations with EGFR TKIs and different molecules as poly (ADP-ribose) polymerase (PARP), Aurora kinase or CDK4–6 inhibitors are currently under investigation and the results are eagerly awaited.

Implementing next generation sequencing in the routine molecular testing

Molecular testing for *EGFR* mutations is a crucial step of the clinical management of lung cancer patients. Until 2015, Sanger sequencing and/or pyrosequencing were the most widespread detection methods used to assess the *EGFR* mutational status in advanced NSCLC (63).

Sanger sequencing was considered as the gold standard approach for a long time, since enabling the evaluation of whole genes sequences as well as the identification of unknown mutations, including the analysis of small DNA fragments sequences (64). Major limitations include laboratory intensiveness, high costs, and low sensitivity, requiring almost 40–50% of tumour cells within the tested sample (64). Therefore several laboratories performed a technological shift to Pyrosequencing, characterized by an inferior limit of detection (5% *vs*. 20%) of mutant alleles, thus allowing the identification of individual bases or short stretches of nucleic acid sequences at predetermined positions. Specifically, the commercially available PS kits properly identifies the most common *EGFR* exons 18–21 mutations, while are not able to adequately cover all *EGFR* uncommon alterations (65). Quantitative real-time polymerase chain reaction (qRT-PCR) was largely adopted to detect either "common" or "uncommon" *EGFR* mutations in the real-world practice scenario (4). However recent work revealed that current commercially available PCR kits could miss around 50% of *EGFR* exon 20 insertion variants as compared to NGS-based molecular profiling (66). Despite its high specificity, an important limitation of RT-PCR consists of detecting only known and well characterized molecular alterations, similarly to other targeted-based approaches. Conversely NGS represents a highly sensitive and specific technology for the molecular assessment of less frequent mutations, allowing the simultaneous detection of several hotspot genes from different patients' samples (67). Differently from targeted-based approaches, NGS is able to identify either known or unknown mutations within the gene panel reference range, offering higher diagnostic accuracy, faster turnaround time for low sample volumes, and lower costs (68). To date, several NGS panels are commercially available enabling the simultaneous analysis of a plethora of clinically relevant hotspots in target genes, including *EGFR* (69). Based on this evidence, ESMO has recently recommended NGS as new standard approach to routinely profile newly diagnosed advanced NSCLC patients with non-squamous histology (70), in order to ensure adequate detection of oncogenic drivers and subsequent assignment to matched targeted treatment. However a significant fraction of NSCLC patients across different countries do not undergo yet NGS-based molecular profiling, because of regulatory, economic, cultural, and logistics barriers which limit patients' access to NGS-platforms (71,72). Particularly in Italy, only one third

of molecular pathology laboratories declared to routinely use NGS for the molecular analysis of advanced NSCLC patients, while RT-PCR still represents the most adopted technique for *EGFR* mutation testing in clinical practice (4).

Since a long time the clinical assessment of *EGFR* mutations by circulating tumor (ct)DNA analysis has been considered as a reliable alternative to tissue genotyping for advanced NSCLC patients who cannot undergo tumor biopsy. Recently a series of diagnostic accuracy clinical studies have definitively demonstrated a high concordance between tissue and ctDNA NGS-based molecular profiling, leading to the subsequent approval of the first ctDNA NGS diagnostic assay for the molecular profiling of advanced NSCLC patients in the United States. The ctDNA NGS analysis has shown to accurately detect molecular mechanisms of acquired resistance under Osimertinib therapy, allowing longitudinal tracking of *EGFR* somatic alterations in *EGFR*-mutant NSCLC patients included in the FLAURA trial (23,73). The phase II ELIOS study (NCT03239340) is prospectively investigating the concordance between tumour tissue and ctDNA-based NGS analysis for the detection of resistance mechanisms to upfront osimertinib in *EGFR*-positive NSCLC patients, and the results are eagerly awaited. In the meantime, the International Association for the Study of Lung Cancer (IASLC) has recently proposed a novel diagnostic algorithm integrating both tissue and liquid biopsy for the molecular profiling of advanced NSCLC (74).

Conclusions

EGFR mutations represent an established therapeutic target in NSCLC, with different generations EGFR-TKIs already approved for the clinical treatment of patients harboring common mutations. Conversely the best therapeutic choices for patients harboring less common *EGFR* molecular alterations are still debated, while novel selective and effective exon 20 insertions inhibitors are coming soon in the clinical setting. With the recent introduction of Osimertinib in first-line, clinical challenges for thoracic oncologist consist of identifying therapeutic strategies to overcome innate and acquired resistance to third generation TKI. Among the different drugs/combinations under clinical investigation, the dual inhibition of EGFR/MET pathways is emerging as an effective strategy, especially for patients harboring MET amplification, while preliminary data regarding HER3 therapeutic targeting, showed a wide spectrum of activity across different

resistance mechanisms, emerging as a potential option for patients who do not harbor MET amplification or may not undergo tumor re-biopsy, which remains currently recommended when clinically feasible. Looking at upfront combinations, available data regarding both chemotherapy and antiangiogenic associations with first-generation EGFR-TKI showed a clinical activity comparable to that observed with Osimertinib monotherapy within the randomized FLAURA trial. Therefore, considering the lack of CNS activity and the worse tolerability profile, characterized by an increased rate of severe AEs, the use of such combinations should be currently limited to specific countries where clinical access to the third generation TKI is still denied. A series of ongoing studies are investigating Osimertinib combinations with both anti-angiogenics and chemotherapeutic agents, and even if preliminary data emerging from second-line setting are quite discouraging, final results from first-line clinical trials are eagerly awaited. Among the most promising upfront combinations studies, the ongoing randomized MARIPOSA trial will inform us whether the combination of amivantamab and lazertinib will be able to further increase the survival plateau set by Osimertinib in the treatment of *EGFR*-mutant disease.

The increasing prevalence of uncommon alterations in the real word scenario and the upcoming advent of new targeted options against the exon20 insertions, highlight the necessity to implement a comprehensive NGS-based molecular profiling in advanced, non-squamous NSCLC patients, in order to adequately detect all *EGFR* molecular alterations and personalize therapeutic strategies. In this regards a recent work has proposed an alternative classification of *EGFR* mutations based on their structure and function, suggesting that functional-based subgroups might predict EGFR-TKI therapeutic response better than classical exon-based groups (75). In conclusion we are currently facing novel diagnostic challenges and therapeutic opportunities, which, if adequately addressed, will allow to further optimize the clinical management *EGFR*-mutant advanced NSCLC patients.

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Footnote

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