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The role of anti-EGFR therapies in EGFR-TKI-resistant advanced non-small cell lung cancer

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ABSTRACT

Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) are the current recommended option for the first-line treatment of patients with EGFR-mutant non-small cell lung cancer (NSCLC). Resistance to first-generation TKIs led to the development of second- and third-generation TKIs with improved clinical outcomes. However, sequential administration of TKIs has led to the emergence of new EGFR resistance mutations and persistent tumor cell survival. This evidence highlights the potential role of EGFR in transducing growth signals in NSCLC tumor cells. Therefore, dual inhibition of EGFR using combinations of anti-EGFR monoclonal antibodies (mAbs) and EGFR-TKIs may offer a unique treatment strategy to suppress tumor cell growth. Several clinical studies have demonstrated the benefits of dual blockade of EGFR using anti-EGFR mAbs coupled with EGFR-TKIs in overcoming treatment resistance in patients with EGFR-mutated NSCLC. However, a single treatment option may not result in the same clinical benefits in all patients with acquired resistance. Biomarkers, including EGFR overexpression, *EGFR* gene copy number, *EGFR* and *KRAS* mutations, and circulating tumor DNA, have been associated with improved clinical efficacy with anti-EGFR mAbs in patients with NSCLC and acquired resistance. Further investigation of biomarkers may allow patient selection for those who could benefit from anti-EGFR mAbs in combination with EGFR-TKIs. This review summarizes findings of recent studies of anti-EGFR mAbs in combination with EGFR-TKIs for the treatment of patients with EGFR-mutated NSCLC, as well as clinical evidence for potential biomarkers towards personalized targeted medicine.

Introduction

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, accounting for 80–90 % of all lung cancer diagnoses, with approximately 60 % of patients having advanced/metastatic disease at diagnosis [1,2]. NSCLC is a heterogeneous disease with respect to its molecular profile and tumor histology [3,4]. Heterogeneity between the NSCLC tumor genome and microenvironment, as well as between primary and metastatic tumors, results in diverse responses to treatment [4]. Historically, platinum-based chemotherapy was the only choice for first-line treatment of NSCLC. However, the identification of epidermal growth factor receptor (EGFR)-activating mutations and overexpression of EGFR protein in epithelial malignancies led to the development of targeted treatment options against EGFR that were hypothesized to be effective [5].

Tyrosine kinase inhibitors (TKIs) have been assessed and recommended by current treatment guidelines, including the National Comprehensive Cancer Network (NCCN), as a first-line treatment option for patients with EGFR-mutant NSCLC [2,6]. EGFR-TKIs have shown improved clinical outcome compared with standard of care (SoC)

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chemotherapy in patients with EGFR-mutant NSCLC, and all are currently approved as first-line treatment regimens [7–20]. The triggering of acquired resistance to first-generation EGFR-TKIs (gefitinib, erlotinib) in EGFR-mutant NSCLC tumors led to the development of second- (afatinib, dacomitinib) and third-generation (osimertinib) agents [21–23].

Sequential administration of first-, second-, and third-generation EGFR-TKIs is associated with the evolution of *EGFR* mutations and development of treatment resistance in NSCLC [24]. The T790M *EGFR* mutation within exon 20 is the most common resistance mechanism in patients with NSCLC who received prior first- or second-generation EGFR-TKIs [25–27]. The third-generation EGFR-TKI, osimertinib, has been approved by both the US FDA and European Commission for patients with T790M-positive NSCLC who have progressed following EGFR-TKI therapy [28], as well as for treatment-naïve patients with sensitive *EGFR* mutations. The use of osimertinib has also led to the emergence of new *EGFR* mutations conferring treatment resistance (e.g., C797S) [29–31]. The mechanism of acquired resistance due to C797S mutation is long-term and occurs approximately one year after initiation of osimertinib administration [32]. There is currently no treatment strategy for C797S-mediated resistance; however, several novel treatment strategies are under investigation, including combining first- and third-generation EGFR-TKIs [33], and approaches using antibody-drug conjugates [34]. Additionally, a Phase I clinical trial of a fourth-generation EGFR-TKI targeting acquired EGFR resistance mutations such as C797S is ongoing (NCT05256290).

The high frequency of *EGFR* mutations, including the diversity of molecular subtypes and incidence rates, in patients with acquired resistance to EGFR-TKIs in NSCLC highlighted the critical role of signaling through the *EGFR* pathway for the survival of EGFR-mutated lung cancer cells [35–37]. This observation shaped the hypothesis that dual EGFR blockade using anti-EGFR monoclonal antibodies (mAbs) coupled with an EGFR-TKI may suppress *EGFR* signaling and trigger cancer cell apoptosis [38]. Several clinical studies have reported the potential benefits of dual inhibition of EGFR with anti-EGFR mAbs, such as cetuximab or panitumumab, in combination with TKIs, such as afatinib and brigatinib, in overcoming EGFR-TKI resistance in EGFR-mutated NSCLC [39–42]. This review summarizes the current clinical evidence for anti-EGFR mAbs in combination with TKIs for the treatment of patients with EGFR-TKI-resistant advanced NSCLC. In addition, the predictive value of potential biomarkers for subgroups of patients with NSCLC who may benefit from dual EGFR inhibition is discussed.

The role of anti-EGFR mAbs in lung cancer treatment

Anti-EGFR mAbs, such as cetuximab, necitumumab and panitumumab, have shown activity in advanced NSCLC in combination with SoC chemotherapy or anti-vascular endothelial growth factor (VEGF) therapy [43–45]. Cetuximab is an anti-EGFR mAb currently indicated for the treatment of *EGFR*-expressing *RAS* wild-type (wt) metastatic colorectal cancer (mCRC) and squamous cell carcinoma of the head and neck [46,47]. Evidence from clinical trials [39–41,48,49] has shown that cetuximab combinations demonstrate potential beneficial activity in the treatment of advanced NSCLC. Phase II and III clinical trials have evaluated the efficacy and safety of adding cetuximab to SoC chemotherapy or anti-VEGF therapy in the first-line setting. These studies have also identified potential predictive biomarkers associated with treatment outcomes with cetuximab-based regimens [45,50–53].

In the Phase III FLEX study, cetuximab plus platinum-based chemotherapy prolonged survival outcomes in patients with EGFR-expressing advanced NSCLC, with an acceptable safety profile [54]. Moreover, meta-analyses pooling data from FLEX and other Phase II and III randomized trials (LUCAS, BMS100, BMS099) confirmed a modest but statistically significant overall survival (OS) with cetuximab plus platinum-based chemotherapy [55–57]. However, the regimen has not gained regulatory approvals and is not currently used in routine clinical practice [6,58].

Both necitumumab and panitumumab have been investigated in combination with platinum-based chemotherapy as first-line treatments in advanced NSCLC in several clinical studies [44,59–62]. However, no Phase III study of panitumumab in NSCLC has been conducted due to its toxicity profile and limited impact on efficacy observed in early-phase studies [59]. In the SQUIRE trial, the combination of necitumumab with gemcitabine and cisplatin improved OS ($p = 0.01$) and PFS ($p = 0.006$) compared with chemotherapy alone. These results confirmed the potential role of agents targeting the EGFR pathway in the treatment of NSCLC [63]. In light of the SQUIRE outcomes, necitumumab in combination with gemcitabine and cisplatin was approved by the US FDA in 2015 as a first-line treatment for squamous metastatic NSCLC. However, necitumumab is not recommended by the NCCN Guidelines® because of its toxicity, cost, and limited improvement in efficacy compared with gemcitabine/cisplatin [6]. A Phase I study of necitumumab in combination with osimertinib in patients with advanced EGFR-mutated NSCLC demonstrated anti-tumor activity, which may suggest a potential future therapeutic role for this regimen [64].

Efficacy of cetuximab–TKI combinations

Of the EGFR mAbs, cetuximab has been the most widely explored in combination with chemotherapy or EGFR-TKIs in preclinical and clinical trials of EGFR-TKI-resistant advanced NSCLC. In an open-label, Phase Ib study, patients with EGFR-mutant NSCLC and acquired resistance to erlotinib/gefitinib were treated with 40 mg afatinib daily until disease progression, and then continued with cetuximab 500 mg/m^2 plus afatinib every 2 weeks (combination phase) [41]. Median PFS and median duration of response in the combination group were 2.9 months (95 % confidence interval [CI]: 1.8–4.8) and 5.7 months (range 3.7–8.3), respectively. Disease control rate (DCR) in these patients was 50.0 % **(**Table 1**).** Notably, patients who received afatinib monotherapy for ≥12 weeks prior to combination treatment showed an improvement in median PFS (4.9 vs 1.8 months; $p = 0.0354$), objective response rate (ORR; 15.8 % vs 5.9 %), and DCR (57.9 % vs 41.2 %), compared with those who received afatinib monotherapy for <12 weeks [41]. Sequential blockade of EGFR family receptors with afatinib followed by cetuximab plus afatinib showed activity in heavily pretreated patients with acquired resistance to erlotinib or gefitinib, with a predictable safety profile. Another open-label, Phase 1b trial of cetuximab plus afatinib in 12 patients with squamous NSCLC also suggested potential benefits of this combination in the treatment of squamous cell carcinoma, including NSCLC [65]. The best overall response was stable disease, which was reported in 75 % of patients. Median PFS was 2.7 months **(**Table 1**).**

The SWOG S1403 randomized Phase II study investigated first-line treatment of cetuximab plus afatinib versus afatinib alone in patients with EGFR-mutant NSCLC harboring *EGFR* mutations exon 19 deletion or L858R. Patients received 40 mg afatinib daily plus cetuximab 500 mg/m^2 every 2 weeks, or afatinib alone (40 mg daily). There was no improvement in median PFS (11.9 vs 13.4 months) and 2-year OS rate (67 % vs 70 %) in the combination group, compared with the afatinib monotherapy group **(**Table 1**)** [40]. Similarly, the ACE-Lung trial was a randomized Phase II study that evaluated first-line treatment with cetuximab plus afatinib in patients with advanced EGFR-mutant NSCLC [39]. Patients in the combination group received afatinib (40 mg daily) and cetuximab (250 mg/m^2 on Day 15 of Cycle 1, followed by 500 mg/m^2 every 2 weeks for 6 months). Patients in the monotherapy group received 40 mg afatinib once daily. There was no significant difference in the proportion of patients who reported treatment failure at 9 months between the groups (59.3 % vs 64.9 %). The median time to treatment failure was 11.1 (95 % CI: 8.5–14.1) and 12.9 (95 % CI: 9.2–14.5) months in the monotherapy and combination groups, respectively **(**Table 1**)** [39].

In a single-arm, Phase II trial, 37 patients with NSCLC harboring an EGFR-exon 20 insertion mutation received cetuximab 500 mg/m^2 every

Table 1
Studies of cetuximab in combination with TKIs in patients with NSCLC.

Study	Design	N	Treatment	Setting	Primary endpoints	Secondary endpoints	Results
Horn 2017 [41]	Phase Ib, open label	171	Afatinib + cetuximab vs afatinib	Second line	DLTs	Safety, PKs, PFS, ORR, DCR	• Median PFS was 2.9 months (95 % CI: 1.8–4.8) in the combination arm PFS was longer in patients with prior afatinib monotherapy (4.9 vs 1.8 months; $p = 0.0354$) ORR was numerically higher in patients with prior afatinib monotherapy (15.8 % vs 5.9 %, $p = 0.3630$) Median DOR was 5.7 months (range 3.7–8.3) in the combination arm DCR was 50 % in the combination arm DCR was higher in patients with prior afatinib monotherapy (57.9 % vs 41.2 %)
Veggel 2018 [67]	Retrospective	4	Afatinib + cetuximab	–	–	–	• Median PFS was 5.4 months (95 % CI: 0.0–14.2) 3 patients showed PR according to RECIST 1.1
Gazzah 2018 [65]	Phase 1b, open label	12	Afatinib + cetuximab vs afatinib	≤Second line	MTD	Safety and tolerability at the MTD, anti-tumor activity ORR, (CR, PR), DCR (CR, PR, SD) according to RECIST 1.1.	• Afatinib 40 mg + cetuximab 250 mg/m^2 was the MTD and approved dose SD was reported in 9 (75.0 %) patients DCR was reported in 9 (75 %) patients Mean duration of DC was 4.1 months Median PFS was 2.7 months (95 % CI: 1.2–4.4)
Goldberg 2020/ SWOG S1403 [40]	Phase II, randomized	168	Afatinib + cetuximab vs afatinib	First line	PFS	ORR, TTD, OS, toxicity	• No improvement in PFS with afatinib + cetuximab compared with afatinib alone (HR 1.01, 95 % CI: 0.72–1.43; $p = 0.94$; median 11.9 vs 13.4 months) No improvement in OS with afatinib + cetuximab compared with afatinib alone (HR 0.82, 95 % CI: 0.50–1.36; $p = 0.44$)
Cortot 2021/ ACE-Lung [39]	Phase II, randomized, open label	117	Afatinib + cetuximab vs afatinib	First line	TTF rate at 9 months	EGFR ctDNA in plasma	• Percentage of patients without treatment failure at 9 months was similar for both groups (59.3 % for group A vs 64.9 % for group A + C) No improvement on PFS, OS, and 12-month survival rate between groups
Wang 2020 [42]	Retrospective	15	Cetuximab + brigatinib (n = 5) vs chemotherapy ± bevacizumab (n = 10)	Second- or subsequent -lines	–	–	• 2 patients, developed into PD, with a PFS of 15 and 13 months Median PFS of patients who received combined targeted therapy was 14 months compared with 3 months for those treated with chemotherapy
Veggel 2023 [66]	Phase II, single arm	37	Afatinib + cetuximab	First line	DCR after 18 weeks	Safety, RR, DOR, PFS	• The primary endpoint was met, with DCR achieved by 54 % of patients Best responses were partial (n = 16), stable (n = 16) or progressive (n = 2) disease ORR was 43 % with confirmed ORR rate of 32 % Median PFS was 5.5 months Median OS was16.8 months

Abbreviations: CI, confidence interval; CR, complete response; ctDNA, circulating tumor DNA; DC, disease control; DCR, disease control rate; DLT, dose-limiting toxicities; DOR, duration of response; EGFR, epidermal growth factor receptor; HR, hazard ratio; MTD, maximum tolerated dose; NSCLCL, non-small cell lung cancer; ORR, overall response rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PK, pharmacokinetics; PR, partial response; RECIST, response evaluation criteria in solid tumors, RR, response rates; SD, stable disease; TKI, tyrosine kinase inhibitor; TTD, time to treatment discontinuation; TTF, time to treatment failure.

2 weeks plus afatinib 40 mg daily [66]. Overall, twenty (54 %) patients achieved the primary endpoint of DCR at 18 weeks. Median PFS and median OS were 5.5 and 16.8 months, respectively. Overall response rate was 43 % and the best response rates were: partial (n = 16) and stable (n = 16), with progressive disease in two patients (Table 1) [66]. In a prior retrospective study of four patients with EGFR-mutant NSCLC and exon 20 insertion, those who received cetuximab in combination with afatinib achieved a median PFS of 5.4 months, and three patients showed partial response according to the response evaluation criteria in solid tumors (RECIST) version 1.1 [67]. These results suggest some clinical potential for the combination of cetuximab and afatinib in patients with EGFR-TKI-resistant NSCLC and exon 20 insertion. The combination of cetuximab plus afatinib is now recommended by NCCN Guidelines® as a treatment option for patients experiencing disease progression following treatment with EGFR-TKIs [6].

The combination of anti-EGFR mAbs with other TKIs, such as anti-anaplastic lymphoma kinase (ALK), has also demonstrated evidence of clinical efficacy in patients with osimertinib resistance mediated by *EGFR cis*-C797S. In a retrospective study by Wang et al. cetuximab plus brigatinib demonstrated efficacy in overcoming osimertinib resistance in patients who had progressed on second and subsequent treatments with osimertinib [42]. Of the 15 patients who progressed from these lines of treatment with osimertinib, five received anti-EGFR/ALK combination therapy, and the remaining patients were treated with SoC chemotherapy [42]. Median PFS for patients in the combination group was 14 months, compared with 3 months for those who received chemotherapy alone (Table 1) [42]. This study provided strong evidence for the efficacy of the anti-EGFR mAb/ALK combination in overcoming acquired resistance; however, these findings were limited by their retrospective nature and small sample size of patients enrolled [42].

Safety of cetuximab–TKI combinations

Combining an anti-EGFR mAb with an EGFR-TKI following EGFR-TKI resistance in NSCLC has resulted in expected safety findings, with studies suggesting a manageable safety and tolerability profile [39–41,65–67].

In an open-label, Phase Ib study by Horn et al. in 36 patients with EGFR-mutant advanced NSCLC who received second-line afatinib plus cetuximab, grade 3/4 adverse events (AEs) were reported in 50 % of patients, with rash as the most frequently reported event (22.2 %) [41]. Notably, the most frequently reported treatment-related AEs (TRAEs) of any grade were rash (69 %), paronychia (39 %), dry skin (36 %), and diarrhea (33 %). Dose reduction and treatment discontinuation due to TRAEs were reported in 8 (22 %) and 3 (8 %) of patients, respectively (Table 2) [41].

In the Phase II randomized SWOG S1403 trial in patients with first-line advanced NSCLC harboring EGFR mutation (exon 19 deletion or L858R), the rates of grade ≥ 3 AEs were higher in 78 patients receiving cetuximab plus afatinib than in those receiving afatinib alone. The most frequently reported TRAEs were acneiform rash (27 %), maculopapular rash (13 %) and diarrhea (15 %). Dose reduction to afatinib 30 mg and treatment discontinuation were observed in 44 (56.7 %) and 12 (14 %) patients, respectively (Table 2) [40].

Similarly, in the Phase II randomized ACE-Lung trial in 58 patients with advanced EGFR-mutant NSCLC who received first-line cetuximab plus afatinib, the most common TRAEs were digestive and skin disorders, in accordance with the known safety profile of EGFR inhibitors. The rates of grade 3 TRAEs were slightly higher in the cetuximab plus afatinib combination than with afatinib monotherapy. No grade 4 TRAEs were reported in the combination group compared with 5.1 % in the afatinib monotherapy group. Treatment discontinuation due to TRAEs was reported in 9 (15.8 %) of patients (Table 2) [39].

This pattern was reflected in smaller clinical trials and retrospective studies investigating the combination of cetuximab plus afatinib in patients with EGFR-TKI-resistant NSCLC, with 31–54 % of patients

Table 2
Safety of cetuximab–TKI combination treatments in patients with NSCLC.

Study	Treatment	N	Grade 3/4 AEs	Other AEs
Horn 2017 [41]	Afatinib + cetuximab	36	• Grade 3 AEs were reported in 16 (44.4 %) patients, with rash (19.4 %) as the most frequent event Grade 4 AEs were reported in 2 (5.6 %) patients	• Most frequent TRAEs (any grade) were rash (69 %), paronychia (39 %), dry skin (36 %), and diarrhea (33 %) 8 (22 %) of patients experiences AEs that led to dose reduction 3 (8 %) of patients experiences TRAEs that led to treatment discontinuation
Veggel 2018 [67]	Afatinib + cetuximab	4	• NR	• Safety profile was acceptable and in line with known toxicity profile of afatinib + cetuximab therapy 2 patients required appropriate skin management and dose reduction
Gazzah 2018 [65]	Afatinib + cetuximab	58	• 18 (31.0 %) patients experienced grade 3 AEs Most frequent grade 3 AEs were acneiform dermatitis and rash (each n = 3; 5.2 %) 2 (3.4 %) patients experienced grade 4 AEs Most frequent grade 4 TRAEs were hypersensitivity and hyperlipasemia (each n = 1; 1.7 %)	• Treatment-related SAEs were reported in 8 (13.8 %) patients 14 (24.1 %) patients experienced AEs that led to dose reduction 19 (32.8 %) patients experienced AEs that led to treatment discontinuation 7 (12.1 %) patients experienced TRAEs leading to discontinuation
Goldberg 2020/ SWOG S1403 [40]	Afatinib + cetuximab	78	• More patients in afatinib + cetuximab arm presented grade ≥ 3 TRAEs compared with group A (72 % vs 40 %; $p = 0001$)	• Most frequent grade ≥ 3 TRAEs were acneiform rash (27 %), maculopapular rash (13 %), and diarrhea (15 %) 44 (56.7 %) of patients experienced dose reduction of afatinib to 30 mg 12 (14 %) pf patients experienced AEs that led to treatment discontinuation
Cortot 2021/ ACE-Lung [39]	Afatinib + cetuximab	58	• Grade 3 TRAEs was slightly higher in the afatinib + cetuximab group compared with afatinib group (52.6 % vs 37.3)	• Diarrhea was reported in 93.2 % of patients in the afatinib group and in 89.5 % of patients in afatinib + cetuximab group

(*continued on next page*)

Table 2 (*continued*)

Study	Treatment	N	Grade 3/4 AEs	Other AEs
			Grade 4 TRAEs were reported only in the afatinib group (5.1 %) Grade 3/4 diarrhea was higher in the afatinib group than afatinib + cetuximab group (18.7 % vs 12.3 %) Grade 3/4 skin rash was higher in the afatinib + cetuximab group than group A (21.1 % vs 10.2 %)	Incidence of skin rash (any grade) was higher in the afatinib + cetuximab group than group A (94.7 % vs 79.7 %) 9 (15.8 %) of patients experience TRAEs that led to treatment discontinuation
Wang 2020 [42]	Brigatinib + cetuximab	15	• NR	• 4 patients from the brigatinib + cetuximab group experienced grade 1/2 AEs Diarrhea and skin reactions were the most frequent grade 1/2 AEs (40 % each) No grade 3/4 AEs were reported
Veggel 2023 [66]	Afatinib + cetuximab	37	• Grade 3 TRAEs were reported in 54 % of patients Grade 3 TRAEs in ≥ 10 % were diarrhea (n = 5; 14 %), rash (n = 5; 14 %) and dry skin (n = 5; 14 %) No grade 4 TRAE was observed	• Most frequent TRAEs were diarrhea (70 %), rash (65 %), dry skin (59 %), paronychia (54 %), and erythema (43 %) 25 (68 %) of patients required dose reduction 6 (16 %) of patients experience AEs that led to treatment discontinuation (one grade 3 allergic reaction after the first infusion of cetuximab)

Abbreviations: AE, adverse event; NSCLC, non-small cell lung cancer; NR, not reported; SAE, serious adverse event; TKI, tyrosine kinase inhibitor; TRAE, treatment-related adverse event.

experiencing grade 3 AEs or TRAEs, and the most frequently reported event were rash, diarrhea, and dry skin. Overall, 24–68 % of patients experienced dose reduction and 12–16 % of patients experienced treatment discontinuation (Table 2) [65–67].

Similar safety findings have been reported with the combination of anti-EGFR and anti-ALK. In the retrospective study by Wang et al. of five patients with advanced NSCLC who received cetuximab plus brigatinib after progression on osimertinib, four (80 %) patients reported grade 1/2 AEs, most frequently with diarrhea (40 %) and skin reactions (40 %). No grade 3/4 AEs were reported (Table 2) [42].

Emerging data for anti-EGFR mAbs in NSCLC: biomarkers and personalized therapy

These clinical findings emphasize the potential of EGFR-targeting mAbs in targeted combination regimens for the treatment of advanced EGFR-resistant NSCLC [45,54,63]. Several potential biomarkers associated with better survival outcomes in patients with NSCLC treated with anti-EGFR mAbs have been identified, including EGFR overexpression, *EGFR* gene copy number, *EGFR* and *KRAS* mutations, and circulating tumor (ct)DNA analysis.

EGFR overexpression

In a retrospective analysis of the FLEX trial, EGFR overexpression, assessed using immunohistochemistry (IHC), was associated with improved clinical responses in patients receiving cetuximab in combination with chemotherapy (cisplatin and vinorelbine), compared with chemotherapy alone [54]. Cetuximab in combination with chemotherapy extended the median OS in patients with squamous (HR 0.62) and non-squamous (HR 0.73) NSCLC in the EGFR-overexpressing group (H-score ≥ 200) [54]; whereas for patients in the low EGFR-expressing group (H-score ⟨200), no difference was observed in median OS between treatment groups (HR 0.99) (Table 3) [54].

In the SQUIRE trial in patients with first-line squamous NSCLC, OS for necitumumab plus gemcitabine and cisplatin versus gemcitabine and cisplatin was more favorable in patients with EFGR-overexpressing tumors (Table 3) [63]. However, the 'interaction tests' did not show a difference in OS HRs between the high versus low EGFR-expressing groups [63]. Similarly, in a parallel trial, INSPIRE, no differences were observed in OS or PFS between patients with high and low EGFR-expressing tumors in either treatment group (necitumumab plus pemetrexed and cisplatin vs pemetrexed and cisplatin) [62]. Notably, further analyses of the SQUIRE results across the range of EGFR IHC (without considering cut-off for IHC level, i.e., EGFR > 0 and EGFR = 0) suggested that patients with detectable EGFR protein benefited from the addition of necitumumab to chemotherapy, regardless of the level of EGFR protein expression (Table 3) [44].

EGFR gene copy number

Several trials have investigated the correlation between *EGFR* copy number (measured by fluorescence *in situ* hybridization [FISH]) and efficacy outcomes to evaluate its potential role as a candidate biomarker for selecting personalized targeted therapy in NSCLC [44,45,53,68]. In the retrospective analysis of clinical outcomes in the SQUIRE trial, the association of EGFR FISH-positive status with efficacy outcomes was not statistically significant; however, OS was longer in EGFR FISH-positive patients who received necitumumab compared with those receiving chemotherapy (Table 3) [44].

In the SWOG S0819 trial, EGFR FISH was evaluated as a predictive biomarker for cetuximab in patients with advanced NSCLC (Table 3) [45]. In this study, 1313 patients were randomly assigned to receive first-line cetuximab in combination with carboplatin and paclitaxel, with or without bevacizumab. This study hypothesized that EGFR FISH positivity could be associated with increased OS or PFS [45]. Adding cetuximab showed no improvement in OS or PFS in patients with non-squamous NSCLC and EGFR FISH-positive status (HR 0.88, $p = 0.34$ and HR 0.99, $p = 0.96$, respectively) [45]. However, EGFR FISH-positive patients with squamous NSCLC who received cetuximab showed an improvement in median OS (HR 0.58, 95 % CI: 0.39–0.86; $p = 0.007$), compared with the EGFR FISH-negative group (HR 1.04, 95 % CI: 0.78–1.40; $p = 0.77$). OS in this group was numerically in favor of cetuximab; however, it was not statistically significant (HR 0.68, 95 % CI: 0.46–1.01; $p = 0.055$) (Table 3) [45]. Although no statistically significant differences were observed in clinical outcomes for the unselected (squamous and non-squamous together) patient populations with positive EGFR FISH, observations in the subgroup of patients with EGFR FISH-positive squamous NSCLC suggested the need to further characterize subpopulations of patients who may benefit from anti-EGFR therapies [45].

A further analysis of SWOG S0819 introduced a combination index by considering the dual association of FISH and IHC (FISH±/IHC±) [53]. OS for unselected NSCLC patients with dual-positive FISH and IHC

Table 3
Potential biomarkers for selecting targeted therapy in NSCLC.

Study	N*	Setting	Treatment	Results	Comments
EGFR overexpression/IHC					
FLEX 2012 [54]	1121	First line†	Cetuximab + CT (cisplatin–vinorelbine) vs CT	**Median OS HR** *EGFR-high (H-score ≥ 200), n = 345* NSCLC: 12.0 vs9.6 (HR 0.73; 95 % CI: 0.58–0.93; $p = 0.011$) SqCLC: HR 0.62; 95 % CI: 0.43–0.88 *EGFR-low (H-score ⟨200), n = 776* NSCLC: 9.8 vs 10.3 (HR 0•99, 95 % CI: 0.84–1.16; $p = 0.88$) SqCLC: HR 0·98; 95 % CI: 0·73–1·30	• High EFGR expression was associated with survival benefits from the addition of cetuximab to first-line CT, and could be a predictive biomarker for personalized targeted treatment
SQUIRE 2015, 2016 [44,63]	982	First line‡	Necitumumab + CT (cisplatin–gemcitabine) vs CT	**Median OS HR** *EGFR-high (H-score ≥ 200), n = 374* HR 0.75; 95 % CI: 0.60–0.94 *EGFR-low (H-score ⟨200), n = 608* HR 0.90; 95 % CI: 0.75–1.07 *Further analysis:* EGFR > 0 OS: 11.7 vs10.0 (stratified HR 0.79, 95 % CI: 0.69–0.92; $p = 0.002$) PFS: 5.7 vs 5.5 (stratified HR 0.84, 95 % CI: 0.72–0.97; $p = 0.018$)	• No significant difference in EGFR-expressing groups, although higher EFGR expression rates are in favor of necitumumab Further analysis of SQUIRE results suggested expression of EGFR, with no cut-off, as a predictive biomarker
EGFR gene copy number/FISH					
SQUIRE 2016 [44]	557	First line‡	Necitumumab + CT (cisplatin–gemcitabine) vs CT	**Median OS** *EGFR FISH-positive* Median OS: 12.6 vs 9.2 months; HR 0.70, 95 % CI: 0.52–0.96 *EGFR FISH-negative* Median OS: 11.1 vs 10.7 months; HR 1.02, 95 % CI: 0.80–1.29	• No significant difference in EGFR-FISH subgroups ($p = 0.066$), although OS rates in these patients with EGFR-FISH-positive status are in favor of necitumumab
SWOG 0819 2018, 2022 [45,53]	976	First line	Cetuximab + CT (carboplatin–paclitaxel) vs CT	**FISH+** *Median OS* SqCLC: 11.8 vs 6.4 (HR 0.58; $p = 0.0071$) Non-SqCLC: 14.3 vs 12.1 (HR 0.88; $p = 0.34$) Unselected: 13.4 vs 9.8 (HR 0.81; $p = 0.054$) *Median PFS* SqCLC: 4.5 vs 2.8 (HR 0.68; $p = 0.055$) Non-SqCLC: 5.7 vs 5.5 (HR 0.99; $p = 0.96$) Unselected: 5.4 vs 4.8 (HR 0.92; $p = 0.40$) **FISH+/IHC+** *OS* SqCLC: 12.6 vs.4.6 (HR 0.32, $p = 0.0002$) Non-SqCLC: (HR 0.9) Unselected: (HR 0.63, $p = 0.01$)	• Higher OS for patients with positive FISH/IHC combination index indicated the predictive value of combination index for personalized targeted treatment
Mutations (*EGFR, KRAS*)					
SWOG 0819 2022 [53]	627	First line	Cetuximab + CT (carboplatin–paclitaxel) vs CT	***KRAS* mt vs *KRAS* wt** OS: HR (95 % CI): 0.86 (0.61–1.20) vs 0.86 (0.70–1.05) PFS: HR (95 % CI): 0.99 (0.72–1.37) vs 0.94 (0.78–1.14)	• *KRAS* mutation status was not associated with a treatment benefit
Horn 2017 [41]	73	Second line§	Cetuximab + afatinib vs afatinib	**T790M + vs T790M−** PFS: 4.8 vs 1.8 months, $p = 0.1306$ ORR: 20.0 % and 0.0 %, $p = 0.0823$ DCR: 60.0 % and 37.5 %, $p = 0.0512$	• Although the difference between clinical outcomes in the subgroups are non-significant, due to the association of T790M + with better survival outcomes, T790M status could be a predictive biomarker for anti-EGFR targeted therapy with cetuximab
ctDNA					
Cortot 2021/ ACE-Lung [39]	81	First line	Cetuximab + afatinib vs afatinib	• Allele frequency of the EGFR gene mutation in ctDNA at baseline was associated with shorter PFS, regardless of the treatment received A bR‖ was observed in 49 (66.2 %) patients: 22/35 (62.9 %) in the afatinib group and 27/39 (69.2 %) in afatinib + cetuximab group. However, this bR was not associated with an improved PFS or OS	• Baseline ctDNA could help identify different patient profiles that would benefit from EGFR inhibition
Mack 2022/ S1403 [70]	106	First line	Cetuximab + afatinib vs afatinib	**Median PFS at baseline** Detectable m*EGFR* in ctDNA: 10.2 months, 95 % CI: 7.3–13.0 Non-detectable m*EGFR* in ctDNA: 11.2 months, 95 % CI: 8.2–15.0 HR 1.46, 95 % CI: 0.90–2.38; $p = 0.12$	• ctDNA clearance was associated with longer PFS and OS, and could be used for monitoring treatment progress. However, further investigation in a larger patient population is warranted

(*continued on next page*)

Table 3 (*continued*)

Study	N*	Setting	Treatment	Results	Comments
				Median PFS after treatment m*EGFR* ctDNA clearance group[III]: 15.1 months (95 % CI:10.6–17.5) Residual m*EGFR* ctDNA group[IIII]: 4.6 months (95 % CI: 1.7–7.5) HR 0.23, 95 % CI: 0.12–0.45; $p < 0.0001$ **Median OS at baseline** Detectable m*EGFR* in ctDNA: 30.2 months, 95 % CI: 25.0–40.8 Non-detectable m*EGFR* in ctDNA: NR, 95 % CI: 25.2–NR HR 2.16, 95 % CI: 1.02–4.58; $p = 0.04$ **Median OS after treatment** m*EGFR* ctDNA clearance group[III]: 32.6 months (95 % CI: 23.5–NE) Residual m*EGFR* ctDNA group[IIII]: 15.6 months (95 % CI: 4.9–28.3) HR 0.44, 95 % CI: 0.21–0.90; $p = 0.02$	

*Number of patients with available molecular assessments. [†]Patients with advanced NSCLC, including those with SqCLC histology. [‡]Patients with advanced SqCLC. [§]Patient who progressed on erlotinib or gefitinib. [||]bR is defined as a decrease in ctDNA at Week 2 compared with the baseline level that was greater than the variability of the digital polymerase chain reaction measurement [39]. [III]m*EGFR* was detectable at baseline, but undetectable after eight weeks of treatment. [IIII]m*EGFR* was detectable at baseline and remained detectable.

Abbreviations: bR, biological response; CI, confidence interval; CT, chemotherapy; ctDNA, circulating tumor DNA; DCR, duration of response; EGFR, epidermal growth factor receptor; FISH, fluorescence *in situ* hybridization; HR, hazard ratio; IHC, immunohistochemistry; m, mutant; mt, mutation; NE: not estimable; NSCLC, non-small cell lung cancer; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; SqCLC, squamous cell lung cancer; wt, wild-type.

was in favor of cetuximab (HR 0.63, 95 % CI: 0.44–0.91; $p = 0.01$) (Table 3) [53]. Additionally, median OS with cetuximab in patients with squamous NSCLC and a positive combination index (FISH+/IHC+) (HR 0.32, 95 % CI: 0.18–0.59; $p = 0.0002$) was 12.6 months (95 % CI: 7.9–15.9), compared with 4.6 months for the control arm (95 % CI: 3.4–7.3) [53]. PFS also improved with the addition of cetuximab in the same group of patients (HR 0.49, 95 % CI: 0.28–0.88; $p = 0.02$) [53].

Mutations in EGFR and KRAS

The potential correlation between mutations in *EGFR* and *KRAS* with clinical responses to treatment with anti-EGFR mAbs has been investigated in several studies on NSCLC [41,53,68]. *KRAS* mutation is regarded as a negative predictive biomarker for clinical outcomes and personalized treatment of mCRC patients with anti-EGFR mAbs [69]. However, further analyses of clinical data from the BMS099 and SQUIRE trials in patients with NSCLC did not find any association between *KRAS* mutation status and treatment benefit of anti-EGFR mAbs [53,68]. In the SWOG 0819 trial, adding cetuximab to first-line chemotherapy was not associated with any improvement in survival outcomes among either *KRAS*-mutant or *KRAS*-wt NSCLC patients (Table 3) [53].

In the trial by Horn et al. the efficacy of cetuximab plus afatinib was also assessed according to *EGFR* T790M mutation. Treatment responses in patients harboring *EGFR* T790M were in favor of cetuximab, although these were not statistically significant [41]. PFS, ORR, and DCR were numerically higher in patients with T790M-positive versus T790M-negative mutations (4.8 vs 1.8 months, 20.0 % vs 0.0 %, and 60.0 % vs 37.5 %, respectively) (Table 3) [41]. Further analysis of responses to cetuximab treatment in a larger study population is required to clarify the predictive potential of *EGFR* T790M mutation status in NSCLC patients.

ctDNA

In the ACE-Lung trial, ctDNA was present at baseline, but was not predictive of objective response or improved PFS (Table 3) [39]. However, patients with an allele frequency greater than the median value (4.3 %) had a shorter PFS compared with patients with a frequency below the median value (HR 1.95, 95 % CI: 1.11–3.41; $p = 0.02$) [39]. ctDNA decreased in 62.9 % of patients in the afatinib monotherapy group and by 69.2 % in the afatinib plus cetuximab group. However, this was not associated with an improved PFS or OS in the afatinib plus cetuximab group compared with the afatinib monotherapy group [39].

Recent findings from the S1403 trial revealed that approximately 90 % (80/98) of patients had undetectable ctDNA after 3 cycles; ctDNA clearance relative to residual ctDNA (after 60 days) was associated with significantly longer PFS ($p < 0.0001$) and OS ($p = 0.02$) [70]. Monitoring ctDNA as part of routine clinical care may therefore provide a valuable platform for evaluating the effectiveness of treatment and may help to identify patients less likely to respond to initial EGFR-TKI therapy. ctDNA can potentially drive precision medicine in the future treatment of NSCLC by reflecting the evolution of *EGFR* mutations during therapy to inform appropriate treatment selection as new mutations arise [24].

In summary, EGFR overexpression, *EGFR* gene copy number, *EGFR* and *KRAS* mutations, and circulating tumor (ct)DNA are biomarkers associated with improved survival outcomes in patients with advanced NSCLC receiving treatment with anti-EGFR mAbs. These biomarkers may support individualized treatment decisions. Positive combination index (FISH+/IHC+) can be considered an appropriate assessment strategy for selecting anti-EGFR mAbs.

Future perspectives

While the current clinical evidence supports the survival benefits of anti-EGFR mAbs, such as cetuximab, in combination with EGFR-TKIs, large prospective Phase II studies are needed to strengthen these findings in hard-to-treat patient populations. Available evidence also suggests that the combination of anti-EGFR mAbs with EGFR-TKIs has an acceptable safety profile in patients with EGFR-TKI-resistant advanced NSCLC [39–41,65–67]. Noting the improved toxicity profile of osimertinib compared with chemotherapy and other EGFR-TKIs [2,71], combining cetuximab with osimertinib may provide a more favorable safety profile due to the potential lower incidence of skin toxicity associated with osimertinib. In addition, further exploration of the role of *EGFR* amplification as a predictive biomarker (e.g., using FISH, ctDNA, or next-generation sequencing [72]) may identify a specific patient population that will benefit most from an EGFR-targeted combination therapeutic regimen that includes cetuximab.

Other emerging concepts and treatment strategies in the management of EGFR-TKI- resistant NSCLC include: (1) natural killer (NK) cell-

based immunotherapies, which are being investigated as a platform for developing new cancer therapies due to the anti-tumor activity of NK cells and their role of bridging innate and downstream adaptive immune responses [73,74]. An ongoing Phase I/IIa clinical trial investigating the anti-tumor activity of cetuximab in combination with NK cell immunotherapies aims to provide clinical evidence supporting this novel approach to treating advanced NSCLC (NCT04872634). (2) Mutations in molecular subtypes in the EGFR pathway, such as *KRAS* and *BRAF*, are associated with resistance and poor response to anti-EGFR agents such as cetuximab [75]. In addition, *KRAS* mutations have been associated with a lack of activity of TKIs [76]. Therefore, understanding the role of such molecular subtypes and harboring mutations associated with poor anti-EGFR response in advanced NSCLC could help to identify biomarkers and select precise treatment strategies. (3) Recent advances in the development of bispecific antibodies, such as amivantamab, provide new targeted therapies to overcome resistance in NSCLC [77]. In two Phase I studies, the combination of amivantamab and lazertinib demonstrated anti-tumor activity with a manageable safety profile in patients with advanced EGFR-mutated NSCLC [78,79]. (4) Tepotinib (a MET inhibitor) is being investigated in combination with osimertinib in patients with NSCLC who acquired resistance to first-line osimertinib due to *MET* amplification (INSIGHT 2 trial, NCT03940703) [80]. The combination of anti-EGFR mAbs and MET inhibitors may be a potential treatment strategy in patients with advanced NSCLC and *MET* amplification following progression on first-line osimertinib [80]. (5) Further, larger scale trials of novel combinations of cetuximab with mAbs against other targets, such as ALK, may confirm the potential of these combinations in hard-to-treat patient populations.

Conclusions

Dual inhibition of EGFR with EGFR-TKIs and anti-EGFR mAbs has shown promising anti-tumor activity in patients with acquired resistance to TKIs mediated by EGFR mutation. Cetuximab/EGFR-TKI combination therapy has shown clinical benefit and a manageable safety profile, suggesting that cetuximab in combination with second- and third-generation TKIs may have a potential role as a second- and/or subsequent-line treatment option for patients with NSCLC who have specific EGFR mutations conferring resistance to prior TKI therapy. In addition, subgroup analyses support the use of EGFR protein expression as a predictive biomarker for selecting patients who may benefit from targeted treatment strategies with anti-EGFR mAbs, such as cetuximab and necitumumab. Finally, further analyses of patient subgroups in studies investigating cetuximab plus afatinib will clarify the potential predictive value of *EGFR* mutation status (e.g., T790M) and ctDNA level for identifying patients who may further benefit from dual EGFR inhibition therapy.

CRediT authorship contribution statement

Fortunato Ciardiello: Conceptualization, Writing – review & editing. **Fred R. Hirsch:** Conceptualization, Writing – review & editing. **Robert Pirker:** Conceptualization, Writing – review & editing. **Enriqueta Felip:** Conceptualization, Writing – review & editing. **Christian Valencia:** Conceptualization, Writing – review & editing. **Egbert F. Smit:** Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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