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Hype or hope – Can combination therapies with third-generation EGFR-TKIs help overcome acquired resistance and improve outcomes in *EGFR*-mutant advanced/metastatic NSCLC?

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ABSTRACT

Three generations of epidermal growth factor receptor - tyrosine kinase inhibitors (EGFR-TKIs) have been developed for treating advanced/metastatic non-small cell lung cancer (NSCLC) patients harboring *EGFR*-activating mutations, while a fourth generation is undergoing preclinical assessment. Although initially effective, acquired resistance to EGFR-TKIs usually arises within a year due to the emergence of clones harboring multiple resistance mechanisms. Therefore, the combination of EGFR-TKIs with other therapeutic agents has emerged as a potential strategy to overcome resistance and improve clinical outcomes. However, results obtained so far are ambiguous and ideal therapies for patients who experience disease progression during treatment with EGFR-TKIs remain elusive.

This review provides an updated landscape of EGFR-TKIs, along with a description of the mechanisms causing resistance to these drugs. Moreover, it discusses the current knowledge, limitations, and future perspective regarding the use of EGFR-TKIs in combination with other anticancer agents, supporting the need for bench-to-bedside approaches in selected populations.

1. Background

Lung cancer is the second most common form of cancer and the leading cause of cancer-related mortality, with a 5-year survival rate of 18.6% for newly diagnosed patients [1]. Non-small cell lung cancer (NSCLC) represents roughly 85% of all lung cancer cases and, based on the World Health Organization (WHO) classification, can be categorized into three main subtypes: adenocarcinoma (40%), squamous cell carcinoma (30%), and large cell carcinoma (10%) [2,3]. Systemic treatment with platinum-based chemotherapy is still the standard treatment for NSCLC patients with advanced-stage disease. The typical median time to progression for patients treated with chemotherapy is around six months, with an average survival time of 10 - 12 months [4,5].

During the nineties and early 2000s, it became clear that several oncogenic mutations are involved in driving NSCLC development and progression. These include mutations affecting genes encoding for the Epidermal Growth Factor Receptor (EGFR), K-RAS, and the Anaplastic Lymphoma Kinase (ALK) [6]. The development of Epidermal Growth Factor Receptor – Tyrosine Kinase Inhibitors (EGFR-TKIs) represented a breakthrough in the treatment of NSCLC. Indeed, these small-molecule drugs replaced older platinum-based chemotherapy regimens for patients harboring specific *EGFR* mutations leading to significant clinical responses and reduced treatment-related toxicities [7–9].

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1.1. The role of the epidermal growth factor receptor (EGFR) in non-small cell lung cancer

EGFR (ErbB1/HER1) is a member of the ErbB receptor family and belongs to the super-family of structurally-related receptor tyrosine kinases (RTKs). The ErbB receptor family also includes the epidermal growth factor receptor 2 (ErbB2; HER2), as well as HER3 (ErbB3) and HER4 (ErbB4) [10].

EGFR signaling regulates the activation of several intracellular signaling pathways responsible for sustaining physiological cellular processes. These include the mitogen-activated protein kinases (MAPK)/extracellular signal-regulated kinases (ERK), phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR, and the interleukin 6 (IL-6)/Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3) pathway (Fig. 1) [11].

EGFR mutations, either resistance or activating mutations, are found in 15% of NSCLC patients (more commonly in non-smokers, women, and Asian populations) and correlate with poor clinical outcomes [12–14]. Typically, *EGFR*-activating mutations cluster in hotspots located between exon 18 and 21 of the *EGFR* gene, encoding the tyrosine kinase domain of the receptor. These mutations promote a dysregulated, ligand-independent activation of EGFR signaling that sustains the proliferation, survival, metabolism, and migration of cancer cells (Fig. 2).

In-frame exon 19 deletions and exon 21 L858R point mutations are the most common *EGFR*-activating mutations (detected in approximately 50% and 40% of *EGFR*-positive NSCLC patients, respectively) [15]. Atypical *EGFR* mutations include G719X (~3%), L861Q (~1%), S768I (~1%) mutations, as well as exon 20 in-frame insertions (~6%) [15]. Mutations that confer resistance to EGFR-TKIs (e.g., *EGFR*-T790M and *EGFR*-C797S) are evaluated in detail later in this review.

2. Epidermal Growth Factor Receptor - Tyrosine Kinase Inhibitors (EGFR-TKIs)

EGFR-TKIs are drugs designed to inhibit the tyrosine kinase domain of the EGFR receptor, thereby interrupting its activation and the subsequent engagement of pro-tumorigenic signaling pathways [16]. Although initial studies failed to demonstrate an advantage of EGFR-TKI monotherapy over platinum chemotherapy, the subsequent re-evaluation of patient subgroups revealed that only patients carrying *EGFR*-activating mutations responded to treatment with EGFR inhibitors [17,18].

In NSCLC patients selected according to the presence of *EGFR*-activating mutations (e.g., exon 19 deletions and exon 21 L858R mutation), the treatment with EGFR-TKI monotherapy has led to significant improvements in progression-free survival (PFS) in comparison with previous platinum-based chemotherapy regimens. Consequently, EGFR-TKIs have become the standard of care for the treatment of advanced/metastatic NSCLC in the presence of actionable *EGFR* mutations [9].

As of 2021, three generations of EGFR-TKIs have been developed and are available in the clinic. The fourth generation of EGFR inhibitors is currently undergoing preclinical evaluation. A list of EGFR-TKIs approved for the treatment of *EGFR*-mutated NSCLC is provided in Table 1.

3. First-generation EGFR-TKIs

First-generation EGFR-TKIs are low molecular weight, reversible, oral EGFR inhibitors which exert their anticancer activity by inhibiting the intracellular phosphorylation of the EGFR receptor. Of note, the development of first-generation EGFR-TKIs started before the discovery of *EGFR*-sensitizing mutations [19]. Three first-generation EGFR-TKIs have received approval for the treatment of *EGFR*-mutant advanced/metastatic NSCLC: gefitinib, erlotinib, and icotinib (Table 2).

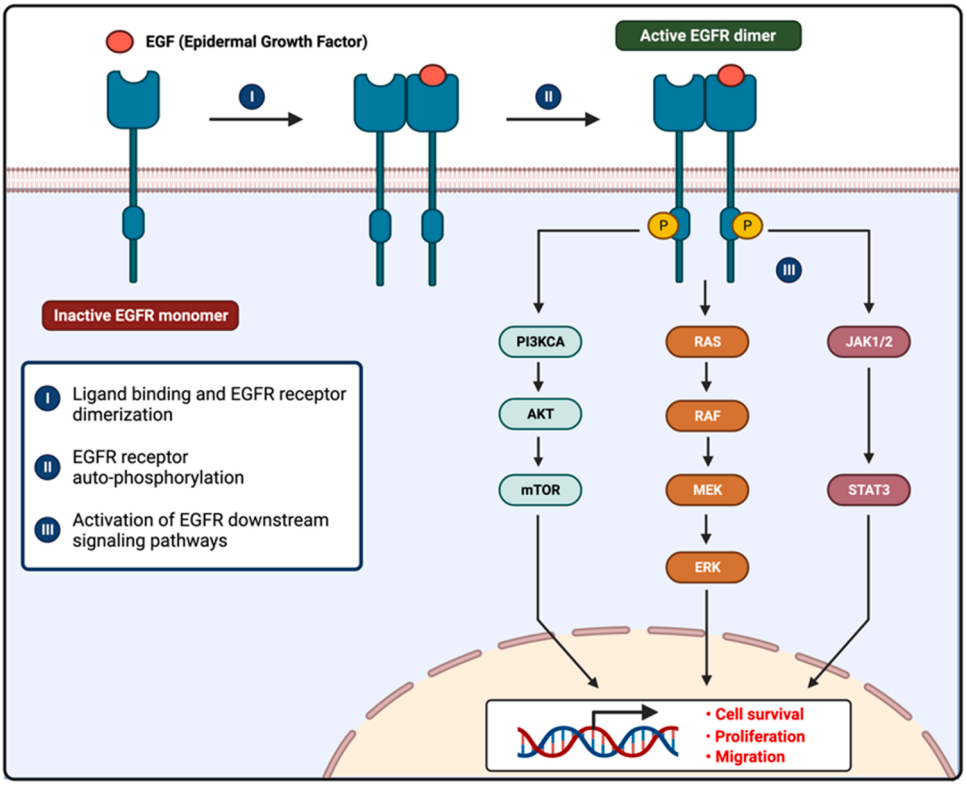

Fig. 1. Schematic representation of EGFR activation and signaling. Upon interaction with its ligand, the EGFR receptor may either homo or heterodimerize with other members of the ErbB family, leading to receptor auto-phosphorylation and the initiation of an intracellular cascade of molecular events that regulates cell proliferation, metabolism, migration, and survival. Image created with BioRender.com

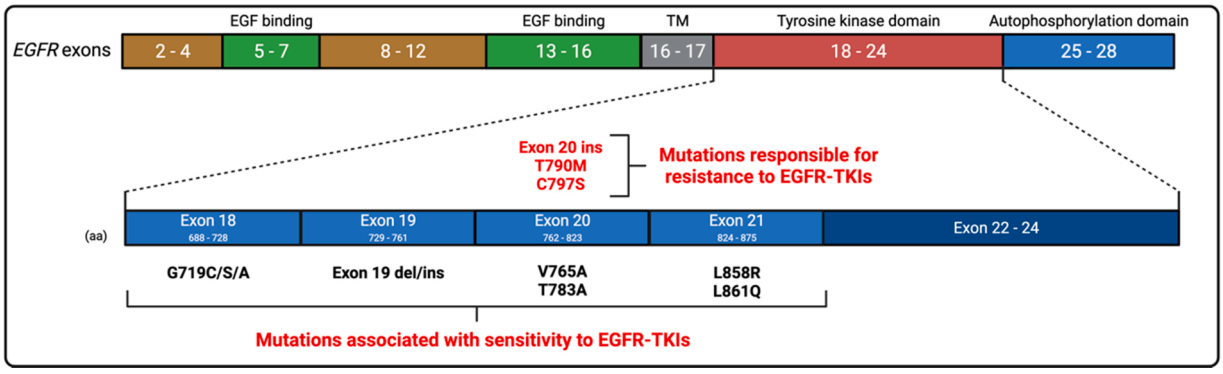

Fig. 2. Structure of the EGFR gene. Activating mutations and resistance mutation are clustered within exons 18 – 21 which encodes the tyrosine kinase domain of the EGFR receptor. Image created with BioRender.com

Table 1
EGFR-TKIs approved for the treatment of *EGFR*-mutant advanced/metastatic NSCLC.

Drug	Manufacturer	Generation	Bond	Spectrum of activity	Approval
Gefitinib (Iressa®)	AstraZeneca	First	Reversible	Mutated and WT-*EGFR*	FDA, EMA
Erlotinib (Tarceva®)	Roche	First	Reversible	Mutated and WT-*EGFR*	FDA, EMA
Icotinib (Conmana®)	Betta Pharmaceuticals	First	Reversible	Mutated and WT-*EGFR*	CNMPA
Afatinib (Gilotrif®)	Boehringer Ingelheim	Second	Irreversible	Pan-ErbB inhibitor	FDA, EMA
Dacomitinib (Vizimpro®)	Pfizer	Second	Irreversible	Pan-ErbB inhibitor	FDA, EMA
Osimertinib (Tagrisso®)	AstraZeneca	Third	Irreversible	Common and T790 M mutations	FDA, EMA
Almonertinib (Amelie®)	Hansoh Pharmaceutical	Third	Irreversible	Common and T790 M mutations	CNMPA

Abbreviations: EGFR-TKI (EGFR-Tyrosine Kinase Inhibitors); NSCLC (Non-Small Cell Lung Cancer); WT (Wild-Type); FDA (U.S. Food and Drugs Administration), EMA (European Medicine Agency); CNMPA (China National Medical Product Administration).

Table 2
Phase III clinical trials of first-generation EGFR-TKIs for *EGFR*-mutant advanced/metastatic NSCLC.

Study name	EGFR-TKI	Comparator arm	ORR (%)	PFS (months)	OS (months)	Reference
IPASS	Gefitinib	Carboplatin + Paclitaxel	67% vs. 41%	9.5 vs. 6.3; $p<0.001$	21.6 vs. 21.9; ns	[20]
WJTOG-3405	Gefitinib	Cisplatin + Docetaxel	62% vs. 32%	9.2 vs. 6.3; $p<0.0001$	34.8 vs. 37.3; ns	[21]
NEJ002	Gefitinib	Carboplatin + Paclitaxel	74% vs. 31%	10.8 vs. 5.4; $p<0.0001$	30.5 vs. 23.6; ns	[22]
EURTAC	Erlotinib	Platinum + Docetaxel or Gemcitabine	64% vs. 18%	9.7 vs. 5.2; $p<0.0001$	22.9 vs. 19.6; ns	[23]
OPTIMAL	Erlotinib	Carboplatin + Gemcitabine	83% vs. 36%	13.1 vs. 4.6; $p<0.0001$	22.8 vs. 27.2; ns	[24]
ENSURE	Erlotinib	Cisplatin + Gemcitabine	63% vs. 34%	11.0 vs. 5.6; $p<0.0001$	21.6 vs. 21.9; ns	[25]
ICOGEN	Icotinib	Gefitinib	27.6% vs. 27.2%	4.6 vs. 3.4; ns	13.3 vs. 13.9; ns	[26]
CONVINCE	Icotinib	Cisplatin + Pemetrexed	N/A	11.2 vs. 7.9; $p=0.006$	30.5 vs. 32.1; ns	[27]

Abbreviations: EGFR-TKI (EGFR-Tyrosine Kinase Inhibitor); NSCLC (Non-Small Cell Lung Cancer); PFS (Progression-Free Survival); OS (Overall Survival); ORR (Objective Response Rate); ns (not significant); N/A (Not Available).

3.1. Gefitinib

Gefitinib (Iressa™; AstraZeneca, London, United Kingdom) initially received approval by the U.S. Food and Drugs Administration (FDA) as second-line therapy for advanced-stage NSCLC patients who progressed after platinum-based chemotherapy [28]. The pivotal phase III IPASS trial (NCT00322452) demonstrated that first-line treatment with gefitinib improves PFS (9.5 months vs. 6.3 months; $p < 0.001$) compared to carboplatin-paclitaxel in a selected cohort of Asian patients with advanced NSCLC [20]. Similar findings were achieved in several phase III trials (NEJ002, WJTOG-3405, IFUM, first-SIGNAL), leading to gefitinib approval by the FDA and the European Medicine Agency (EMA) as first-line therapy for advanced/metastatic NSCLC patients harboring *EGFR*-activating mutations [21,22,29,30].

3.2. Erlotinib

Erlotinib (Tarceva™; F. Hoffman-La Roche, Basel, Switzerland) was initially approved by the FDA for use in unselected advanced/metastatic NSCLC patients who progressed while receiving platinum chemotherapy [31]. Based on results obtained in several randomized phase III clinical trials (EURTAC, OPTIMAL, and ENSURE), the FDA and EMA later extended erlotinib approval to include its use as first-line therapy for *EGFR*-mutated advanced/metastatic NSCLC. These trials demonstrated erlotinib superiority over platinum-containing chemotherapy concerning PFS, along with a more favorable toxicity profile, in NSCLC patients with *EGFR* mutations [23–25].

3.3. Icotinib

Icotinib (Conmana™; Zhejiang Beta Pharmaceutical Co Ltd, Hangzhou, People's Republic of China) is a first-generation EGFR-TKIs that received approval from the Chinese National Medical Products Administration (CNMPA) for the second-line treatment of *EGFR*-mutant advanced/metastatic NSCLC. Approval was granted after the phase III ICOGEN trial (NCT01040780) established that icotinib is non-inferior to gefitinib in patients who progressed after at least one platinum-based chemotherapy regimen and that treatment with icotinib is associated with fewer adverse events [26]. Based on the positive results obtained in the phase III CONVINCE trial (NCT01719536), the CNMPA later extended icotinib approval to include its use as first-line treatment for *EGFR*-mutant advanced/metastatic NSCLC [27].

3.4. Comparison between first-generation EGFR-TKIs

Several clinical trials evaluating the three available first-generation EGFR-TKIs yielded similar results in terms of clinical efficacy. It was therefore presumed that these drugs could be interchangeable when treating NSCLC patients harboring *EGFR* mutations. Nevertheless, while first-generation EGFR-TKIs share a similar mechanism of action, they possess different chemical structures, resulting in different target affinity, metabolism, and toxicity [32]. A summary of the pharmacokinetic profiles of first-generation EGFR-TKIs is provided in Table 3.

Erlotinib is 3-fold less lipophilic than gefitinib, possibly explaining, at least in part, some of the dissimilarities observed in the pharmacokinetic and pharmacodynamic profiles of these two EGFR-TKIs [32]. Remarkably, the bioavailability of erlotinib increases from 60 to 100% when taken with food. Analogously, food absorption increases the bioavailability of icotinib, whereas it does not affect gefitinib bioavailability [32–34].

After oral administration, first-generation EGFR-TKIs undergo extensive hepatic metabolism mediated by enzymes belonging to the cytochrome P450 family and are primarily excreted in feces, with minor contributions in urine [35,36]. Tobacco smoking increases the metabolic clearance of erlotinib due to the induction of enzymes responsible for its metabolism, resulting in decreased therapeutic exposure [37]. Furthermore, a recent study reported that the treatment of PC9 and HCC827 NSCLC cell lines with serum obtained from smokers induced resistance to erlotinib. This observation suggests that nicotine is involved in promoting erlotinib resistance in smokers [38].

The meta-analysis published by Liang et al. was the first study to provide an indirect comparison of gefitinib, erlotinib, and icotinib for the treatment of advanced/metastatic NSCLC patients with *EGFR* mutations based on information obtained from several phase III randomized controlled trials. The authors reported that first-generation EGFR-TKIs share comparable efficacy but presented different efficacy-toxicity patterns [39]. Liu et al. later obtained similar results in their meta-analysis that compared the effectiveness and the rate of adverse events in NSCLC patients treated with first-generation EGFR-TKIs. The authors concluded that these drugs exhibit similar efficacy, although treatment with erlotinib was associated with a higher frequency of adverse events as compared with gefitinib and icotinib [40].

4. Second-generation EGFR-TKIs

Despite the remarkable clinical responses observed in *EGFR*-mutant advanced/metastatic NSCLC patients treated with first-generation EGFR-TKIs, acquired resistance to these compounds inevitably develops after a median period of 10 – 14 months, principally due to the selection of resistant clones harboring the secondary *EGFR*-T790M mutation [41]. Consequently, a second-generation of EGFR-TKIs was developed to address the issue of acquired resistance to first-generation EGFR-TKIs (Fig. 3).

Second-generation EGFR-TKIs are irreversible pan-ErbB inhibitors [42]. Pros of this generation of EGFR inhibitors include a broader inhibitory profile on the ErbB receptor family and a more robust inhibition of downstream signaling [43]. Second-generation EGFR-TKIs approved for the treatment of *EGFR*-mutant advanced/metastatic NSCLC include afatinib and dacomitinib (Table 4) [44].

4.1. Afatinib

Afatinib (Gilotrif™; Boehringer Ingelheim, Ingelheim, Germany) is the first irreversible second-generation EGFR-TKI approved for the treatment of *EGFR*-mutant advanced/metastatic NSCLC [45].

The efficacy and safety of afatinib as first-line therapy for *EGFR*-mutated advanced/metastatic NSCLC was explored in the phase III LUX-Lung 3 (NCT00949650) and LUX-Lung 6 (NCT01121393) trials in comparison with cisplatin-pemetrexed chemotherapy and cisplatin-gemcitabine chemotherapy, respectively [46,47]. In both trials, patients who received afatinib achieved a significantly longer PFS than patients treated with platinum-based chemotherapy, although without substantial improvements in OS [48]. A later subgroup analysis revealed that afatinib-treated patients harboring atypical *EGFR* mutations (L861Q, G719X, or S768I) had a longer OS than chemotherapy-treated patients. This finding led to the extension of afatinib approval to include patients expressing atypical *EGFR* mutations [49,50].

The phase IIb LUX-Lung 7 study (NCT01466660) was the first direct comparison between a first- and a second-generation EGFR-TKI for the treatment of *EGFR*-mutant advanced/metastatic NSCLC in the first-line setting [51]. Clinical results demonstrated that afatinib treatment significantly improves PFS and ORR and has a longer median duration of response (DoR) than gefitinib, although without significant differences in OS between the two treatment arms [52,53].

4.2. Dacomitinib

Dacomitinib (Vizimpro™; Pfizer Inc, New York, USA) is a second-generation EGFR-TKI approved by EMA and FDA for the first-line treatment of patients with *EGFR*-mutant advanced/metastatic NSCLC [44,54]. Dacomitinib was initially investigated as salvage therapy in the phase II/III ARCHER 1028 (NCT00769067) and ARCHER 1009 (NCT01360554) studies in unselected NSCLC patients who progressed while on platinum-based chemotherapy. Results failed to demonstrate the superiority of dacomitinib over erlotinib concerning PFS and OS. However, a subsequent analysis of patient subgroups revealed that dacomitinib has efficacy comparable to that of erlotinib in patients harboring *EGFR*-activating mutations [55–57].

Dacomitinib as first-line treatment was assessed in the phase III ARCHER 1050 trial (NCT01774721) in a selected population of NSCLC patients harboring *EGFR*-activating mutations. Dacomitinib showed benefits over gefitinib concerning both PFS and OS, although with a higher incidence of treatment-related adverse events [58–60]. Since the ARCHER 1050 trial excluded patients with brain metastases, evidence regarding the efficacy of dacomitinib in treating CNS lesions is lacking. Clinical studies performed in China and Japan suggest that dacomitinib might be more active against brain metastases than first-generation EGFR-TKIs. Nevertheless, given the small sample size of these studies, the efficacy of dacomitinib for the treatment of CNS lesions remains unclear [61–63].

5. Third-generation EGFR-TKIs

Afatinib and dacomitinib showed partial efficacy against NSCLC cell lines expressing the *EGFR*-T790M mutation. Nevertheless, these second-generation EGFR-TKIs ultimately failed to overcome resistance in

Table 3
Pharmacokinetic profiles of first-generation EGFR-TKIs approved for treating *EGFR*-mutant NSCLC.

Drug	Dosage	Metabolism	CYP enzymes	Tmax	T1/2	BA	Excretion
Gefitinib (Iressa®)	250 mg/die	Hepatic	CYP3A4, CYP2D6, CYP1A1	3 – 7 h	~ 40 h	~ 60%	~ 86% feces, ~ 4% urine
Erlotinib (Tarceva®)	150 mg/die	Hepatic	CYP3A4, CYP3A5, CYP1A1/2	3 – 4 h	~ 36 h	~ 60%	~ 83% feces, ~ 9% urine
Icotinib (Conmana®)	125 mg thrice/die	Hepatic	CYP3A4, CYP3A5, CYP1A2	1 – 3 h	~ 6 h	~ 60%	>90% feces, ~ 9% urine

Abbreviations: EGFR-TKIs (EGFR – Tyrosine Kinase Inhibitors); NSCLC (Non-Small Cell Lung Cancer); CYP (Cytochrome P450 isoenzymes), Tmax (Time to Maximum Plasma Concentration); T1/2 (Half-Life); BA (Bioavailability).

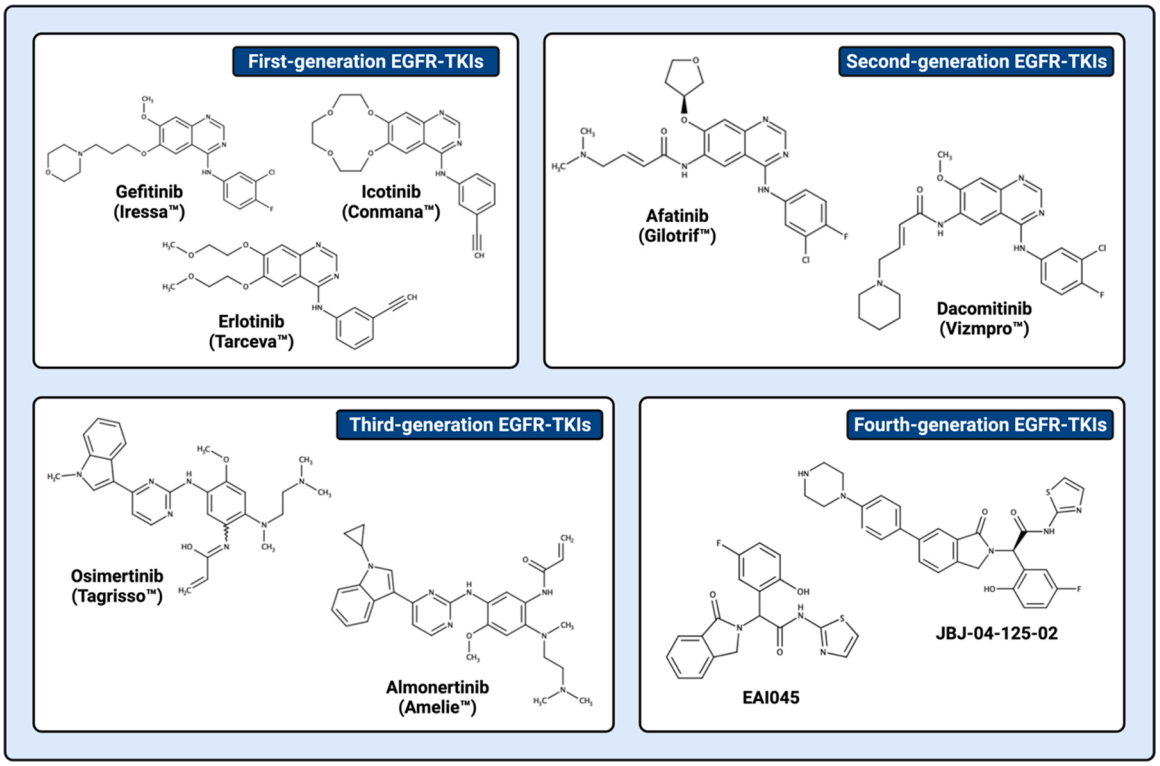

Fig. 3. Overview of the chemical structures of EGFR-TKIs. Three generations of EGFR-TKIs are currently available in the clinic for the treatment of EGFR-mutant advanced/metastatic NSCLC. The fourth generation of allosteric EGFR-TKIs is currently under preclinical evaluation. MarvinSketch™ (ChemAxon) was used for drawing chemical structures. Image created with Biorender.com.

Table 4
Selected clinical trials of second-generation EGFR-TKIs for *EGFR*-mutant advanced/metastatic NSCLC.

Study	Trial design	EGFR-TKI	Comparison	ORR (%)	PFS (months)	OS (months)
LUX-Lung 3 (NCT00949650)	Phase III first-line	Afatinib	Cisplatin + Pemetrexed	56% vs. 23%; $p = 0.001$	11.1 vs. 6.9; $p = 0.001$	*Ex19del*: 33.3 vs. 21.1; $p = 0.0015$ *L858R*: 27.6 vs. 40.3; ns
LUX-Lung 6 (NCT01121393)	Phase III first-line	Afatinib	Cisplatin + Gemcitabine	67% vs. 23%; $p<0.0001$	11.0 vs. 5.6; $p<0.0001$	*Ex19del*: 31.4 vs. 18.4; $p = 0.023$ *L858R*: 19.6 vs. 24.3; ns
LUX-Lung 7 (NCT01466660)	Phase IIb first-line	Afatinib	Gefitinib	70% vs. 56%; $p=0.0083$	11.0 vs. 10.9; $p = 0.017$	*Exon19del*: 30.7 vs. 26.4; ns *L858R*: 25.0 vs. 21.2; ns
ARCHER 1009 (NCT01360554)	Phase III second-line	Dacomitinib	Erlotinib	11% vs. 8%; ns	14.6 vs. 9.6; ns	26.6 vs. 23.2; ns
ARCHER 1028 (NCT00769067)	Phase II second-line	Dacomitinib	Erlotinib	17% vs. 5.3%; $p=0.011$	2.8 vs. 1.9; $p<0.012$	9.53 vs. 7.44; ns
ARCHER 1050 (NCT01774721)	Phase III first-line	Dacomitinib	Gefitinib	75% vs. 72%; ns	14.7 vs. 9.2; $p<0.001$	34.1 vs. 27.0; $p = 0.0438$

Abbreviations: EGFR-TKIs (EGFR – Tyrosine Kinase Inhibitors); NSCLC (Non-Small Cell Lung Cancer); PFS (Progression-Free Survival); OS (Overall Survival); ORR (Objective Response Rate); ns (not significant).

patients since the dosage required to achieve the complete inhibition of T790M-mutant tumors is associated with unacceptable toxicity in patients [64,65].

Third-generation EGFR-TKIs have been specifically designed to overcome the *EGFR*-T790M-mediated resistance by forming an irreversible bond with the C797 residue in the receptor kinase domain, thereby preventing receptor autophosphorylation and engagement of downstream signaling pathways [66]. Two third-generation EGFR-TKIs

Table 5
Selected clinical trials of third-generation EGFR-TKIs for *EGFR*-mutant advanced/metastatic NSCLC.

Study name	Design	EGFR-TKI	Comparator arm	ORR (%)	PFS (months)	OS (months)
AURA3 (NCT02151981)	Phase III (second-line)	Osimertinib	Cisplatin/ Carboplatin + Pemetrexed	71% vs. 31%; $p<0.001$	10.1 vs. 4.4; $p<0.001$	26.8 vs. 22.5; ns
FLAURA (NCT02296125)	Phase III (first-line)	Osimertinib	Gefitinib	80% vs. 76%; ns	18.9 vs. 10.2; $p<0.001$	38.6 vs. 31.8; $p = 0.046$
APOLLO (NCT02981108)	Phase I/II (second-line)	Almonertinib	No	68.9%	12.3	N/A

Abbreviations: EGFR-TKIs (EGFR – Tyrosine Kinase Inhibitors); NSCLC (Non-Small Cell Lung Cancer); PFS (Progression-Free Survival); OS (Overall Survival); ORR (Objective Response Rate); ns (not significant); N/A (Not Available).

received approval for clinical use in NSCLC patients harboring classical *EGFR* or *EGFR*-T790M mutations: osimertinib and almonertinib (Table 5).

5.1. Osimertinib

Osimertinib (Tagrisso™; AstraZeneca, Cambridge, United Kingdom) is an orally administered and irreversible third-generation EGFR-TKI targeting common *EGFR*-activating mutations, as well as the *EGFR*-T790M mutation [66]. In preclinical studies, osimertinib demonstrated remarkable inhibitory activity against NSCLC cell lines harboring the *EGFR*-T790M mutation. This inhibition translated into profound and sustained tumor regression in *EGFR*-mutant tumor xenograft and transgenic models without significant off-target effects due to a lower affinity for the wild-type EGFR receptor [66].

The efficacy and safety of osimertinib as salvage therapy for NSCLC patients who progressed during treatment with first- or second-generation EGFR-TKIs due to the emergence of the *EGFR*-T790M mutation was initially documented in the phase I/II AURA trial and later confirmed in the phase III AURA3 trial (NCT02151981) [67]. In the AURA3 trial, patients treated with osimertinib exhibited longer PFS (10.1 months vs. 4.4 months; HR = 0.30; 95% CI 0.23 to 0.41; $p < 0.001$) and higher ORR (71% vs. 31%) than patients treated with platinum-doublet chemotherapy (cisplatin/carboplatin plus pemetrexed). There were no significant differences in OS among the two treatment arms [67,68].

The phase III FLAURA trial (NCT02296125) explored the efficacy of safety of osimertinib as first-line therapy for *EGFR*-mutant advanced/metastatic NSCLC [69,70]. First-line treatment with osimertinib was associated with a significantly longer PFS (18.9 months vs. 10.2 months; HR = 0.46; 95% CI 0.37–0.57; $p < 0.001$) and OS (38.6 months vs 31.8 months; 95% CI 26.6–36.0; HR = 0.80; 95% CI 0.64–1.00; $p = 0.046$), although without significant differences in ORR as compared with first-generation EGFR-TKIs [70,71]. Despite a longer duration of exposure, patients treated with osimertinib had lower rates of serious adverse effects than patients who received first-generation EGFR-TKIs (34% vs. 45%) [70]. As a result, osimertinib received approval by EMA and FDA as first-line therapy and as second-line therapy for patients who progressed during treatment with EGFR-TKIs due to the acquisition of the *EGFR*-T790M mutation [9].

Insights obtained from the AURA3 trial highlighted that osimertinib possesses remarkable activity against brain metastases in *EGFR*-T790M-positive patients who progressed during treatment with first-generation EGFR-TKIs [72]. Patients with brain metastases who received osimertinib achieved a longer median CNS PFS than patients treated with platinum-pemetrexed chemotherapy (11.7 vs. 5.6 months; 95% CI 0.15–0.69; $p = 0.004$), irrespective of prior brain radiotherapy [72].

The FLAURA study further established the CNS activity of osimertinib. In this study, the risk of CNS progression in osimertinib-treated patients was consistently lower as compared with patients treated with first-generation EGFR-TKIs (20% vs. 39%), as well as the onset of new brain metastases (12% vs. 30%), hence supporting the protective role of osimertinib in the development of CNS lesions [73]. Besides, osimertinib treatment has demonstrated higher ORR and survival benefits in *EGFR*-T790M-positive NSCLC patients harboring brain metastases and leptomeningeal metastases who progressed on prior therapy with EGFR-TKIs [74].

5.2. Almonertinib

Almonertinib (Amelie™, Hansoh Pharmaceutical Group, Jiangsu, People's Republic of China) is a novel orally-available, pyrimidine-based, third-generation EGFR-TKI with high selectivity and potent inhibitory activity against both *EGFR*-sensitizing and *EGFR*-T790M mutations [75].

The efficacy and safety of almonertinib for NSCLC patients with *EGFR* mutations who had previously received treatment with first- or second-generation EGFR-TKIs was explored in the phase I/II APOLLO trial (NCT02981108) [75]. Results demonstrated that almonertinib is safe and well-tolerated in patients. Median PFS in almonertinib-treated patients was 11.8 months, and ORR was 68.9% [76]. Consequently, the CNMPA approved almonertinib for the second-line treatment of *EGFR*-mutant advanced/metastatic NSCLC in patients harboring the *EGFR*-T790M mutation [77].

Almonertinib as first-line treatment for *EGFR*-mutant advanced/metastatic NSCLC is currently under investigation in the AENEAS phase III study (NCT03849768). Preliminary results showed that almonertinib significantly prolonged median PFS (19.3 vs. 9.9 months; HR = 0.46; $p < 0.0001$) and DoR (18.1 vs. 8.3 months; HR = 0.38; $p < 0.0001$) over gefitinib treatment. Despite an overall longer treatment duration, almonertinib was well-tolerated in patients and exhibited a favorable safety profile [78].

The APOLLO study yielded insights concerning the efficacy of almonertinib in treating patients with CNS metastases. Median PFS in patients with CNS lesions was 10.8 months, with a median DoR of 11.3 months. CNS disease control rate (DCR) and CNS ORR were 91.3% and 60.9%, respectively [76]. High-dose almonertinib as first-line therapy for *EGFR*-mutant NSCLC patients with CNS metastases will be further investigated in the phase II ACHIEVE trial (NCT04808752).

6. Fourth-generation EGFR-TKIs

Despite the impressive clinical responses observed in patients treated with the third-generation of EGFR inhibitors, either as first-line therapy or as second-line treatment for patients who progressed while on treatment with first- or second-generation EGFR-TKIs, the selection of resistant clones expressing additional mutations in the tyrosine kinase domain of the EGFR receptor limits the efficacy of *EGFR*-T790M-selective EGFR-TKIs. Treatment failure and disease progression after treatment with third-generation EGFR-TKIs usually occur within a year, mainly due to the emergence of tertiary *EGFR*-C797S mutation [79].

A novel class of allosteric, mutant-selective, fourth-generation EGFR inhibitors has been developed with the intent to overcome acquired resistance to third-generation EGFR-TKIs [80–82]. Allosteric EGFR inhibitors target the allosteric site of the EGFR receptor, which is situated away from the ATP binding site commonly targeted by classical EGFR inhibitors. Remarkably, the *EGFR*-T790M and *EGFR*-C797S mutations do not affect the allosteric site of the EGFR receptor. Therefore, these mutations do not influence the efficacy of allosteric EGFR-TKIs [83].

EAI045 is the first allosteric EGFR inhibitor designed to target drug-resistant *EGFR*-T790M and *EGFR*-C797S mutants [84]. EAI045 exhibited potent inhibitory activity in L858R/T790M-mutant NSCLC H1975 cell line, although it failed to block EGFR autophosphorylation. Furthermore, EAI045 did not show activity in a keratinocyte cell line expressing wild-type EGFR, suggesting that this drug is selective for mutant EGFR only [85]. Since EGFR dimerization is a mandatory step for activating EGFR signaling, investigators hypothesized that EAI045 was inactive for asymmetric dimers between wild-type and mutant EGFR. These confirmed that EAI045 is active and selective for EGFR mutants in a monomer state and dimerization-defective EGFR mutants [85].

Remarkable synergy was observed *in vitro* between EAI045 and cetuximab (an anti-EGFR monoclonal antibody that blocks EGFR dimerization by physically binding the receptor) in NSCLC cell lines bearing the double L858R/T790 M mutation. Furthermore, EAI045 combined with cetuximab potently inhibited Ba/F3 cells harboring the triple L858R/T790 M/C797S mutation [85]. *In vivo*, treatment with EAI045 in combination with cetuximab led to significant tumor shrinkage in a genetically engineered mouse model of double L858R/T790 M and triple L858R/T790 M/C797S mutants, while mice treated with EAI045 alone failed to respond to treatment [85]. These results indicate that EAI045, combined with cetuximab, is active against lung cancers resistant to all EGFR-targeted therapies.

JBJ-04-125-02 is another allosteric EGFR-TKI reported to be more potent than EAI045 in inhibiting the proliferation of NSCLC cells harboring L858R/T790 M/C797S mutations [86]. Dual EGFR targeting with the combination of JBJ-04-125-02 and osimertinib enhanced apoptosis and delayed the onset of drug resistance compared to either single agent alone, both *in vitro* and *in vivo* [86].

While it is unlikely that allosteric EGFR inhibitors will be effective as single-agent treatment as they do not abrogate mutated EGFR signal transduction due to receptor dimerization, preclinical data suggest that the combination of a traditional EGFR-TKI with an allosteric EGFR inhibitor could enhance the overall antitumor response and possibly prevent the emergence of acquired resistance [85,86]. Still, more research is required to validate the use of allosteric EGFR inhibitors for the treatment of *EGFR*-mutant advanced/metastatic NSCLC.

7. Resistance to EGFR-TKIs

Several mechanisms responsible for driving acquired resistance to EGFR-TKIs have been identified. These can be categorized into either *EGFR*-dependent or *EGFR*-independent resistance mechanisms (Fig. 4) [87].

Mutations conferring resistance to EGFR-TKIs usually arise due to the treatment-dependent selective pressure exerted on cancer cells, leading to the selection of resistant clones harboring additional mutations which confer a survival advantage. However, these molecular aberrations have also been detected in EGFR-TKI-naïve patients. Furthermore, some of these mechanisms may overlap in resistant clones, depending on whether the EGFR-TKI treatment is administered as first or second-line therapy [88–91].

8. EGFR-dependent resistance

8.1. Primary EGFR mutations – intrinsic resistance to first- and second-generation EGFR-TKIs

Approximately 20-30% of patients with *EGFR* mutations treated with first-generation EGFR-TKIs fail to respond or respond only for a short time due to intrinsic resistance to EGFR inhibitors [92].

In-frame exon 20 insertions account for 5-10% of all *EGFR* mutations detected in NSCLC patients and are associated with intrinsic resistance to first-generation EGFR-TKIs [91]. Interestingly, patients harboring exon 20 insertions do not respond to first-generation EGFR-TKIs but have a similar OS to patients with common *EGFR*-activating mutations [93]. Exon 20 insertions have also been associated with reduced response to third-generation EGFR-TKIs such as osimertinib [94].

8.2. Secondary EGFR mutations – acquired resistance to first- and second-generation EGFR-TKIs

The secondary EGFR-T790M mutation is detected in approximately 50-60% of patients treated with first- or second-generation EGFR-TKIs at progression [41]. This mutation causes a threonine-to-methionine substitution at position 790 in exon 20 of the *EGFR* gene, determining a conformational change in the ATP-binding pocket of the kinase domain that sterically hinders the binding of first- and second-generation EGFR-TKIs. *EGFR*-T790M mutation also enhances the catalytic activity of the EGFR receptor by increasing its affinity for ATP, thus conferring a survival advantage to mutant cells [95]. Other less common *EGFR* mutations associated with secondary resistance to EGFR inhibitors include L747S, D761Y, and T854A [96].

Loss of *EGFR*-T790M is present in roughly half of the patients treated with second-line osimertinib at the time of progression. *EGFR*-T790M loss has been linked with early resistance to second-line osimertinib (usually associated with the emergence of EGFR-independent resistance mechanisms such as *MET/HER2* amplification, *KRAS* mutations, small-cell transformation, and gene fusions) [79,97,98]. In contrast, patients who develop late resistance to third-generation EGFR-TKIs are more likely to have conserved the *EGFR*-T790M mutation and acquired additional *EGFR* mutations (e.g., tertiary *EGFR*-C797S mutation) or *EGFR* amplification [98].

8.3. Tertiary EGFR mutations - resistance to third-generation EGFR-TKIs

EGFR-C797S mutation is the most common tertiary *EGFR* mutation, causing resistance to third-generation EGFR-TKIs [79]. This mutation replaces the cysteine in position 797 with a serine, resulting in the loss of

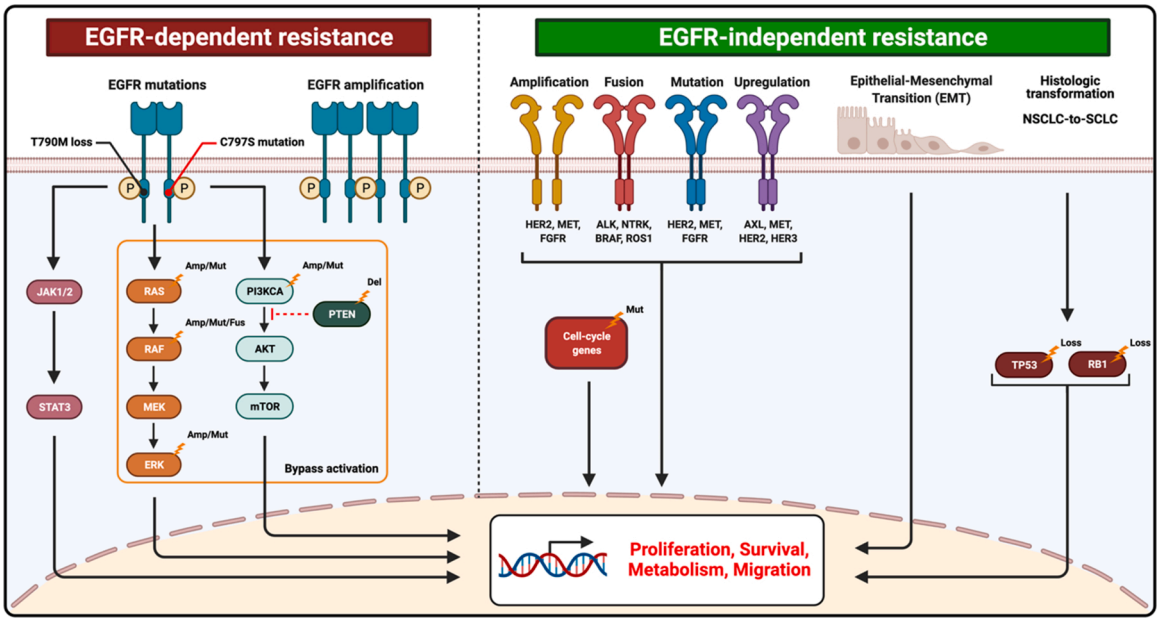

Fig. 4. Schematic representation of molecular mechanisms involved in resistance to EGFR-TKIs. Acquired resistance to EGFR-TKIs may be divided either into EGFR-dependent (e.g., EGFR amplification or mutations) or EGFR-independent (e.g., additional gene amplifications and mutations, oncogenic fusions, bypass pathway activation of RAS-MAPK and PI3K/Akt/mTOR, mutations affecting cell-cycle genes, oncogene fusions, activation of the epithelial-to-mesenchymal (EMT) transition, and histologic transformation) [79]. Image created with BioRender.com.

the covalent bond between the mutant receptor and third-generation EGFR-TKIs. *EGFR*-C797S mutation accounts for 10-25% of cases of resistance to second-line osimertinib and approximately 7% of cases resistance to first-line osimertinib [79]. Additional tertiary *EGFR* mutations associated with resistance to third-generation EGFR-TKIs include G796X, L729X, L718Q, G719X, G724S, and S768I mutations [99].

The allelic context in which the *EGFR*-C797S mutation arises has consequences for treatment with EGFR-TKIs. In this regard, the rare emergence of the C797S mutation in *trans* with T790 M mutation confers susceptibility to EGFR-TKIs, making it possible to hit both C797S and T790 M alleles using first- and third-generation EGFR-TKIs, respectively. In contrast, the development of the *EGFR*-C797S mutation in *cis* with the *EGFR*-T790M mutation confers resistance to all available EGFR inhibitors [100–102].

9. EGFR-independent mechanisms

EGFR-independent mechanisms resistance to EGFR-TKIs may include the activation of alternative bypass pathways due to the emergence of mutations downstream of EGFR, oncogenic gene fusions, gene amplification, mutations affecting genes encoding for cell-cycle proteins, activation of the epithelial-to-mesenchymal transition (EMT), as well as the NSCLC to SCLC histologic transformation [91].

Multiple competing EGFR-independent resistance mechanisms may co-exist within the same tumor, reflecting the peculiar complexity and heterogeneity of NSCLC in response to treatment with EGFR-TKIs [103,104].

9.1. MET and HER2 amplification

MET gene amplification is the most common cause of bypass pathway activation involved in acquired resistance to EGFR-TKIs since it provokes the EGFR-independent phosphorylation of the ErbB3 receptor, resulting in the subsequent bypass activation of the PI3K/Akt/mTOR pathway [105,106]. *MET* amplification is detected in approximately 5-22% of NSCLC patients who progress on first-line treatment with first- or second-generation EGFR-TKIs and is associated with poor prognosis [105]. Recent data from the AURA3 and FLAURA studies revealed that *MET* amplification is present in roughly 15% of patients treated with first-line osimertinib at the time of progression [97,107].

HER2 gene amplification is another EGFR-independent mechanism causing acquired resistance to EGFR-TKIs. This alteration has been detected in approximately 2% of patients who acquired resistance after treatment with first-line osimertinib and in 5% of patients treated with second-line osimertinib [97,107]. In a small number of cases, HER2 amplification was found to co-exist with EGFR-L792X + C797X + PIK3CA amplification (in 1% of cases), EGFR-G796S + MET amplification (1%), and PIK3CA amplification (1%) [97]. Recently, HER2 amplification has been reported in a patient who experienced intrinsic resistance to osimertinib [108].

9.2. Bypass activation – RAS-MAPK and PI3K/Akt/mTOR pathways

Aberrations involving the RAS-MAPK pathway are known to confer resistance to third-generation EGFR-TKIs in patients with *EGFR*-mutant NSCLC. Accordingly, several *KRAS* mutations (G12S, G12D, G13D, Q61R, and Q61 K) have been associated with the emergence of acquired resistance to osimertinib, both in the first- and second-line settings [79]. *BRAF V600E* is another mutation that affects the activation of the RAS-MAPK pathway. This aberration has been detected in 3% of patients at the time of progression with first- or second-line osimertinib [107,109,110].

Bypass activation of the PI3K/Akt/mTOR pathway may occur either through *PI3KCA* mutations or amplification but also as a result of *PTEN* deletion [111]. In NSCLC, *PI3KCA* mutations are often present in concomitance with mutations affecting other oncogenic driver genes (e.g., *EGFR* and *KRAS* mutations) and represent a poor prognostic factor [112]. Several *PI3KCA* mutations are associated with resistance to second-line osimertinib. These include the E545 K, E542 K, R88Q, N345 K, and E418 K mutations, which occur at a frequency of 4–11% [79].

9.3. Cell-cycle gene alterations and gene fusions

Aberrations affecting genes encoding for cell-cycle proteins such as cyclin D1, cyclin D2, cyclin E1, cyclin-dependent kinase (CDK) 4 and CDK6, and the CDK inhibitor 2A have been detected in approximately 10-12% of patients treated with first- or second-line osimertinib at the time of progression, and associate with poor clinical outcomes [79].

Oncogenic gene fusions have been identified in 3-10% of cases of acquired resistance to second-line osimertinib and can co-exist with *EGFR*-C797S, *BRAF* mutations, and *MET* amplification [79]. Examples of these molecular aberrations include *GFR3–TACC3* and *RET–ERC1* [97]. Furthermore, several other oncogenic gene fusions (e.g., *CCDC6–RET*, *NTRK1–TPM3*, *NCOA4–RET*, *GOPC-ROS1*, *AGK–BRAF*, and *ESYT2–-BRAF*) are potentially involved in acquired resistance to second-line osimertinib [79].

The *SPTBN1-ALK* fusion has been reported in a patient who developed resistance after treatment with first-line osimertinib, but not in patients treated with osimertinib as second-line therapy [97,107]. The oncogenic *EML4-ALK* gene fusion is associated with resistance to second-line osimertinib [113]. Lastly, another study reported the novel oncogenic *PLEKHA7-ALK* gene fusion as a determinant of resistance following treatment with second-line osimertinib [114].

9.4. Histologic and phenotypic transformation

The histologic transformation from NSCLC to SCLC (small-cell lung cancer) is a known mechanism of resistance to first-generation EGFR-TKIs that arises in approximately 4-15% of patients treated with EGFR-TKIs [79,115]. This transformation dramatically impacts patients' prognosis and has also been reported to confer resistance to osimertinib [116]. Although a comprehensive understanding of the underlying mechanisms responsible for histologic transformation is missing, Lee et al. found that the complete inactivation of tumor suppressor genes such as *TP53* and *RB1* represent a predisposing factor for histologic transformation [117].

Resistance to osimertinib is associated with the expression of EMT transcription factor TWIST-1 in NSCLC cells harboring *EGFR*-activating mutations [118]. In accordance, cells samples obtained from advanced/metastatic NSCLC patients who acquired resistance to first- and third-generation EGFR-TKIs exhibit EMT features without the presence of additional *EGFR* mutations. EMT features include a reduction in the expression of epithelial cell junction proteins (e.g., E-cadherin) and a concomitant increase expression of mesenchymal markers (e.g., vimentin) [119].

9.5. Other EGFR-independent resistance mechanisms

Additional EGFR-independent resistance mechanisms may include fibroblast growth factor 2 (FGF2) amplification, AXL overexpression, as well as amplification of SRC family kinases (SFKs) and focal adhesion kinase (FAK) [111,120].

10. What's next? - EGFR-TKIs combination therapies

Acquired resistance to EGFR-TKIs poses a significant challenge in long-term clinical responses given the scarcity of effective pharmacological interventions for patients who progress after the failure of treatment with third-generation EGFR-TKIs. Therefore, effective therapies for patients who experience disease progression due to the emergence of resistance continue to remain elusive.

The combination of EGFR-TKIs with other anticancer agents (e.g., chemotherapy, radiotherapy, and targeted therapies) has recently emerged as a strategy to avoid, or at least delay, the emergence of acquired resistance to EGFR-TKIs with the intent to improve clinical outcomes in lung adenocarcinoma patients whose tumors express *EGFR*-activating mutations (Fig. 5).

11. EGFR-TKIs in combination with chemotherapy

Several double-blind, randomized, phase III studies (INTACT-1, INTACT-2, TALENT, and TRIBUTE) evaluated the efficacy of EGFR-TKIs (gefitinib or erlotinib) with concomitant cytotoxic platinum-based chemotherapy as first-line therapy for patients with advanced/metastatic NSCLC. However, results obtained were disappointing as these trials failed to demonstrate clinical benefits in patients treated with this combination [121–124].

Two hypotheses have been proposed to explain the failure of these trials: lack of adequate patient selection based on the *EGFR* status and the administration schedule of chemotherapeutic agents [125,126]. Unfortunately, no proper optimization of scheduling was performed before the initiation of these randomized studies.

The hypothesis that patient selection based on *EGFR* status influences clinical outcomes is supported by subgroup analyses of several phase III studies. These reported improvements in PFS and OS for the combination treatment over chemotherapy alone in patients with adenocarcinoma histology and never-smokers (characteristics usually associated with *EGFR*-activating mutations) [122–124].

11.1. Preclinical studies

Pemetrexed is a multitargeted antifolate drug commonly used in NSCLC treatment, either as a single agent or in combination with other chemotherapeutics, in the first-line, second-line, or maintenance settings [127,128]. Pemetrexed and EGFR-TKIs have different mechanisms of action and minimal overlapping toxicity profiles. Therefore, the combined treatment with these two drugs with proper scheduling is expected to exert a synergistic anticancer effect with minimal additional toxicity.

The sequence of administration of erlotinib and pemetrexed influences the cytotoxicity of this combination. A rational explanation for the antagonistic effect is the erlotinib-dependent induction of cell cycle arrest in the G1 phase that confers protection against the cytotoxic activity of pemetrexed [129–131]. The synergistic interaction between EGFR-TKIs and pemetrexed was postulated to be related to the modulation of EGFR and Akt phosphorylation and the inhibition of thymidylate synthase (TS) expression and activity [132–134].

Paclitaxel is another well-established anticancer drug used for treating a variety of solid tumors, including NSCLC [135]. Similar to the experience on the combination of pemetrexed with EGFR-TKIs, the sequential administration of paclitaxel followed by gefitinib exhibits a synergistic effect in lung adenocarcinoma cell lines expressing mutant EGFR receptor [136]. In contrast, the reverse sequence (gefitinib followed by paclitaxel) resulted in an antagonist effect as the exposure of cancer cells to gefitinib induces cell cycle arrest in the G1 phase and therefore protects cells from subsequent paclitaxel-mediated cytotoxicity. Moreover, the sequential combination of paclitaxel plus gefitinib determines an increase in the levels of phospho-EGFR that makes cancer cells more susceptible to treatment with EGFR inhibitors [136].

11.2. EGFR-TKIs plus chemotherapy in unselected NSCLC patients

The phase II/III FASTACT-1 and FASTACT-2 (NCT00883779) trials explored the efficacy of the sequential combination of EGFR-TKIs with platinum-based doublet chemotherapy (gemcitabine plus carboplatin or cisplatin) in an unselected population of chemotherapy-naïve advanced/metastatic NSCLC patients [137]. In FASTACT-1, patients who received the combination exhibited a significantly longer PFS, and this benefit was consistent across all clinical subgroups. There were no differences between the two treatment arms concerning OS [137].

These results were later confirmed in the FASTACT-2 study. Patients treated with the combination had a significantly longer PFS (7.6 months vs. 6.0 months, HR = 0.57; 95% CI 0.47–0.69; $p < 0.0001$) and OS (18.3 months vs. 15.2 months; HR = 0.79; 95% CI 0.64–0.99; $p = 0.0420$) than patients receiving chemotherapy plus placebo [138]. A later biomarker analysis confirmed that only patients harboring *EGFR* mutations benefited from the combination treatment concerning PFS (16.8 months

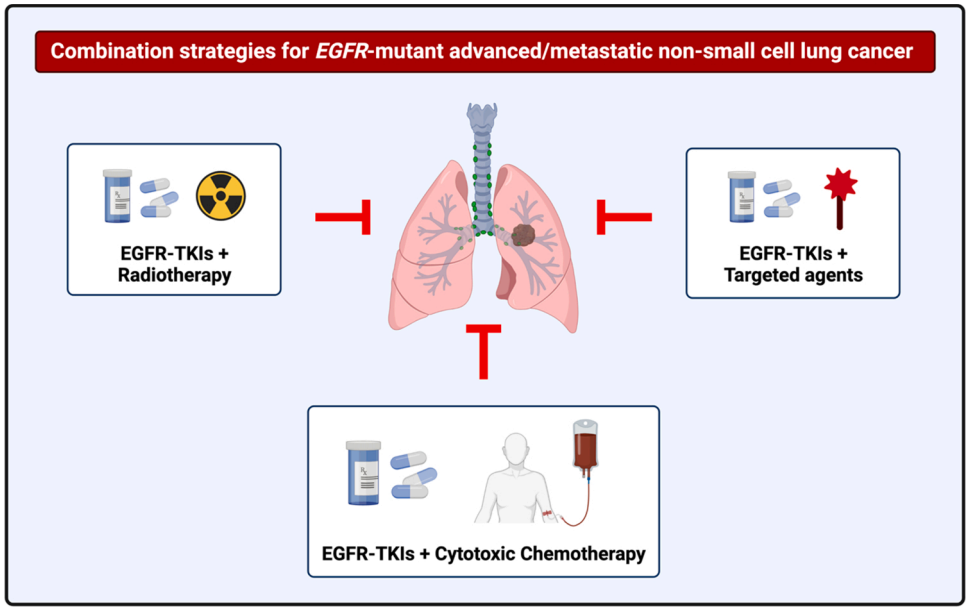

Fig. 5. Overview of possible combination therapies for the treatment of EGFR-mutant advanced/metastatic NSCLC. Several clinical trials are exploring the feasibility of these combination strategies for treating advanced/metastatic NSCLC patients whose tumors express EGFR-activating mutations. Image created with BioRender.com

vs. 6.9 months; HR = 0.25; 95% CI 0.16–0.39; $p < 0.0001$) and OS (31.4 months vs. 20.6 months; HR = 0.48; 95% CI 0.27–0.84; $p = 0.0092$) [139].

Several clinical trials reported promising results concerning the efficacy and safety of EGFR-TKIs in combination with platinum/pemetrexed chemotherapy as first-line therapy for *EGFR*-mutant advanced/metastatic NSCLC (Table 6) [140–146].

A recent meta-analysis by Wu et al. reviewed the results obtained in eight randomized clinical trials (involving 1349 patients with *EGFR*-mutant NSCLC) exploring the efficacy of first-generation EGFR-TKIs combined with platinum-doublet chemotherapy (carboplatin or cisplatin plus pemetrexed) versus EGFR-TKI monotherapy as first-line therapy [147]. Combination treatment was associated with better PFS and OS than EGFR-TKI monotherapy (pooled HR of PFS and OS for the combination therapy was 0.56; 95% CI 0.50–0.64; $p < 0.00001$, and 0.70 (95% CI 0.54–0.90; $p = 0.005$, respectively). ORR in the combination group was significantly higher than in the EGFR-TKI monotherapy group (RR 1.18; 95% CI 1.10–1.26). Although clinically manageable, the addition of chemotherapy to EGFR-TKI monotherapy was associated with a higher incidence of chemotherapy-induced toxicity [147].

11.3. Third-generation EGFR-TKIs plus chemotherapy

To date, very little preclinical data is available about the efficacy and safety of the combination of third-generation EGFR-TKIs (e.g., osimertinib and almonertinib) with chemotherapy. Recently, La Monica et al. showed that the combination of osimertinib with cisplatin-pemetrexed chemotherapy inhibits cell proliferation and induces cell death in *EGFR*-T790M-positive PC9 and HCC827 NSCLC cell lines and NSCLC *EGFR*-T790M PC9 nude mice xenografts. Moreover, combination treatment delayed the emergence of acquired resistance to EGFR-TKIs [148].

EGFR-TKIs, have been reported to interact with ABC transporters, either as substrates or inhibitors [149–152]. ABC transporters are responsible for the efflux of chemotherapeutic agents and the emergence of multidrug-resistant (MDR) phenotypes. In this regard, Wu et al. reported that almonertinib reverses the MDR mediated by the ABCB1 (P-glycoprotein) drug transporter by inhibiting the transporter at submicromolar concentrations, hence re-sensitizing ABCB1-overexpressing cells to conventional cytotoxic agents [153]. This finding is in line with a previous report by Hsiao et al. that found that osimertinib inhibits the ABCB1 transporter without affecting its expression levels, enhances drug-induced apoptosis, and reverses the MDR phenotype in ABCB1-overexpressing cancer cells [154].

Overall, these findings have implications for the choice of chemotherapy as the expression of drug transporters might be able to predict synergism between EGFR-TKIs and cytotoxic drugs.

Recently, Okamoto et al. reported the results of the first randomized phase II study comparing the efficacy and safety of the combination of osimertinib with carboplatin-pemetrexed as second-line therapy for patients who progressed after developing acquired resistance to EGFR-TKIs due to the emergence of the secondary *EGFR*-T790M mutation [155]. The combination of osimertinib and carboplatin-pemetrexed failed to significantly prolong PFS (HR = 1.09; 95% CI 0.51–2.32; $p = 0.83$). Median PFS was 15.8 months (95% CI 7.6–25.9 months) for patients in the osimertinib group and 14.6 months (95% CI 12.9–26.4 months) in the combination group. Analysis of OS and ORR did not yield results supporting the combination treatment. Despite an expected higher frequency of adverse events, the combination of osimertinib with chemotherapy was generally tolerable [155].

The combination of osimertinib with platinum-based chemotherapy holds the promise to represent a realistic strategy to delay the emergence of resistance and achieve long-term clinical responses, especially for patients harboring CNS metastases given the higher CNS activity of third-generation EGFR-TKIs. However, available clinical data concerning the combination of osimertinib plus chemotherapy is limited and mostly refers to anecdotal case reports, emphasizing the necessity of additional evaluation in randomized phase III clinical trials [156–160].

12. EGFR-TKIs combined with radiotherapy (RT)

Brain metastases (BMs) are a common life-threatening complication of NSCLC, causing neurological symptoms and severely impacting both the quality of life and survival of patients. Up to 70% of patients whose tumors harbor *EGFR*-activating mutations develop BMs [161–163]. Radiotherapy (RT) is a treatment commonly used in NSCLC therapy [164]. NSCLC patients with multiple BMs are treated with whole-brain radiation therapy (WRBT) to reduce neurological symptoms, whereas patients with few BMs may be eligible to receive ablative treatment with either stereotactic radiosurgery (SRS) or surgery [165].

Treating and preventing the development of BMs represents a challenge since most traditional cytotoxic agents and novel tyrosine kinase inhibitors do not pass the blood-brain barrier (BBB) and do not accumulate within the brain due to increased efflux. First- and second-generation EGFR-TKIs have limited activity against BMs due to poor drug diffusion within the CNS. Interestingly, the BBB permeability of first-generation EGFR-TKIs is increased during brain RT due to radiation-induced disruption of the barrier, leading to higher drug concentration in the brain [166]. Third-generation EGFR-TKIs (e.g., osimertinib and almonertinib) have demonstrated noticeable activity against BMs in NSCLC patients with *EGFR* mutations due to their higher diffusion within the brain [167].

Since the combination of EGFR-TKIs with RT might exert a synergistic antitumor effect, this strategy is receiving increasing attention for the treatment of *EGFR*-mutant NSCLC patients with BMs.

12.1. Preclinical studies of EGFR-TKI plus radiotherapy

Several preclinical studies indicated that EGFR-TKIs possess radiosensitizing effects on cancer cells [168–172]. Wang et al. recently reported that osimertinib, in combination with radiotherapy, significantly

Table 6
Trials involving first-line treatment with EGFR-TKIs plus chemotherapy in *EGFR*-mutant advanced/metastatic NSCLC.

Study	Phase	Treatment	Combination	Comparison	ORR	PFS (months)	OS (months)
CALGB 30406	II	Paclitaxel/Carboplatin + Erlotinib	Concurrent	Erlotinib	73% vs. 70%	17.2 vs. 14.1	38.1 vs. 31.3
Han et al	II	Pemetrexed/Carboplatin + Gefitinib	Sequential	Gefitinib	82.5% vs. 65.9%	17.5 vs. 11.9	32.6 vs. 25.8
Xu et al.	II	Pemetrexed/Carboplatin + Icotinib	Sequential	Icotinib	77.8% vs. 64.0%	16.0 vs. 10.0	36.0 vs. 34.0
An et al	II	Pemetrexed + Gefitinib	Sequential	Gefitinib	80.0% vs. 73.3%	18 vs. 14	34 vs. 32
Cheng et al.	II	Pemetrexed + Gefitinib	Concurrent	Gefitinib	80.2% vs. 73.8%	15.8 vs. 10.9	43.4 vs. 36.8
NEJ009	III	Pemetrexed/Carboplatin + Gefitinib	Concurrent	Gefitinib	84% vs. 67%	20.9 vs. 11.9	50.9 vs. 38.8
Noronha	III	Pemetrexed/Carboplatin + Gefitinib	Concurrent	Gefitinib	75.3% vs. 62.5%	16.0 vs. 8.0	NR vs. 17.0
Yang et al.	III	Pemetrexed/Cisplatin + Gefitinib	Sequential	Gefitinib	65.4% vs. 70.8%	12.9 vs. 16.6	32.4 vs. 45.7

Abbreviations: EGFR-TKIs (EGFR – Tyrosine Kinase Inhibitors); NSCLC (Non-Small Cell Lung Cancer); PFS (Progression-Free Survival); OS (Overall Survival); ORR (Objective Response Rate); NR (Not Reached).

decreased the proliferation of NSCLC cells harboring the T790 M/L858R *EGFR* mutations, reduced G2/M phase cell cycle arrest, and blocked the irradiation-induced DNA double-strand breaks (DSBs) repair, both *in vitro* and *in vivo* [172].

Baumann et al. reviewed and summarized the principal mechanisms behind the synergistic interaction between EGFR-TKIs and RT. These include the direct killing of cancer stem cells (CSCs), cellular radiosensitization via impaired signal transduction, suppression of DSBs repair, reduced repopulation, and improved reoxygenation during fractionated radiotherapy [173].

12.2. EGFR-TKIs plus radiotherapy in the clinical practice

Insights gathered in preclinical studies suggest that the addition of RT to EGFR-TKI treatment might potentiate the antitumor activity of EGFR-TKIs in advanced/metastatic NSCLC patients with *EGFR* mutations, hence providing a rationale for employing this combination in the clinical setting. Initial retrospective study of EGFR-TKIs plus brain RT found no survival benefit over EGFR-TKI monotherapy in *EGFR*-mutant NSCLC patients with BMs [174,175]. Nonetheless, several later studies indicated that first-generation EGFR-TKIs in combination with brain RT might indeed be more effective than EGFR-TKI monotherapy for treating NSCLC patients with BMs [176–179].

Dong et al. reviewed the efficacy of this combination as first-line therapy in a meta-analysis that analyzed the results obtained in 12 retrospective studies performed in 1533 *EGFR*-mutant NSCLC patients with BMs who received either EGFR-TKIs plus brain RT (mainly WBRT) or EGFR-TKI monotherapy [180]. Combination treatment was associated with statistically significant improvements in OS (HR = 0.64, 95% CI 0.52–0.78; $p < 0.001$) and iPFS (HR = 0.62, 95% CI 0.50–0.78; $p < 0.001$) as compared to EGFR-TKI monotherapy [180]. Although promising, these findings need to be interpreted with caution as they are not based on randomized controlled trials and have several limitations, including insufficient subgroup analysis, bias towards Asian populations, and lack of assessment of treatment-related adverse events. Therefore, additional randomized clinical trials are needed to clarify the feasibility of this strategy.

Yang et al. recently published the results of a randomized phase III clinical trial (NCT01887795), assessing the efficacy and safety of erlotinib with concurrent WBRT as first-line treatment in NSCLC patients harboring BMs and expressing either *EGFR*-mutant or *EGFR*-wt receptor. Results failed to demonstrate clinical benefits in patients treated the combination over patients treated with erlotinib alone [181]. In comparison with the WBRT-alone group, median PFS and OS in the combination group were 5.3 vs. 4.0 months ($p = 0.825$) and 12.9 vs. 10.0 months ($p = 0.545$), respectively. Median iPFS for WBRT concurrent erlotinib was 11.2 months vs 9.2 months for WBRT-alone ($p = 0.601$). In *EGFR*-mutant patients, iPFS (14.6 vs. 12.8 months; $p = 0.164$), PFS (8.8 vs. 6.4 months; $p = 0.702$), and OS (17.5 vs. 16.9 months; $p = 0.221$) [181].

12.3. Third-generation EGFR-TKIs plus radiotherapy

Third-generation EGFR-TKIs are more effective in treating BMs due to higher penetration and activity within the CNS. Results obtained in the AURA3 and FLAURA studies demonstrate that osimertinib prolongs the median CNS PFS compared with platinum-based chemotherapy, regardless of prior brain radiotherapy [72,73,182]. Since osimertinib has radio-sensitizing effects on *EGFR*-mutant NSCLC cell lines, combining RT with osimertinib might be a feasible strategy to improve the efficacy of this combination [172]. Nevertheless, clinical data concerning this combination is limited, emphasizing the need for large randomized controlled trials in selected populations of metastatic NSCLC patients harboring *EGFR*-activating mutations.

Several clinical trials will evaluate the efficacy and safety of third-generation EGFR-TKIs plus brain RT. Examples include the randomized phase II NORTHSTAR study (NCT03410043), which will investigate the efficacy and safety of osimertinib plus brain RT, and the randomized phase II OUTRUN trial (NCT03497767), which will explore the combination of osimertinib with or without SRS in both first-line and second-line settings for *EGFR*-mutant NSCLC. Moreover, two phase II trials will be conducted in China to explore the efficacy and safety of almonertinib in combination with thoracic RT as first-line therapy in *EGFR*-mutant locally advanced NSCLC patients (NCT04636593) and to evaluate the combination of SRS with sequential almonertinib in EGFR-TKI-naïve NSCLC patients harboring BMs (NCT04643847).

13. EGFR-TKIs combined with anti-angiogenic agents

The VEGF signaling pathway plays a pivotal role in regulating angiogenesis and is associated with increased proliferation and metastatic dissemination in many human cancers, including NSCLC [183]. Consequently, several compounds were developed to disrupt the activation of the VEGF pathway. Examples include monoclonal antibodies directed against VEGF-A (e.g., bevacizumab) and VEGF-R (e.g., ramucirumab), and small-molecule TKIs with activity against VEGF receptor (e.g., vandetanib, axitinib, nintedanib, sunitinib, and sorafenib) [184].

13.1. Preclinical assessment of combined VEGF-EGFR in NSCLC

NSCLC cells harboring *EGFR* mutations express higher levels of VEGF compared to cells expressing wild-type *EGFR*, as the activation of EGFR signaling up-regulates the expression of HIF-1α in a hypoxia-independent manner, leading to increased tumor angiogenesis [185–187]. The activation of VEGF signaling supports tumor growth through the engagement of PI3K/Akt/mTOR and MAPK pathways [188]. In a xenograft mouse model, VEGF expression increased after the development of resistance to erlotinib. Moreover, despite the erlotinib-mediated suppression of EGFR signaling, phosphorylated ERK and Akt were up-regulated as compared with the erlotinib-sensitive phase, suggesting that VEGF is directly involved in acquired resistance to EGFR-TKIs [189,190].

Evidence obtained from preclinical studies suggests that the EGFR and VEGF pathways are linked and may replace each other to sustain the growth and proliferation of cancer cells. Furthermore, anti-angiogenic drugs may increase the delivery of EGFR-TKIs within the tumor tissue by normalizing tumor vasculature, hence resulting in increased anticancer effect [191]. As a result, the dual inhibition of EGFR-VEGF pathways may represent a viable strategy to suppress tumor growth and delay the emergence of drug resistance, potentially supporting its translation into clinical practice [190].

13.2. Combined VEGF-EGFR inhibition in the clinic

Initial evidence supporting the efficacy of dual VEGF-EGFR inhibition in patients with *EGFR*-mutant advanced NSCLC was provided by retrospective group analyses from the BeTa and ATLAS clinical trials [188]. A recent meta-analysis by Chen et al. concluded that first-generation EGFR-TKIs, combined with anti-angiogenic drugs such as bevacizumab or ramucirumab, are more effective than EGFR-TKI monotherapy in the first-line setting for *EGFR*-mutant advanced/metastatic NSCLC, although with an increase in the number of treatment-related adverse events [192].

Conforti et al. analyzed the results of several randomized controlled trials combining EGFR-TKIs with anti-angiogenic drugs [193]. PFS was significantly longer in patients treated with EGFR-TKIs combined with an anti-angiogenic drug (e.g., bevacizumab or ramucirumab) than in patients who received EGFR-TKI monotherapy, with a pooled median PFS of 17.8 months (95% CI 16.5–19.3) for the combination versus 11.7 months (95% CI 11.1–12.7) for EGFR-TKI as monotherapy. There were no substantial differences in OS and ORR between the two treatment arms [193].

The randomized, multicenter, phase III CTONG-1509 trial (NCT02759614) investigated the efficacy and safety of bevacizumab with or without erlotinib in a cohort of Chinese patients with *EGFR*-mutant advanced/metastatic NSCLC. Results demonstrate that the combination of erlotinib and bevacizumab has superior efficacy to erlotinib monotherapy with minimal additional toxicity [194]. Median PFS was 18.0 months (95% CI 15.2–20.7) in the combination group and 11.2 months (95% CI 9.7–12.5) in patients treated with erlotinib monotherapy (HR = 0.57; 95% CI 0.44–0.75; $p < 0.001$). ORR was 86.3% and 84.7% for the combination and erlotinib monotherapy groups, respectively ($p = 0.741$).

EMA approved the combination of erlotinib plus bevacizumab as first-line treatment for *EGFR*-mutant advanced/metastatic NSCLC based on the positive results obtained in the pivotal phase II JO25567 trial. In this study, patients treated with erlotinib plus bevacizumab achieved a longer median PFS (16.0 months; 95% CI 13.9–18.1) as compared with patients treated with erlotinib alone (9.7 months; 95% CI 5.7–11.1) for erlotinib alone (HR = 0.54; 95% CI 0.36–0.79; $p = 0.0015$) [195]. The median OS was 47.0 months for the combination group and 47.4 months for the erlotinib monotherapy group (HR = 0.81; 95% CI 0.53–1.23; $p = 0.3267$) [196]. The combination of erlotinib plus bevacizumab was well tolerated in patients [197].

The efficacy and safety of erlotinib plus ramucirumab were investigated in the phase III RELAY trial (NCT02411448) in NSCLC patients expressing *EGFR*-mutant tumors. Patients in the combination arm had a longer median PFS (19.4 vs. 12.4 months; HR = 0.59; $p < 0.0001$) and DoR (18.0 vs. 11.1 months, $p = 0.0003$) than patients treated with erlotinib plus placebo [198]. Based on the results obtained in the RELAY study, both EMA and FDA approved ramucirumab plus erlotinib as first-line treatment for *EGFR*-mutant advanced or metastatic NSCLC.

13.3. Third-generation EGFR-TKIs in combination with angiogenesis inhibitors

Very little data is available concerning the efficacy of osimertinib in combination with angiogenesis inhibitors. The open-label, multicentre phase I study (NCT02789345) investigated the safety of osimertinib in combination with necitumumab (a human IgG1 anti-EGFR antibody) or ramucirumab in patients with advanced *EGFR*-T790M-positive NSCLC who had progressed following treatment with first- or second-generation EGFR-TKIs [199]. Median PFS was 11.0 months (90% CI 5.5–19.3), and the median DoR was 13.4 months (90% CI 9.6–21.2). Remarkably, efficacy was observed both in patients with and without CNS metastasis (ORR 60% and 87%; median PFS 10.9 and 14.7 months, respectively) [199].

Part of the patients with *EGFR*-mutant advanced/metastatic NSCLC enrolled in the NEJ026 trial received osimertinib plus bevacizumab after disease progression while on therapy with erlotinib plus bevacizumab [200]. Median PFS between enrollment and progressive disease of second-line treatment was 28.6 months (95% CI 22.1–35.9) in the group that previously received the combination of erlotinib plus bevacizumab and 24.3 months (95% CI 20.4–29.1) in the erlotinib monotherapy group (HR = 0.80; 95% CI 0.59–1.10). In both arms, median OS of patients with osimertinib second-line treatment was longer than other second-line chemotherapy groups [50.7 months (95% CI, 38.0–50.7) versus 40.1 months (95% CI, 29.5 to not reached), (HR = 0.645; 95% CI, 0.40–1.03)], respectively [200].

Moreover, results obtained in a randomized phase II study performed in Japan, exploring the combination of osimertinib plus bevacizumab against osimertinib monotherapy in NSCLC patients previously treated with EGFR-TKIs, failed to show benefit concerning PFS (9.4 months vs. 13.5 months; HR = 1.44; 80% CI 1.00–2.08; $p = 0.2$) and OS (not reached vs. 22.1 months; $p = 0.96$) in patients treated with the combination versus patients treated with osimertinib monotherapy [201]. Since the results obtained from the previously mentioned studies failed to show a clear benefit in PFS and OS with the combination of osimertinib and bevacizumab, the use of this combination for the treatment of *EGFR*-mutant NSCLC patients who progressed while on therapy with first-generation EGFR-TKIs is not recommended.

Overall, clinical data concerning the efficacy of third-generation EGFR-TKIs plus angiogenesis inhibitors as first-line therapy for *EGFR*-mutated NSCLC is lacking. Additional insights will be provided by two studies currently ongoing: the phase II FLAIR trial, which will explore the efficacy of the combination of osimertinib plus bevacizumab for the first-line treatment of advanced/metastatic NSCLC patients harboring *EGFR* exon 21 L858R substitution, and by the phase II RAMOSE study (NCT03909334), which is exploring the efficacy and safety of the combination of osimertinib and ramucirumab for treatment-naïve *EGFR*-mutant NSCLC patients [202,203].

14. EGFR-TKI combined with MET inhibitors

The mesenchymal-epithelial transition receptor (c-MET) is a transmembrane tyrosine kinase receptor encoded by the MET proto-oncogene located on human chromosome 7 (7q21-31) [204]. c-MET, through the interaction with its ligand - the hepatocyte growth factor (HGF) - plays a pivotal role in regulating several physiological processes, including embryogenesis, liver regeneration, and wound healing through the engagement of pathways such as RAS-MAPK, PI3K/Akt/mTOR, JAK/-STAT, and Wnt/β-catenin pathways [204].

Several molecular aberrations (e.g., MET amplification, MET point mutations, and exon 14 skipping mutations) cause a dysregulated activation of c-MET signaling and play a crucial role in the development and progression of several human cancers, including NSCLC [205]. As a result, various HGF/c-MET-targeted therapies have been developed. Examples of drugs able to disrupt c-MET signaling include small molecule inhibitors (e.g., crizotinib, tivantinib, savolitinib, tepotinib, cabozantinib, and foretinib), monoclonal antibodies directed against c-MET (e.g., onartuzumab), and antibodies against HGF (e.g., ficlatuzumab and rilotumumab) [206]. In this regard, Moosavi et al. recently provided a comprehensive review regarding HGF/MET-targeting agents in association with other therapeutic strategies for cancer treatment [207].

In EGFR-mutated NSCLC, MET amplification emerged as a critical resistance mechanism to EGFR-TKIs due to bypass activation of the PI3K/Akt/mTOR pathway [105,106]. Patients who develop MET-mediated resistance after treatment with osimertinib show inferior PFS and OS than those without MET amplification [208]. Therefore, the combination of small-molecule inhibitors, able to simultaneously target EGFR and c-MET, has been suggested as a potential strategy to achieve a more robust inhibition of pro-tumorigenic signaling and avoid the emergence of acquired resistance in patients treated with EGFR-TKIs [207].

14.1. Preclinical studies involving combined EGFR-MET inhibition

Engelman et al. firstly reported that EGFR-mutant NSCLC cell line HCC827 developed resistance when exposed to increasing concentrations of gefitinib due to emergence of the MET amplification and that the combined EGFR-MET inhibition abrogates PI3K/Akt/mTOR and ERK signaling, effectively restoring sensitivity to EGFR-TKIs, both in vitro and in vivo [106]. Tang et al. later confirmed the efficacy of dual EGFR-MET inhibition in inducing the regression of H1975 erlotinib-resistant tumor xenografts treated with a combination of erlotinib plus the small-molecule MET inhibitor SU11274 [209]. Van der Steen et al. reported that the interaction between erlotinib and crizotinib could depend on lysosomal redistribution of the drugs and inhibition of phosphor-PRAS40 [210,211].

Furthermore, Shi and collaborators recently demonstrated that MET amplification and c-MET hyperactivation confer resistance to both first and third-generation EGFR-TKIs in the lung adenocarcinoma HCC827 cell line [212]. Besides, c-MET inhibition, obtained either with a small-molecule c-MET inhibitor or gene knockdown, effectively restores

sensitivity to osimertinib, both in vitro and in vivo [212]. The complete suppression of ErbB3 phosphorylation – obtained through the combined EGFR-MET inhibition - is essential to restore sensitivity to osimertinib in cell lines that acquired resistance to third-generation EGFR-TKI through MET amplification [212].

14.2. Combined EGFR-MET inhibition in the clinic

Preclinical results obtained by several authors seem to confirm the efficacy of combined MET-EGFR inhibition in overcoming resistance to EGFR-TKIs [213–215]. Therefore, the combined inhibition of EGFR and c-MET signaling emerged as a potential treatment strategy for EGFR-mutant advanced/metastatic NSCLC patients who experience tumor progression after the failure of first- or second-line EGFR-TKI treatment due to acquisition of MET aberrations [216]. Nevertheless, results obtained in several phase III trials, which combined c-MET inhibitors with first-generation EGFR-TKIs, have shown conflicting results [217]. However, most of these studies were performed in unselected cohorts of patients, further emphasizing the need to select patients based on *EGFR* and *MET* status.

Several case reports suggested that combined inhibition of EGFR and c-MET signaling could help overcome *MET*-induced resistance in patients experiencing progression after treatment with third-generation EGFR-TKIs. In this regard, osimertinib, in combination with the c-MET inhibitor crizotinib, was tested in two patients who developed resistance to osimertinib due to *MET* amplification (detected through NGS-based ctDNA profiling) with positive results [208]. In another case report, a patient who developed MET-mediated resistance after treatment with erlotinib was treated with a combination of osimertinib and crizotinib and achieved partial response [218].

In their retrospective analysis, Wang et al. provided further confirmation regarding the feasibility of the EGFR-TKIs/crizotinib combination for targeting *MET*-amplified, *EGFR*-mutant NSCLC. Nine out of eleven patients with *MET* amplification (acquired during therapy with first- or third-generation EGFR-TKIs) and treated with a combination of either first- or third-generation EGFR-TKIs plus crizotinib achieved partial response, with an ORR of 81.8% and a median PFS of 5.8 months [219]. Interestingly, patients treated with this combination experienced the loss of *MET* amplification at disease progression [219].

Updated results from the phase I CHRYSALIS study (NCT02609776), which explored the safety and efficacy of the combination of amivantamab with lazertinib (a third-generation EGFR-TKI), in both chemotherapy-naïve and osimertinib-relapsed patients with *EGFR*-mutated NSCLC were presented at the American Society of Clinical Oncology (ASCO) 2021 [220]. Combination treatment with amivantamab and lazertinib yielded responses in 36% of chemotherapy-naïve patients who progressed while on osimertinib. NGS-based ctDNA biomarker analyses identified a subgroup of patients harboring both *EGFR* and *MET* alterations more likely to respond to combination treatment. In this regard, median PFS for biomarker-positive and negative patients was 6.7 months (CI 95%, 3.4–NR) and 4.1 months (CI 95%, 1.4–9.5), respectively [220].

The efficacy and safety of the combination of amivantamab (an EGFR-MET bispecific antibody) and lazertinib will be investigated in the phase III MARIPOSA study (NCT04487080) as first-line treatment for *EGFR*-mutant advanced/metastatic NSCLC patients harboring *EGFR* exon19del or exon 21 L858R substitution [221]. Another phase Ib/II trial (NCT02335944) evaluated the efficacy of third-generation EGFR-TKI nazartinib in combination with the MET inhibitor capmatinib [222]. Results do not support the use of this combination over EGFR-TKI monotherapy in unselected *MET* patients. Nevertheless, capmatinib plus nazartinib demonstrated clinical efficacy in *MET*-positive patients, confirming the role of *MET*-related aberrations in driving resistance to EGFR-TKIs. The combination treatment was well tolerated, with a similar safety profile across the groups [222].

The multi-arm, open-label, multicentre phase Ib TATTON study (NCT02143466) assessed the safety and tolerability of osimertinib in combination with other targeted therapies such as selumetinib (MEK1/2 inhibitor), savolitinib (MET-TKI), or durvalumab [anti-programmed cell death ligand 1 (anti-PD-L1) monoclonal antibody] for the treatment of *MET*-amplified, *EGFR*-mutant advanced/metastatic NSCLC patients who had progressed during treatment with third-generation EGFR-TKIs [223]. Clinical results demonstrated the safety of combining osimertinib with selumetinib or savolitinib, whereas the combination of osimertinib plus durvalumab was deemed not feasible due to increased reports of interstitial lung disease [223].

In the TATTON study, two expansion cohorts were considered: parts B and D. Part B included three cohorts of patients: patients who had previously received a third-generation EGFR-TKI (B1) and EGFR-TKI-naïve patients who were either *EGFR*-T790M-positive (B2) or negative (B3). In contrast, part D enrolled patients who had not previously received third-generation EGFR-TKIs and were *EGFR*-T790M-negative. Final data for the two expansion cohorts (parts B and D) were presented at the World Conference on Lung Cancer (WCLC) 2020 [224]. The primary endpoint was safety tolerability, and secondary endpoints included ORR, PFS, and pharmacokinetics. Median PFS was 5.5 months (95% CI 4.1–7.7), 9.1 months (95% CI 5.5–12.8), 11.1 months (95% CI 4.1–22.1), and 9.0 months (95% CI 5.6–12.7) for cohorts B1, B2, B3, and D, respectively. ORR was 33%, 65%, 67%, and 62% for cohorts B1, B2, B3, and D, respectively [224].

Overall, results from the TATTON study demonstrate that the combination of osimertinib and savolitinib has promising antitumor activity in *MET*-amplified, *EGFR*-mutant advanced/metastatic NSCLC who had progressed on previous treatment with third-generation EGFR-TKIs, warranting further investigation in randomized phase III trials [225].

15. EGFR-TKIs combined with MEK inhibitors

MEK is a key downstream component of the RAS/RAF/MEK/ERK signaling pathway, a tightly regulated intracellular pathway involved in cell proliferation, differentiation, and apoptosis [226]. Since MEK is a convergence point for many signaling cascades, it represents a relevant molecular target for therapies aimed at preventing the activation of signaling pathways responsible for sustaining cancer cell proliferation and survival [227]. Several small-molecule MEK1/2 inhibitors have received approval from the FDA. Examples of these drugs include trametinib, binimetinib, selumetinib, and cobimetinib [228].

16. Preclinical evaluation of dual EGFR-MEK inhibition

Preclinical studies highlighted that combined EGFR-MEK inhibition might be a feasible strategy to postpone the emergence of acquired resistance to EGFR-TKIs and improve treatment outcomes in *EGFR*-mutant advanced/metastatic NSCLC [229–231]. The concomitant ERK1/2 - EGFR inhibition by trametinib and the third-generation EGFR-TKI WZ4002 prevents the reactivation of ERK1/ERK2 and enhances the antitumor activity of EGFR inhibitors [230]. Intriguingly, combined EGFR-MEK inhibition prevented the emergence of both T790M-dependent and independent drug resistance in several NSCLC models, both *in vitro* and *in vivo* [230].

Dual MEK-EGFR inhibition provides a higher inhibition of protumorigenic signaling pathways, potentially delaying the onset of resistant clones [230]. However, resistance to combined MEK-EGFR inhibition ultimately arises due to the compensatory activation of the PI3K/AKT/mTOR pathway [230]. The combined inhibition of the MEK and the PI3K/AKT/mTOR pathway with trametinib plus taselisib is effective in overcoming acquired resistance and restoring sensitivity to EGFR-TKIs in both *in vitro* and *in vivo* NSCLC models, in particular in cases where resistance to EGFR inhibitors was due to the activation of the c-MET pathway, activation of EMT, or acquisition of the secondary *EGFR*-T790M mutation [232].

Recently, Qu et al. reported that the dual targeting of MEK and PI3K

efficiently inhibited the cell proliferation, induced apoptosis and the G0/G1 cell cycle arrest in EGFR-TKI-resistant NSCLC cell lines [233]. These results provide a rationale for translating the dual targeting of MEK/PI3K signaling in the clinical setting as a potential strategy to overcome resistance to EGFR-TKIs, especially in NSCLC patients harboring *KRAS* and *PI3KCA* mutations.

Furthermore, the combined inhibition of the Fibroblast Growth Factor Receptor (FGFR) and Akt pathway has recently emerged as a strategy to disrupt resistance to EGFR-TKIs mediated by the activation of signaling pathways downstream of Akt. In this regard, Terp et al. provided compelling evidence that the dual FGFR/Akt inhibition could be exploited to overcome resistance to EGFR-TKIs, both *in vitro* and *in vivo* [234]. Although still preliminary, these results provide a rationale for future clinical trials.

16.1. Combined EGFR-MEK inhibition in the clinic

The phase Ib TATTON study (NCT02143466) assessed the safety and tolerability of osimertinib in combination with selumetinib (MEK1/2 inhibitor) for the treatment of *EGFR*-mutant advanced/metastatic NSCLC patients who progressed during treatment with third-generation EGFR-TKIs [223]. Primary endpoints included safety, tolerability, and preliminary efficacy (ORR, DoR, and pharmacokinetics). The combination of osimertinib plus selumetinib exhibited an acceptable safety profile and demonstrated antitumor activity in patients, warranting further investigation [235].

17. Discussion

First- and second-generation EGFR-TKIs demonstrated their superiority over first-line platinum-based chemotherapy in NSCLC patients harboring *EGFR*-activating mutations, especially regarding PFS and ORR [43]. However, their efficacy is reduced by the emergence of acquired resistance. The vast majority of patients treated with EGFR-TKIs experience disease progression within 9 - 15 months due to the selection of drug-resistant clones harboring the secondary *EGFR*-T790M mutation [99]. Third-generation EGFR-TKIs have been designed to address the issue of acquired resistance to first- and second-generation EGFR-TKIs. Nevertheless, patients still develop resistance, wither due to the selection of clones harboring the tertiary *EGFR*-C797S mutation or through EGFR-independent mechanisms [87]. Fourth-generation allosteric EGFR-TKIs have shown promising anticancer activity in resistant tumor models, both in vitro and in vivo. However, results obtained so far are preliminary and require further confirmation [236].

Acquired resistance to EGFR-TKIs represents a significant hurdle in achieving long-term clinical response, also given the scarcity of effective pharmacological interventions for patients who progress after the failure of EGFR-TKI treatment. NGS data played an important role in unraveling the resistance mechanisms responsible for resistance to EGFR-TKIs. Furthermore, NGS data and provided a broader picture concerning the molecular events responsible for disease progression after treatment failure [237,238]. Resistance to EGFR-TKIs may arise either through EGFR-dependent mechanisms or EGFR-independent mechanisms [79]. Though several resistance mechanisms have been identified, there is still much to learn about how resistant cells evade the inhibition of EGFR signaling mediated by EGFR-TKIs.

In this regard, the clinical implementation of more comprehensive and sensitive molecular techniques is paramount to monitor clinical response to EGFR-TKIs and clarify the mechanisms of clonal selection and evolution of resistant cells [239,240]. In particular, liquid biopsies might represent a feasible and reliable alternative to standard biopsy sampling, which can be potentially biased and misleading given the high NSCLC intertumoral and intratumoral heterogeneity [237,238]. The TATIN trial (NCT04148066) will explore the feasibility of ctDNA liquid biopsy to guide treatment in *EGFR*-mutant advanced or metastatic NSCLC patients treated with EGFR-TKIs. Hopefully, this approach will help identify resistance mechanisms and devise tailored treatment approaches based on the mutational profile of patients.

For years, cytotoxic platinum-based chemotherapy has been the only treatment option for NSCLC patients who progressed while on therapy with EGFR-TKIs. EGFR-TKIs combined with chemotherapy may potentially prevent, or at least postpone, the emergence of acquired resistance. However, first-line treatment with EGFR-TKI plus chemotherapy has not been widely used in the clinical setting mainly due to a lack of demonstrated survival advantage, controversial results between clinical trials, meta-analyses, systematic reviews, and concerns about potential toxicity [241]. Recent clinical trials performed in selected patients populations have shown promising results, albeit without establishing a clear and definitive role for this combination in the clinical practice [242].

At present, there is a lack of definitive data concerning the efficacy of third-generation EGFR-TKIs in combination with platinum-based chemotherapy. The phase II OPAL trial was the first study to evaluate the safety and feasibility of osimertinib in combination with cisplatin/carboplatin plus pemetrexed in treatment-naïve *EGFR*-mutated advanced/metastatic NSCLC patients [243]. Two phase III clinical trials have been initiated to determine the efficacy and safety of this combination: the FLAURA2 study (NCT04035486), which is assessing osimertinib in combination with cisplatin/carboplatin plus pemetrexed; and the ACROSS 1 trial (NCT04500704), which is evaluating the combination of almonertinib plus platinum chemotherapy (carboplatin-pemetrexed) [244].

Since EGFR-TKIs have demonstrated radiosensitizing effects when combined with radiotherapy, this combination has emerged as a strategy for treating *EGFR*-mutant NSCLC patients with brain metastases. However, the efficacy of first-generation EGFR-TKIs in treating BMs is limited due to poor drug diffusion within the CNS and although some promising results have been obtained, the evidence available thus far concerning the use of this combination is controversial and insufficient to support its broad use in the clinic. The combination of third-generation EGFR-TKIs plus brain RT might represent a rational strategy to improve response to treatment, especially considering the higher CNS activity of third-generation EGFR-TKIs. To fill the gap between preclinical studies and their clinical translation, several clinical trials such as the phase II NORTHSTAR and OUTRUN studies have been initiated and will evaluate the efficacy and safety of this combination, both as first-line or second-line therapy for NSCLC patients with brain metastases.

The inhibition of EGFR signaling alone is likely insufficient to achieve long-term responses in patients due to the emergence of additional *EGFR* mutations and activation of alternative resistance mechanisms. Therefore, the combined inhibition of multiple pathways involved in sustaining pro-tumorigenic signaling has been proposed as a strategy to overcome resistance to EGFR-TKIs [245]. Recently, Fernandes Neto et al. suggested that inhibition of an oncogenic pathway at multiple nodes could represent an effective strategy to prevent the development of acquired resistance to EGFR-TKIs [246]. Treatment with multiple-low dose (MLD) therapy, using four drugs inhibiting a different node in the MAPK pathway, prevented the development of resistance with minimal toxicity and was shown to be effective in PDX models harboring a range of resistance mechanisms to EGFR-TKI [246]. Although promising, additional studies are required before this approach can be translated into the clinic.

Dual EGFR-VEGF inhibition already represents an established strategy for treating advanced/metastatic NSCLC patients with *EGFR* mutations. Still, there is little evidence regarding the efficacy and safety of third-generation EGFR-TKIs in combination with anti-angiogenic agents. Since third-generation EGFR-TKIs have shown higher antitumor activity in *EGFR*-mutated NSCLC patients, it might be reasonable to expect that the dual EGFR-VEGF inhibition, obtained combining osimertinib or almonertinib with anti-angiogenic drugs, would translate into a more robust antitumor effect. However, the combination of

osimertinib with bevacizumab as second-line therapy failed to show significant improvements in PFS and OS in a selected population of *EGFR*-mutant advanced/metastatic NSCLC patients. The efficacy and safety of osimertinib in combination with either bevacizumab or ramucirumab for the first-line treatment of NSCLC patients harboring *EGFR* mutations is currently under assessment in two phase II clinical trials (FLAIR and RAMOSE). Hopefully, these trials will provide compelling evidence regarding the efficacy and safety of this combination in the first-line setting.

MET amplification is an important mechanism involved in acquired resistance to EGFR-TKIs. Therefore, the combined EGFR-MET inhibition has emerged as a promising strategy to overcome resistance in NSCLC patients harboring these genetic aberrations [247]. Initial trials involving this combination in unselected patient populations failed to demonstrate improvements in treated patients. Nevertheless, recent data seem to support a positive role of third-generation EGFR-TKIs in association with c-MET inhibitors in selected NSCLC populations harboring *MET* and *EGFR* aberrations [221,222,225].

Still, no consensus exists on the optimal combination of EGFR-TKIs and MET-TKIs to overcome the *MET*-mediated resistance to EGFR inhibitors. Moreover, the resistance mechanisms responsible for the failure of combined EGFR-MET-TKI therapy have not been fully clarified. Preliminary results from the TATTON study suggest that osimertinib plus savolitinib may be effective in overcoming *MET*-induced resistance to EGFR-TKIs in patients with *EGFR*-mutant NSCLC whose disease has progressed on prior treatment with third-generation EGFR-TKIs [223]. Further investigation of this combination is ongoing in the SAVANNAH (NCT03778229) and ORCHARD (NCT03944772) phase II studies [248].

In conclusion, results obtained so far in preclinical and clinical studies indicate that the combination of EGFR-TKIs with other therapeutic agents (e.g., chemotherapy, radiotherapy, and targeted therapies) has the potential to avoid, or at least delay, the emergence of acquired resistance to EGFR inhibitors. Nonetheless, available data is either immature or insufficient to conclusively support the use of these combinations in the clinical setting. In particular, there is a need to perform more research in randomized controlled trials enrolling patients based on the availability of targetable molecular alterations. Several clinical trials are currently exploring the efficacy and safety of the combination of third-generation EGFR-TKIs with other anticancer therapeutics. Hopefully, these studies will provide more information regarding the feasibility of these combinations for the treatment of advanced/metastatic NSCLC patients harboring *EGFR*-activating mutations.

Author Contributions

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