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REVIEW

Emerging drugs for EGFR-mutated non-small cell lung cancer

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ABSTRACT

Introduction: Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) represent the standard of care for patients with metastatic non-small-cell lung cancer (NSCLC) harboring sensitizing *EGFR* mutations. However, these agents are associated with inevitable treatment resistance. Newer generations of TKIs are under development that may prevent or overcome resistance and enhance intracranial activity.

Areas covered: In this review, we will discuss newer generations of EGFR TKIs for *EGFR*-mutated NSCLC. We will also address resistance mutations and escape pathways associated with these agents such as secondary mutations, downstream signaling, bypass pathways, phenotypic transformation, anti-apoptotic signaling, immune evasion, and angiogenesis. Furthermore, this article encompasses emerging data from combination trials with next-generation TKIs that are being pursued to delay or prevent the occurrence of resistance.

Expert opinion: The promise and challenge of precision oncology is encapsulated in the treatment of *EGFR*-mutated NSCLC with TKIs. Third generation TKIs have shown superior efficacy in the front-line setting and have become standard of care. A better understanding of mechanisms of treatment failure and disease relapse will be required to develop novel therapeutic strategies to further improve patient outcomes in the future.

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NSCLC; EGFR; tyrosine kinase inhibitors; resistance mechanisms

1. Background

Lung cancer is the leading cause of cancer-related mortality worldwide [1]. Non-small-cell lung cancer (NSCLC) accounts for 80–85% of all lung cancers diagnosed. Molecularly targeted therapies against specific oncogenic drivers have had a significant impact on the treatment paradigm and clinical outcome in patients with NSCLC carrying these mutations [2]. One of the most commonly encountered drivers is the activating mutation in epidermal growth factor receptor (*EGFR*), which has been observed in approximately 17% of patients [3]. Signaling through EGFR, a member in the ErbB family of tyrosine kinase receptors, is instrumental for cell growth and proliferation [4]. Once activated by its various ligands, including epidermal growth factor (EGF) and TGF-α, it dimerizes and auto-phosphorylates intracellular tyrosine residues. This activated complex activates several signaling pathways, most notably MAPK, PI3K-AKT, and JAK-STAT [5]. The activation of these pathways drives cell growth and proliferation and inhibits apoptosis, leading to oncogenesis. Several *EGFR* mutations, which render this pathway constitutively active have been identified. The in-frame deletion of exon 19 and substitution of leucine for arginine in exon 21 (L858R) together account for 85–90% of all *EFGR* mutations [6,7]. These mutations have been the target for tyrosine kinase inhibitors (TKIs) ever since responses were initially demonstrated with targeting of mutant EGFR[8].

Three generations of TKI therapy are now FDA approved for the treatment of *EGFR*-mutated NSCLC. The first generation, such as gefitinib and erlotinib, binds non-covalently to EGFR tyrosine kinase sites, competing with ATP [9]. The second generation, e.g. afatinib and dacomitinib, binds covalently. The third generation TKIs, such as osimertinib, have shown promising efficacy against tumors expressing the exon 20 T790M mutation, which is the most common cause of acquired resistance mutation seen with first or second generation TKIs [10]. Osimertinib is the first third generation TKI to be approved by the FDA to treat *EGFR* mutant NSCLC.

2. Medical needs

Despite the successes of targeted therapy for *EGFR*-mutated NSCLC, the majority of patients develop resistance to first and second generation TKIs in 8–12 months [11]. Multiple mechanisms of resistance have been identified, such as the development of mutations directly altering EGFR (most importantly the T790M mutation), the activation of bypass signaling pathways such as MET and HER2, histological transformation of the tumor, and activation of downstream pathways. The incidence of these mechanisms varies, but in one genotypic analysis of TKI resistant tumor cells, the T790M mutation was present in 49% of samples, with MET amplification accounting for 5% and transformation to small cell reported in 14% of

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patients [10]. Development of newer generation TKIs with activity against emerging acquired resistance mutations such as C797S, better CNS activity and multi-drug combination strategies are areas of active research interest.

3. Existing treatment

The first generation of TKIs, erlotinib and gefitinib, are reversible inhibitors. Among patients with *EGFR* mutations, several large randomized trials demonstrated superior progression-free survival (PFS) with single agent erlotinib or gefitinib over platinum-based chemotherapy ranging from 8 to 10 months, but without an overall survival (OS) benefit, likely due to a large percentage of post-treatment cross-over [12–14]. Overall, gefitinib and erlotinib seem to have a similar efficacy profile [15].

Afatinib, a second generation TKI binds irreversibly to the EGFR TK domain and demonstrates similar PFS benefit over chemotherapy as well. Patients with exon 19 deletions were found to have improved OS of 10 months in both Lux-Lung 3 and 6 trials of afatinib; a benefit that was not found in patients with L858R mutation [16]. Afatinib was compared to gefitinib in a head-to-head Phase IIb trial with improved numerical OS in the afatinib group irrespective of mutation subgroup, that however failed to reach statistical significance [17]. Afatinib also seems to be effective in uncommon mutations of *EGFR*. A post-hoc pooled analysis of Lux-lung 2, 3, and 6 showed response rates greater than 50% among patients with uncommon mutations such as G719X, L861G, and S768I [18].

Osimertinib is a third generation EGFR TKI, targeting both sensitizing mutations and T790M, the most common resistance mutation encountered with first and second generation TKIs. In the AURA3 trial, which randomized patients with T790M mutation after progression with a first generation TKI, osimertinib showed a superior PFS of 10.1 months versus 4.4 months in the chemotherapy group [19]. The FLAURA trial comparing osimertinib to first generation TKIs in the front-line setting showed a better PFS of 18.9 months versus 10.2 months [20]. Although OS data remain immature, osimertinib has been approved as a first-line drug by FDA given the impressive PFS advantage.

Most studies of resistance mechanisms to EGFR TKIs centered on first-generation agents. The mechanisms may be broadly classified into secondary *EGFR* mutations, bypass signaling and phenotypic transformation. In a comprehensive study of 155 patients with progression on first generation TKIs, T790M was reported in 60% of patients. Other potential resistance mechanisms identified were HER2 amplification (13%), MET amplification (5%), and small cell transformation (3%) [21]. A smaller report of 37 patients identified *PIK3CA* mutations in 5% of patients who acquired resistance, with SCLC transformation accounting for 14% [10]. The T790M mutation results in reduced binding affinity for first and second generation TKIs leading to resistance [22]. For patients with other activated bypass pathways, successful treatment has been reported with the administration of another targeted therapy, either alone or in combination. Resistance mediated through *MET* amplification after treatment with erlotinib may be overcome by crizotinib and responses lasting around four months have been reported [23]. A series of 12 patients who developed MET overexpression by IHC showed that the combination of gefitinib with a MET inhibitor achieved partial response and stable disease in 7 and 2 patients, respectively [24]. Unfortunately, the treatment of *HER2* alterations with Trastuzumab has hitherto been disappointing [25]. *BRAF* mutations with increased downstream signaling has also been described [26].

Phenotypic transformation from adenocarcinoma to small cell lung carcinoma (SCLC) may also be seen with the development of resistance to TKIs [27]. Targeted EGFR inhibition may select for sub-clones which possess features of epithelial-to-mesenchymal transition (EMT) facilitating differentiation into small cell histology [10]. At resistance, these tumors were shown to express the original EGFR mutation, indicating their sub-clonal origin. AXL and Hedgehog pathways have been shown to be involved in such transformation in preclinical studies [28,29].

CNS progression is an important reason for treatment failure, particularly among the first-generation inhibitors which occurs mainly due to limited blood–brain barrier (BBB) penetration and differences in pharmacokinetics . With better intracranial penetrance, the intracranial activity of osimertinib has been prospectively assessed with reported CNS response rate of 54% and disease control rate of 92% [30].

Resistance to the third generation TKIs, especially osimertinib, has been explored. It may be mediated through a tertiary mutation C797S at the drug's binding site on the EGFR receptor. The activation of alternative pathways is also associated with acquired resistance to third generation agents. A report of 23 patients with resistance to osimertinib showed that 7 had acquired *MET* amplifications and crizotinib, a MET inhibitor had encouraging activity in patients with *MET* amplification [31]. A study of 43 patients with T790M mutations treated with rociletinib reported that 46% of patients harbored multiple resistance mutations as assessed by ctDNA, highlighting the complexity and heterogeneity of tumor biology in *EGFR*-mutated NSCLC [32].

4. Current research goals

A major focus of research in *EGFR* mutant NSCLC is to delineate mechanisms underlying resistance. With the use of osimertinib in the front-line setting, the spectrum of resistance mutations may be different from those reported previously in the second line setting. Novel therapeutic approaches will be needed to target emerging resistance mutations. The optimal combination strategy to target downstream and bypass signaling is still unclear, especially with co-occurring resistance mutations and pathways. Moreover, transformation to SCLC is associated with aggressive disease course, and a better ability to predict the genomic risk of transformation will need to be developed. Further study is also required to determine the role of immunotherapy in patients with *EGFR* mutations, including the efficacy and safety of combining immunotherapeutic agents with EGFR-targeted therapy.

5. Scientific rationale

As osimertinib has been approved as front-line treatment for EGFR-mutated NSCLC, we will focus on describing the approaches for overcoming resistance to osimertinib and

other next generation TKIs. Broadly, resistance mechanisms to newer generation TKIs include secondary EGFR mutations, downstream activation, up-regulation of bypass signaling, phenotypic transformation, engagement of anti-apoptosis machinery, angiogenesis, compensatory JAK-STAT signaling, and SFK pathway activation (Figure 1).

6. Emerging treatment

6.1. Dacomitinib

Dacomitinib is a second-generation, irreversible EGFR TKI. In the Phase 3 ARCHER 1050 study, dacomitinib was compared with gefitinib in the front-line setting for the treatment of Stage IIIB/IV *EGFR* mutated (exon 19 del or exon 21 L858R ± exon 20 T790M) NSCLC without CNS metastases. Dacomitinib showed a significant improvement in OS compared to gefitinib (HR 0.76; 95% CI, 0.582–0.993; 2-sided P = 0.044) with a median OS of 34.1 months vs 26.8 months [33].

6.2. Avitinib (AC0010)

Avitinib is a third-generation TKI, active against both *EGFR* sensitizing mutations and T790M. A Phase I trial in 52 reported patients with *EGFR* mutations who had progressed on first-line treatment with or without T790M mutations showed an ORR of 36.5%, median PFS ranging from 14.0 to 35.6 weeks and an acceptable toxicity profile. Resistance is mediated by *BRAF*-V600E mutation, *ROS1* fusion, *MET* amplification, and *ERBB2* amplifications. No *EGFR* C797S mutations were detected [34]. Avitinib however has a low BBB penetration rate of 0.046–0.146% which may be a significant barrier to meaningful CNS activity [35].

6.3. Nazartinib (EGF816)

Nazartinib is a third generation TKI with activity against *EGFR* mutants and T790M, with high selectivity over wild-type EGFR. A Phase I trial of nazartinib in patients with T790M mutation demonstrated an ORR of 44%, DCR 91% and PFS of 9.2 months among 127 evaluable patients [36]. Nine patients with progression were evaluated for resistance and the C797S mutation with a concurrent *mTOR* deletion, *BRAF* fusions, *MET* amplification, and concurrent *TP53* and *RB1* truncating mutations were found [37].

In a Phase II expansion cohort of TKI naïve patients (NCT02108964); an unconfirmed ORR and DCR of 75% and 96%, respectively, was reported in a preliminary analysis among 24 evaluable patients [38]. Agents being assessed in combination trials with Nazartinib include capmatinib (INC280), an oral cMET inhibitor (NCT02335944) and nivolumab (NCT02323126).

6.4. AZD3759

AZD3759 is a unique EGFR TKI with a high capability of penetrating the BBB, resulting in approximately equal free concentrations in the blood, cerebrospinal fluid, and brain tissue [39]. The Phase I BLOOM study (NCT02228369) was conducted with two phases to evaluate the safety, pharmacokinetics and efficacy of AZD3759 in patients with *EGFR*-mutated NSCLC carrying brain and leptomeningeal metastases

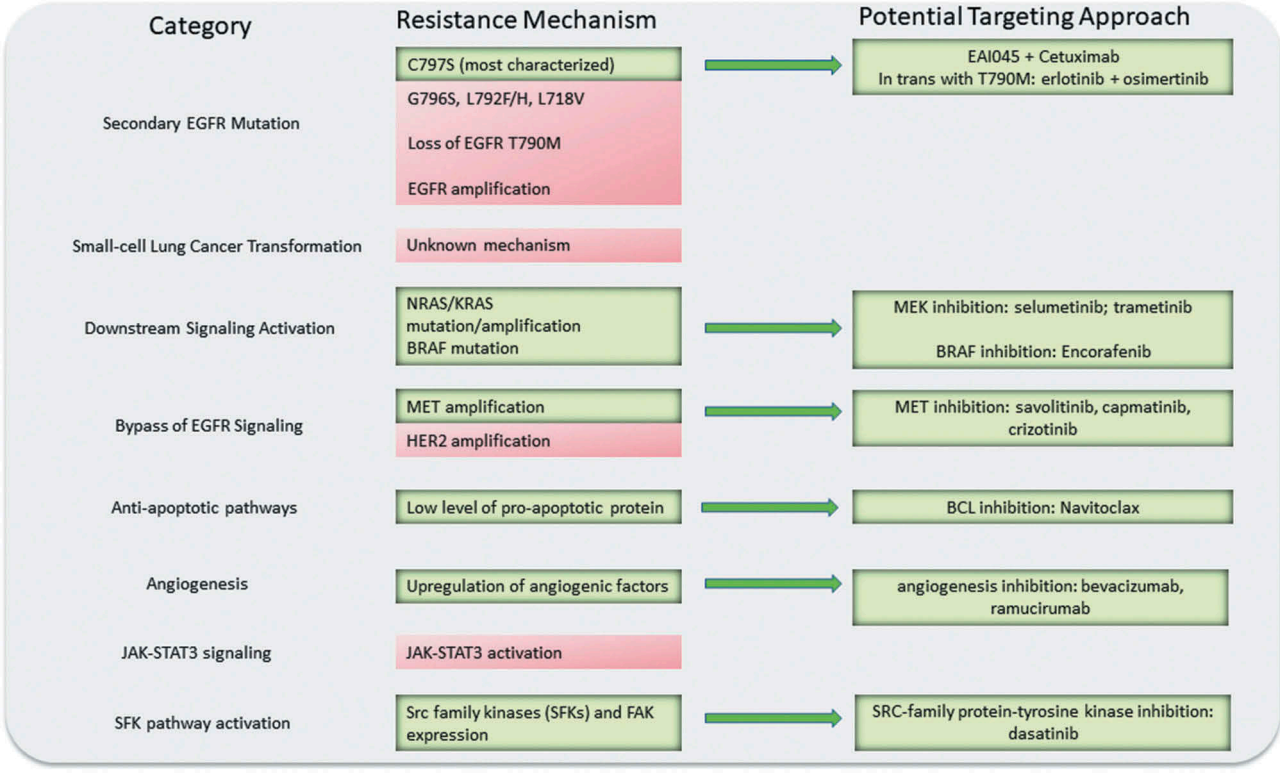


Figure 1. Mechanisms of resistance to osimertinib and targeted approaches to prevent or overcome resistance.

[40]. The dose-escalation phase enrolled patients who had progressed on prior TKI therapy. The dose-expansion phase included patients who were TKI naïve with either brain or leptomeningeal metastases and patients with leptomeningeal metastases who had been pretreated with an EGFR TKI. In the dose-expansion phase, among 18 TKI naïve patients with assessable CNS target lesions, a CNS-specific ORR of 83% and DCR of 89% were observed. For extracranial disease, the ORR and DCR were 72 and 94%, respectively. Overall, the combined ORR and DCR were 65 and 90%, respectively. In the subset of pre-treated patients with leptomeningeal involvement, an assessment by MRI revealed a RR of 28% and DCR of 78% with no confirmed extracranial responses [40].

6.5. Mavelertinib (PF-06747775)

Mavelertinib is a third generation EGFR TKI with pre-clinical activity against T790M. A Phase I/II study of mavelertinib as a single agent and in combination with other agents is ongoing (NCT02349633). Common adverse events reported were diarrhea (57%), rash (59%), paronychia (52%), dermatitis acneiform (34%), and stomatitis (32%) [41]. Among 26 evaluable patients in the dose escalation cohort, responses were observed at all dose levels with a PR in 11 patients and stable disease in 6 patients. Disease progression was noted in two patients and symptomatic deterioration in one patient. The AE profile was acceptable with no dose limiting toxicities [42].Other cohorts include Cohort 1, a Phase II study to assess activity in treatment naïve patients with EGFR mutant NSCLC. Cohort 2A is a Phase Ib evaluation of a combination of palbociclib with mavelertinib; followed by Cohort 2B, a randomized Phase II evaluation of the same combination with single agent mavelertinib in previously-treated patients with T790M mutations. Finally, Cohort 3 is a Phase Ib evaluation of the combination of mavelertinib with avelumab, an anti-PD-L1 antibody in the same population profile. The results of these cohorts are awaited.

6.6. YH25448 (GNS-1480)

YH25448 is a selective irreversible, third-generation EGFR TKI with good BBB penetrance. It targets the activating EGFR mutations Del19 and L858R, as well as the T790M mutation. In a Phase I/II study (NCT03046992), AEs of Grade 3 or higher were observed in only 5% of the patients indicating a good safety profile. Among 91 evaluable patients, ORR was 64% for the entire cohort. A higher ORR noted for T790M-positive patients (67% vs 47%). In nine patients with brain metastases, the overall intracranial ORR was 56%, indicating promising CNS activity [43].

6.7. Anlotinib

Anlotinib is a novel multi-targeted TKI targeting VEGFR, FGFR, PDGFR, and c-Kit. It was tested in the third-line setting for the treatment of advanced NSCLC in a randomized Phase III double-blind placebo-controlled trial (ALTER-0303). A significant improvement in OS was found; 9.6 versus 6.3 months (HR 0.68, 95% CI 0.54–0.87 p = 0.0018) [44]. A subset analysis among 138 patients with *EGFR* mutations revealed that this survival benefit extended to patients who had progressed on one or two prior lines of EGFR TKIs including osimertinib [45].

6.8. TAK-788 (AP32788) and other novel TKIs

TAK-788 is an investigational agent with activity against *EGFR* and *HER2* mutations, including exon 20 insertions. An ongoing Phase I/II trial reported interim results on 14 evaluable patients: three patients had PR, six experienced SD with an acceptable toxicity profile. All the partial responses were seen in patients with the *EGFR* exon 20 insertion making them attractive candidates for future expansion cohorts [46]. Other novel EGFR TKIs currently in Phase I trials include BPI-15086 (NCT02914990), DBPR112 (NCT03246854), FCN411 (NCT03420079), BPI-7711 (NCT03386955), and ZN-e4 (NCT03446417). Olmutinib, a third generation TKI with activity against T790M mutant NSCLC was approved in South Korea in 2016 based on a Phase I/II trial HM-EMSI-101 [NCT01588145]. Among 69 evaluable patients, objective responses were observed in 62% of patients. Development of the drug was halted and the drug withdrawn from the market in 2018 due to serious toxicity including two cases of fatal Steven-Johnson 's syndrome. Multiple agents targeting EGFR and novel combination strategies against specific resistance mechanisms are currently undergoing active investigation.

Selected novel agents active in EGFR mutant NSCLC in Phase 1 trials are summarized in Table 1.

7. Strategies to prevent primary resistance and overcome secondary resistance to third generation TKIs

7.1. Tertiary EGFR mutations

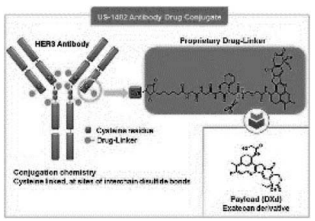
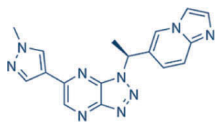
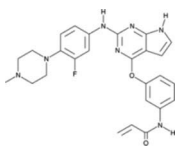
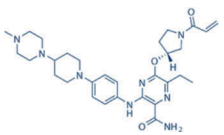
Osimertinib is a pyrimidine-based compound that forms an irreversible covalent bond at the ATP-binding site of the EGFR kinase domain via the C797 residue located on exon 20. Tertiary mutations at this site account for up to 40% of acquired resistance to third generation TKIs [47].

An analysis of 61 plasma samples of patients with acquired C797S resistance mutations revealed that 82% of patients had C797S/T790M in *cis* configuration compared to 10% in *trans*. 6% also had a loss of the founder T790M clone. 84% had at least one additional described resistance mechanism in addition to C797S such as *EGFR* amplification (48%) *MET* amplification (6%) *BRAF* V600E (5%), and *PIK3CA* mutation (15%) [48].

Interestingly, C797S and T790M mutations in *trans* configuration have been found to be sensitive to a combination of first- and third-generation TKIs whereas if these mutations are in *cis* configuration, EGFR combinations are generally ineffective [49].

Next-generation sequencing of plasma ctDNA from 93 Chinese patients who had progressed on osimertinib, 90 of whom had been pre-treated with first or second generation TKIs showed secondary mutations in L798I (binding interference), G796 (solvent front mutations), L792 (hinge pocket mutation) and L718 (steric hindrance) residues on *EGFR* that were confirmed in vitro to confer resistance [50]. Another study of 110 Chinese patients with exposure to prior generation of TKIs who had developed resistance to osimertinib showed that 52.7% of patients had resistance mutations, which were more prevalent in patients with exon 19 Deletion than in L858R (62.5% vs 39.1%,

Table 1. Selected novel agents active in EGFR mutant NSCLC in Phase 1 trials. Note: Structure of volitinib, ASP8273, SNX-5422 from www.Selleckchem.com; Structure of AC0010 from caymanchem.com; Structure of U3-1402 from https://www.daiichi-sankyo.eu/oncology/adc-franchise/; N/A: Not available in public databases.

Compound	Company	Structure	Indication	Stage of development	Target	NCT Number	Status
FCN-411	Ahon	N/A	PD on 1st gen – T790M	Phase 1	Unknown	NCT03420079	Not yet recruiting
ZN-e4	Zeno	N/A	PD on 1st gen – T790M	Phase 1	Unknown	NCT03446417	Not yet recruiting
U3-1402	Daiichi Sankyo		PD on 1st gen	Phase 1	HER3-targeting antibody drug conjugate	NCT03260491	Recruiting
BPI-7711	Betta Pharma	N/A	PD on 1st gen – T790M	Phase 1	EGFR (including T790M)	NCT03386955	Recruiting
DBPR112	National Health Research Institutes, Taiwan	N/A	PD on 1st gen	Phase 1	EGFR	NCT03246854	Recruiting
BPI-15086	Betta Pharm	N/A	PD on 1st gen – T790M	Phase 1	EGFR (including T790M)	NCT02914990	Recruiting
AZD6094	AstraZeneca		PD on 1st gen	Phase 1	MET	NCT02374645	Active, not recruiting
AC0010 (Avitinib)	ACEA Pharmaceutical		PD on 1st gen – T790M	Phase 1	EGFR (including T790M)	NCT02330367	Recruiting
ASP8273	Astellas Pharma		EGFR sensitive mt	Phase 1	EGFR (including T790M)	NCT02113813	Active, not recruiting

p = 0.015). The authors hypothesized that this may due to prolonged drug selection pressure from longer treatment of the deletion 19 group [51].

Mechanisms of resistance to osimertinib in the front-line setting are currently incompletely characterized, though sequencing of ctDNA from nine TKI naive patients at the time of progression in the Phase 1 cohort of the AURA trial showed several potential mechanisms: *MET* amplification (n = 1); amplification of *EGFR* and *KRAS* (n = 1); *MEK1, KRAS, or PIK3CA* mutation (n = 1 each); *EGFR* C797S mutation (n = 2); *JAK2* mutation (n = 1); and *HER2* exon 20 insertions (n = 1). Acquired EGFR T790M was not detected [52].

The development of 4th generation TKIs with activity against C797S mutations is in progress. EAI001 and EAI045 are fourth-generation EGFR TKIs that are non-ATP binding and allosterically inhibit mutant EGFR. EAI045 is not equally efficacious against the two EGFR subunits in their dimerized form and therefore needs to be combined with an EGFR dimerization inhibitor such as cetuximab for potent synergistic activity [53]. Novel agents targeting compound mutations involving T790M (T790M/C797S/L858R), (del 19/T790M/C797S) and (T790M/L858R) are in pre-clinical development [54–56]. Brigatinib, a dual EGFR and ALK kinase inhibitor also has been reported to have activity against triple mutated *EGFR* [57]. Necitumumab is a humanized antibody that binds to the extracellular region of EGFR, preventing dimerization and downstream signaling of the EGFR receptor. A Phase I trial of necitumumab with osimertinib is recruiting (NCT02496663).

7.2. Primary resistance mediated by exon 20 mutations of HER pathway

Insertion mutations in exon 20 of *EGFR* (*HER1*) or *HER2* occur in ~3% of lung adenocarcinomas and are associated with primary resistance to first and third generation TKIs. These mutations limit the size of the binding pocket through steric hindrance and prevent the binding of almost all TKIs to their site of activity. Poziotinib is a quinazoline-based pan-HER inhibitor that irreversibly blocks HER1 (ErbB1/EGFR), HER2 (ErbB2), and HER4 (ErbB4), including those with activating *EGFR* or *HER2* exon 20 mutations through its small and flexible nature that helps overcome steric hindrance. It is selective to exon 20 mutations with 65 times more potency in inhibiting cell lines with *EGFR* exon 20 insertions compared to *EGFR* T790M mutant cell lines [58]. A Phase II trial of poziotinib among 50 patients with *EGFR* exon 20 mutations and 13 patients with HER2 exon 20 mutations showed response rates of 58% and 50% respectively. 60% of patients had ≥grade 3 adverse events; most commonly skin-rash (27.5%) and diarrhea (12.5%) while 62.5% patients required dose reductions [59].

U3-1402 is an anti-HER3 antibody drug conjugate with a linker and topoisomerase I inhibitor payload that targets EGFR mutant lung cancers with HER3/ERBB3 oncogene overexpression and is currently in Phase I trials [60].

7.3. Acquired resistance through upregulated downstream signaling

The upregulation of signaling pathways downstream of EGFR is a major mechanism of secondary resistance to EGFR TKIs. Increased RAS signaling, through *NRAS* mutations (E63K)/amplifications and *KRAS* mutations (G12A/D, Q61H or A146T) or amplifications have been reported with secondary resistance to third generation TKIs [32,61]. Acquired *BRAF* V600E mutations are also associated with resistance to osimertinib [62]. Novel *BRAF* fusion products AGK/BRAF and PJA2/BRAF were described at the onset of resistance to the first and third generation TKIs, which were functionally validated in patient-derived cell lines [63].

The occurrence of acquired resistance mediated by downstream signaling may be prevented or delayed by certain up-front combination strategies. Selumetinib, a MEK inhibitor when combined with osimertinib, delayed the emergence of resistance in an EGFRm/T790M cell line by exploiting the dependence of resistant sub-clones to RAS signaling and is being tested in the ongoing multi-arm Phase Ib trial TATTON [61].

On the other hand, another MEK inhibitor, trametinib was shown to be effective in overcoming acquired resistance mediated by novel *BRAF* fusion products; *AGK/BRAF* and *PJA2/BRAF* [63]. In a similar vein, secondary resistance mediated by acquired *BRAF V600E* mutations was shown to be sensitive to a combination of encorafenib (BRAF inhibitor) and osimertinib in a patient-derived cell-line [62].

7.4. Acquired resistance through bypass parallel signaling

Blockade of the EGFR pathway with the third generation TKIs may activate bypass mechanisms to restore pro-growth signaling resulting in treatment resistance.

Acquired resistance through parallel MET and PI3K-mTOR signaling may be prevented by up-front combination regimens. Secondary *MET* and *HER2* amplifications are encountered with the third-generation TKIs in up to 30% and 10% of patients, respectively [31,32]. The multi-arm Phase Ib TATTON trial is currently testing the addition of savolitinib, a MET inhibitor to osimertinib in the second-line setting to prevent bypass signaling. Capmatinib is another MET inhibitor that is currently in combination Phase I/II trials in combination with EGF-816. Mutations in *PIK3CA* or the loss of *PTEN*, a negative regulator of PI3K signaling were associated with a poor response to EGFR TKI and the addition of rapamycin, an mTOR inhibitor slowed the progression of T790M mutant NSCLC in a mouse model [64,65]. Currently, sapanisertib (INK-128), an mTOR inhibitor is being studied in combination with osimertinib in a Phase I trial to prevent resistance mediated by up-regulated bypass signaling (NCT02503722).

Resistance acquired through FGF2-fibroblast growth factor receptor 1 (*FGFR* 1) amplification was implicated at progression on osimertinib in one patient with FGF2 mRNA level approximately 20-fold higher than normal [66]. Novel agents with activity against FGFR kinase have shown promising activity in urothelial cancer with response rates ranging from 24% with rogaratinib to 42% with erdafitinib in Phase I and Phase II trials, respectively [67,68]. The efficacy of these agents in overcoming secondary resistance mediated by bypass FGFR signaling in NSCLC remains yet to be elucidated.

7.5. Resistance through transformation to small cell histology

Histological transformation to small cell lung cancer has been described with third Generation TKIs. As such, no combination strategy currently exists to prevent this occurrence [69].

7.6. Resistance acquired through augmented anti-apoptosis

BIM is a pro-apoptotic protein that inhibits BCL-2. In patients with germline *BIM* deletions, low levels of BIM protein were correlated with reduced survival in patients treated with EGFR TKIs indicating that anti-apoptotic processes may mediate resistance to TKI therapy [70]. Primary resistance through apoptotic escape may be prevented by rationally combining TKI therapy with pro-apoptotic agents up-front. The efficacy of the BH-3 mimetic navitoclax (which inhibits both BCL-2 and BCL-XL) in combination with osimertinib is being studied in an ongoing Phase Ib study (NCT02520778).

7.7. Resistance acquired through augmented angiogenesis

The efficacy of frontline TKI therapy may be enhanced by targeting augmented angiogenesis upfront to prevent resistance. Results from a Phase III study of 228 patients with *EGFR* mutations in the front line setting showed a PFS benefit (16.9 months vs 13.3 months, HR 0.61, p = 0.0157) with the addition of bevacizumab to erlotinib [71]. An ongoing, Phase I study (NCT02789345) of ramucirumab in combination with osimertinib in 25 patients with T790M-positive EGFR-mutant NSCLC showed an ORR of 76% and a 6-month PFS of 64% with no additive toxicities [72]. A randomized Phase II trial of osimertinib with bevacizumab (BOOSTER) in the second-line setting is in progress (NCT03133546). Apatinib is a highly selective VEGFR2 inhibitor that is being studied in combination with EGFR TKIs including osimertinib in a Phase I trial (NCT03050411).

7.8. Resistance mediated by JAK-STAT3 signaling

The upregulation of JAK-STAT3 signaling occurs as part of an early adaptive response to treatment with TKI therapy and is augmented via IL-6 driven autocrine loops [73]. However, trials of ruxolitinib in combination with erlotinib in patients with acquired resistance failed to show meaningful clinical benefit [74]. An ongoing trial seeks to assess the efficacy of itacitinib, a JAK-STAT3 inhibitor in combination with osimertinib in patients with T790M mutation to target the reflex up-regulation of JAK-STAT3 signaling.

7.9. Other pathways of signal transduction

SRC is a non-receptor intracellular tyrosine kinase involved in cell survival and differentiation that functions downstream of EGFR [75]. EGFR TKI-resistant cell lines show elevated SRC levels, which were abrogated by dasatinib [76]. The expression of several other non-receptor tyrosine kinases is elevated in response to EGFR TKI therapy. Increased Src family kinases (SFKs) and FAK expression drives the activation of YAP1, which upregulates AXL, MET and CDCP1; all of which have important mitogenic and downstream signal transduction properties that can result in EGFR TKI resistance.

Acquired resistance mediated through non-receptor tyrosine kinase activity may be sensitive to combination strategies. In a Phase II trial of patients with progression of disease with first-generation TKIs, the combination of dasatinib and erlotinib showed modest response rates of 15% with an acceptable toxicity profile [77]. A Phase I/II trial of the combination of dasatinib with osimertinib is currently on-going. Pre-clinical evidence of promising efficacy exists for the efficacy of combining osimertinib with TPX-0005, a small molecule actively against Src and FAK, with a Phase I trial currently in the planning stages [78]. DS-1205c is an AXL inhibitor that is currently being tested in combination with osimertinib for patients with disease progression on first-generation TKIs. (NCT03255083)

8. Immunotherapy in *EGFR* mutated NSCLC

Single-agent response rates with immune checkpoint inhibitors in *EGFR*-mutated NSCLC tend to be very low (3–4%) likely due to the relatively low tumor mutational burden [79]. A number of trials are on-going to ascertain optimal strategies for harnessing the immune system in the treatment of these malignancies. However, caution needs to be exercised when combining different agents due to the risk of augmented toxicities with limited survival benefit. In the Phase I TATTON trial, when osimertinib was combined with durvalumab, 13/34 patients (38%) developing interstitial pneumonitis when compared to osimertinib monotherapy (3%) and durvalumab monotherapy (2%) leading to the termination of the combination arm [80]. A recent meta-analysis of randomized trials that compared checkpoint inhibitors to docetaxel in the second line setting showed no survival benefit with checkpoint inhibitors in EGFR-mutant advanced NSCLC [81]. A Phase II trial of pembrolizumab in *EGFR* mutant NSCLC (sensitizing or non-sensitizing), TKI naive patients with PD-L1–positivity (≥1%) was stopped prematurely after lack of responses (0/10) were demonstrated in patients with documented *EGFR* mutations despite 73% of those patients having PD-L1 expression ≥50% [82].

However, preliminary signals of efficacy do exist in certain selected groups of patients with *EGFR* mutations. A subset analysis of the single arm Phase II ATLANTIC trial (NCT02087423) of durvalumab in advanced NSCLC in the ≥ third-line setting showed encouraging efficacy in the EGFR+/ALK+/≥25% TC PD-L1 population (N = 77) with a median OS of 13.3 months and 53.3% of the cohort being alive at 1 year; which was similar to the EGFR-/ALK- population (N = 149) (10.9 months and 47.8%, respectively). These results will need prospective validation in larger cohorts of patients [83]. EGF-816 is currently being tested in two ongoing trials with anti-PD-1 checkpoint inhibitors PDR001 and nivolumab in the trials NCT02900664 and NCT02323126, respectively.

Selected early stage trials of third generation TKIs in combination with other agents are summarized in Table 2.

Table 2. Selected early stage trials of 3rd Generation TKIs in combination with other agents.

Compound	Company	EGFR-TKI	Indication	Stage of development	Target	Status	NCT
Selumetinib/ Durvalumab/ Savolitinib	AstraZeneca	Osimertinib	EGFR mutation	Phase 1	MEK/PD-L1/ MET	Recruiting	NCT02143466
DS-1205c	Daiichi Sankyo	Osimertinib	PD on 1st gen – non T790M	Phase 1	AXL	Recruiting	NCT03255083
Ramucirumab/ Necitumumab	Eli Lilly and AstraZeneca	Osimertinib	T790M	Phase 1	VEGFR/EGFR	Active, not recruiting	NCT02789345
Bevacizumab	Hoffmann-La Roche	Osimertinib	PD on prior TKI	Phase 2	VEGFR	Recruiting	NCT03133546
Sapanisertib	Millennium Pharmaceuticals	Osimertinib	PD on 1st gen	Phase 1	mTOR	Recruiting	NCT02503722
Apatinib	Jiangsu Hengrui Medicine	Erlotinib, geftinib, osimertinib	PD on 1st gen	Phase 1	VEGFR	Recruiting	NCT03050411
Navitoclax	Abbvie	Osimertinib	T790M	Phase 1	Bcl-2 and Bcl-XL	Recruiting	NCT02520778
Necitumumab	Eli Lilly	Osimertinib	PD on 1st gen – nonT790M	Phase 1	EGFR	Recruiting	NCT02496663
Capmatinib (IC280)	Novartis	EGF-816	PD on prior TKI	Phase 1/2	MET	Recruiting	NCT02335944
PDR001	Novartis	EGF-816	EGFR mutation	Phase 1	PD-1	Recruiting	NCT02900664

9. Combination of third generation EGFR TKI with platinum-based chemotherapy and other EGFR TKIs

A Phase III trial of 344 Japanese patients with newly diagnosed/ recurrent *EGFR* mutant Stage III/IV NSCLC compared the combination of gefitinib, carboplatin, and pemetrexed to gefitinib alone. The chemotherapy combination arm reduced the risk of death by 30% and improved median OS by 13.4 months (52.2 months vs 38.8 months, HR 0.695 $p < 0.01$) [84]. An earlier RCT of 121 untreated patients with *EGFR* mutant lung adenocarcinoma randomized to the same regimen also showed a survival benefit to upfront combination chemotherapy compared with EGFR TKI alone [85]. It is unknown whether this survival benefit will hold true in combination chemotherapy with third generation TKIs such as osimertinib. A randomized Phase II trial is currently ongoing [86]. Case reports exist of response to a combination of osimertinib and gefitinib in a case of a patient with osimertinib resistance mediated by a C797S mutation in *trans* position to T790M [87].

10. Potential development issues

10.1. Tumor heterogeneity

Up to 75% of early stage NSCLCs have significant tumor heterogeneity at baseline associated with a lesser duration of response to TKIs [88,89]. Over time, the sub-clonal architecture shifts and expands, which increases the complexity of sub-clonal hierarchy. Ultimately, resistance may develop due to the competitive selection pressure applied by TKI therapy, which necessitates re-biopsies to ascertain the mechanism of resistance. This carries significant procedural risks to patients and may delay the initation of the next line of therapy. Besides, resistance mutations may be spatially dispersed within the tumor reducing the clinical yield of a biopsy.

Liquid biopsy with sequencing of circulating tumor DNA (ctDNA) is a non-invasive method of detecting genomic changes that may circumvent some of the pitfalls of repeat biopsies. The non-invasive nature of liquid biopsies offers the advantage of serial tracking of emerging subclones of mutations in near real-time. Rising frequencies of *EGFR* T790M have been observed before the onset of clinical resistance to EGFR TKIs [90,91]. The incorporation of mutational data from ctDNA for serial monitoring of the development of resistance at the molecular level can stratify patients destined to relapse, months to even years prior to radiologic progression. This opens a window of opportunity to intervene with salvage therapies tailor-made to the patient's resistance profile.

10.2. Combinatorial complexity and rational strategies for polytherapy

With a better understanding of resistance mechanisms associated with the use of EGFR TKIs and the ability to serially sequence resistant sub-clones through liquid biopsies, there has been an explosion in the combinatorial approaches available to target molecular alterations that drive resistance. Blocking 'truncal' mutations in the evolutionary tree might slow down the evolution of sub-clonal genetic heterogeneity and reduce the complexity of downstream branches. But the efficacy of this approach in improving overall outcomes is unproven. On the other hand, convergence of a myriad number of mutated pathways into common signaling cascades may reduce the permutations into a more manageable set of actionable pathways. Thus, there remains a lack of clarity on the optimal approach to targeting sub-clonal heterogeneity.

In a similar vein, significant controversy exists as to whether a strategy of targeting upstream bypass pathways is superior to blocking downstream signaling or vice versa. Inhibition of down-stream MEK in combination with EGFR inhibition was found to be a better strategy to delay the occurrence of resistance than targeting bypass MET signaling in EGFR-mutant cell lines [92].

The analysis of high throughput sequencing data using computational models that employ machine learning techniques may further improve precision in predicting and targeting the pathways and molecular alterations most amenable to achieving durable responses [93].

11. Conclusion

The promise of precision oncology is encapsulated by the paradigm shift that has occurred with the advent of molecularly targeted treatments for *EGFR*-mutated NSCLC. Osimertinib, a third-generation EGFR TKI, has moved into the front-line setting with robust activity against sensitizing *EGFR* mutations, T790M and at sanctuary sites such as brain and leptomeninges. Resistance and relapse, which invariably occur with EGFR TKIs indicate that different combination strategies that target both EGFR signaling and bypass pathways will need to be pursued sequentially or concurrently. Advances in sequencing ctDNA make it possible to assess the serial evolution of mutational sub-clones in real-time; opening a window of opportunity to implement personalized therapies specific to individual patients when the tumor burden is still sub-threshold. Moving forward, rational biomarker-driven approaches will be required to ascertain the optimal strategies of incorporating immune therapy and other combinations to combat resistance and improve clinical benefit.

12. Expert opinion

With the approval of osimertinib as first-line therapy for *EGFR*-mutated NSCLC, a better understanding of the resistance mechanisms in the front-line setting is now a central focus of research. Resistance to osimertinib in the second-line setting is associated with the occurrence of gatekeeper *EGFR* C797S mutations in up to one-third of TKI pre-treated patients and rational approaches to combining novel fourth generation TKIs with cetuximab and necitumumab are being explored. Development of agents that target other acquired *EGFR* resistance mutations such as L798I, G796, L792, and L718 are ongoing. A strategy to overcome resistance mediated through downstream signaling is to combine third generation TKIs with inhibitors of MEK pathway, which is being studied in numerous ongoing Phase I/II trials. Novel agents that target non-EGFR resistance mutations including *FGFR1* alterations, *BRAF* fusion, *HER2* Exon 20 mutations and RET fusions are under active development. The efficacy of abrogating bypass signaling, such as the MET pathway, awaits confirmation from large randomized trials. Many combination strategies, such as targeting anti-apoptotic signaling, angiogenesis, JAK-STAT3, and SFK/FAK pathway have been shown to augment efficacy and prevent resistance to third-generation TKIs in early studies. Though immune checkpoint inhibitor monotherapy does not lead to significant clinical benefit in relapsed/refractory *EGFR* mutant NSCLC; with a more sophisticated understanding of the determinants of immune response, it may be possible to identify subsets of patients who will derive benefit with combination immune strategies. Furthermore, advances in liquid biopsy with circulating tumor DNA have the potential to enhance the ability to detect resistance and relapse in a timely manner, and improve the speed and precision of molecularly targeted therapies.

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