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REVIEW ARTICLE

Targeting Acquired and Intrinsic Resistance Mechanisms in Epidermal Growth Factor Receptor Mutant Non-Small-Cell Lung Cancer

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Abstract

Over the past 2 decades, rapid advances in molecular profiling and the development of targeted therapies have dramatically improved the clinical course of advanced non-small-cell lung cancer (NSCLC). Mutations in the epidermal growth factor receptor (*EGFR*) gene are found in about a third of patients with advanced NSCLC, and the approval of first-generation EGFR targeted kinase inhibitors significantly improved survival when compared with platinum-based doublet chemotherapy (PBC), the previous standard of care. Inevitably, selective pressure from first-generation EGFR inhibitors led to acquired resistance mechanisms, such as the T790M mutation. The advent of third-generation EGFR inhibitors (e.g., osimertinib) successfully overcame the T790M resistance mechanism, and osimertinib subsequently became the first-line therapy for *EGFR* mutant NSCLC. Currently, research in *EGFR* mutant NSCLC is primarily focused on targeting resistance mechanisms to osimertinib. Over the past several years, many important acquired and intrinsic mechanisms of resistance to osimertinib have been identified. Acquired resistance mechanisms include C797X, mesenchymal epithelial transition factor (*MET)* amplification, *HER2/HER3* amplification, phosphoinositide 3-kinase (PI3K) pathway mutations, RAS/mitogen-activated protein kinase (MAPK) pathway mutations, cell–cycle gene alterations, oncogenic fusions, and histologic transformations. An important intrinsic resistance mechanism to osimertinib is the *EGFR* exon 20 insertion mutation, which is sensitive to the newly Food and Drug Administration (FDA)-approved tyrosine kinase inhibitor mobocertinib and the EGFR/MET bispecific antibody amivantamab. This review article aims to (1) summarize the advances in the treatment of *EGFR* mutant NSCLC, (2) delineate known resistance mechanisms to the current first-line therapy, osimertinib, and (3) describe the development of targeted drugs that aim to overcome these resistance mechanisms.

1 Background

The clinical course of advanced non-small-cell lung cancer (NSCLC) has swiftly evolved over the past 20 years. Platinum-based doublet chemotherapy (PBC) was the standard of care for all patients with advanced NSCLC and a good performance status. However, PBC yielded disappointing results: an objective response rate (ORR) of about 30%, median progression-free survival (PFS) of 5–6 months, and median overall survival (OS) of 11–12 months [1, 2]. Fortunately, improvements in molecular profiling and the approval of various targeted therapies have drastically improved the prognosis for patients with targetable mutations [3–6].

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The detection of oncogenic driver mutations in the epidermal growth factor receptor (*EGFR*) gene was a pivotal milestone in the diagnosis and treatment of NSCLC [7]. EGFR is a receptor tyrosine kinase that is commonly expressed in normal tissue and participates in cellular pathways leading to cell proliferation, migration, and survival. Activating mutations affecting the kinase domain of EGFR lead to ligand-independent downstream signaling of EGFR, thereby promoting cancer growth. Such mutations occur in up to half of patients with NSCLC, with the peak incidence in East-Asian, non-smoking, and female patients [8].

The classical *EGFR* L858R point mutation and exon 19 deletions comprise up to 90% of *EGFR* mutant NSCLC and lead to conformational changes that destabilize the dormant form of the EGFR protein, effectively shifting the equilibrium towards the active form [9, 10]. This conformational change in the adenosine triphosphate (ATP) pocket of EGFR is the target of first- and second-generation tyrosine kinase inhibitors (TKIs) [7]. EGFR TKIs bind the ATP pocket

Key Points

Osimertinib, a third-generation epidermal growth factor receptor (EGFR) inhibitor, is the standard of care for advanced *EGFR* mutant non-small-cell lung cancer in the first-line setting.

Identified mechanisms of resistance to osimertinib can be classified as EGFR-dependent versus EGFR-independent and acquired versus intrinsic.

The *EGFR* exon 20 insertion mutation is more sensitive to novel tyrosine kinase inhibitor and antibody therapies than osimertinib or the earlier generation EGFR inhibitors.

Several targeted therapies aiming to overcome resistance mechanisms to osimertinib are currently in development or being tested in clinical trials.

of EGFR, leading to inhibition of kinase phosphorylation and downstream pathways. The approval of first-generation reversible EGFR TKIs (e.g., gefitinib, erlotinib) for advanced and metastatic *EGFR* mutant NSCLC dramatically improved ORR as high as 80% and median PFS to greater than 10 months, exceeding that observed from PBC [1, 2]. The success of these agents was pivotal in transforming the management of NSCLC from a histology-based approach to a personalized, targeted approach. However, despite such advancements in molecular profiling and targeted therapeutics, selective pressure on the cancer cells inevitably led to drug resistance and disease progression [11]. While some of this was overcome in the landmark FLAURA trial with the T790M-active inhibitor osimertinib, up to 10% of *EGFR* mutant NSCLC had de novo or primary resistance either due to different *EGFR* mutations, such as *EGFR* exon 20 insertion (ex20ins), or poorly understood initial tumor biology [6]. This article will review the tumor biology of resistance (Fig. 1) and current research to overcome this common clinical challenge (Table 1).

2 Acquired Resistance: EGFR-Dependent Mechanisms

2.1 T790M

Soon after the advent of first-generation TKIs, a somatic mutation in *EGFR*, p.Thr790Met (T790M), was discovered. T790M alters a residue situated deep inside the ATP pocket of EGFR and thereby blocks the binding of first- and second-generation TKIs to the ATP-binding site [12]. Therefore, the presence of the T790M mutation prior to treatment with first- and second-generation TKIs led to intrinsic resistance and therefore poorer outcomes [13]. Additionally, 50–60% of patients who initially responded to first- or second-generation TKIs ultimately developed T790M mutations, leading to acquired resistance to therapy [14, 15].

The development of third-generation TKIs, particularly osimertinib, was essential in overcoming this resistance mechanism. Osimertinib covalently bonds with the C797 residue of the ATP-binding site of EGFR regardless of the T790M mutation. Osimertinib was initially approved for treatment of *EGFR* mutant, T790M-positive NSCLC after progression on first-line TKI. Subsequently, the FLAURA trial in 2018 showed that osimertinib led to increased PFS (19 vs. 10 months) when compared to gefitinib and erlotinib in the first-line setting for advanced *EGFR* mutant NSCLC [6]. As osimertinib improved the median OS for advanced *EGFR* mutant NSCLC to 38.6 months and demonstrated a favorable toxicity profile compared to earlier generation TKIs, it was approved as first-line therapy for all advanced *EGFR* mutant NSCLC regardless of T790M status. [6, 16]

Notably, osimertinib also demonstrated improved efficacy against central nervous system (CNS) disease, which is present in about 30% of *EGFR* mutant NSCLC at diagnosis [16]. First- and second-generation EGFR TKIs yielded variable activity against brain metastases [17–19], whereas in the FLAURA trial, front-line osimertinib demonstrated a PFS benefit in patients with CNS disease [6, 20]. In the phase I BLOOM study, 160 mg of osimertinib daily, which is double the normal dose, yielded an ORR of 62% and a median OS of 11 months in patients with leptomeningeal disease [21].

Recently, another third-generation TKI, lazertinib, also demonstrated a favorable safety profile and anticancer activity in a phase I/II trial of *EGFR* mutant, T790M-positive NSCLC [22], and there are other agents with similar activity in development worldwide. Because of these advances, T790M as a resistance mechanism has become less clinically relevant. When resistance develops to first-line osimertinib, plasma genotyping shows no evidence of emergence of T790M mutations [23]. Instead, due to selective pressure from osimertinib, acquired resistance is often associated with development of other EGFR-dependent and EGFR-independent bypass pathways.

2.2 C797S

Since osimertinib overcomes T790M resistance by binding to the C797 residue in the ATP pocket, it is not surprising that the most common *EGFR*-dependent mechanism of resistance to osimertinib are mutations at C797 [23]. C797 mutations also confer resistance to similar third-generation EGFR TKIs (e.g., rociletinib, olmutinib, and nazartinib)

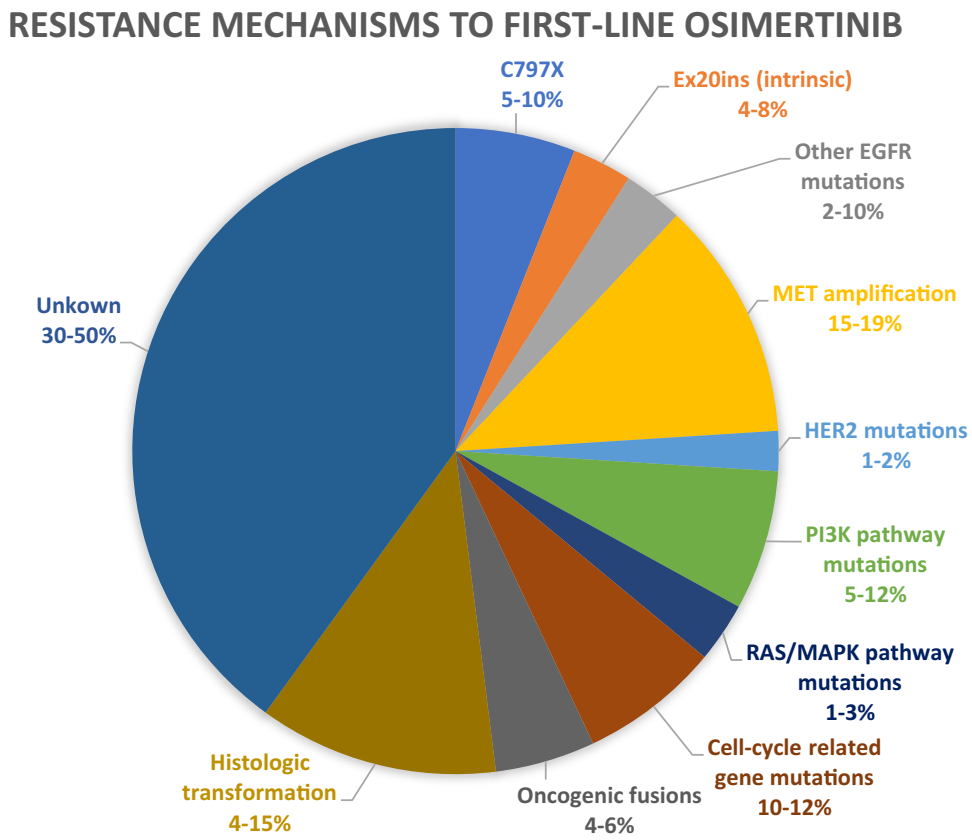

Fig. 1 Approximate distribution of resistance mechanisms to first-line osimertinib. *EGFR* epidermal growth factor receptor, *ex20ins* exon 20 insertion, *MAPK* mitogen-activated protein kinase, *MET* mesenchymal epithelial transition factor, *PI3K* phosphoinositide 3-kinase

[24]. Mutations at C797 were detected in 15% of patients at disease progression to osimertinib in the second-line setting and 7% of patients at progression to first-line osimertinib [22]. Notably, similar resistance mutations at C797, including C797G, have also been reported. [24]

In the absence of a T790M mutation, tumors resistant to osimertinib due to C797S mutations retain sensitivity to first- and second-generation EGFR TKIs (e.g., gefitinib, erlotinib, afatinib) [25, 26]. In the presence of a T790M mutation, which is only observed in patients who had a prior earlier generation EGFR TKI, if the T790M and C797S mutations are on different alleles (*trans*), then the tumor will likely retain sensitivity to first- and second-generation EGFR TKIs [27]. Retrospective data show that two-thirds of progressed cases have *cis* presentations, which would remain resistant to both first- and second-generation EGFR TKIs [28].

Adding first- or second-generation EGFR TKIs to osimertinib in the first-line setting may prevent the clonal selection of C797S mutations [29]. Additionally, "fourth-generation" EGFR TKIs in development (e.g., EAI045, JBJ-04-125-02, BLU-945) may overcome both C797S and T790M mutations in vitro and in vivo, but have not been assessed in clinical trials yet and may be dependent on the underlying core driver mutation [30]. Additionally, a novel anaplastic lymphoma kinase (ALK)/EGFR inhibitor, brigatinib, in combination with a fourth-generation EGFR TKI, has also demonstrated in vivo efficacy against triple-mutant (EGFR mutant, T790M positive, C797S mutant) NSCLC [31]. BBT-176 is another novel EGFR TKI designed to allosterically inhibit EGFR with C797S mutations (NCT04820023).

2.3 Other EGFR-Dependent Acquired Resistance Mechanisms

While mutations at C797 are the most common on-target resistance mechanisms in *EGFR* mutant NSCLC, other tertiary *EGFR* mutations have also been detected. For example, G796D/R/S and L792H mutations in exon 20 of *EGFR* lead to conformational changes that sterically hinder osimertinib [31–33]. On exon 18 of *EGFR,* rare mutations at G719, L718, and G724 have been associated with osimertinib resistance, though in the absence of T790M mutation, they may also remain sensitive to first- and second-generation EGFR TKIs [24, 32]. Interestingly, G724S mutations generally only lead to osimertinib resistance in the presence of exon 19 deletion. but not in the presence of L858R [33]. *EGFR* amplification, which is correlated with EGFR immunohistochemistry (IHC), is also associated with osimertinib resistance, though this association may be confounded by concurrent off-target bypass pathways [34, 35]. A phase I trial employing the combination of osimertinib

Table 1 Selected trials addressing resistance mechanisms to third-generation EGFR inhibitors

NCT number	Phase	Title	Resistance mechanism involved	Treatment arms
NCT04820023	1/2	Phase 1/2 Study of BBT-176 in Advanced NSCLC With Progression After EGFR TKI Treatment	C797X	BBT-176 ± cetuximab
NCT02496663	1	Osimertinib and Necitumumab in Treating Patients With EGFR-Mutant Stage IV or Recurrent Non-small Cell Lung Cancer Who Have Progressed on a Previous EGFR Tyrosine Kinase Inhibitor	C797X	Osimertinib + necitumumab
NCT02609776	1	Study of Amivantamab, a Human Bispecific EGFR and cMet Antibody, in Participants With Advanced Non-Small Cell Lung Cancer (CHRYSALIS)	*MET* and ex20ins; broadly efficacious in *EGFRm* NSCLC	Amivantamab + lazertinib ± carboplatin/pemetrexed
NCT03944772	2	Phase 2 Platform Study in Patients With Advanced Non-Small Lung Cancer Who Progressed on First-Line Osimertinib Therapy (ORCHARD)	*MET, EGFR* C797X, *EGFR* amplification	Osimertinib + savolitinib; osimertinib + gefitinib; osimertinib + necitumumab; durvalumab + carboplatin + pemetrexed
NCT03778229	2	Osimertinib Plus Savolitinib in EGFRm+/MET+ NSCLC Following Prior Osimertinib (SAVANNAH)	*MET*	Osimertinib + savolitinib
NCT03940703	2	A Study of Tepotinib Plus Osimertinib in Osimertinib Relapsed MET Amplified NSCLC (INSIGHT 2)	*MET*	Tepotinib ± osimertinib
NCT02099058	1	A Study Evaluating the Safety, Pharmacokinetics (PK), and Preliminary Efficacy of ABBV-399 in Participants With Advanced Solid Tumors	*MET*	Telisotuzumab vedotin ± (erlotinib OR osimertinib OR nivolumab)
NCT03516214	1	EGF816 and Trametinib in Patients With Non-small Cell Lung Cancer Harboring Activating EGFR Mutations (EATON)	*MET*	EGF816 + trametinib
NCT03784599	2	T-DM1 and Osimertinib Combination Treatment to Target HER2 Bypass Track Resistance in EGFR Mutation Positive NSCLC (TRAEMOS)	*HER2*	Trastuzumab emtansine + osimertinib
NCT03260491	1	U3-1402 in Metastatic or Unresectable Non-Small Cell Lung Cancer	*HER3*	U3-1402 (consists of an antibody component, patritumab, U3-1287)
NCT02503722	1	Testing the Combination of MLN0128 (TAK-228) and AZD9291 in Advanced EGFR (Epidermal Growth Factor Receptor) Mutation Positive Non-small Cell Lung Cancer	*mTOR*	Osimertinib + sapanisertib
NCT03255083	1	DS-1205c With Osimertinib for Metastatic or Unresectable Epidermal Growth Factor Receptor (EGFR)-Mutant Non-Small Cell Lung Cancer	*AXL*	DS-12505c + osimertinib
NCT03455829	1b/2	G1T38, a CDK 4/6 Inhibitor, in Combination With Osimertinib in EGFR-Mutant Non-Small Cell Lung Cancer	CDK 4/6	Lerociclib + osimertinib
NCT02716116	1/2	A Study of TAK-788 in Adults With Non-Small Cell Lung Cancer	*EGFR* ex20ins	Mobocertinib ± carboplatin/pemetrexed
NCT03318939	2	Phase 2 Study of Poziotinib in Patients With NSCLC Having EGFR or HER2 Exon 20 Insertion Mutation	*EGFR* ex20ins	Poziotinib

Table 1 (continued)

NCT number	Phase	Title	Resistance mechanism involved	Treatment arms
NCT03974022	1/2	Assessing an Oral EGFR Inhibitor, DZD9008 in Patients Who Have Advanced Non-small Cell Lung Cancer With EGFR or HER2 Mutation (WU-KONG1)	*EGFR* ex20ins, *HER2*	DZD9008
NCT04036682	1/2a	A Phase 1/2a Trial of CLN-081 in Patients With Non-Small Cell Lung Cancer	*EGFR* ex20ins	CLN-081

EGFR epidermal growth factor receptor, *EGFRm* epidermal growth factor receptor mutant, *ex20ins* exon 20 insertion, *MET* mesenchymal epithelial transition factor, *NCT* National Clinical Trial, *NSCLC* non-small-cell lung cancer, *TKI* tyrosine kinase inhibitor

and necitumumab for patients demonstrated clinical activity against EGFR-dependent resistance (T790M+/C797S+) after progression on third-generation TKI (NCT02496663). Most recently, a phase I study utilizing the combination of amivantamab, a bispecific EGFR and c-mesenchymal epithelial transition factor (c-MET) antibody, with lazertinib, a third-generation EGFR TKI, demonstrated promising results with an ORR of 36% in patients who progressed on osimertinib and an ORR of 100% in TKI-naïve patients [36]. Overall, the lack of specific agents to target these EGFR-dependent acquired resistance mechanisms is an important area of future research and drug development.

3 Acquired Resistance: EGFR-Independent Mechanisms

3.1 *MET* Amplification

In classical *EGFR* mutant NSCLC, the most common EGFR-independent mechanism that confers resistance to osimertinib is *MET* amplification, which bypasses EGFR by leading to constitutive activation of downstream signaling pathways, such as those mediated by mitogen-activated protein kinase (MAPK), signal transducer and activator of transcription (STAT), and phosphoinositide 3-kinase (PI3K)-Akt [37, 38]. Like *EGFR* amplification, *MET* amplification is strongly correlated with MET IHC [39]. *MET* amplifications can also be identified through routine circulating tumor DNA (ctDNA) analysis [38].

In the AURA3 study, *MET* amplification was found through plasma next-generation sequencing (NGS) in 19% of patient samples at disease progression [40]. Through NGS ctDNA analysis, after progression on first-line osimertinib, *MET* amplification was found in 15% of patient samples [41]. Because it is more challenging to detect amplifications than mutations diagnostically, the incidence of *MET* amplification may be underestimated by these data. Based on existing retrospective data, *MET* amplification occurs regardless of the presence or loss of the T790M mutation [42–44] and co-occurs with *EGFR* C797S in 5–10% of cases. [45]

To overcome resistance to osimertinib due to *MET* amplification, c-Met inhibitors may be utilized. Given the availability for other indications, crizotinib with osimertinib was initially tested and found to be efficacious against tumors that acquire resistance to osimertinib through *MET* amplification [46, 47]. In the phase Ib TATTON trial, the combination of the MET TKI savolitinib with osimertinib yielded an ORR of 30% and a PFS of 5.4 months in patients with acquired resistance to third-generation EGFR TKIs in the setting of *MET* amplification [48]. The phase II trial for this combination is currently underway (NCT03778229). The combination of another MET TKI (capmatinib) with

gefitinib has also yielded favorable results in a phase II trial of patients with *MET* amplification who were previously treated with an EGFR TKI. In this trial, ORR for all patients was 27%, but it increased to 47% in the subset of patients who had six or more *MET* gene copies [49]. In another phase Ib/II trial of patients with *MET* overexpression or amplification who had progressed on a previous EGFR TKI, the combination of gefitinib and the MET TKI, tepotinib, led to higher ORR compared with standard PBC [50]. As previously mentioned, the combination of the bispecific EGFR and c-MET antibody amivantamab and the third-generation EGFR TKI lazertinib has recently demonstrated an ORR of 36% in patients who progressed on osimertinib and an ORR of 100% in TKI-naïve patients. [36]

3.2 *HER2* and *HER3* Amplification

The ErbB2 tyrosine kinase receptor is encoded by *HER2* and is responsible for activating downstream PI3K-Akt and MAPK pathways. *HER2* amplification is found in about 2% of patients with resistance to first-line osimertinib [41]. The anti-HER2 antibody-drug conjugated (ADC) trastuzumab-emtansine (T-DM1) has shown efficacy in preclinical models and in patients harboring concurrent *HER2* amplification and *EGFR* mutation after progressing on an EGFR TKI [51]. Further clinical studies are needed to optimize the role of HER2 inhibitors in overcoming osimertinib resistance in *EGFR* mutant NSCLC.

HER3 (ERBB3) is another receptor that is often overexpressed in *EGFR* mutant NSCLC, and it leads to cell growth and proliferation through dimerization with either EGFR or HER2 [52]. Patritumab deruxtecan is a novel HER3 directed ADC that is demonstrating favorable results in patients previously treated with an EGFR TKI, yielding an ORR of 25% and a disease control rate of 70%. Interestingly, the performance of patritumab deruxtecan was not affected by the presence or absence of other oncogenic mutations, suggesting that HER3 antagonism may serve as a therapeutic approach that is relatively agnostic to the mechanism of resistance (NCT03260491).

3.3 PI3K Pathway Mutations

Activation of the PI3K pathway, either through *PIK3CA* mutation or *PTEN* deletion, is implicated in up to 5% of patients who develop resistance to first-generation EGFR TKIs and 5–12% of patients who develop resistance to osimertinib [30]. Though *PIK3CA* mutations commonly co-occur with other driver mutations in NSCLC and generally portend worse prognosis, evidence suggests that in *EGFR* mutant NSCLC, the presence of a concurrent *PIK3CA* mutation has no significant impact on the clinical benefit from EGFR TKI monotherapy [53]. Targeted therapies against *PIK3CA* mutations have not demonstrated clinical benefit thus far.

3.4 RAS-MAPK Pathway Mutations

Mutations along the RAS-MAPK pathway have also been implicated in TKI resistance in patients with *EGFR* mutant NSCLC. In the FLAURA trial, variable mutations in *NRAS* and *KRAS* were found in 1% of patients who progressed on first-generation TKIs and 3% of patients who progressed on first-line osimertinib [41]. *NRAS* mutations include the E63K mutation, while *KRAS* mutations include the G12S, G13D, Q61R, and G12D mutations [44]. *BRAF*V600E mutations were found in 3% of patients who progressed on first- or second-line osimertinib [54, 55]. There has also been a reported case of *MAPK1* mRNA overexpression in one patient who progressed on second-line osimertinib [56]. BRAF inhibitors or the vascular endothelial growth factor receptor (VEGFR)/MET/AXL inhibitor cabozantinib may confer efficacy against osimertinib resistance due to such mutations, but robust clinical trial data are lacking [54, 57]. Similarly, MEK inhibitors such as selumetinib may help overcome this resistance mechanism to osimertinib. Indeed, the combination of selumetinib and osimertinib overcame TKI resistance attributed to *NRAS* mutations both in vitro and in vivo, but further clinical evidence supporting these combination strategies with EGFR and MAPK active TKIs are needed [58].

3.5 Cell-Cycle–Related Gene Mutations

In the AURA3 and FLAURA trials, alteration of cell-cycle–related genes was found in about 10% of patients who progressed on first-line osimertinib and 12% of patients who progressed on second-line osimertinib [40, 41]. The most common cell-cycle gene alterations are mutations or amplifications of genes encoding cyclin D1, D2, and E1, cyclin-dependent kinase (CDK) 4 and 6, and CDK inhibitor 2A. Such mutations have been reported in other studies and are associated with poorer prognosis after progression on osimertinib [59]. There is currently one phase Ib/II trial utilizing lerociclib, a CDK4/6 inhibitor, in conjunction with osimertinib in patients with *EGFR* mutant NSCLC (NCT03455829).

3.6 Oncogenic Fusions

Chromosomal rearrangements involving driver oncogenes, also known as oncogenic fusions, are rare events that have been identified in about 5% of patients who progress on first-line osimertinib [40]. These include *FGFR3–TACC3*, *RET–ERC1*, *CCDC6–RET*, *NTRK1–TPM3*, *NCOA4–RET*, *GOPC-ROS1*, *AGK–BRAF*, *ESYT2–BRAF*, and *SPTBN1–ALK*. [40,

44] In two patients with acquired resistance attributed to *CCD6-RET* fusion, the combination of osimertinib with the Ret inhibitor pralsetinib (BLU-667) was well-tolerated and led to rapid response in both patients [60]. The other fusions, while unusual, might be amenable to combination TKI therapy as well.

3.7 Histologic and Phenotypic Transformations

Histologic transformation from *EGFR* mutant NSCLC to small-cell lung cancer (SCLC) has been observed in up to 14% of patients who progressed on first-generation TKIs and between 4% and 15% of patients who progressed on first- or second-line osimertinib [61–64]. At time of transformation, the founder *EGFR* mutation is generally preserved [62, 65]. While the mechanism of transformation is unclear, concurrent loss of function mutations in *TP53* and *RB1* are associated with a significantly increased risk of transformation [66–68]. Therefore, patients with *EGFR* mutant NSCLC and concurrent pretreatment alterations in *TP53* or *RB1* may warrant monitoring for transformation into SCLC [66]. Unlike gene mutations, the occurrence of histologic or phenotypic transformation is not apparent through plasma analysis and therefore necessitates tissue biopsy. Unfortunately, there are no targeted therapies for such transformations, and treatment with standard PBC generally yields modest outcomes with chemotherapy and little to no observed efficacy with immunotherapy [65]. The combination of osimertinib with carboplatin and etoposide is being studied in a phase 1 study aiming to prevent transformation to SCLC in patients with *EGFR* mutant NSCLC and concurrent *TP53* and *RB1* alterations (NCT03567642). Transformation to squamous cell cancer has been similarly noted in about 15% of patients who progress on first- or second-line osimertinib, and the *EGFR* mutation is generally preserved in this scenario as well [69–71]. Lastly, resistance to osimertinib has also been attributed to epithelial-to-mesenchymal transition (EMT) and over-expression of TWIST-1 (an EMT transcription factor) by NSCLC cells, leading to active investigation of TWIST-1 inhibitors in animal models [72, 73].

3.8 Strategies to Prevent Resistance to Osimertinib

Simultaneously targeting EGFR as well as known bypass pathways may prevent *EGFR*-independent resistance. There are multiple clinical trials testing EGFR TKIs in combination with targeted inhibitors, and many more rational combinations, as described in the previous sections.

For commonly emerging mechanisms of resistance, moving the combination to the frontline may improve PFS. Since chemotherapy is a standard second-line approach now, there are trials combining chemotherapy in the first-line setting with EGFR TKIs. Concurrent use of chemotherapy with gefitinib versus gefitinib alone did not confer survival benefit in patients with untreated *EGFR* mutant NSCLC [74, 75]. However, for selected patients in the second-line setting, the combination of various chemotherapies with osimertinib appears to be tolerable and may better control CNS disease than chemotherapy alone [76]. Now, the same concept is being tested in the phase III FLAURA2 trial, comparing PBC plus osimertinib versus osimertinib alone in untreated *EGFR* mutant NSCLC (NCT04035486).

Increased vascular endothelial growth factor (VEGF) has been associated with EGFR TKI resistance in preclinical models [77], and some Japanese studies have shown that the combination of VEGF inhibitors with first-generation TKIs increase PFS [78, 79]. However, the combination of osimertinib with VEGF inhibitors has failed to prolong PFS or survival when compared to osimertinib alone [80]. The phase III EA5182 study is testing the combination of bevacizumab plus osimertinib with osimertinib alone in the frontline setting (NCT04181060).

Immune checkpoint inhibitors appear generally less effective in *EGFR* mutant NSCLC, without clear predictive biomarkers of response [81]. In pre-clinical studies, EGFR activation led to upregulated programmed death-ligand 1 (PD-L1), but the combination of EGFR inhibitors and programmed cell death protein 1 (PD-1) inhibitors did not lead to synergistic effects [82]. Early combination trials of osimertinib and durvalumab were halted due to high rates of immune-related adverse events, particularly pneumonitis, so only chemotherapy combinations are now being investigated [83]. Identifying effective combinations of targeted therapy and immunotherapy is an unmet need in treating *EGFR* mutant NSCLC, and the reduced efficacy of checkpoint inhibitors is likely from lower tumor immunogenicity, but may be augmented by future combination therapies [84]. In the IMpower130 trial, the addition of atezolizumab to PBC in the first-line setting conferred no benefit when compared to PBC alone [85]. Interestingly, in the IMpower150 trial, the addition of both atezolizumab and bevacizumab to PBC (ABCP regimen) demonstrated improved PFS and OS when compared to other arms, suggesting a synergistic efficacy of VEGF and immune checkpoint inhibitors [86].

Concurrent local radiotherapy with a third-generation EGFR inhibitor versus third-generation EGFR inhibitor alone has improved PFS and OS in patients with oligometastatic disease in the first-line setting (NCT02893332). This suggests that radiotherapy, when appropriate, may prevent or delay the development of resistance mechanisms.

4 Intrinsic Resistance to EGFR TKIs

Each of the acquired mechanisms of resistance to EGFR TKIs described in this paper can also present as intrinsic mechanisms of resistance prior to any treatment. For example, though T790M is considered an acquired mechanism of resistance to earlier generation TKIs, germline T790M mutations have been observed in 1% of NSCLC cases [87]. Though third-generation TKIs like osimertinib have made germline T790M mutations less clinically relevant, finding this mutation warrants a genetics evaluation and counseling for the patient and their family [88]. Another rare but well-described mechanism of intrinsic resistance to third-generation EGFR TKIs is *MET* amplification [89]. Beyond these, there are other types of *EGFR* mutations that have been relatively resistant to TKI therapy until recently.

4.1 *EGFR* Exon 20 Insertion

A rare but important subset of *EGFR* mutant NSCLC with intrinsic resistance to third-generation TKIs is *EGFR* ex20ins NSCLC. *EGFR* ex20ins comprises about 4% of all *EGFR* mutant NSCLC and is associated with intrinsic resistance to currently available *EGFR* TKIs and poorer outcomes for patients [90, 91]. EGFR proteins with ex20ins mutations have binding pockets that are inaccessible to existing EGFR TKIs [92]. Retrospective studies of first-generation EGFR TKIs in *EGFR* ex20ins NSCLC demonstrated ORR between 8% and 27% and median PFS of less than 3 months [93]. Third-generation *EGFR* inhibitors, such as osimertinib, show only slightly better activity against *EGFR* ex20ins NSCLC, and most patients have a short duration of response [37]. Interestingly, a few variants of *EGFR* ex20ins NSCLC, such as A763_Y764insFQEA insertion, are significantly more responsive to existing EGFR TKIs [94].

Therefore, most *EGFR* ex20ins NSCLC patients are treated with PBC with or without antiangiogenic therapy or immunotherapy as first-line therapy, though some patients may be prescribed first-line osimertinib, with variable results. In clinical trials of EGFR inhibitors versus PBC in classical *EGFR* mutant NSCLC, PBC yields an ORR of about 30% and median PFS of about 5–6 months [1, 10, 95]. Existing literature suggests that immunotherapy is relatively ineffective against *EGFR* mutant NSCLC, whereas data on the utility of antiangiogenic therapy in *EGFR* mutant NSCLC are mixed [96, 97]. Retrospective studies have described the clinical course of *EGFR* ex20ins NSCLC treated with first-line PBC, finding ORR of 20–30% and PFS of 6–7 months, similar to the course of classical *EGFR* mutant NSCLC treated with first-line platinum-based chemotherapy [98–100].

Amivantamab, a novel bispecific antibody targeting EGFR and MET receptor, was recently approved for patients with locally advanced or metastatic *EGFR* ex20ins NSCLC after progression on or after platinum-based chemotherapy [101]. This accelerated approval was based on results from the multicenter, multicohort, non-randomized, open-label clinical trial CHRYSALIS. In the subset of 81 patients with *EGFR* ex20ins NSCLC who had progressed on platinum-based chemotherapy, the ORR was 40% and the median duration of response was 11.1 months (NCT02609776).

TKIs targeting the ex20ins EGFR protein are also being tested against PBC in the first-line setting. Mobocertinib (TAK-788) is a novel TKI with higher affinity binding to the ex20ins mutant EGFR than other available TKIs. A phase II, open-label, cohort expansion demonstrated that mobocertinib leads to an ORR of 28% with a median duration of response of 17.5 months, leading to the recent Food and Drug Administration (FDA) approval of this agent (NCT02716116). Other TKIs are in clinical development as well, such as poziotinib, DZD9008, and CLN-081 (NCT03318939, NCT03974022, and NCT04036682)

While currently the EGFR ex20ins agents are approved in the second-line setting, both have ORR similar to what we observe with platinum-based chemotherapy in the first-line setting. In the EXCLAIM-2 study, mobocertinib is being tested against PBC in the frontline setting (NCT04129502). A phase III study of combination amivantamab and carboplatin-pemetrexed therapy compared with carboplatin-pemetrexed therapy in advanced *EGFR* ex20ins NSCLC is also currently underway (NCT04538664).

5 Future Directions

Upon the development of resistance to third-generation TKIs, most *EGFR* mutant NSCLC is treated with standard PBC. However, as targeted therapies against specific resistance mechanisms are developed, there will likely be a myriad of agents and their combinations that may be used to overcome resistance, as summarized in Fig. 2. Notably, the phase II ORCHARD trial follows a biomarker-driven approach to assigning targeted therapies to be given simultaneously with osimertinib when specific resistance mechanisms arise (e.g., add savolitinib for *MET* alteration, add gefitinib for C797X mutation) [102].

To facilitate a biomarker-driven approach, we anticipate widespread utilization of liquid biopsy as a complement to repeat tissue biopsy or empiric PBC. Monitoring ctDNA, released from tumor cells into the bloodstream, is a non-invasive and feasible method of detecting tumor alterations

pertinent to NSCLC [103]. Compared with tissue biopsy, monitoring ctDNA for *EGFR* mutations has a 67% sensitivity and 94% specificity [104]. Polymerase chain reaction (PCR)-based and NGS-based analysis of ctDNA has high specificity, but lower sensitivity due to lack of tumor shedding in up to 20% of patients with NSCLC [105]. The other limitations of NGS-based analysis are reduced sensitivity in detecting gene amplifications, which is ideally assessed through fluorescence in situ hybridization (FISH), and inability to detect histologic transformation, which requires tissue biopsy. Despite these limitations, NGS-based liquid biopsy is clinically useful and relatively feasible for not only detecting initial driver mutations, but also predicting recurrence and identifying genetic modifiers of resistance [106]. One study profiling ctDNA in patients with stage I–III lung cancer found that post-treatment ctDNA reliably identified minimal residual disease and preceded radiographic recurrence by a median of 5.2 months, suggesting that ctDNA profiling may allow for personalized adjuvant therapy while disease burden is at its lowest [107]. Importantly, NGS-based biopsy for patients with NSCLC appears to be more time efficient for personnel and more cost-effective for patients [108]. Standardization of NGS-based liquid biopsy in monitoring for resistance in NSCLC is likely to become more standard in the future as costs continue to drop.

Ultimately, further molecular profiling, active surveillance of resistance mechanisms, and development of targeted therapeutics will continue to transform the landscape of *EGFR* mutant NSCLC. Concurrent investigation of immune checkpoint inhibitor, antiangiogenic therapy, and radiation therapy will likely augment the efficacy of targeted treatment regimens and move toward the goal of personalized, gene-directed therapy in most patients with NSCLC.

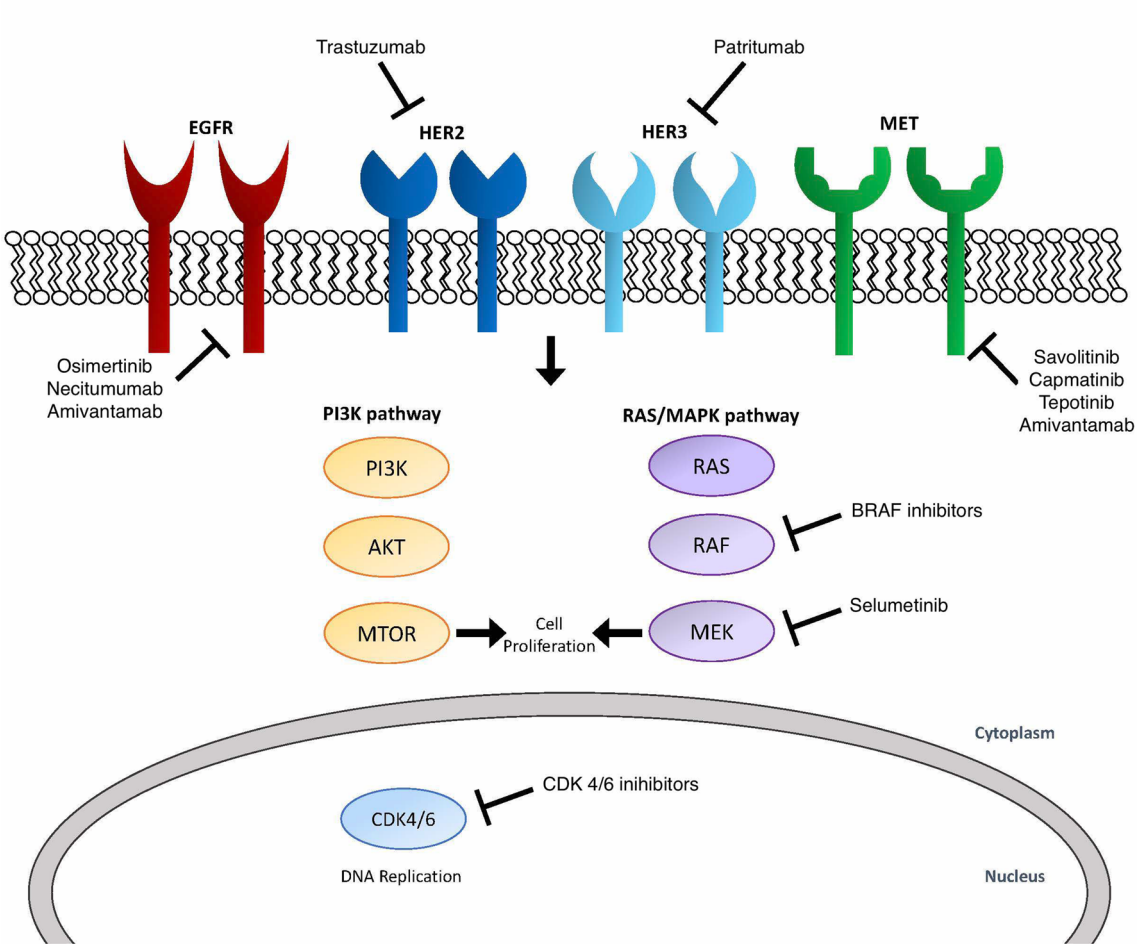

Fig. 2 Overview of oncogenic pathways and examples of targeted inhibitors to overcome resistance to treatment in *EGFR* mutant NSCLC. *CDK* cyclin-dependent kinase, *EGFR* epidermal growth factor receptor, *MAPK* mitogen-activated protein kinase, *MET* mesenchymal epithelial transition factor, *NSCLC* non-small-cell lung cancer, *PI3K* phosphoinositide 3-kinase

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Authors' contributions MPS and JWN outlined the manuscript. MPS conducted literature review and composed the manuscript. MPS and JWN revised the manuscript.

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