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# Overcoming therapy resistance in *EGFR*-mutant lung cancer

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**Tyrosine kinase inhibitors (TKIs) have dramatically changed the clinical prospects of patients with non-small cell lung cancer harboring epidermal growth factor receptor (EGFR)-activating mutations. Despite prolonged disease control and high tumor response rates, all patients eventually progress on EGFR TKI treatment. Here, we review the mechanisms of acquired EGFR TKI resistance, the methods for monitoring its appearance, as well as current and future efforts to define treatment strategies to overcome resistance.**

The *EGFR* gene encodes the epidermal growth factor receptor (EGFR) tyrosine kinase, which is broadly expressed across normal tissues[1]. A potential role for EGFR signaling in cancer was postulated soon after its discovery in 1962[2], and attempts to target this protein were made in several cancer subtypes, including the use of monoclonal antibodies as part of the standard of care in the treatment of squamous head and neck cancers and colorectal tumors[3]. Initial clinical studies of EGFR tyrosine kinase inhibitors (TKIs) enrolled broad populations of patients with non-small cell lung cancer (NSCLC), but it was the discovery of *EGFR* mutations associated with dramatic responses to EGFR TKI treatment what paved the way to precision medicine in lung cancer[4,5].

Somatic activating mutations causing alterations to the kinase domain of EGFR are observed in 10–15% of Caucasian patients and up to 50% of East-Asian patients with NSCLC, with a higher incidence in females and those who have either never smoked or are former light smokers[6,7]. These genetic alterations are responsible for constitutive ligand-independent receptor activation and downstream signaling promoting cell survival and proliferation[4]. Small-molecule EGFR TKIs bind the adenosine triphosphate pocket (ATP) of EGFR, therefore inhibiting its autophosphorylation and downstream signal transduction. Exon 19 deletions and exon 21 L858R point mutations represent the majority of *EGFR* mutations sensitive to targeted treatment with EGFR TKIs[8].

Several phase III clinical trials have investigated the role of first- (gefitinib and erlotinib) and second-generation (afatinib and dacomitinib) EGFR TKIs, which have shown similar median response rates of 70–75% and significant improvements in progression-free survival (PFS) ranging from 10–14 months compared with platinum-based chemotherapy in treatment-naive, *EGFR*-mutated, advanced NSCLC[9–13]. More recently, the third-generation EGFR TKI osimertinib showed superior efficacy, with improved PFS (18.9 versus 10.2 months) and overall survival (38.6 versus 31.8 months)[14,15]. Despite these major therapeutic advances and in-depth understanding of the genetic determinants, resistance to EGFR TKIs inevitably occurs, resulting in disease progression[16–18].

Drug resistance arises from the evolutionary pressure exerted on cancer cells through spatial and temporal clonal selection[19,20] and is fueled by the random acquisition of genetic mutations[20,21]. The pace at which clonal selection occurs is conditioned by the functional impact of sequentially acquired alterations on cell fitness, and different evolutionary paths are strongly influenced by exogenous selective pressure[19,22–24]. However, mechanisms of acquired resistance (arising in persisting, drug-tolerant cells following initial responses to targeted therapy) have recently been suggested to be more common than the selection preexisting drug-resistant subclones[25–27].

In *EGFR* mutation-positive disease, selective pressure from TKI therapy may result in the elimination of targeted clones and subsequent selection of cells lacking the original actionable driver mutation, or in the de novo acquisition of on- and off-target resistance mechanisms[24,28]. In the framework of tumor heterogeneity, the preexistence of resistant clones and the onset of induced adaptive resistance or tolerance mechanisms determines the timing of EGFR TKI resistance and defines innate (defined as disease progression within the first 3 months after TKI initiation) or acquired resistance mechanisms[29].

A deeper understanding of tumor heterogeneity and the identification of specific resistance mechanisms might help to prevent the emergence of resistant clones. For example, gatekeeper mutations (such as $EGFR^{T790M}$ mutations arising in patients treated with first- or second- generation EGFR TKIs) act by limiting drug accessibility to the kinase ATP-binding pocket[17] and by increasing the ATP affinity of the mutant *EGFR*, thus preventing noncovalent first- and second-generation EGFR TKIs from outcompeting ATP without affecting drug affinity itself[30]. Next-generation TKIs have been developed with more potent and irreversible binding properties, thus avoiding the ATP-competitive TKI binding to mutated domains and delaying the occurrence of resistance[31].

Other mechanisms involved in the development of TKI resistance include off-target resistance mutations and nongenetic adaptive changes, including activation of the aurora kinase A–interleukin-6–STAT3 (signal transduction and activator of transcription 3) pathway, nuclear factor-κB or induction of type I interferon[17,32–37] (Fig. 1).

Combinations of EGFR TKIs with different drugs (including other TKIs, monoclonal antibodies, chemotherapy and vaccines) are currently under investigation. These combination strategies

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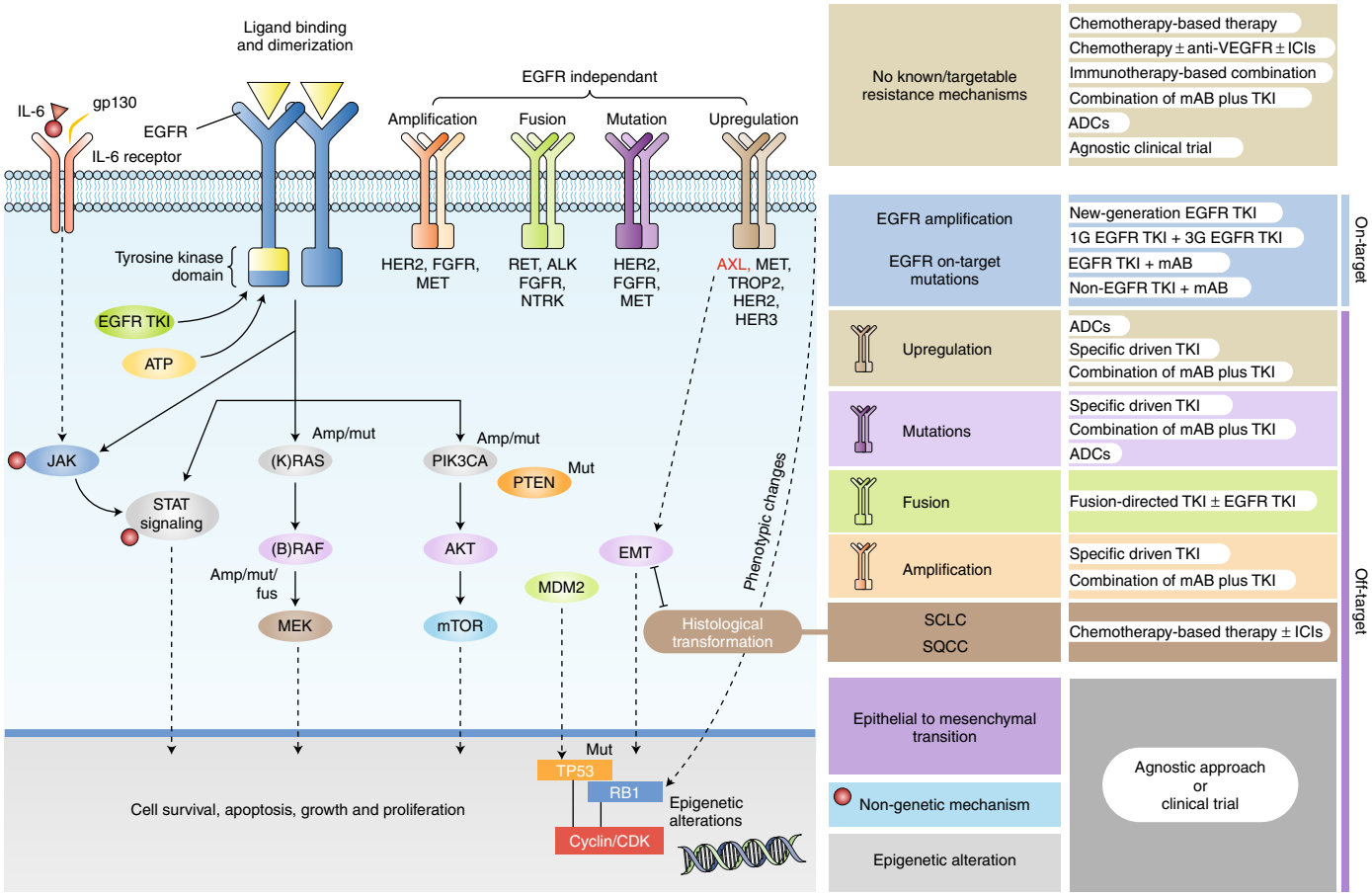


**Fig. 1 | Overview of the EGFR signal transduction pathway model, with a focus on the acquired resistance mechanisms and related potential treatment strategies.** Displayed are the genetic mechanisms of therapy resistance, including on- and off-target mechanisms. Nongenetic mechanisms are also included. The right panel summarizes the current therapeutic approaches to circumvent therapy resistance in each context. 1G, first-generation; 3G, third generation; amp, amplification; EMT, epithelial–mesenchymal transition; FGFR, fibroblast growth factor receptor; fus, fusion; gp130, glycoprotein 130; ICI, immune checkpoint inhibitor; IL-6, interleukin-6; JAK, Janus kinase; mAB, monoclonal antibody; MDM2, mouse double minute-2 homolog; mTOR, mechanistic target of rapamycin; mut, mutation; NTRK, neurotrophic tyrosine receptor kinase; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PTEN, phosphatase and tensin homolog; RB1, retinoblastoma protein; SQCC, lung squamous cell carcinoma; TP53, tumor protein 53; TROP-2, trophoblast cell-surface antigen 2; VEGFR, VEGF receptor.

might delay resistance preemptively by targeting specific subclones that might emerge through selective pressure. Alternatively, comprehensive genetic profiling of the tumor at disease progression permits identification of the resistance mechanisms in place and allows selection of the most appropriate combination approach.

Finally, phenotypic transformation into new histologic subtypes leads to target independence, which needs to be investigated at the occurrence of TKI resistance and eventually treated. Indeed, being able to predict histologic transformation in order to prevent its occurrence could be the optimal strategy to pursue.

In this Review, we appraise the current approaches to preventing and overcoming therapy resistance in *EGFR*-mutant lung cancer. We describe new combinatorial approaches and their impact on central nervous system (CNS) involvement in NSCLC harboring *EGFR*-sensitive mutations, and focus on current and experimental treatment strategies to circumvent therapeutic resistance.

## Mechanisms of resistance to EGFR TKIs

The relative incidences of on-target (*EGFR*-dependent) and off-target (*EGFR*-independent) resistance mechanisms substantially differ according to the specific EGFR TKI used. Patients receiving first- or second-generation EGFR TKIs predominantly develop *EGFR*-dependent resistance, whereas only about 20% of patients receiving the third-generation TKI osimertinib as second-line therapy reveal on-target resistance mechanisms[28,38]. *EGFR*-dependent resistance occurs in 10–15% of patients treated with first-line osimertinib[39]. Evidence supporting different resistance patterns according to treatment line suggests potential variability in the presentation of mutations in the adjuvant setting. Indeed, a recent report has shown significant improvement in disease-free survival in patients who received 3 years of adjuvant osimertinib after radical surgery, with or without standard adjuvant chemotherapy, in stage IB-IIIA *EGFR* mutation-positive NSCLC[40], although overall survival data are still awaited (Fig. 2).

**EGFR target-dependent mechanics of resistance.** Alterations in EGFR arise quickly following targeted resistance and are located in critical amino acid residues that allow bypass of the mechanisms of action of the different EGFR TKIs. The order and context of the appearance of different mutations condition responses to different lines of therapy.

*T790M mutations.* The amino acid substitution p.Thr790Met results from a gatekeeper mutation in exon 20 of *EGFR* and is responsible for steric hindering to the binding of first- and second-generation EGFR TKIs to their cognate ATP-binding site on EGFR[30,41].

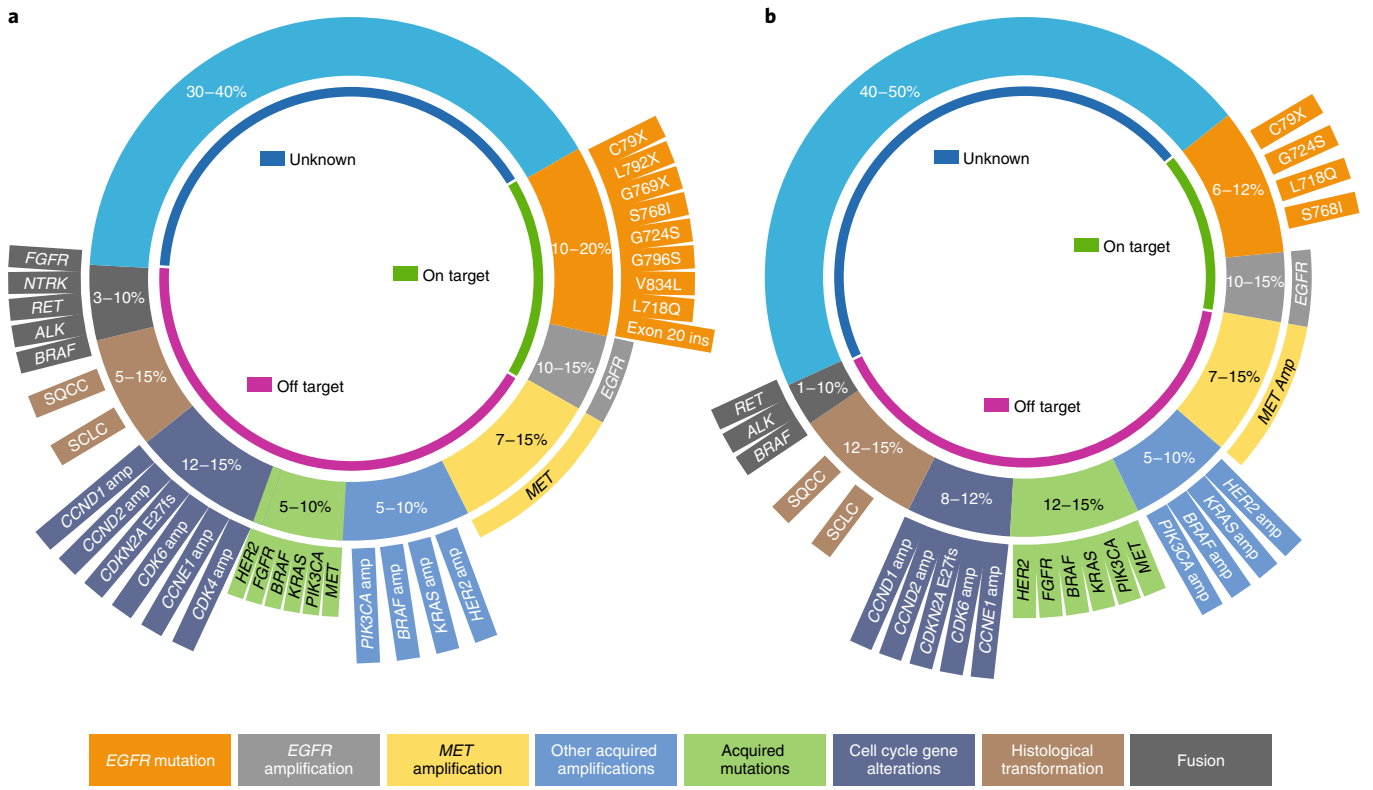


**Fig. 2 | Mechanisms of resistance to osimertinib. a**,**b**, Resistance mechanisms arising after second-line (**a**) and first-line (**b**) osimertinib therapy. The specific mutations are indicated, along with the incidence of each alteration and the broad classes of alterations driving resistance. Amp, amplification; Ins, insertion.

The T790M mutation is found in 50–60% of patients receiving gefitinib, erlotinib or afatinib and preserves sensitivity to third-generation TKIs[28,42–44] (Fig. 3). Intriguingly, presence of the pretreatment T790M mutation has been reported widely, with highly variable incidence rates (<1–65%), and is potentially related to the worst clinical outcomes[32,45]. In the phase III randomized AURA3 trial, osimertinib was shown to prolong PFS compared with platinum-based chemotherapy (median PFS = 10.1 versus 4.4 months; hazard ratio (HR) = 0.30; 95% confidence interval (CI) = 0.23–0.41), even in patients with brain metastasis (median PFS = 8.5 versus 4.1 months; HR = 0.32; 95% CI = 0.21–0.49). It also showed a higher response rate (71 versus 31%)[42]. However, no statistically significant overall survival benefit was determined in this study (median overall survival = 26.8 versus 22.5 months; HR = 0.87; 95% CI = 0.67–1.12)[46], given that over 70% of patients in the control arm were crossed over to osimertinib. Recently, lazertinib, another third-generation EGFR TKI, showed a good safety profile and antitumor activity in a phase I/II trial in *EGFR*-mutant NSCLC bearing T790M resistance mutations[47].

Data from plasma genotyping of T790M-positive patients receiving second-line osimertinib within the randomized phase III AURA3 trial reveal that about 50% of patients retained the T790M mutation at the time of disease progression, including the totality of patients with tertiary *EGFR*-dependent osimertinib resistance mechanisms[38]. The remaining patients showed loss of the T790M mutation at the time of progression to osimertinib, suggesting that $EGFR^{T790M}$ existed as a subclone. This event is associated with the occurrence of off-target resistance mechanisms, including modulation other oncogenic signaling pathways, emergence of secondary driver oncogene mutations or chromosomal rearrangements and histologic transformation[38,48]. Of note, loss of T790M in patients was associated with earlier resistance and shorter survival[48–50].

When osimertinib was administered in the front-line setting, no evidence of T790M mutation emerged at resistance from plasma genotyping[39]. This was expected considering the selective activity of osimertinib on *EGFR*-sensitizing mutations as well as T790M mutations.

Given the efficacy results obtained in the first-line setting, osimertinib swiftly moved to the front-line setting[14,15]. Therefore, the incidence of T790M mutation as a resistance mechanism is expected to become less relevant over time, despite it remaining as one of the patterns defining on-target resistance mechanisms.

*C797X mutations.* Cys 797 is the site of covalent binding of osimertinib, regardless of the presence of T790M mutations[51], and it overcomes the increased ATP affinity mediated by T790M. C797X mutations have emerged as the most frequent *EGFR* resistance mechanism to osimertinib, occurring at the time of progression after exposure to third-generation EGFR TKIs, with different incidence rates according to the treatment setting[38,39,52]. This mutation at position 797 in exon 20 of *EGFR* is located within the irreversible EGFR TKI binding site[52]. Serine is the most frequently substituted amino acid (p.Cys797Ser), whereas glycine (p.Cys797Gly) has been reported anecdotally[38,39,53,54].

The C797X mutation was detected in 15% of blood samples from patients at disease progression to second-line osimertinib within the AURA3 trial[38], whereas higher incidences (22–25%) were observed in smaller series of tissue rebiopsies of T790M-positive patients receiving third-generation EGFR TKIs[48,55].

In the front-line setting, the incidence of C797X mutation at the time of disease progression is lower; it was found in only 7% of a small patient cohort (91 patients) within the FLAURA trial[39].

Two aspects are crucial for the management of C797X-mediated resistance. First, in the absence of a coexisting T790M mutation,

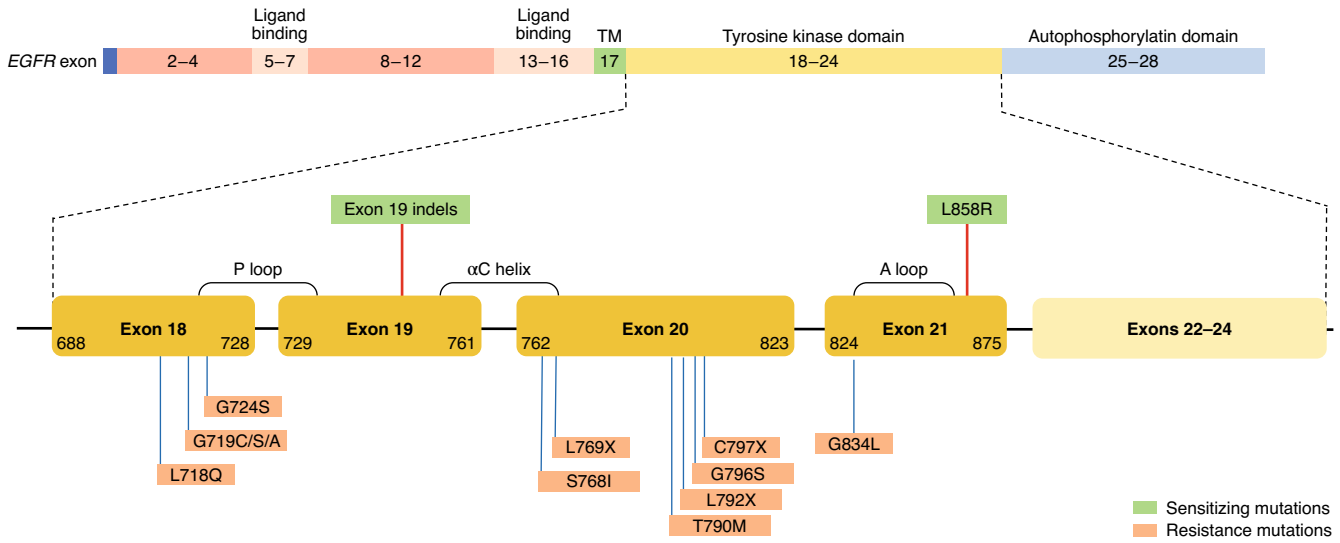


**Fig. 3 | Specific *EGFR*-dependent (on-target) mutations acquired after osimertinib treatment.** Schematic depicting the different exons in *EGFR* and the functional domains in which the sensitizing or resistance-conferring mutations occur. Indels, insertions/deletions; TM, trans-membrane domain.

NSCLC with an *EGFR*-activating mutation alone that develops resistance through C797S may potentially retain sensitivity to quinazoline-based EGFR inhibitors, including gefitinib, erlotinib and afatinib[56,57]. Second, when the C797S and T790M mutations are in *trans* (on different alleles), the tumors retain sensitivity to first- and second-generation EGFR TKIs[58], and the rationale for combining them with third-generation TKIs has been demonstrated in vitro and in some case reports[59]. However, no evidence of efficacy of such combinations emerged when the mutations occurred in the *cis* position[58]. Clinical data have revealed that 66% of cases have in *cis* presentation compared with 34% presenting in *trans*[52,60,61]. Combinations of mutant-selective and first- or second-generation EGFR inhibitors could be used as initial treatment for *EGFR*-mutant NSCLC, given that these EGFR inhibitors are effective against non-cross-resistant mutations, and when combined they might prevent the emergence of resistant clones[62–64].

**Additional EGFR-dependent resistance mechanisms.** Less frequent tertiary *EGFR* mutations have been described as EGFR-dependent mechanisms of resistance to third-generation EGFR TKIs[38,39,53,65]. G796R, G796S and G796D are solvent-front mutations occurring in exon 20 of *EGFR*, determining steric hindering to osimertinib[53,66,67]. Similarly, L792X mutations involving the hinge pocket have been identified as being responsible for steric interference in accessibility to the EGFR kinase domain, and these might coexist with in *trans* G796/C797X (ref. [53,68]). Drug sensitivity against these newly on-target EGFR resistance mechanisms requires further exploration. S768I mutations and exon 20 insertions, which are usually identified at baseline, have also been anecdotally associated with osimertinib resistance, although their specific roles have not yet been defined[38,39,69].

Other rare EGFR TKI resistance mutations involve exon 18 of the *EGFR* gene, including the L718 and (although the data are less solid) G719 residues at the ATP-binding site[39,53], as well as the G724S mutation in the kinase P-loop domain[70,71]. Interestingly, these mutations have been reported to retain in vitro sensitivity to first- and second-generation EGFR TKIs in the absence of T790M mutation[53,57,72].

Finally, wild-type *EGFR* gene amplification has been described after failure of third-generation EGFR TKIs (osimertinib and rociletinib)[73,74].

**EGFR target-independent mechanisms of resistance.** Rewiring of cell signaling irrespective of EGFR is also a key mechanism to circumvent EGFR TKI therapy in the absence of *EGFR* mutations. Here, we cover the most frequent alterations and the clinical actionability of these.

***MET* amplification**. MET receptor tyrosine kinase signaling is the most frequently altered pathway involved in *EGFR* resistance following EGFR TKI treatment, irrespective of the EGFR TKI used or line of therapy. Although the occurrence of *MET* mutations or increased MET ligand (hepatocyte growth factor) levels has been described anecdotically[74,75], the *MET*-mediated resistance mechanism frequently occurs trough *MET* gene amplification[38,39,74]; this results in bypass of EGFR downstream signaling through STAT, mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways.

*MET* amplification was found in 5–22% of patients experiencing disease progression after receiving first-generation EGFR TKIs[28,65,76]. The historical lack of consensus on the definition of *MET* amplification contributes to such discordant findings[77–79]. Currently, the most widely adopted definition for *MET* amplification is the presence of a *MET* gene copy number of ≥5 or a *MET*/*CEP7* ratio of ≥2 (refs. [77,78,80,81]), but MET overexpression by immunohistochemistry[81,82] has also been adopted in ongoing clinical trials. Of note, the strategy to co-target EGFR and MET with an upfront combination of a first-generation EGFR TKI and an anti-MET monoclonal antibody failed to demonstrate a survival advantage compared with EGFR TKI alone; however, this was assessed in an unselected population of patients with *EGFR*-mutant advanced NSCLC[83].

Plasma genotyping of patients at disease progression to osimertinib revealed *MET* amplification in 10–19 and 15% of patients receiving second- and first-line therapy, respectively[38,39,48]. To date, there has been a lack of consensus on the definition of *MET* amplification using liquid biopsy followed by hybridization-capture-based next-generation sequencing (NGS), which enables the simultaneous detection of single-nucleotide variants, insertions/deletions, rearrangements and copy number alterations, and the establishment of different cut-offs based on different validation cohorts[84–87]. Additionally, tissue analysis could reveal much higher rates because of a potential underestimation or a lower sensitivity of gene amplification assessment in blood[54]. Combinatorial

approaches with concurrent EGFR TKIs and MET TKIs are currently under investigation in patients with *EGFR*-mutant NSCLC with *MET* amplification.

*HER2* **amplification**. The *HER2* gene encodes the ErbB2 receptor tyrosine kinase. It mediates EGFR TKI resistance through alternative activation of the MAPK and PI3K pathways. *HER2* amplification was detected in 12% of tumor samples from patients with no coexisting T790M mutations experiencing disease progression on first-generation EGFR TKIs[88]. Similar to *MET* amplification testing, NGS strategies have been developed with variable criteria for *HER2* amplification definition according to different platforms' reports and validation cohorts, including tissue or plasma[84,89–91].

Data on osimertinib resistance derive from plasma genotyping using comprehensive gene-profiling platforms. This approach identified *HER2* amplification in 5% of patients in the second-line AURA3 trial[38] (in some cases coexisting with other *EGFR*-dependent and -independent resistance mechanisms) and in 2% of patients in the front-line setting of the FLAURA study[39]. Interestingly, *HER2* copy number gain/amplification and $EGFR^{T790M}$ mutation were mutually exclusive in all of the observed findings[38,39,88].

*Oncogenic fusion/chromosomal rearrangements*. Several gene fusions involving driver oncogenes have been identified in 4–7% of patients, mainly as second-line osimertinib resistance mechanisms[38,48]. These include *RET* (*RET–ERC1*, *CCDC6–RET* and *NCOA4–RET*)[38,48,54], *BRAF* (*AGK–BRAF*, *ESYT2–BRAF*, *PCBP2–BRAF* and *BAIAP2L1–BRAF*)[48,54], *NTRK* (*TPM3–NTRK1*)[54], *ROS1* (*GOPC–ROS1*)[92] and *FGFR* (*FGFR3–TACC3*) among others[38]. In the front-line osimertinib setting, a single case of an *ALK* fusion (*SPTBN1–ALK*) has been reported[39].

These gene fusions represent rare events. Similar to the case of *EGFR* mutation-positive patients with *RET* fusions treated with concurrent administration of osimertinib and the RET inhibitor BLU-667, there is evidence for using TKIs in combination to bypass these mechanisms of resistance[54].

**Additional mechanisms of EGFR TKI resistance.** The *EGFR*-independent resistance mechanisms described above act through the activation of alternative bypass tracks, reactivating MAPK and PI3K signaling pathways. Alterations in genes upstream of these pathways have also been found to drive EGFR TKI resistance.

*RAS* mutations have been described involving *NRAS* (1% of patients in the first-generation TKI-treated cohort of the FLAURA trial)[39]. Variable *KRAS* mutations were identified in 1% of patients progressing on first-generation TKIs[39], 3% of patients after front-line osimertinib[39] and 1–7% of patients after second-line osimertinib[38,48]. $BRAF^{V600E}$ mutations were observed in 3% of patients at disease progression to first- or second-line osimertinib[38,39,48], whereas other variants have been reported occasionally with different TKIs[39,48].

*PIK3CA* mutations and *PTEN* loss are responsible for increased PI3K signaling. *PIK3CA* amplifications or mutations represent 3–5% of the identified resistance mechanisms after first-generation EGFR TKI therapy[28,39] and 5–12% of cases at progression to third-generation TKIs (as determined by plasma genotyping)[38,39,48,74], with this latter value reaching 17% in a tissue analysis series[55]. In vitro studies showed that the addition of PI3K inhibitors to EGFR TKIs overcomes EGFR TKI resistance[93]. The loss of *PTEN* is mainly described as a mechanism of primary EGFR TKI resistance[94,95], but it has been described as an acquired mechanism as well[55].

Plasma analyses from the AURA3 and FLAURA trials revealed a consistent rate of alteration of cell cycle-related genes in terms of amplification or mutations in cyclin D1, D2 and E1 genes, cyclin-dependent kinase 4 and 6 genes (*CDK4* and *CDK6*, respectively) and the cyclin-dependent kinase inhibitor 2A gene (*CDKN2A*), representing 10% of resistance mutations in response to osimertinib in the front-line setting and 12% in the second-line setting[38,39].

Other rare *EGFR*-independent mechanisms that have been reported involve the *FGFR* gene family (mainly through gene amplification), and alterations in common signaling pathway mediators such as Src family kinases, AXL receptor tyrosine kinase and the transmembrane protein CUB domain-containing protein 1 have been identified in in vitro studies[96,97].

In some cases, these alterations co-occur at baseline with *EGFR* mutations and are associated with poor response to EGFR TKIs, as has been described for *AXL* and *CDCP1* messenger RNA overexpression, both with first- and third-generation drugs in vitro and in patients[97]. The potential to co-target these specific resistance mechanisms showed promising evidence in preclinical studies[98] and is currently under evaluation in a clinical trial (Table 1).

*Histologic and phenotypic transformations*. Unlike gene mutational status, the presence of histologic and phenotypic transformation as an acquired resistance mechanism to EGFR TKI cannot be evaluated by plasma analysis and requires the investigation of tissue samples.

Histologic transformation into small cell lung cancer (SCLC) has been reported in up to 14% of *EGFR*-mutant lung cancers at disease progression after first-generation EGFR TKIs[28]. A similar proportion (4–15%) of SCLC transformation occurs in patients experiencing disease progression on third-generation EGFR TKIs, either in the first- or second-line setting[48,54,55,99]. The founder *EGFR* mutation is usually retained at the moment of SCLC transformation[28,100]. Among the proposed mechanisms responsible for small cell transformation, particular interest is set on the potential role of *RB1* and *TP53* genes. Indeed, SCLC transformation occurred in 18% of patients with *EGFR*-mutant lung cancer with preexisting concurrent *RB1* and *TP53* mutations, whereas no cases of SCLC were observed in *EGFR* mutation-positive patients with wild-type *RB1* and *TP53*, confirming previous evidence[101–103]. Therefore, the presence of *RB1* or *TP53* alterations in plasma genotyping may suggest, in the absence of other resistance mechanisms, that further investigation of the tissue/rebiopsy should be performed to look for SCLC transformation. This category of patients would be expected to have a poorer prognosis due to intrinsinc resistance mechanisms. Indeed, despite some reports of initial response to platinum and etoposide[104], conventional systemic chemotherapy has shown limited efficacy[100,105].

Squamous cell transformation has also been identified recently as an acquired EGFR TKI resistance mechanism, occurring in about 15% of patients receiving both front- and second-line osimertinib[99]. Similar to SCLC transformation, the primary *EGFR* mutation is preserved[106,107].

Any histologic transformation hypothesis raises the question of a preexisting initial mixed histology, which usually cannot be accurately assessed in small biopsy/cytological samples. To date, no specific approaches have been validated in *EGFR*-mutant NSCLC with transformed histology, and histology-driven treatment remains the standard of care in this patient subgroup.

## Treatment strategies to overcome resistance

The identification of specific resistance mechanisms led to the development of biomarker-driven therapies[108,109]. Agnostic combinatorial approaches targeting multiple nodes of resistance (Fig. 1), as well as targeted strategies preemptively preventing the selection of resistant clones, are currently under evaluation (Tables 1–3).

**Biomarker-driven approaches.** Fourth-generation EGFR inhibitors (overcoming C797S and T790M mutations), such as EAI045 (ref. [110]), JBJ-04-125-02 (ref. [111]) and BLU-945 (ref. [31]), have demonstrated in vitro and in vivo activity alone or in combination with

**Table 1 | Ongoing clinical trials in pretreated *EGFR*-mutant metastatic NSCLC**

| NCT identifier | Phase | Previous EGFR TKI | Required alteration | Includes uncommon *EGFR* mutations | Treatment arm(s) |
|---|---|---|---|---|---|
| NCT03944772 (ORCHARD) | II | Mandatory (osimertinib) | *EGFR* mut and either *MET* amp/mut, $EGFR^{C787X}$, *EGFR* amp or no biomarker | NR | Biomarker driven: Osimertinib + savolitinib (MET inhibitor); Osimertinib + gefitinib; Osimertinib + necitumumab (anti-EGFR mAb); Durvalumab + carboplatin + pemetrexed |
| NCT03778229 (SAVANNAH) | II | Mandatory (osimertinib) | *EGFR* mut and high *MET* amp | Yes | Osimertinib + savolitinib |
| NCT03940703 (INSIGHT 2) | II | Mandatory (osimertinib) | *EGFR* mut and high *MET* amp | NR | Osimertinib + tepotinib (MET inhibitor) |
| NCT03784599 | II | Mandatory (any) | *EGFR* mut and high *HER2* amp | NR | Osimertinib + T-DM1 |
| NCT03133546 | II | Mandatory (first or second generation) | *EGFR* mut (T790M+) | No | Osimertinib ± bevacizumab |
| NCT03532698 | II | Mandatory (osimertinib) | *EGFR* mut | NR | Osimertinib + aspirin |
| NCT02759835 | II | Allowed | *EGFR* mut | NR | Osimertinib + local ablative therapy |
| NCT04484142 | II | Mandatory (any) | *EGFR* mut | NR | DS-1062a (TROP2 ADC) |
| NCT03455829 | Ib/II | Allowed | *EGFR* mut | NR | Osimertinib + G1T38 (CDK4/6 inhibitor) |
| NCT03831932 | I/II | Mandatory (any) | *EGFR* mut | No | Osimertinib + telaglenastat (glutaminase inhibitor) |
| NCT02917993 | I/II | Mandatory (any) | *EGFR* mut | Yes | Osimertinib + itacitinib (JAK inhibitor) |
| NCT04001777 | Ib | Mandatory (any) | *EGFR* mut | NR | Osimertinib + APG-1252 (Bcl-2 inhibitor) |
| NCT02520778 | Ib | Mandatory (any) | *EGFR* mut | Yes | Osimertinib + navitoclax (Bcl-2 inhibitor) |
| NCT04085315 | I/Ib | Mandatory (osimertinib) | *EGFR* mut | Yes | Osimertinib + alisertib (aurora A kinase inhibitor) |
| NCT02099058 | I/Ib | Mandatory (arm E: osimertinib) | *EGFR* mut | NR | Osimertinib + telisotuzumab vedotin (c-MET ADC) |
| NCT02496663 | I | Mandatory (any) | *EGFR* mut | Yes | Osimertinib + necitumumab |
| NCT02789345 | I | Mandatory | *EGFR* mut (T790M−) | NR | Osimertinib + necitumumab; Osimertinib + ramucirumab |
| NCT03891615 | I | Mandatory (osimertinib) | *EGFR* mut | NR | Osimertinib + niraparib (PARP inhibitor) |
| NCT03516214 | I | Allowed | *EGFR* mut | No | EGF816 (third-generation EGFR TKI) + trametinib (MEK inhibitor) |
| NCT02503722 | I | Mandatory (any) | *EGFR* mut | No | Osimertinib + sapanisertib (TAK-228; mTOR inhibitor) |
| NCT02609776 | I | Allowed | *EGFR* mut (cohort MET-1: *EGFR* mut and *MET* amp/mut) | *EGFR* exon 20 ins (cohort D) | Lazertinib; Amivantamab (bispecific EGFR and c-MET mAb); Lazertinib + amivantamab |
| NCT03255083 | I | Mandatory (any) | *EGFR* mut | Yes | Osimertinib + DS-1205c (AXL inhibitor) |
| NCT03260491 | I | Mandatory (any) | *EGFR* mut | Yes | U3-1402 (HER3 ADC) |
| NCT03054038 | I | Mandatory (any) | *EGFR* mut (T790M−)[a] | Yes | Afatinib + necitumumab |

[a]If osimertinib was not received. Amp, amplification; ins, insertion; mAb, monoclonal antibody; mTOR, mechanistic target of rapamycin; mut, mutation; NR, not reported.

**Table 2 | Ongoing clinical trials in treatment-naive *EGFR*-mutant metastatic NSCLC**

| NCT identifier | Phase | Previous EGFR TKI | Required alteration | Includes uncommon *EGFR* mutations | Treatment arms |
|---|---|---|---|---|---|
| NCT03392246 | II | Not allowed | *EGFR* mut | No | Osimertinib + selumetinib (MEK inhibitor) |
| NCT03909334 | II | Not allowed | *EGFR* mut | No | Osimertinib + ramucirumab |
| NCT02971501 | II | Not allowed | *EGFR* mut and BM+ | Yes | Osimertinib ± bevacizumab |
| NCT02803203 | I/II | Not allowed | *EGFR* mut | NR | Osimertinib + bevacizumab |
| NCT02954523 | I/II | Not allowed | *EGFR* mut | Yes | Osimertinib + dasatinib |
| NCT03122717 | I/II | Not allowed | *EGFR* mut | No | Osimertinib + gefitinib |
| NCT03810807 | I | Not allowed | *EGFR* mut | NR | Osimertinib + dacomitinib |
| NCT03567642 | I | Not allowed | *EGFR* mut with concurrent *RB1* and *TP53* mut | NR | Osimertinib + platinum + etoposide |

BM, brain metastasis; mut, mutation; NR, not reported.

osimertinib but have not yet reached the clinical trial stage. The use of first- or second-generation EGFR TKIs after osimertinib failure in the presence of C797X and the absence of T790M and the combination of osimertinib with previous-generation TKIs for C797X and T790M in *trans* have been reported[56,57,62]. Interestingly, the ALK inhibitor brigatinib in addition to a fourth-generation EGFR TKI demonstrated in vivo activity against triple-mutant *EGFR* (sensitive mutation/T790M/C797S)[112].

Several trials have targeted *MET* amplification—one of the most represented EGFR TKI resistance mechanisms. Interim results from the phase Ib TATTON trial investigating the combination of osimertinib and the MET TKI savolitinib have been presented recently[113]. In this trial, the objective response rate (ORR) was 30% (95% CI = 20–43) in patients previously treated with third-generation EGFR TKIs, with a median PFS of 5.4 months. The ORR was 64–67% in third-generation EGFR TKI-naive patients, with the median PFS ranging from 7.6–11 months in the different subcohorts evaluated[113]. A phase II trial with this combination in the same setting is currently ongoing (NCT03778229). The combination of capmatinib (a MET inhibitor) with the first-generation EGFR TKI gefitinib also demonstrated favorable results in a phase Ib/II trial in patients with *MET* amplification pretreated with EGFR TKI; an ORR of 27% was obtained, but this value reached 47% in patients with a *MET* gene copy number of ≥6 (ref. [114]). Another MET TKI, tepotinib, was evaluated in combination with gefitinib in a phase Ib/II trial, resulting in a higher ORR compared with standard chemotherapy[115]. Very recently, a bispecific EGFR and c-MET monoclonal antibody, amivantamab, was safely combined with the third-generation EGFR TKI lazertinib in a phase I study, reaching an ORR of 36% (95% CI = 22–51) in osimertinib-resistant patients and 100% (95% CI = 83–100) in EGFR TKI-naive patients (NCT02609776). Ongoing phase II trials in this setting are reported in Table 1.

*HER2* amplification-driven resistance was sensitive to the combination of osimertinib and the anti-human epidermal growth factor receptor 2 (HER2) antibody–drug conjugate (ADC) trastuzumab–emtansine (T-DM1) in preclinical models[116]. This approach is currently under evaluation in clinical trials (Table 1). T-DM1 monotherapy was reported to have some activity in patients with *HER2* amplification and concurrent *EGFR* mutation who had progressed on a previous EGFR TKI[117]. In the same study, the combination of T-DM1 and the pan-HER inhibitor neratinib was tested in *HER2* amplification in preclinical models, revealing marked tumor regression similar to that obtained with trastuzumab–deruxtecan (another HER2-targeting ADC)[117]. These results support further studies using this novel drug in the setting of *HER2*-mediated EGFR TKI resistance[118].

The efficacy of combining osimertinib with specific TKIs according to the identified pattern of resistance has been reported in preclinical models[119,120]; however, few clinical reports are available[54] and no specific clinical trials have been designed yet.

Of interest, the phase II ORCHARD trial[121] was designed following a biomarker-driven strategy in which a different combinatorial partner was assigned to osimertinib in each treatment subgroup according to the identified EGFR TKI resistance mechanism (savolitinib, gefitinib, necitumumab or others in the case of *MET* alteration, C797X mutation, *EGFR* amplification or no biomarkers, respectively).

Other combinations with EGFR TKIs include different drug classes targeting different molecules and pathways, including MEK, poly (ADP-ribose) polymerase (PARP), CDK4–6 and JAK (Tables 1 and 2).

**Agnostic strategies.** In the absence of a specific resistance mechanism, biomarker-driven approaches are not feasible. Chemotherapy is often used in these instances, as patients with *EGFR*-mutant NSCLC are sensitive to platinum chemotherapy doublets, such as platinum plus pemetrexed in patients with adenocarcinoma histology[42,122]. Continued EGFR TKI administration during chemotherapy remains controversial. For instance, in a phase III clinical trial following first-line gefitinib, there was no difference in PFS and worse overall survival in patients who continued gefitinib, despite considering that randomization at first radiographic progression in this trial does not reflect commonly adopted strategies in clinical practice[123,124]. Whether the same will apply to osimertinib is unknown, and the decision to continue osimertinib during chemotherapy may be influenced by the presence of CNS metastases.

Immune checkpoint inhibitor monotherapy did not demonstrate superiority to chemotherapy in *EGFR* mutation-positive disease[125], while immune checkpoint inhibitor combination with chemotherapy only showed a benefit when associated with an anti-angiogenic drug in this setting[126]. Exploratory analyses in patients with *EGFR* mutations in the IMpower150 trial revealed improved PFS and overall survival in favor of the combination of atezolizumab plus bevacizumab plus chemotherapy compared with bevacizumab plus chemotherapy alone. Specific data on immunotherapy in *EGFR*-mutant disease will be discussed in detail in the section "Immunotherapy strategies."

Novel compounds with agnostic targets are currently evaluated in the EGFR TKI resistance setting. As HER3 is frequently overexpressed in *EGFR*-mutant tumors, patritumab deruxtecan (U3-1402; a novel HER3-directed ADC) showed promising results in patients pretreated with EGFR TKI, with an ORR of 25% (95% CI = 14.4–38.4) and a disease control rate of 70% (95% CI = 55.9–81.2).

**Table 3 | Ongoing clinical trials of immunotherapy in *EGFR*-mutant metastatic NSCLC**

| NCT identifier | Phase | Previous EGFR TKI | Required alteration | Includes uncommon *EGFR* mutations | Treatment arm(s) |
|---|---|---|---|---|---|
| NCT03515837 (KEYNOTE 789) | III | Mandatory (any) | *EGFR* mut (T790M–)[a] | No | Platinum + pemetrexed ± pembrolizumab |
| NCT02864251 (CheckMate722) | III | Mandatory (any) | *EGFR* mut (T790M–)[a] | NR | Platinum + pemetrexed; Platinum + pemetrexed + nivolumab; Ipilimumab + nivolumab |
| NCT03991403 | III | Mandatory (any) | *EGFR* mut (T790M–)[a] | NR | Carboplatin + paclitaxel + bevacizumab + atezolizumab; Platinum + pemetrexed |
| NCT03256136 | II | Mandatory (any third generation) | Cohorts A and C: *EGFR* mut | No | Cohort A (previous chemo): nivolumab + ipilimumab; Cohort C (chemo naive): platinum + pemetrexed + nivolumab |
| NCT03786692 | II | Mandatory (any) | *EGFR* mut | No | Carboplatin + pemetrexed + bevacizumab ± atezolizumab |
| NCT03647956 | II | Mandatory (any) | *EGFR* mut | Yes | Carboplatin + pemetrexed + bevacizumab + atezolizumab |
| NCT04099836 | II | Mandatory (osimertinib) | *EGFR* mut | No | Atezolizumab + bevacizumab |
| NCT04426825 | II | Mandatory (any) | *EGFR* mut (T790M–)[a] | NR | Atezolizumab + bevacizumab |
| NCT04245085 | II | Mandatory (any) | *EGFR* mut (T790M–)[a] | Yes | Arm A: carboplatin + paclitaxel + bevacizumab + atezolizumab; Arm B: pemetrexed + bevacizumab + atezolizumab |
| NCT04147351 | II | Mandatory (any) | *EGFR* mut (T790M–)[a] | Yes | Platinum + pemetrexed + bevacizumab + atezolizumab |
| NCT02323126 | II | Mandatory (group 1: one previous line) | *EGFR* mut (T790M+) | NR | EGF816 + nivolumab |
| NCT04120454 | II | Mandatory (any) | *EGFR* mut | Yes | Ramucirumab + pembrolizumab |
| NCT03994393 | II | Mandatory (any) | *EGFR* mut (T790M–)[a] | No | Platinum + pemetrexed + durvalumab + tremelimumab |
| NCT04517526 | II | Mandatory (osimertinib) | *EGFR* mut | NR | Platinum + pemetrexed + durvalumab + bevacizumab + SBRT |
| NCT04538378 | II | Mandatory (TKI and chemo) | *EGFR* mut and SCLC transformation | NR | Olaparib + durvalumab |
| NCT03381274 | Ib/II | Mandatory (arm A (one previous line) or arm B (2–4 previous lines)) | *EGFR* mut (T790M–) (arm A) | NR | Arm A: osimertinib + oleclumab (CD73 monoclonal antibody); Arm B: oleclumab + AZD4635 (A2aR antagonist) |
| NCT02364609 | I | Mandatory (erlotinib) | *EGFR* mut | Yes | Afatinib + pembrolizumab |
| NCT03846310 | I/Ib | Mandatory (arms 1 and 2; any) | *EGFR* mut | NR | Arm 1: carboplatin + pemetrexed + zimberelimab (anti-PD-1); Arm 2: carboplatin + pemetrexed + zimberelimab + etrumadenant (anti-A2aR/A2bR) |

[a]If osimertinib was not received. Chemo, chemotherapy; mut, mutation; NR, not reported; PD-1, programmed cell death protein 1; SBRT, stereotactic body radiation therapy.

Interestingly, efficacy was observed regardless of the presence or absence of $EGFR^{C797S}$ mutation, *MET* amplification, *HER2* mutation, *BRAF* fusion or *PIK3CA* mutation (NCT03260491).

Trophoblast cell-surface antigen 2 (TROP2) is an intracellular calcium signaling transducer that is often overexpressed in NSCLC. A phase I study (NCT03401385) of DS-1062a—a TROP2 ADC—showed encouraging results in NSCLC, and a phase II study is currently enrolling *EGFR*-mutant patients at EGFR TKI failure (Table 1).

**Preventing the emergence of resistance.** The main unselective approach to delaying EGFR TKI resistance is a combination of EGFR TKIs with chemotherapy. Previous attempts to combine first-generation EGFR TKIs with chemotherapy in patients with NSCLC not selected for *EGFR* mutations showed no additional survival benefit compared with chemotherapy alone[127–130], regardless of *EGFR* mutational status[131].

However, in selected patients with mutant *EGFR*, the combination of gefitinib with carboplatin plus pemetrexed was initially

compared with sequential alternative regimens in the phase II NEJ005 trial, demonstrating prolonged PFS and overall survival[132,133]. The phase III NEJ009 trial was then conducted to compare the same combination of chemotherapy plus gefitinib versus gefitinib alone. The PFS (the primary endpoint in this study) was significantly longer in the combination arm (20.9 versus 11.9 months; HR = 0.490; $P < .001$) and an overall survival advantage was also observed[134]. Comparable results were observed in a similar phase III trial (PFS = 16 versus 8 months (HR = 0.51; $P < 0.001$); overall survival = not reached versus 17 months (HR = 0.45; $P < 0.001$))[135]. Whether these results will extend to osimertinib is being tested in the phase III trial FLAURA 2, which is comparing carboplatin (or cisplatin) pemetrexed plus osimertinib with osimertinib alone as a first-line therapy[136].

The combination of EGFR TKIs with antiangiogenetic drugs has been investigated in the front-line setting with the aim of delaying resistance. Vascular endothelial growth factor (VEGF)–EGFR crosstalk is well described[137], and increased VEGF was associated with erlotinib resistance in preclinical models, although the mechanistic basis of how VEGF/VEGF receptor inhibition enhances the effects of EGFR inhibitors clinically remains unknown[138]. The combination of erlotinib and the anti-VEGF monoclonal antibody bevacizumab showed activity in the single-arm phase II trial BELIEF[139] and demonstrated significant PFS benefit in a randomized phase II trial (JO25567) compared with erlotinib alone[140]. PFS benefit was confirmed in the phase III NEJ026 trial[141], although it did not translate into an overall survival advantage[142]. Similarly, PFS benefit was obtained when combining erlotinib with ramucirumab, a monoclonal antibody targeting VEGF receptor 2 (ref. [143]), although overall survival remains immature in this trial. The association of anti-VEGF and osimertinib is under evaluation. Of note, recently published data from a phase I/II trial show a median PFS of 19 months with osimertinib plus bevacizumab[144], which is identical to single-agent osimertinib in the front-line setting[15]. Phase II and III trials are currently investigating osimertinib plus anti-VEGF both in first-line and EGFR TKI pretreatment settings, where recently presented data from a phase II trial (UMIN000023761) showed that the osimertinib and bevacizumab combination failed to prolong PFS compared with osimertinib alone in pretreated T790M-positive patients (Tables 1 and 2).

*EGFR*-dependent osimertinib resistance mechanisms often retain sensitivity to previous-generation EGFR TKIs in the absence of T790M mutation. Therefore, the front-line combination of osimertinib with first- or second-line EGFR TKIs could be a strategy to prevent on-target resistance. Initial results from a phase I/II trial of osimertinib plus gefitinib showed encouraging activity (ORR = 85.2%; 95% CI = 67.5–94.1), an acceptable safety profile and rapid plasma clearance of the *EGFR* mutation[63].

Similarly, co-targeting EGFR and alternative pathways to prevent the occurrence of *EGFR*-independent resistance mechanisms was successful in preclinical settings, and various clinical trials are ongoing with EGFR TKIs plus MET, MEK, Src, PARP or CDK4–6 inhibitors (Tables 1 and 2). Of particular interest, the combination of lazertinib (a third-generation EGFR TKI) and the bispecific anti-EGFR and anti-MET antibody amivantamab resulted in an ORR of 100% (95% CI = 83–100) in treatment-naive patients in the phase I CHRYSALIS study.

Additionally, the combination of osimertinib plus platinum and etoposide is under evaluation in *EGFR* mutation-positive patients harboring concurrent *RB1* and *TP53* mutations, with the aim to prevent the development of SCLC histology (Table 2).

Another appealing approach in the front-line context is treating drug-tolerant disease through radiation. An upfront combination of local radiotherapy with first-line EGFR TKI demonstrated improved PFS and overall survival compared with EGFR TKI alone in oligometastatic patients (NCT02893332), confirming that radically treating metastatic sites, whenever feasible, has the potential to reduce the proportion of persister subclones.

## Immunotherapy strategies

The role of immune oncology agents in the treatment of *EGFR*-positive NSCLC has not been clearly established and, to date, the efficacy of anti-programmed cell death protein 1 or anti-programmed death-ligand 1 inhibitors is minimal in *EGFR*-mutant patients. Subgroup analysis of pretreated patients harboring *EGFR*-sensitizing mutations in the main immune oncology clinical trials revealed a strongly reduced benefit from immune checkpoint inhibitors as monotherapy in this special population[145–147]. Consistently, a meta-analysis including 186 *EGFR*-mutant patients from three clinical trials confirmed the absence of an overall survival advantage with immune oncology monotherapy compared with docetaxel[125]. Recently, data from a multicenter registry of patients harboring driver mutations and receiving immune oncology monotherapy (that is, IMMUNOTARGET) were published. The report included 125 *EGFR* mutation-positive patients. Among the evaluable patients ($n = 115$), the ORR was 12.2%, whereas 67% of patients experienced progressive disease as the best radiological response and the median PFS was 2.1 months (95% CI = 1.8–2.7 months)[148].

*EGFR*-mutant patients were excluded from most first-line clinical trials with immune oncology agents, with the exception of IMpower130 and IMpower150 (refs. [126,149,150]). No benefit was observed from the addition of atezolizumab to platinum-based chemotherapy in the *EGFR*-mutant subgroup in the IMpower130 trial compared with chemotherapy alone[149]. The addition of atezolizumab and bevacizumab to platinum doublet chemotherapy (the ABCP regimen) was the only immune oncology regimen demonstrating a benefit in the subgroup of *EGFR*-mutant patients within the IMpower150 trial[150]. Out of 124 *EGFR* mutation-positive patients included in this study, 91 harbored *EGFR*-sensitizing mutations and 86% received previous EGFR TKI treatment. In the subgroup of patients with *EGFR*-sensitizing mutations, overall survival and PFS were significantly improved in the ABCP arm, suggesting synergistic activity of immune oncology and antiangiogenic drugs in *EGFR*-mutant settings[150].

Different phase II and III trials of chemotherapy–immunotherapy combinations are ongoing in patients pretreated with an EGFR TKI (Table 3) as a strategy to delay EGFR TKI resistance by modulating the tumor microenvironment and increasing sensitivity to immune oncology agents[151]. However, the high rate of pulmonary toxicities (38% pneumonitis) observed with the combination of osimertinib and the anti-programmed death-ligand 1 durvalumab in the phase Ib TATTON trial[152] raised safety concerns; further studies in the same setting have therefore been interrupted prematurely[153].

## CNS disease

The incidence of brain metastasis in *EGFR*-mutant NSCLC ranges from 24–32% at diagnosis[154,155], with a high cumulative lifetime risk that is partially imputable to the long survival of this patient category. Indeed, the 2-year cumulative incidence of CNS metastasis has been reported to be 20% among *EGFR* mutation-positive patients with NSCLC treated with first-generation EGFR TKIs[156,157], with this value reaching 47% among patients with preexisting CNS disease[156].

The high incidence of CNS disease reflects a pharmacological sanctuary due to the blood–brain barrier exclusion of several drugs[158]. In this setting, the addition of brain radiation therapy showed an improvement of intracranial PFS[159–161].

First- and second-generation EGFR TKIs showed variable activity on brain metastasis, with erlotinib showing better CNS data[162–164]. In particular, high-pulse dosing of erlotinib with the aim of increasing the drug concentration in the cerebrospinal fluid resulted in high intracranial response rates (75%) in an early-phase clinical trial[165].

Third-generation osimertinib demonstrated CNS activity in preclinical models[166], and patients with asymptomatic or clinically stable brain metastasis were included in pivotal clinical trials investigating this drug, representing up to 37% of the study population in the EGFR TKI pretreatment setting[14,42,167,168].

A pooled analysis of the phase II trials AURA Extension and AURA2 included 128 patients with NSCLC and CNS disease pretreated with EGFR TKI. The ORR was 54% and the disease control rate was 92% in the 50 evaluable patients, regardless of previous brain radiotherapy[169]. CNS efficacy was specifically evaluated in the AURA3 trial, in which the ORR in patients with measurable CNS lesions was 70% with osimertinib compared with 31% with platinum–pemetrexed (odds ratio = 5.13; 95% CI = 1.44–20.64)[170].

The front-line osimertinib FLAURA trial included about 20% of patients with stable or asymptomatic CNS metastasis, with a confirmed PFS benefit over first-generation TKIs (15.2 versus 9.6 months; HR = 0.47; 95% CI = 0.30–0.74; $P < 0.001$)[14]. Of note, CNS progression was observed in 6% of patients in the osimertinib arm compared with 15% of patients receiving gefitinib or erlotinib[14]. Consistently, in the ADAURA trial, adjuvant osimertinib demonstrated an 82% risk reduction of CNS disease recurrence compared with the placebo (HR = 0.18; 95% CI = 0.10–0.33)[40].

Regarding leptomeningeal disease, the 160 mg daily osimertinib schedule (double the standard dose) showed an ORR of 62% and a median overall survival of 11 months in patients with cytologically confirmed leptomeningeal disease in the phase I BLOOM study[171]. Similar results were obtained in a phase II trial, with intracranial complete responses in 12.5% of patients with leptomeningeal disease who were pretreated with EGFR TKI[172].

To date, front-line osimertinib is the standard of care in *EGFR*-positive advanced NSCLC, irrespective of CNS involvement. The activity of osimertinib on CNS disease allows a reduction or delay of CNS progression. Recently, interesting results have been presented by Piper-Valillo and colleagues[173] about osimertinib dose intensification to 160 mg in patients developing CNS progression, especially when associated with chemotherapy or radiotherapy. Treating CNS progression remains an unmet need in *EGFR*-mutant disease, and despite the role of local treatments including radiotherapy and neurosurgery, further research should specifically focus on biomarkers of response, including liquid biopsy[174].

## Monitoring resistance through circulating tumor DNA

Blood-based tumor analyses are an attractive opportunity for cancer diagnostics[175]. In contrast with tissue biopsy, liquid biopsy is minimally invasive and accessible. Circulating tumor DNA (ctDNA) defines short DNA sequences shed by tumor cells into the systemic circulation, as identified by the presence of mutations not found in normal tissues[176]. The sensitivity and specificity of plasma genotyping compared with tumor genotyping ranges across different tumors and is related to tumor burden as well as anatomical tumor sites[85,177]. Solid data on comparative testing are eagerly needed to assess the capability of liquid biopsy to detect spatial and temporal heterogeneity. Although the development of ctDNA detection and analyses methods is expanding quickly, available data show 80% sensitivity in detecting tumor alterations of interest in NSCLC, with higher concordance in patients with extrathoracic disease[178].

A meta-analysis by Luo et al.[179] investigating the diagnostic performance of ctDNA showed 67.4% sensitivity and 93.5% specificity for the detection of *EGFR* mutations on ctDNA compared with tissue. In contrast, the sensitivity to detect T790M mutation was 61.4% in the phase II AURA Extension and AURA2 trials[180]. Besides PCR-based tests that have been developed and approved for initial diagnosis and identification of the $EGFR^{T790M}$ mutation, NGS-based platforms are currently used in the investigational setting to identify multiple resistance-associated mutations. Despite the high specificity of plasma NGS-based techniques, their sensitivity is lower across different platforms[181–183]. This is due to the absence of tumor shedding in 15–20% of patients[184], their inferior sensitivity to detecting gene amplification compared with fluorescence in situ hybridization[185] and their failure to detect histologic transformation. Conversely, the main advantage of plasma NGS evaluation is the potential to capture tumor clonality and heterogeneity. To date, most of the available data on resistance mechanisms to osimertinib have been obtained through plasma NGS analysis, although comparisons between tissue and plasma samples are limited in this setting. Comprehensive analyses are awaited in order to evaluate the concordance between ctDNA and tissue data at the occurrence of resistance[186]. In the clinical context, plasma and tissue profiling should be adopted as complimentary methods when available.

The chance to detect early subclonal events[177] supports the dynamic monitoring of ctDNA as a potential tool to investigate the emergence of resistance mutations. This strategy in clinical practice is under evaluation in the APPLE trial, the aim of which is to compare the initiation of treatment of $EGFR^{T790M}$ based on cfDNA versus radiological evidence of disease progression[187].

The utility of ctDNA might be translated to early-stage lung cancer as a potential marker of minimal residual disease, which is not detectable by conventional diagnostic methods[188].

The identification of circulating biomarkers to monitor radically treated lung cancers carrying *EGFR* mutations could help identify patients at higher risk for disease relapse and overall worse prognosis. *EGFR* mutation status monitoring through plasma-derived ctDNA at baseline and at disease recurrence is currently under investigation in the ADAURA trial, with the aim of improving the management of patients with radically resected lung cancer[40].

## Conclusion

Increased understanding of the complexity of EGFR TKI resistance remains a priority in clinical practice as this will facilitate the development of therapies to circumvent disease progression. Despite notable advances in tackling therapy resistance in experimental settings, platinum-based chemotherapy is the only approved regimen for patients experiencing disease progression on osimertinib to date.

Understanding the dynamics of the different alterations associated with EGFR resistance and the interplay with the different lines of therapy will help to guide clinical decisions, with anticipation and eventual circumvention of disease progression. For instance, the presence of *TP53* mutations, the co-occurrence of *TP53* and *RB1* mutations or the lack of plasma clearance of mutant *EGFR* could help to identify patients who will not derive a durable response from EGFR TKIs and who might benefit from front-line or early combinatorial approaches. Dynamic plasma monitoring through serial liquid biopsies might help to identify the occurrence of acquired *EGFR*-dependent and -independent resistance mutations earlier[175], while its impact on patient management and related outcomes remains to be assessed. Unlike tissue samples, plasma genotyping has the potential to detect determinant mutations of spatial heterogeneity, at the cost of lower sensitivity and inability to detect histologic transformation[189,190]. All of these aspects have an impact on clinical decision-making in daily practice, especially taking into account the additional costs associated with comprehensive genomic profiling of tumors. Guaranteeing access to clinical trials, including comprehensive genomic profiling evaluation, should be a priority in order to allow patients to access potentially tailored, effective mutation-driven treatments, to expand knowledge and to accumulate evidence on the determinants of EGFR TKI resistance and strategies to overcome disease progression.

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## Competing interests

A.P. received honoraria for consulting, advising or lecturing from AstraZeneca, Agilent/Dako, Boehringer Ingelheim, Bristol Myers Squibb, Eli Lilly, Janssen, Merck Sharp & Dohme, Novartis, Pfizer and Roche/Genentech. P.A.J. is a consultant at AstraZeneca, Boehringer Ingelheim, Daiichi Sankyo, Novartis, Sanofi Oncology, Takeda Oncology, Pfizer, Roche/Genentech, Biocartis, Silicon Therapeutics, Esai, Bayer, ACEA Biosciences, Eli Lilly, Araxes Pharma and SFJ Pharmaceuticals; is a Scientific Advisory Board member for Voronoi, Biocartis, Mirati Therapeutics, Ignyta and Loxo Oncology; reports receiving commercial research grants from AstraZeneca, Boehringer Ingelheim, Daiichi Sankyo, Astellas, Eli Lilly, Revolution Medicines and Takeda Oncology; has ownership interest (including patents) in Gatekeeper Pharmaceuticals and Loxo Oncology; and reports receiving other remuneration from LabCorp. T.M. reports grant/research support from AstraZeneca, Bristol Myers Squibb, Clovis Oncology, G1 Therapeutics, MSD, Merck Serono, Novartis, Pfizer, Roche, SFJ Pharmaceuticals, Takeda and Xcovery; speaker's fees from ACEA Pharma, Alpha Biopharma, Amgen, Amoy Diagnostics, AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, Eli Lilly, InMed Medical Communication, MSD, Novartis, Pfizer, prIME Oncology, Roche/Genentech, Taiho and Takeda Oncology; honoraria from AbbVie, ACEA Pharma, Alpha Biopharma, Amgen, Amoy Diagnostics, AstraZeneca, Bayer, Boehringer Ingelheim, Blueprint Medicines Corporation, Bristol Myers Squibb, Celgene, CStone Pharmaceuticals, Daiichi Sankyo, Eli Lilly, Fishawack Facilitate, Hengrui Therapeutics, Ignyta, Incyte Corporation, InMed Medical Communication, IQVIA, Janssen, Loxo Oncology, Merck Serono, MSD, MORE Health, Novartis, OncoGenex Pharmaceuticals, OrigiMed, PeerVoice, Pfizer, prIME Oncology, Roche/Genentech, Sanofi-Aventis R&D, SFJ Pharmaceuticals, Takeda Pharmaceuticals (Hong Kong), Vertex Pharmaceuticals, Yuhan Corporation, Medscape/WebMD (medical education/continuing medical education activities), PeerVoice (independent medical education) and prIME Oncology (medical education); shares in Hutchison Chi-Med and Sanomics; stock options in Clearbridge Biomedics (now Biolidics), Loxo Oncology, OrigiMed and Virtus Medical Group; an advisory role for AbbVie, ACEA Pharma, Amgen, AstraZeneca, Bayer, Blueprint Medicines Corporation, Boehringer Ingelheim, Bristol Myers Squibb, Celgene, Cirina, CStone Pharmaceuticals, Daiichi Sankyo, Eli Lilly, Fishawack Facilitate, G1 Therapeutics, GeneDecode (uncompensated), Hengrui Therapeutics, Hutchison Chi-Med, Ignyta, Incyte Corporation, IQVIA, Janssen, Loxo Oncology, Lunit, Merck Serono, Merck Sharp & Dohme, Novartis, OncoGenex Technologies, OrigiMed, Pfizer, Roche/Genentech, Sanofi-Aventis R&D, SFJ Pharmaceutical, Takeda Oncology, Vertex Pharmaceuticals, Virtus Medical Group and Yuhan Corporation; board of directors/leadership roles (remunerated) for AstraZeneca and Hutchison Chi-Med; and board of directors/leadership roles (non-remunerated) for the American Society of Clinical Oncology (ASCO), Asian Thoracic Oncology Research Group (ATORG), Chinese Lung Cancer Research Foundation Limited (CLCRF), Chinese Society of Clinical Oncology (CSCO), Hong Kong Cancer Fund

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## Additional information

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