Critical Reviews in Oncology / Hematology 196 (2024) 104295

Contents lists available at ScienceDirect

Critical Reviews in Oncology / Hematology

journal homepage: www.elsevier.com/locate/critrevonc

The study of primary and acquired resistance to first-line osimertinib to improve the outcome of EGFR-mutated advanced Non-small cell lung cancer patients: the challenge is open for new therapeutic strategies

Alessandra Ferro [a,1], Gian Marco Marinato [a,b,1], Cristiana Mulargiu [a,b], Monica Marino [a,b], Giulia Pasello [a,b], Valentina Guarneri [a,b], Laura Bonanno [a,b,*]

[a] Medical Oncology 2, Veneto Institute of Oncology IOV – IRCCS, Padua, Italy
[b] Department of Surgery, Oncology and Gastroenterology, University of Padova, Padua, Italy

ARTICLE INFO

Keywords:
Epidermal growth factor receptor
Non-small cell lung cancer
Osimertinib
Targeted therapy
Cancer resistance mechanism
Personalized medicine

ABSTRACT

The development of targeted therapy in epidermal growth factor receptor (EGFR)-mutated non-small cell lung cancer (NSCLC) patients has radically changed their clinical perspectives. Current first-line standard treatment for advanced disease is commonly considered third-generation tyrosine kinase inhibitors (TKI), osimertinib. The study of primary and acquired resistance to front-line osimertinib is one of the main burning issues to further improve patients' outcome.

Great heterogeneity has been depicted in terms of duration of clinical benefit and pattern of progression and this might be related to molecular factors including subtypes of EGFR mutations and concomitant genetic alterations. Acquired resistance can be categorized into two main classes: EGFR-dependent and EGFR-independent mechanisms and specific pattern of progression to first-line osimertinib have been demonstrated.

The purpose of the manuscript is to provide a comprehensive overview of literature about molecular resistance mechanisms to first-line osimertinib, from a clinical perspective and therefore in relationship to emerging therapeutic approaches.

1. Introduction

Epidermal growth factor receptor (*EGFR*) sensitizing mutations are detected in 10–15% of advanced non-small cell lung cancer (aNSCLC) in Western Countries and about 40% in Asian Countries (Zhang et al., 2016). The two most common activating *EGFR* mutations are in-frame deletions in exon 19 (ex19Del) and amino acid substitution (leucine to arginine at codon 858 [L858R]) in exon 21 (Rosell et al., 2009; Douillard et al., 2014). *EGFR* mutation predicts response to EGFR-tyrosine kinase inhibitors (TKIs), a class of molecules capable of binding the adenosine triphosphate (ATP) binding domain of EGFR protein thus interrupting the downstream signalling pathway (Mok et al., 2009; Wu et al., 2014).

In the last decades, molecular analysis and identification of driver genetic alterations have led to outstanding improvement in the outcome of aNSCLC and EGFR inhibitors have been the forerunners of this therapeutic revolution. Several phase III studies demonstrated the superiority of front-line first- and second-generation (1st and 2nd gen) TKIs – gefitinib, erlotinib, afatinib – over standard chemotherapy, both in terms of overall response rate (ORR) and progression-free survival (PFS) (Mok et al., 2009; Rosell et al., 2012; Sequist et al., 2013). Anyway, inevitably, after a median time of 9–15 months, all patients develop acquired resistance (Yu et al., 2013). Third generation (3rd gen) TKI Osimertinib, demonstated its efficacy in patients progressing to EGFR-TKIs in the presence of a secondary T790M mutation and it was characterized by improved activity at central nervous system (CNS) site (Mok et al., 2017a). More recently, in the phase III FLAURA trial, osimertinib has shown to improve survival outcomes over 1st gen TKI in first-line setting and it is currently mainly considered as the standard of care for first-line treatment of patients carrying common EGFR sensitizing mutations (Soria et al., 2018; Anon, 2018). Median progression-free survival (mPFS) to first-line osimertinib in the clinical trial was 18.9 months (Soria et al., 2018), while in real-life setting the treatment is often used beyond radiological progression and locoregional treatment strategies are employed wherever possible (Le et al.,

* Correspondence to: via Gattamelata, 64, Padua 35128, Italy.
E-mail address: laura.bonanno@iov.veneto.it (L. Bonanno).
[1] These authors contributed equally to this work and share first authorship

https://doi.org/10.1016/j.critrevonc.2024.104295
Received 14 September 2023; Received in revised form 25 January 2024; Accepted 7 February 2024
Available online 20 February 2024

2018; Gomez et al., 2016) An updated overall survival (OS) analysis, with a median follow-up of 39 months, showed a median survival (mOS) of 38.6 months for osimertinib and 31.8 months for the control arm (Ramalingam et al., 2020). CNS activity, in particular, was found to be greatly improved leading to an OR of 91% and a longer mPFS (13.9 months *versus* non reached) (Reungwetwattana et al., 2018).

Although limited, a proportion of patients (20–30%) do not respond to osimertinib despite the presence of *EGFR* sensitizing mutation and great heterogeneity has been observed in terms of duration of clinical benefit (Vendrell et al., 2021). No clinical or molecular predictive markers are currently available to identify patients who could benefit from different therapeutic approaches, possibly including osimertinib in combination with other agents (Cho et al., 2023; Planchard et al., 2023). In parallel, while increasing amount of data is available concerning acquired resistance mechanisms, different therapeutic strategies are under evaluation and no standardized detection methods are yet available for clinical practice.

In our manuscript, we aim to summarize primary and acquired resistance mechanisms to Osimertinib, collecting available date concerning first-line Osimertinib and classifying them according to temporal and molecular criteria. We also put them into a clinical prespective, depicting and classifying potential therapeutic strategies to overcome them. The purpose of our review is to provide evidence-based answers to the new therapeutic challenges in order to optimize the clinical management of aNSCLC harboring *EGFR* alterations.

2. Methods

Literature research was conducted using MEDLINE/PubMed, EMBASE, Scopus and Cochrane Library databases, up to October 2023. The literature recovery was supplemented by manual searches of abstracts meeting proceedings, including the European Society of Medical Oncology (ESMO) congress, the American Society of Clinical Oncology (ASCO), the European Lung Cancer Congress (ELCC) and the World Conference on Lung Cancer (WCLC). Non-English language literature was excluded. The following keywords were used as literature search terms: lung cancer or NSCLC and EGFR and/or EGFR-TKI and/or osimertinib and/or EGFR-TKI resistance, etc. Two authors (AF and GMM) independently selected studies and disagreements were discussed and solved with a third author (LB).

3. Primary resistance to first -line osimertinib

Primary (or intrinsic) resistance is defined by the demonstration of no clinical benefit and/or radiological progressive disease in *EGFR*-mutated aNSCLCs within six months since the beginning of EGFR-inhibitor. It concerns about 20–30% of patients and it is supposed to be related to molecular alterations existing before the start of targeted therapy (Jackman et al., 2010; Santoni-Rugiu et al., 2019; Wang et al., 2016).

A molecular perspective considers primary resistance to front-line osimertinib mainly in relationship to molecular heterogeneity of *EGFR* mutations and to co-existing molecular alterations in other genes (Leonetti et al., 2019).

Several real-world studies showed that some clinical features (such as smoking history and high baseline neutrophile-to-lymphocyte ratio) in parallel with the presence of co-mutations in genes other than *EGFR* might have a role in defining heterogeneity in clinical benefit (Park et al., 2022; Guo et al., 2020; Kim et al., 2019a). Another potential predictive biomarker is programmed death-ligand 1 (PD-L1) status: a recent meta-analysis concluded that high PD-L1 expression is likely to be a negative predictive biomarker for EGFR-TKIs in *EGFR*-mutant NSCLC patients, whereas a post-hoc analysis of the FLAURA trial did not confirm the role among patients treated with osimertinib (Brown et al., 2020; Peng et al., 2021).

3.1. Heterogeneity in EGFR genetic alterations

Collectively, excluding classical *EGFR* mutations (L858R or ex19del), about 20% of *EGFR*-mutated patients carry a mutations considered as atypical and the rate of uncommon mutations is likely to increase as long as next-generation sequencing (NGS) is used to detect a larger spectrum of genetic alterations in clinical practice (Arcila et al., 2013).

Being a rare and heterogeneous group of diseases, most of the data we use to face clinical practice stem from case reports, retrospective analyses and, more recently, real-world evidence.

Robichaux *et al.*, in their pre-clinical work, suggested that *EGFR* mutations could be divided into four distinct subgroups based on structure and function, and that structure–function-based groups can predict ORR and patient outcomes better than exon-based groups (Robichaux et al., 2021). These subtypes of EGFR kinase domain mutations are: 1) classical-like mutations that are far from the ATP-binding pocket; 2) T790M-like mutations that are located in the hydrophobic core; 3) exon 20 insertions in the αC–β4 Loop following the C-terminal end of the αC-helix (Ex20ins-L); 4) P-loop and αC-helix compressing (PACC) mutations that are located on the interior surface of the ATP-binding pocket or C-terminal portion of the αC-helix (Robichaux et al., 2021). On the other hand, clinical data collected and analyzed till now are not based on biological classification and only two groups are clearly established according to clinical results: Non-exon 20 insertions and exon-20 insertions.

3.1.1. Uncommon EGFR mutations (other than Exon 20 insertions)

Uncommon classical-like EGFR mutations, though occurring in different exons, confer similar sensitivity to EGFR TKIs. For example, favourable responses were observed in patients harboring G719X, S768I and L861Q, which are classified as sensitizing EGFR mutations. Second- and 3rd-gen EGFR TKIs should be preferred over 1st-gen TKIs that have shown limited activity (Russo et al., 2019). In particular, afatinib (2nd-gen TKI) was the first drug with data focusing on rare mutations: the inclusion of patients with uncommon *EGFR* mutations in LUX-Lung 2, LUX-Lung 3 and LUX-Lung 6, allowed for a combined post hoc analysis providing evidence that afatinib is strongly active especially in the presence of G719X, L861Q and S768I mutations (Yang et al., 2015). Osimertinib activity in uncommon EGFR mutations has been evaluated mostly through retrospective studies. In a multi-center retrospective study collecting 62 aNSCLC patients with uncommon *EGFR* mutation treated with first-line osimertinib, the drug demonstrated activity allowing a 91% disease control rate (DCR) and a median duration of response (mDOR) of 17.4 months (Bar et al., 2022). Moreover, a wide Italian experience recently showed a large use of osimertinib in real-world setting, even in the presence of uncommon mutations, and relevant activity has been observed (Pizzutilo et al., 2022). Consistent data arise from the phase II retrospective UNICORN study, which is investigating the efficacy and tolerability of osimertinib as first-line therapy for uncommon or compound *EGFR*-positive NSCLC patients: a total of 60 patients have been included so far and osimertinib has been found to be effective in atypical *EGFR* mutations with a high rate of ORR (61%) also confirming intracranial activity (Bar et al., 2023).

Recently the first prospective randomized trial specifically concerning uncommon EGFR mutations was presented: Achilles/TORG1834 trial demonstrated the superiority of afatinib *versus* chemotherapy. The most common included mutatations were G719X and L861Q, being PACC and classical-like mutations, respectively (Robichaux et al., 2021; Miura et al., 2023).

3.1.2. Exon 20 insertions

EGFR exon 20 insertion (ex20ins) mutations account for around half of the uncommon mutations (approximately 10% of *EGFR* mutations) and, although highly heterogeneous, the vast majority of exon 20 alterations are located between amino acids 766 and 775 and involve insertions or duplications of 1–4 amino acids, altering the alpha-C helix

conformation (Arcila et al., 2013; Yasuda et al., 2013). Given the wide spectrum of *EGFR* ins20 mutations, each mutation response to specific therapies significantly differ in available preclinical studies. Even though several published case reports suggest a potential role for osimertinib, clinical data are mainly inconsistent and in a retrospective analysis, including 21 patients carrying *EGFR* ex 20ins, only one partial response was observed following osimertinib and mPFS was only 3.6 months (95% CI: 2.6–4.5) (van Veggel et al., 2020). A prospective phase I/II trial focused on the role of osimertinib for *EGFR* ex20ins-positive NSCLC: 12 patients were evaluated, no radiological response was observed and DCR was 58% with mPFS of only 3.8 months. The main interest of the study lays on the demonstration of different pharmacokinetic data and sensitivity according to ex20ins subtype (Yasuda et al., 2021).

Nowadays, multiple inhibitors have been developed for the treatment of *EGFR* ex20ins–positive NSCLC patients: these include poziotinib, mobocertinib, amivantamab, CLN-081, tuxobertinib, tarloxotinib, luminespib and pyrotinib (Meador et al., 2021; Hou et al., 2022; Song et al., 2022). These new TKIs generally showed promising activity in exon 20 *EGFR*-mutated patients but at cost of considerable toxicity, with the potential exception of CLN-081 (Piotrowska et al., 2021). The FDA granted approval to amivantamab in May 2021 and to mobocertinib in September 2021 (Park et al., 2021a; Zhou et al., 2021). Nevertheless, EMA did not consider the risk/benefit assessment of second-line mobocertinib favourable, also taking into account the tolerability profile and the limited intracranial activity.

More recently, the phase III randomized trial including 308 patients carrying EGFR exon 20 insertions was presented. The study demonstrated statistically and clinically significant improvement in PFS for amivantamab plus chemotherapy *versus* chemotherapy in first-line setting, thus potentially establishing for the first time a real evidence-based standard of care for first-line treatment of this subset of EGFR mutated patients (Zhou et al., 2023).

3.2. Heterogeneity in the presence of concomitant genetic alterations

Although far less frequently, primary *EGFR*-independent resistance mechanisms might occur, even among patients carrying classical *EGFR* sensitizing mutations (Leonetti et al., 2019).

Blakely *et al.* sequenced cell-free DNA (cfDNA) from 1122 *EGFR*-mutant aNSCLC patients and found widespread occurrence of additional genetic alterations (92.9% had at least one other cancer-related variant). More importantly, some co-occurring genetic alterations are linked to therapeutic response, underlining how a wider genomic picture is required to understand tumor heterogeneity beyond a single driver oncogene (Blakely et al., 2017a).

The most common co-occurring genomic alterations involve *TP53* (54.6–64.6%), *RB1* (9.6–10.33%), *ERBB2* (8–11%), *CTNNB1* (which encodes β-catenin; 5.3–9.6%), *PIK3CA* (9–12.4%), *NKX2–1* (12.2–16.7%), *CDK4* (7–10%), *CDK6* and *CCNE1* as shown in Fig. 1 (Skoulidis and Heymach, 2019).

3.2.1. TP53 mutations

Mutations in *TP53* represent the most prevalent co-alteration: reports indicated that up to 60% of advanced *EGFR*-mutant lung cancers harbour TP53 mutations (Rachiglio et al., 2019). Furthermore, *TP53* co-mutation is considered a negative prognostic marker in *EGFR*-mutated NSCLC and a predictor of worse response to EGFR-TKI therapy, including osimertinib treatment (Canale et al., 2020). TP53 mutations are independently associated with worse PFS in both first-, second-, and third-generation *EGFR*-TKIs (Hazard ratio, HR: 2.02; 95% CI: 1.04–3.93, p= 0.038 and HR: 2.23, 95% CI 1.16–4.29, p= 0.017, respectively) (Kim et al., 2019b). The negative predictive effect of TP53 mutations might be related to tumor-suppressive function loss, genomic instability, and

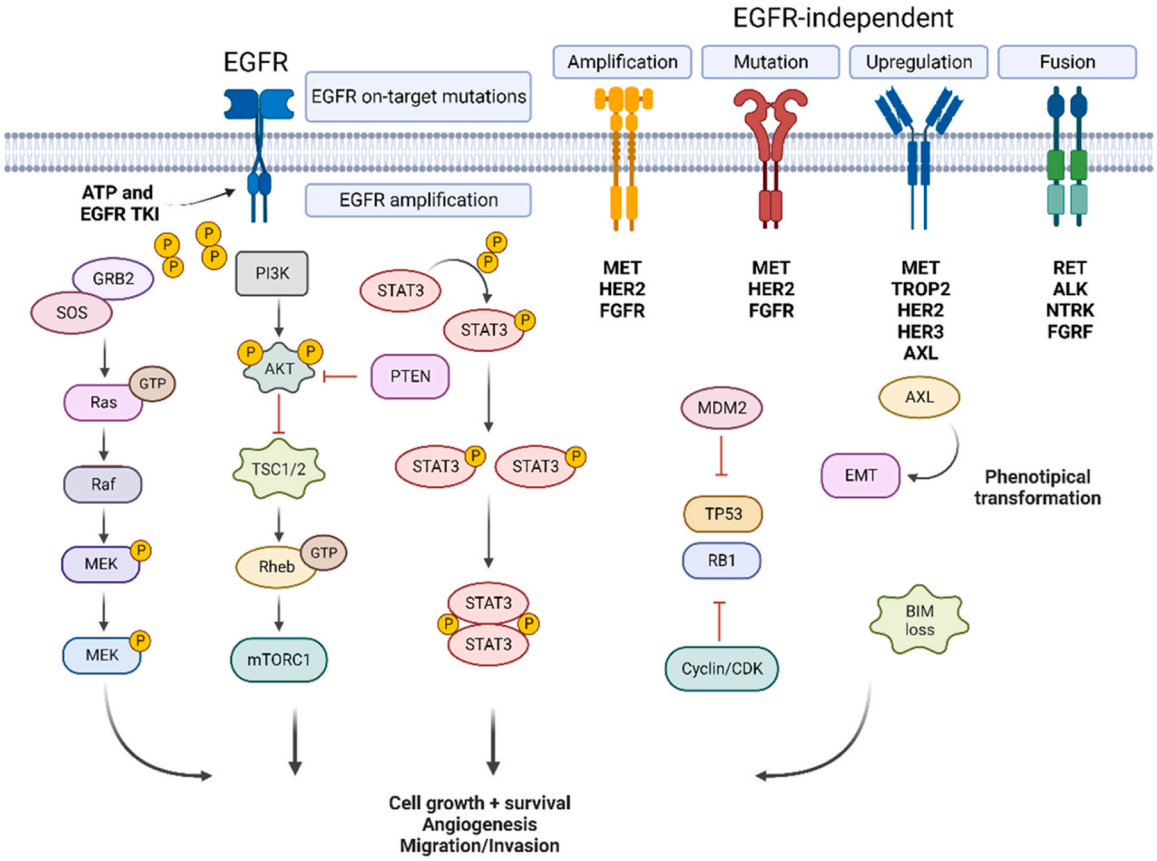

Fig. 1. molecular pathways driving Osimertinib resistance.

cancer cell's impairment in transcriptome and phenotype regulation (Oren and Rotter, 2010). According to the functional effects on the p53 protein, TP53 mutations were divided into disruptive mutations and non-disruptive mutations with disruptive mutations leading to complete or almost complete loss of p53 protein activity (Poeta et al., 2007). The two groups could have differential role in *EGFR* mutated patients and nondisruptive mutations demonstrated to be associated with worse outcome among patients treated with *EGFR* first and second generation TKIs (Molina-Vila et al., 2014).

3.2.2. *KRAS mutations*

Although generally considered mutually exclusive, a concomitant genetic alteration in *KRAS* gene is found in about 6–35% of *EGFR*-positive patients by using NGS and the most frequent alterations detected are G12C, G12V and G12D mutation (Rachiglio et al., 2019; Gristina et al., 2021). Reports on the efficacy of EGFR-TKIs in these double-positive patients have inconsistent results. In a large database study, metastatic NSCLC patients with *EGFR/PIK3CA* and *EGFR/KRAS* co-mutations experienced worse PFS with TKIs compared to patients who harbour only *EGFR* mutation in a panel of 50 cancer-related genes (Guibert et al., 2017), while potential predictive impact was not confirmed in a retrospective analysis in Asiatic population (Zhuang et al., 2019).

In parallel, a smaller study on 14 patients with *EGFR/KRAS* co-mutations suggested potential impact of dominant variant allelic frequency (VAF) on outcome. Eight out of 14 patients had dominant VAF of *EGFR* mutations relative to *KRAS* mutations and their outcome following TKIs was significantly improved when compared with patients with dominant VAF of *KRAS*: median 11.09 *versus* 2.42 months; $p = 0.0081$ and ORR 57.1 *versus* 16.7% (Rachiglio et al., 2019). On the other side, when using liquid biopsy, we recently demonstrated that even low-frequency KRAS co-mutations detected by droplet digital polymerase chain reaction (ddPCR) analysis could reduce the efficacy of targeted *EGFR* therapy (Nardo et al., 2021).

3.2.3. *Other known concomitant alterations*

PIK3CA mutations frequently coexist with other oncogenic driver mutations, especially involving *EGFR* and *KRAS* (Wang et al., 2014). Activating *PIK3CA* mutations have been found in approximately 3.5% of *EGFR*-mutated patients and have been associated with poor survival for NSCLC patients treated with EGFR-TKIs (Guo et al., 2020). However, *PIK3CA* alterations are highly heterogeneous and the available studies have limited sample sizes and inconsistent results, so additional evidences on *PIK3CA* mutations in different domains and their impact on EGFR-TKI efficacy are warranted.

Other less common concurrent genetic alterations involve *PTEN* (deletion 3%, mutation 3.7%). Both deletion and low protein expression result to be independent predictors of worse PFS for patients treated with EGFR-TKIs (Wang et al., 2019a). In particular, Wang *et al.* showed that among 169 *EGFR*-positive NSCLC patients treated with EGFR-TKIs, those with concurrent *PTEN* deletion had a shorter PFS and OS (HR for PFS, 3.64; 95% CI, 1.47–9.00; HR for OS, 2.86; 95% CI, 1.04–7.89).

Among other co-alterations involving downstream proliferation pathways, the most relevant one probably concerns *RB1*, a gene encoding for a negative regulator of cell cycle. *RB1* alterations in *EGFR*-mutant lung cancers almost always occur concurrently with *TP53* alterations. *RB1* and *TP53* biallelic loss in *EGFR*-mutant lung cancers might define a subset of patients at risk for histologic transformation at the time of progression (Lee et al., 2017a).

Other co-alterations of cell cycle genes, such as *CCND1/2*, *CCNE1*, *CDK4/6* were significantly associated with intrinsic resistance to Osimertinib (Blakely et al., 2017b). Preclinical data suggest the rationale for using a CDK4/6 inhibitor, abemaciclib, to treat *EGFR*-mutated NSCLC patients progressed to osimertinib either as single treatment or combined with osimertinib (La Monica et al., 2020).

Furthermore, a B-cell lymphoma-2 (BCL-2)-like 11 (BIM) deletion polymorphism causes reduced expression of proapoptotic BH3-only BIM protein, thus precluding TKI-induced apoptosis of *EGFR*-mutated cells (Costa et al., 2007). Lv *et al.* conducted a systematic review and meta-analysis concluding that the presence of *BIM* deletion polymorphism was significantly associated with shorter PFS, lower ORR and DCR and a trend towards unfavourable OS (Lv et al., 2021). Moreover, *BIM* plays a crucial role in tissue vascularization: a recent retrospective analysis evaluating the efficacy of EGFR-TKI plus bevacizumab, a vascular endothelial growth factor (VEGF) inhibitor, versus EGRK-TKI alone in aNSCLC patients harboring *EFGR* mutations and *BIM* deletion showed that the association of bevacizumab resulted in significantly better outcome (Morrison et al., 2013; Cardona et al., 2021). On the other side, data about a potential role for BIM in modulating sensitivity to EGFR inhibition have not been confirmed in a retrospective analysis on 141 *EGFR*-mutated NSCLC patients with or without BIM deletion polymorphism (Liu et al., 2020). Also mesenchymal-epithelial transition factor (*MET*) amplification -the most common acquired resistance mechanism to first-line osimertinib- could represent a mechanism of intrinsic resistance: concomitant *MET* amplification significantly reduces PFS in patients treated with EGFR-TKIs, thus suggesting the potential to define customized combinatory treatment strategy (Ortiz-Cuaran et al., 2016; Yu et al., 2018). Its amplification results in the activation of numerous signaling pathways, physiologically activated by *EGFR* (MAPK, STAT, PI3K–AKT). Therefore, despite the pharmacological inhibition of *EGFR*, collateral pathways of carcinogenic activity do not cease to operate (Martinez-Marti et al., 2017).

In conclusion, primary resistance molecular mechanisms demonstrated to be heterogeneous and molecular characterization of *EGFR*-mutated patients are warranted both in the frame of translational studies and biomarker analyses of interventional trials, in order to lay the basis for future customized approach to *EGFR* mutant disease.

4. Acquired resistance following first-line osimertinib

Data concerning molecular mechanisms of acquired resistance to first-line treatment are limited when compared to analyses concerning patients progressing to second-line osimertinib (Zhao et al., 2019; Yu et al., 2015; Ahn et al., 2016), while substantial differences in resistance mechanisms between the two settings have been observed (Schmid et al., 2020).

Acquired resistance mechanisms are usually classified according to biological criteria:

1) On-target resistance is caused by a mutation in *EGFR* that alters the TKI-protein linkage or allows EGFR to work despite TKI inhibition
2) Off-target resistance is led by the activation of an alternative molecular pathway able to fuel cancer cell survival and proliferation despite EGFR inhibition
3) Histological transformation or linear plasticity.

The first evidence of Osimertinib activity in first line stems from two cohorts of treatment-naïve patients included in AURA study (Ramalingam et al., 2018). In this context, 38 plasma samples were collected at the time of progression and analysed with NGS in order to explore potential resistance mechanisms. However, the most valuable information about resistance to first-line osimertinib derives from translational analyses performed in patients enrolled in FLAURA study: 109 patients had paired plasma samples analyzed by NGS, before treatment and at progression. There was no evidence of acquired T790M mutation, while the most common resistance mechanism was *MET* amplification (16% of patients), followed by *EGFR* C797S mutation (6%). Compared to 1st-gen TKI resistance, off-target mechanisms seemed to be more frequent, possibly in relationship with the increased capacity to inhibit the target. Notably, in 65% of patients, resistance mechanisms could not be identified in plasma analysis (Chmielecki et al., 2023).

Moreover, several real-world data about tissue molecular

characterization at the time of progression are now available (Table 1). Overall, emerging insights on the first-line osimertinib resistance mechanisms confirm the increased frequency of off-target alterations and *MET* amplification as the most frequent (Cardona et al., 2022; Akli et al., 2022; Ahn et al., 2022a; Mazieres et al., 2022). In particular, a recent study including 95 biopsies performed in *EGFR*-mutated patients at the time of osimertinib progression identified the following off-target alterations: *MET* amplification (9 cases), *HER2* amplification (3 cases), *MYC*, *MDM2/CDK4*, *CCND1*, *PIK3CA* and *KRAS* G12A mutations, *RET* and *BRAF* fusions, *RB1* loss of function; on-target alterations were less frequent and comprehended: *EGFR* amplification (7 cases), C797S and G724S. Moreover, there were 14 cases of histological transformation (Choudhury et al., 2023; Schoenfeld et al., 2020).

4.1. On target (EGFR-dependent) resistance mechanisms

In patients treated with first-line osimertinib, on target acquired resistance mechanisms account for about 10% of cases (Schmid et al., 2020). In the FLAURA series, the most frequent acquired resistance *EGFR* alteration is C797S, followed by L718Q, G796S and S768I; in another retrospective study also G724S and EGFR amplification were described (Chmielecki et al., 2023; Schoenfeld et al., 2020).

Osimertinib selectively blocks EGFR protein by irreversibly binding to its C797 residue and, as expected, the most common EGFR-dependent mechanisms of resistance are based on mutations involving this spot and the protein modification hinders the covalent bond of osimertinib to the mutant *EGFR* (Yu et al., 2007; Passaro et al., 2021). In particular, C797S accounts for about 7% of cases of acquired resistance after first-line osimertinib, making it the second most frequent acquired resistance mechanism, behind *MET* amplification (Leonetti et al., 2019). Interestigly, its rate of detection is similar both in tissue and in plasma (15–22%) (Papadimitrakopoulou et al., 2020; Yang et al., 2018; Oxnard et al., 2018).

Mutations involving G724, located in exon 18, are less common and mutually exclusive with C797S. They appear in about 1–4% of first-line Osimertinib resistance cases (Choudhury et al., 2023; Schoenfeld et al., 2020). G724 is located in the glycine rich N-lobe of the kinase domain of EGFR; rigidifies the loop and prevents osimertinib binding with F723 amino-acid (Lazzari et al., 2019; Tumbrink et al., 2021). *In vivo and in vitro* analyses confirm that this mutation limits the activity of Osimertinib (Fassunke et al., 2018).

Although less studied in clinical setting, G796 residue, adjacent to C797, can be involved in acquired resistance mechanisms since its modifications sterically interfere with Osimertinib aromatic ring and reduce binding affinity (Tumbrink et al., 2021). There are various types of G796 mutations (G796R, G796S, G796D) and main evidence is available about the role of G796R (Zhang et al., 2018; Ou et al., 2017), that in preclinical models affects the osimertinib/EGFR complex binding (Yang et al., 2018).

Other rare *EGFR* mutations, including genetic alterations at positions L718, L792, G724, and G796, were also reported as mechanisms leading to tumor cells' resistance to front-line osimertinib (Chmielecki et al., 2023; Dong et al., 2021). In particular, the group of mutations involving L792 (L792F, L792Y, and L792H) add a benzene or imidazole ring to the side chain L792 residue, affecting the correct orientation of Osimertinib and preventing the linkage with the active site (Chen et al., 2017). Functional studies *in vitro* demonstrated that the mutations activate the pathways MAPK/ERK and JAK-STAT thus conferring resistance to Osimertinib at all doses (Fairclough et al., 2019). Mutations involving L718 position were already known as uncommon *EGFR* mutation in treatment-naïve patients and subsequently identified as possible first-line osimertinib resistance mechanism, in about 2% of cases (Chmielecki et al., 2023; Bersanelli et al., 2016; Ramalingam et al., 2018 Oct). The presence of the mutation reduces the covalent bond of osimertinib and therefore the Therapeutic Index (Yang et al., 2018; Shen et al., 2021; Yang et al., 2020).

Table 1
Main molecular characterization studies in NSCLC patients progressing to first-line osimertinib.

Study	Number of patients	Material	On-target resistance mechanism	Off-target resistance mechanism
Ramalingam S. et al. 2018 (Ramalingam et al., 2018)	19	Plasma ctDNA	C797S: 2 G719S: 1EGFR amp: 1	MEK1 G128V: 1, HER2: 2 (exon 20 ins: 1, E1247K: 1), JAK2 V617F: 1, PIK3CA E545K: 1, MET amp: 1, KRAS: 2 (G12D: 1, ampl: 1), TP53: 9, CTNNB1: 2 (G34V: 1, S37F: 1), PTEN Q171*: 1, RB1: 4 (R255*: 1, pLys427fs: 1, c407fs: 1, L158*: 1)
Chmielecki J. et al. 2023 (Chmielecki et al., 2023)	109	Plasma ctDNA	C797X: 7, L718Q: 2, S768I: 1, G796S: 1, Exon 20 ins: 1	MET amp: 17, HER2 amp: 2, KRAS mut: 3 (A146T: 1, G12C: 1, G12D: 1), BRAF V600E mut: 3, PIK3CA mut: 6 (E545K: 4, E453K: 1, H1047R: 1), ALK fusion: 1, CCND1 amp: 4, CCND2 amp: 1, CCND3 amp: 1, CCNE1 amp: 3, CDK4 amp: 2, CDK6 amp: 5
Schoenfeld et al. 2020 (Schoenfeld et al., 2020)	27	Tissue samples	EGFR amp: 9, G724S: 1.	TP53: 20, PIK3CA: 3, PTEN: 3, KRAS: 2 (G12A: 1, amp: 1), MET: 3 (H1094Y: 1, ampl.: 2), BRAF-TRIM24 fusion: 1, RET-RUFY2 fusion: 1, CDKN2A deletion: 4, CDKN2B deletion: 4
Cardona A. et al. 2022 (Cardona et al., 2022)	94	Tissue samples and plasma ctDNA	EGFR amplification: 8,4%, T790M loss: 15,4%, acquired EGFR mutations: 11,6%	MET mutations: 2,7%, MET amp. 0,6%, HER2 amp: 6,2%, KRAS mut: 4,8%, BRAF mut 3,4%, BRAF fusions: 1,4%, PIK3CA mut. 3,4%, JAK mut: 4,8%, TP53 mut: 29,2%, CTNNB1 mut: 1,4%, RET fusion: 2,7%, PTEN loss: 0,6%

(*continued on next page*)

Table 1 (*continued*)

Study	Number of patients	Material	On-target resistance mechanism	Off-target resistance mechanism
Akli A. et al. 2022 (Akli et al., 2022)	27	Tissue samples and plasma ctDNA	C797S: 3 (11%), L730V: 1 (4%), EGFR amp. 1 (4%), L718V: 1 (4%), L747P: 1 (4%), L861G: 1 (4%), G719A: 1 (4%)	MET amp: 4 (15%), HER2 amp: 1 (4%), TP53 mut: 5 (18%), KRAS mut: 1 (4%), STK11 mut: 1 (4%), CCDC6-RET fusion: 1 (4%), SCLC transf: 1 (4%)
Choudhury N. et al. 2023 (Choudhury et al., 2023)	95	Tissue samples	EGFR amp.: 7, C797S: 3, G724S: 2	MET amp.: 9, HER2 amp: 3, PIK3CA mut: 3, KRAS G12A: 1, RET/RUFY2 fusion: 2, BRAF/TRIM24 fusion: 1, RB1 loss: 2, MDM/CDK4 amp: 1, CCND1 amp: 1, MYC amp: 1, SCLC transf: 7, SQCC transf: 4, LCNEC transf: 2

Abbreviation: ctDNA, circulating tumor DNA.

4.2. *Off target (EGFR-independent) resistance mechanisms*

Off target mechanisms imply the activation of alternative pathways to overcome *EGFR* inhibition. Other transmembrane proteins might activate parallel downstream pathways, in order to by-pass EGFR inhibition, while downstream intracellular GTP-ase proteins might directly activate signalling downstream *EGFR* (Leonetti et al., 2019).

4.2.1. *MET amplification*

The most frequently altered pathway involves MET. MET is a transmembrane protein activating MAPK, STAT and PI3K/AKT proteins, thus able to bypass EGFR blockade (Le et al., 2018; Ortiz-Cuaran et al., 2016; Coleman et al., 2021).

MET amplification is the most frequent and well described off-target resistance mechanism, accounting for at least 7–15% of acquired resistance to first line osimertinib (Chmielecki et al., 2023) (Fig. 2). First information concerning the role of MET in acquired resistance to osimertinib stems from translational analyses in patients included in clinical trials, but the acquisition of increased data led to the knowledge of the high heterogeneity of *MET* alterations and the incomplete consistence between plasma and tissue analysis results (Oxnard et al., 2018). In addition, MET alterations can co-occur with other acquired alterations such as *CDK6* and *BRAF* amplification and their impact on outcome is not yet understood (Le et al., 2018; Oxnard et al., 2018).

Initially, similar frequency of *MET* amplification had been depicted after failure of first-line or subsequent-line osimertinib (Ramalingam et al., 2018; Papadimitrakopoulou et al., 2020; Yang et al., 2018; Ramalingam et al., 2018 Oct; Coleman et al., 2021; Reita et al., 2021), but, as already mentioned, much higher rate has been described when analysing patients with systematic tissue rebiopsies as performed in interventional clinical trials, such as SAVANNAH and INSIGHT2 studies, where *MET* amplification has been detected in 30–60% of patients (Mazieres et al., 2022; Ahn et al., 2022b).

On the other side, different methods are used to detect *MET* amplification thus influencing the incidence of its identification. Fluorescent in situ hybridization (FISH), performed in tissue, is the gold standard for detection of *MET* amplification, being able to distinguish between amplification and polysomy by using the mean MET per cell and CEP7 ratio (Kawakami et al., 2014); most studies use a cut-off MET/CEP7 ratio of $\geq$ 2, but standardized cut-off to define *MET* amplification is not available yet (Passaro et al., 2021; Coleman et al., 2021). IHC seems not to be a reliable predictor of *MET* amplifications. In a small cohort study of 181 patients, the use of IHC to diagnose *MET* alterations was compared to FISH and NGS: a total of 3 out of 181 patients were diagnosed with *MET* amplification by FISH or NGS, and two out of three had negative IHC for MET amplification (Guo et al., 2019). Finally, NGS is increasing its role both in tissue and in plasma even though standardized cut-offs are not available yet: generally, a cutoff with GCN $\geq$10 is preferred as it corresponds to a high level of *MET* amplification. The higher cut off has been shown to have greater concordance with FISH (Schubart et al., 2021). On the other side, *MET* amplification detection might be suboptimal in plasma due to technical limits (Coleman et al., 2021).

MET exon 14 skipping mutation occurs in 3–4% of treatment-naïve lung cancers (Awad et al., 2016), but it is very rarely associated with *EGFR* mutation, even though its potential role in acquired resistance has been demonstrated in preclinical models (Suzawa et al., 2019; Li et al., 2017).

4.2.2. *Alterations of HER2 and other transmembrane receptors*

Human epidermal growth factor receptor 2 (HER2) is a member of transmembrane tyrosine kinase ErbB family implicated in signal transduction through the PI3K intracellular pathway. Its hyperactivation could result in the bypass of EGFR inhibition. *HER2* amplifications were found in 5% of patients enrolled in AURA trial and in 2% of FLAURA trial in patients treated with first line osimertinib (Ramalingam et al.,

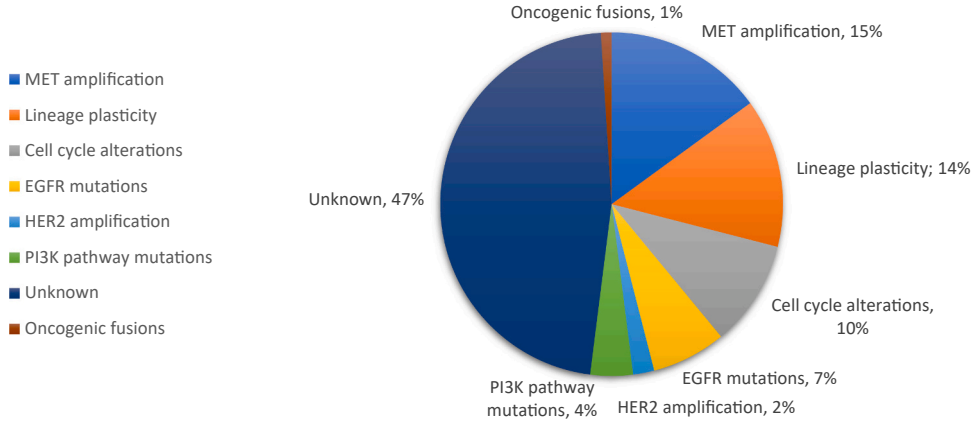

Fig. 2. distribution of the resistance mechanisms after 1st line Osimertinib.

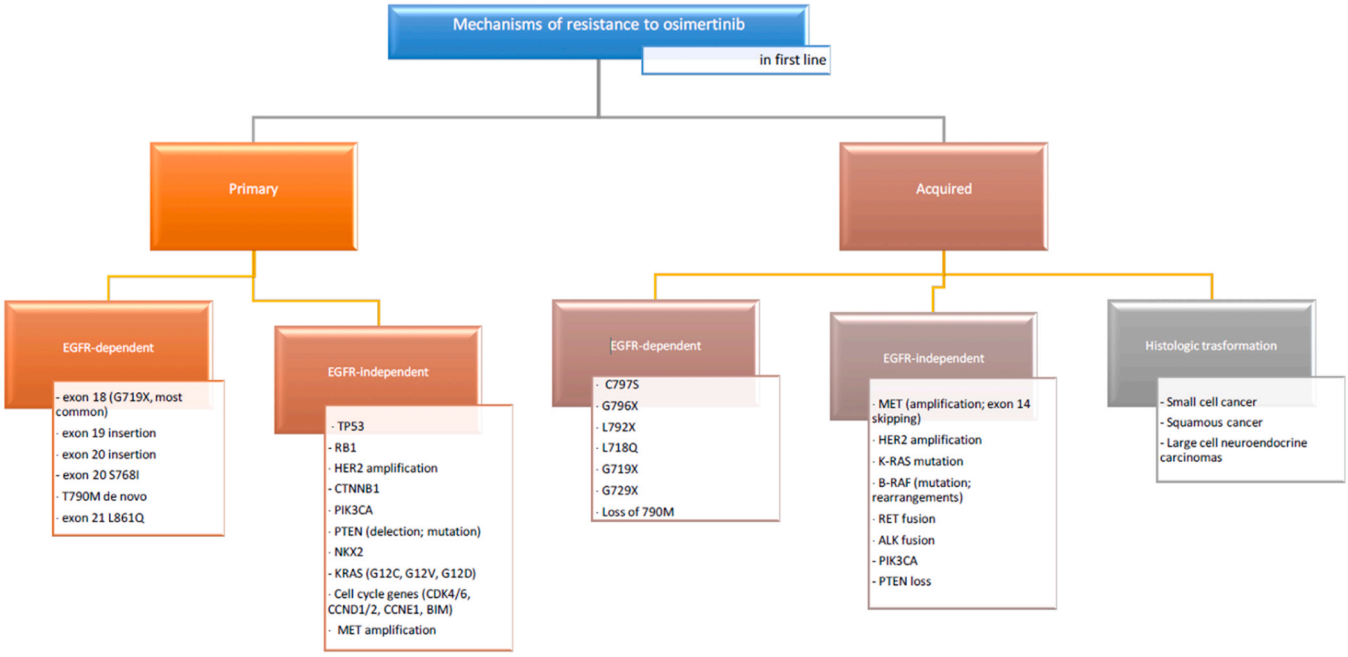

Fig. 3. overview of possible resistance mechanisms after 1st line Osimertinib.

2018; Chmielecki et al., 2023). Other possible *HER2* mediated resistance mechanisms comprehend exon 20 insertion and exon 16 skipping-mutation (Chmielecki et al., 2023; Hsu et al., 2020). The main methods used to assess *HER2* amplification are NGS (both in plasma samples and tissue) and FISH, with good concordance (Le et al., 2018; Michels et al., 2019; Hondelink et al., 2021).

As part of transmembrane tyrosine kinase family, *RET* and *ALK* fusions, although rarer than *MET* and *HER2* amplifications, could represent other potentially targetable resistance mechanisms. Heterogeneous *RET* and *ALK* fusions are rarely found at the time of acquired resistance (Le et al., 2018; Piotrowska et al., 2018). Gene fusions represents a technical challenge for NGS detection (Belli et al., 2021). Both DNA and RNA NGS can be performed, either with some limitations: DNA sequencing is affected by lower sensitivity while RNA approach can be limited by RNA quality and quantity (Bruno and Fontanini, 2020).

G protein-coupled superfamily receptors (GPCR) are a large group of seven-pass transmembrane receptors able to detect extracellular ligands and promote intracellular signaling through the associated G proteins (Rosenbaum et al., 2009).Increasing research efforts are focusing on the interactions between GPCRs and tyrosine kinase receptors (RTK). In this context, GPCRs can have a role in stimulating EGFR and participating in cancer evolution (Moody et al., 2023; Zhang et al., 2022). *In vitro* analysis have explored the role of GPCR in eliciting gefitinib resistance (Kuzumaki et al., 2012).From this point of view, GPCR represent a future promising potential target for precision medicine in the context of EGFR mutated NSCLC disease (Zhang et al., 2022).

4.2.3. *KRAS and BRAF alterations*

Other different off-target resistance mechanisms occur when gain- or loss-of-function genetic alterations hit critical genes involved in the *EGFR* downstream signaling pathway. *KRAS* is a downstream component of the *EGFR* network and promotes pro-survival and growth signals. If mutated, it becomes constitutively activated independently from *EGFR* status (Ortiz-Cuaran et al., 2016; Normanno et al., 2009). Various studies reported different acquired *KRAS* mutations after progression to first-line osimertinib (mainly G12A, G12C, G12D) (Ramalingam et al., 2018; Chmielecki et al., 2023; Choudhury et al., 2023; Schoenfeld et al., 2020). They are found in about 3–4% of cases both in plasma and tissue by using NGS panels and they are frequently associated with *PI3KCA* and *BRAF* mutations (Le et al., 2018; Yang et al., 2018; Oxnard et al., 2018; Michels et al., 2019; Zhao et al., 2020).

Mutations and rearrangements in *BRAF* are other possible acquired resistance mechanisms (Aboubakar Nana and Ocak, 2021). The most frequent mechanism is V600E mutation, which accounts for about 3% of them (Chmielecki et al., 2023). Rearrangements causing loss of N-terminal auto-inhibitory domain account for about 2% of osimertinib resistance mechanisms (Aboubakar Nana and Ocak, 2021).

4.2.4. *Alterations of PI3K pathway*

Downstream in the essential oncogenic pathway, PIK3CA constitutes the catalytic subunit of *PI3K* and activating alterations were found in 4–10% of patients after progression following second-line osimertinib (Papadimitrakopoulou et al., 2020) and 3–7% following first line (Ramalingam et al., 2018; Chmielecki et al., 2023; Cardona et al., 2022; Choudhury et al., 2023). E545K is the most common gain of function mutation (Leontiadou et al., 2018): already found in patients progressing to later-line osimertinib, it was also detected in patients treated with first-line osimertinib among other rarer variants (E453K, H1047R) (Le et al., 2018; Chmielecki et al., 2023; Zhao et al., 2020).

A different mechanism leading to PI3K/AKT up-regulation is *PTEN* loss. *PTEN* is a negative regulator of PI3K/AKT by dephosphorylation of PIP-3 to PIP-2 and it has been described as a possibly involved in acquired resistance development (Ramalingam et al., 2018; Cardona et al., 2022; Schoenfeld et al., 2020; Kim et al., 2015; Bordi et al., 2019; Yang et al., 2021).

4.3. *Histological transformation*

The histological transformation of *EGFR*-mutated aNSCLC concerns about 3–15% of all *EGFR*-mutated patients and it seems to be more common after front-line osimertinib (as shown in Fig. 2) (Schoenfeld et al., 2020; Niederst et al., 2015; Sequist et al., 2011). Commonly, *EGFR*-mutant small cell lung cancers (SCLCs) continue to harbor the original *EGFR*-mutation indicating the direct evolution from the original NSCLC (Marcoux et al., 2019). Tissue biopsy at the time of progression remains the standard way to identify this transformation; however, in several cases, *RB1* and *TP53* liquid biopsy mutations at the time of progression could suggest neuroendocrine evolution (Vendrell et al.,

2020; Pizzutilo et al., 2021; Bonanno et al., 2021). However, *RB1* loss, detected in 9.5%−12.5% of *EGFR*-mutant NSCLCs, is necessary but not sufficient for SCLC development and additional pathways, such as epigenetic changes, are supposed to play an essential role (Shaurova et al., 2020). A possible explanation could be found in the selective pressure driven by TKI inhibition, leading to the selection of pre-existing resistant clones, even though additional mechanisms are probably required and remain unknown (Niederst et al., 2015; Lee et al., 2017b).

From a clinical point of view, patients whose tumours undergo histological transformation are characterized by poor prognosis; treatments are currently decided on the basis of the knowledge concerning de-novo SCLC patients and duration of the derived clinical benefit is rather limited (Marcoux et al., 2019; Roca et al., 2017). Currently, the main challenge in this context is the early identification of patients at risk, in order to personalize treatment and potentially improve their outcome. We are performing multicentric retrospective analysis among patients developing histological transformation and preliminary data suggest a potential role for molecular characterization in plasma in order to anticipate the risk of histological transformation before tissue rebiopsy (Bonanno et al., 2021).

In addition, several reports have described lineage plasticity to other rare histological phenotypes, such as squamous cell carcinomas (SCCs) and large cell neuroendocrine carcinomas (LCNECs) (Izumi et al., 2018; Moriya et al., 2017). *EGFR*-mutated adenocarcinoma to SCC lineage transformation is a rare type of acquired resistance to EGFR TKIs and the incidence of SCC transformation during front-line osimertinib results to be higher than with first-/second-generation EGFR TKIs or later-line Osimertinib (Park et al., 2019). The etiopathogenesis of histological transformation to SCC remains poorly defined and it could reveal a previous coexistence of two components (both adenocarcinoma and SCC) and a switch to the predominant histology during targeted therapy (Pathak and Villaflor, 2021). No consistent molecular signature has been identified in SCC transformation; but in genetically engineered mouse models, live kinase B1 (LKB1) inactivation has shown to promote gradual transition from adenocarcinoma to SCC, leading to drug resistance through metabolic alterations (Han et al., 2014).

5. Therapeutic strategies in Osimertinib-resistant patients – primary resistance

As already mentioned, a quote of *EGFR*-mutated patients (about 20–30%) doesn't benefit from first-line osimertinib even in the presence of *EGFR* sensitizing mutations (Santoni-Rugiu et al., 2019). In addition, approximately 20% of patients with *EGFR*-mutated NSCLC can't receive a second-line therapy, which has prompted the researchers to examine the possibility of preventing the emergence of resistance to third-generation EGFR TKI by combining EGFR inhibition with other therapeutic strategies, such as chemotherapy, MET inhibitors and anti-angiogenesis agents. Ongoing clinical trials in this setting are listed in Table 2.

5.1. EGFR TKI plus chemotherapy

Preclinical data suggest that EGFR-TKIs combined with chemotherapy may act synergistically to delay the development of acquired resistance (La Monica et al., 2019). The phase III study, NEJ009, evaluated gefitinib alone *versus* gefitinib in combination with carboplatin and pemetrexed in naïve *EGFR*-mutated NSCLC patients and demonstrated improved PFS and OS (HR, 0.695; 95% CI: 0.52–0.93) in patients in the combination arm while toxicity profile was overall acceptable (Nakamura et al., 2018; Hosomi et al., 2020).

Several prospective trials are ongoing with the aim of evaluating the efficacy and safety of the combination of third-generation TKI with chemotherapy in previously untreated patients: the ongoing phase II

Table 2
Ongoing clinical trials in front-line setting for EGFR mutated advanced non-small cell lung cancer.

ClinicalTrial.gov Identifier (Study Title)	Phase	Drug	Mechanism of action	Status	Primary and secondary endpoint
NCT04772235 (TOTEM)	I	Repotrectinib + Osimertinib	ALK, ROS1 and TRK inhibitor EGFR inhibitor	Recruiting	AEs and DLT ORR and DCR
NCT04811001 (CAPLAND)	II	Arm A: Osimertinib -> Dacomitinib Arm B: Dacomitinib -> Osimertinib	EGFR inhibitor	Recruiting	OS PFS
NCT04769388 (FLAME)	II	Arm A: Osimertinib + Carboplatin + PemetrexedArm B: Osimertinib	NA	Recruiting	PFSOS ORR and DCR
NCT05493501	III	Arm A: AumolertinibArm B: Aumolertinib + Chemotherapy Arm C: Osimertinib	EGFR inhibitor	Recruiting	PFSOS, ORR and DCR
NCT04743505	I-II	APL-101 + Osimertinib	c-MET inhibitor	Recruiting	AEs and PFS
NCT04141644	I	Ipilimumab + Osimertinib	Anti-CTLA4 mAb	Recruiting	AEsORR and PFS
NCT05382728 (FLETEO)	III	Arm A: TY-9591Arm B: Osimertinib	EGFR inhibitor	Not yet recruiting	PFSiORR and iPFS
NCT05401110	I	Carotuximab + Osimertinib	monoclonal antibody directed against endoglin(CD105)	Not yet recruiting	AEsORR and DCR
NCT03909334	II	Arm A: Ramucirumab + OsimertinibArm B: Osimertinib	Anti- VEGFR mAb	Recruiting	PFSORR, DCR and OS
NCT04780568	I	Tegavivint + Osimertinib	inhibitors of Wnt/β-catenin signaling	Recruiting	AEsORR, PFS, OS and DOR
NCT05163249 (FLOWERS)	II	Arm A: Savolitinib + osimertinib Arm B: Osimertinib	c-MET inhibitor	Not yet recruiting	ORRPFS, DOR and DCR
NCT04988607	II	Arm A: Bevacizumab + OsimertinibArm B: Osimertinib	Anti-VEGF mAb	Not yet recruiting	PFSOS, TTF and ORR
NCT04770688(AUTOMAN)	I-II	Anlotinib + Osimertinib	EGFR TKIs	Recruiting	ORRDLT, DCR and DOR
NCT03516214 (EATON)	I	Nazartinib + Trametinib	EGFR + MET inhibitor	Recruiting	DLTAEs and ORR
NCT03392246	II	Osimertinib + Selumetinib	EGFR TKIs	Not recruiting	BORPFS and OS
NCT02954523	I-II	Osimertinib + Dasatinib	ABL1/SRC TKI	Not recruiting	Safety
NCT05507606	II	Osimertinib + Bevacizumab + CT	EGFR inhibitorAnti-VEGF mAb	Recruiting	ORR OSand PFS
NCT03567642	I	Osimertinib + CT platinum-based	EGFR inhibitor	Recruiting	SafetyToxicity profile (MTD)

Abbreviations: AE, adverse event; Anti-CTLA4 mAb, anti- cytotoxic T lymphocyte antigen-4 monoclonal antibody; Anti-VEGF mAb, anti-vascular vascular endothelial growth factor monoclonal antibody; BOR, best overall response; CT, chemotherapy; DCR, disease control rate; DLT, dose limiting toxicities; DOR, duration of response; EGFR, Epidermal growth factor receptor; iORR, immune objective response rate; iPFS, immune progression-free survival; MTD, maximal tolerated dose; NA, not applicable; PFS, progression-free survival; ORR, overall response rate; OS, overall survival; TKI, tyrosine kinase inhibitor; TTF, time to treatment failure.

OPAL trial (Asahina et al., 2021) and the phase III FLAURA2 study (NCT04035486) explore the association of osimertinib and chemotherapy (Planchard et al., 2023). In the FLAURA2 safety run-in period, 30 patients received osimertinib and pemetrexed with carboplatin or cisplatin, and the reported results showed that the safety profile of these combinations were as expected and manageable (Planchard et al., 2021). Recently published results demonstrated that Investigator-assessed PFS was significantly longer in the osimertinib-chemotherapy arm than in the osimertinib arm (HR, 0.62; 95% CI, 0.49–0.79; p<0.001). At 24 months, 57% (95% CI, 50–63) of the patients in the combination arm and 41% (95% CI, 35–47) of those in the osimertinib arm were alive and progression-free (Planchard et al., 2023).

Interestingly, the phase III TOP study (NCT04695925) compares efficacy and safety of osimertinib alone *versus* osimertinib plus pemetrexed and carboplatin in treatment-naïve patients with advanced NSCLC and concurrent EGFR and TP53 mutations. Additionally, another study has been designed to prevent SCLC transformation through the combining of platinum-etoposide chemotherapy with osimertinib in the first-line setting for EGFR-mutated NSCLC with concurrent RB1 and TP53 mutations (NCT03567642).

5.2. TKIs combinations

There is increasing interest regarding the effect of concomitant genetic alterations on the efficacy of EGFR-TKIs (CHEVALLIER et al., 2020; Canale et al., 2017), indeed some ongoing studies are designed to investigate the impact of specific co-mutations. Co-existing *MET* amplification or MET over-expression and *EGFR*-mutation at baseline has been associated with shorter PFS on EGFR-TKI monotherapy (Shi et al., 2016). In a preclinical study by Peng *et al.* dual targeted therapy with osimertinib plus savolitinib showed anti-tumor activity in the lung cancer organoids drug testing assay (Peng et al., 2022). On the basis of preclinical rationale, a phase II clinical trial is ongoing with the aim to evaluate the efficacy of osimertinib with or without savolitinib as first-line treatment in patients with de novo *MET*-amplified/over-expressed, *EGFR*-mutated NSCLC (Li et al., 2023).

In the same contest, MARIPOSA is a phase III trials comparing the targeted agents combination lazertinib and amivantamab *versus* lazertinib alone *versus* osimertinib in first-line setting in *EGFR*-mutated patients, thus testing the hypothesis that inhibiting EGFR and MET frontline could improve outcome (Cho et al., 2022). PFS is the primary endpoint of the study and one of the main challenges is tolerability, given the fact that a combination therapy, potentially more toxic than a single agent, is proposed frontline to the all-comer *EGFR*-mutated patients, for which an effective and well tolerated therapy already exists. Anyway, clinically meaningful improvements over standard care have been observed in first line, as reported at ESMO Congress 2023: at a median follow-up of 22.0 months, the combination of amivantamab and lazertinib showed a 30% reduction in the risk for disease progression or death *versus* osimertinib alone (HR, 0.70; 95% CI, 0.58–0.85; p<0.001), with mPFS of 23.7 months (95% CI, 19.1–27.7) *versus* 16.6 months (95% CI, 14.8–18.5), respectively. ORR was 86% (95% CI, 83–89) for the combination *versus* 85% (95% CI, 81–88) for osimertinib alone. Moreover, at interim OS analisys, there was a favourable trend for amivantamab plus lazertinib over osimertinib (HR, 0.80; 95% CI, 0.61–1.05; P=0.1). As regarding the safety profile, the incidence of most EGFR- and MET-inhibition related AEs was higher with amivantamab–lazertinib than with Osimertinib. Treatment-related adverse events (TRAEs) leading to discontinuations of all agents occurred in 10% of patients treated with amivantamab plus lazertinib and 3% with osimertinib. Notably, any venous thromboembolism occurred in 37% of patients in the combination arm and 9% on osimertinib arm, leading the investigators to recommend prophylactic anticoagulation in ongoing trials of amivantamab–Lazertinib (Cho et al., 2023).

5.3. EGFR TKI plus antiangiogenetic drugs

Adding VEGF inhibitors to first-generation EGFR TKIs has been shown to prolong PFS but not OS in front-line setting (Nakagawa et al., 2019; Yamamoto et al., 2021; Maemondo et al., 2020). A Phase I/II trial single arm investigating the combination of osimertinib with bevacizumab showed a 12-month PFS rate of 76% and a median PFS of 19 months (Yu et al., 2020). Another phase II study evaluating the efficacy and safety of osimertinib plus bevacizumab for previously untreated patients with NSCLC harboring *EGFR*-sensitizing mutations failed to exhibit the efficacy of the combination for improving the PFS (Kenmotsu et al., 2022). A randomized phase II trial (NCT02971501) will determine the efficacy of first-line osimertinib plus bevacizumab against osimertinib alone in previously untreated *EGFR* mutated patients with brain metastases: primary endpoint is PFS and among the secondary endpoints we highlight intracranial response rate and time to intracranial progression. Moreover, another phase II study (NCT03909334) will determine the activity of osimertinib plus ramucirumab against osimertinib alone in previously untreated *EGFR* mutated metastatic patients. Furthermore, NCT05507606 trial has been designed to evaluate the efficacy of the combination of osimertinib, bevacizumab and platinum-doublet chemotherapy in previously untreated *EGFR*-mutated NSCLC patients.

5.4. EGFR TKI + immune checkpoint inhibitor (ICI)

The combination of EGFR and immunotherapy has also been investigated. A phase II trial was conducted to evaluate the efficacy of pembrolizumab in this setting in patients whose tumors have high PD-L1 expression (Lisberg et al., 2018 Aug). The trial enrolment was interrupted due to the lack of efficacy after 11 of the 25 planned patients received immunotherapy. Some ongoing trials are evaluating safety and efficacy of the ICI plus EGFR-TKI combination strategy: for example, the phase Ib NCT04141644 aims to determine the activity of osimertinib plus ipilimumab in patients with *EGFR*-mutated NSCLC. While outcomes of these trials are immature, clinical data available in different settings overall discourage the use of ICI-containing combinations in EGFR mutated NSCLC (Attili et al., 2023; Yang et al., 2023). Moreover, the possible risk of combined toxicity poses a major challenge for the drug combinations (To et al., 2021).

6. Therapeutic strategies in Osimertinib-resistant patients – acquired resistance

Osimertinib has dramatically improved the outcome of *EGFR*-mutated patients, but inevitably all patients progress and chemotherapy is currently the standard of care in this setting (Di Noia et al., 2021).

Primarily, post-osimertinib treatment approaches depend on different clinical and radiologic patterns of disease progression: in case of oligo-progression in asymptomatic patients, continuing osimertinib beyond-progression, possibly associating locoregional treatment in the sites of oligo-progression, is considered as a valid option (Le et al., 2018). Some ongoing clinical trials, such as SARON trial, are investigating this strategy (NCT02417662), while real-world evidences already highlighted the role of local treatment in clinical practice (Lorenzi et al., 2022).

CNS progression appears in about 30% of osimertinib progressing patients (Reungwetwattana et al., 2018) and represents a clinical challenge due to the lower effectiveness of chemotherapy in CNS. If the progression is limited to CNS, sampling the progressing site represents a further difficulty and, if surgery is not possible, no histological and molecular data are available (De Mattos-Arruda et al., 2015). Surgery and stereotactic radiotherapy, when feasibile, represent the main therapeutic options (Suh et al., 2020), but they do not grant protective effect on further CNS progression. Overall, CNS activity is one of the main aims when testing the efficacy of a new therapeutic strategies in this context.

When progression is widespread and symptomatic, a switch of systemic therapy is required. The development of new treatment strategies includes two different approaches. The first approach is based on the concept of personalized precision medicine: each patient progressing to first-line osimertinib is tested in order to detect the involved mechanism of acquired resistance and new treatment options are tested in molecularly selected patients. The second approach aims to study targeted treatment options in molecularly unselected patients, mainly targeting the most frequent resistance mechanisms.

Anyway, in several clinical trials osimertinib is maintained after first-line treatment in combination with an additional treatment. This strategy is based on a biological rationale: osimertinib continues to exert his inhibitor effect in the pool of cells that are still sensible, and slows down the progression of disease (Chaft et al., 2011). As known, EGFR pathway blockade is still crucial even if the tumoral cells have developed alternative pathways of resistance, and stopping osimertinib could lead to a faster disease progression (Chmielecki et al., 2011). In particular, osimertinib continuous effect on CNS even in the presence of extracranial progression, might be crucial when turning into chemotherapy or another different approach with limited brain activity (Reungwetwattana et al., 2018; Popat et al., 2023).

See Table 3 for novel therapeutic strategies in *EGFR* mutated aNSCLC patients post-progression to first-line osimertinib.

6.1. Molecular selected therapeutic strategies

6.1.1. Overcoming on-target resistance

Among on-target resistance mechanisms, the point mutation C797S in exon 20 is the most frequent alteration, detected in about 6% of patients progressing to first-line Osimertinib (Chmielecki et al., 2023). Brigatinib showed potential to overcome C797S and T790M *in cis* mutations in preclinical studies (Uchibori et al., 2017) and clinical activity has been reported, alone or associated with other TKIs (Zhao et al., 2018; Wang et al., 2019b; Lin et al., 2021; Wang et al., 2020). Moreover, allosteric EGFR inhibitors were designed to overcome on-target Osimertinib resistance: they showed a synergistic activity with osimertinib and their mechanism of action is independent of common ATP-binding site mutations (Koulouris et al., 2022). These novel mutant-selective inhibitors were designed to bind to a different EGFR site than existing ATP-competitive EGFR TKIs, inducing the stabilization of the inactive "C-helix out" conformation of the kinase and, thus, its inhibition. However, this allosteric pocket is not available when EGFR dimerises. This partially explains why the early allosteric inhibitors (EAI001, EAI045, and DDC4002), used in monotherapy, failed to demonstrate robust antitumor activity (Beyett et al., 2022). With the intent to prevent the allosteric pocket occlusion that is induced by the EGFR dimerization, the combination treatment of the EAI045 and the dimerization-disrupting antiEGFR monoclonal antibody, cetuximab, was recently evaluated. Indeed, the combination showed antitumor activity against L858R/T790M and L858R/T790M/C797S EGFR mutant NSCLC cellular assays (Jia et al., 2016). Therefore, emerging treatment strategies including combination of ATP-competitive and allosteric inhibitors are under evaluation and, on the other hand, new, more potent allosteric EGFR inhibitors, such as JBJ-04–125–02 and JBJ-09–063, have already demonstrated single-agent activity against L858R/T790M and L858R/T790M/C797S mutations, in preclinical models (Jia et al., 2016; To et al., 2019).

BLU-701 is an investigational, reversible, brain-penetrant, wildtype-sparing oral TKI with nanomolar potency on common activating mutations (exon 19 deletion and L858R) and C797X resistance mutations (Tavera et al., 2022). The HARMONY trial (NCT05153408) is an ongoing phase I/II study aiming to evaluate the safety and antitumor activity of BLU-701 as monotherapy or in combination with osimertinib or with platinum-based chemotherapy in patients with EGFR-mutated NSCLC progressed to a previous EGFR-TKI. In HARMONY's monotherapy arm patients must harbour an EGFR C797X resistance mutation (Spira et al., 2022).

Unfortunately, in the setting of on-target acquired resistance, we already have negative results: the combination of Osimertinib plus necitumumab in patients carrying secondary alterations in EGFR at the time of disease progression after first-line Osimertinib stopped its development since futility criteria were met (Riess et al., 2022).

6.1.2. Overcoming off-target resistance

In the presence of off-target acquired resistance mutations, combining EGFR-TKIs with a drug able to inhibit the parallel resistance pathway involved has a clear biological rationale. The first published data in this context are mainly case reports and case series of osimertinib-progressed patients, treated with targeted agents selected on the basis of the detected resistance mechanism, alone or in combination with osimertinib (Bertoli et al., 2022).

Combination strategies with EGFR-TKIs may overcome the genomic heterogeneity of drug resistance; however, the risk of overlapping toxicities and resulting dose adjustments can often hamper effective drug combinations and must be taken into account when planning clinical trials (Coleman et al., 2021).

In parallel with this first spontaneous clinical experience, clinical trials have been designed to formally demonstrate the activity and efficacy of this molecularly tailored strategies and thereafter to compare them with standard chemotherapy. *MET* amplification is the most common mechanism of osimertinib acquired resistance and therefore it was the first to be formally investigated as a therapeutic target.

The main options to target the resistance mechanism are: 1) small molecules that act as tyrosine kinase inhibitors, 2) antibodies that block the extracellular domain of the target, or 3) antibody-drug conjugates (ADCs) that, once linked to the target, are internalized and release the chemotherapic component inside the cell (Coleman et al., 2021).

Some case reports suggested that combining crizotinib (a TKI with both anti-ALK and -MET activity) with osimertinib might get over *MET*-induced resistance (Deng et al., 2018; Zhu et al., 2019) but also highlighted the issue of increased and sometimes unacceptable toxicity of combination strategies, underlying the importance of specifically designed studies and dosage modifications (Deng et al., 2018). After these first reports, *EGFR*-mutant NSCLC patients included in phase I/II studies with a MET TKI and EGFR TKI combination experienced demonstrated clinical benefit (Sequist et al., 2020; Yu et al., 2021). The most studied drugs in this context are savolitinib, tepotinib and capmatinib, being competitive tyrosine kinase inhibitors specifically targeting MET and inhibiting downstream signalling through the ERK and AKT pathways (Santarpia et al., 2021).

TATTON study investigated various possible combinations of treatments with osimertinib after an EGFR-TKI progression, including two arms (B and D) with savolitinib in patients with *MET* amplification confirmed at the time of disease progression. Allowed testing modalities included local tissue FISH (MET gene copy number $\geq$5 or MET–CEP7 ratio $\geq$2 was required), local tissue immunohistochemistry (MET +3 expression in $\geq$50% of tumour cells was required), or NGS ($\geq$20% tumour cells, coverage of $\geq$200 $\times$ sequencing depth and $\geq$5 copies of MET over tumour ploidy were required). In arm B, 138 patients received osimertinib plus savolitinib 600 mg (n=130) or 300 mg (n=8). In arm D, 42 patients received osimertinib plus savolitinib 300 mg. In part B, 66 (48%; 95% CI 39–56) and in part D, 23 (64%; 95% CI 46–79) patients had an objective response (Sequist et al., 2020).

These favourable results let to the development of the SAVANNAH trial, a phase II trial based on osimertinib plus savolitinib therapy post osimertinib progression in *EGFR*-mutated patients with *MET* overexpression and/or amplification. Patients were included according to molecular characterization in tissue detected either with IHC (cut-off: 3+ in $\geq$50% of tumour cell [IHC50+]) and FISH (cut-off: MET copy number $\geq$5 and/or MET:CEP7 signal ratio $\geq$2 [FISH5+]). First results, presented at IASLC 2022, showed that 62% of enrolled patients had MET amplification at the time of progression to osimertinib and results of

Table 3
Novel therapeutic strategies in EGFR mutated advanced non-small cell lung cancer patients post-progression to first-line osimertinib.

Mechanism ofresistance	*ClinicalTrial.gov Identifier (Study Title)*	*Treatment*	*References*
MET	NCT03778229 II (SAVANNAH)NCT03940703 (INSIGHT-2)NCT04816214 (GEOMETRY-E)NCT02609776 (CHRISALYS)NCT04077463 (CHRISALYS-2)NCT05261399 (SAFFRON) NCT03944772 (ORCHARD)	Savolitinib + OsimertinibTepotinib + OsimertinibCapmatinib + OsimertinibLazertinib vs Lazertinib + AmivantamabAmivantamab + Lazertinib + CTSavolitinib + Osimertinib vs Platinum-based DoubletSavolitinib + Osimertinib	*MJ Ann, IASLC, 2022Mazieres J, ESMO 2022Yi-Long Wu, JCO 2022*Park K, J Clin Oncol, 2021*Marmarelis M, IASLC 2022Shun L, IASLC 2022*De Langen J, Ann of Oncol 2022
HER2	NCT03784599 (TRAEMOS)NCT04619004 (HERTHENA-Lung01)NCT03297606 (CAPTUR)	T-DM1 + OsimertinibPatritumumab deruxtecanTrastuzumab-PertuzumabTKIs + CT + anti-VEGF	*M.Jebbink, Journal of T Oncology, 2021PA Janne, Cancer Disco, 2022 Schrock et al, Batra et al, 2021 Piotrowska, Cancer discovery, 2018*
ALK fusion		Crizotinib + OsimertinibAlectinib + Osimertinib	
RET fusion		BLU667 + Osimertinib	
BRAF	NCT02143466 (TATTON)	Trametinib + OsimertinibSelumetinib + OsimertinibDabrafenib + Trametinib + Osimertinib	I Dagogo-Jack, JTO 2019JCH Yang, Ckin Canc Research, 2022Meng P, Lung Cancer, 2020
KRAS		Selumetinib + OsimertinibTrametinib + Osimertinib	
CDK4/6		Abemaciclib	La Monica et al, Cancers 2021
EGFR C797SL718QG724XT790M	NCT04862780 (SIMPHONY)NCT03944772 (ORCHARD)NCT04029350NCT04862780 (SIMPHONY)	TKIs + Osimertinib (mutation in trans)TKI 4° gen (EAI-001–045 + Cetuximab; JBJ04–125–02)BLU-945 vs BLU-945 + OsimertinibGefitinib + OsimertinibDacomatinibAfatinibAnlotinib + OsimertinibBLU-945 vs BLU-945 + Osimertinib	Zhou Z, J Thorac Oncol, 2019Jia Y, Nature, 2016Shum E, ASCO Annual Meeting, 2022Zhou B, J Clin Pharm Ther 2022Shum E, ASCO Annual Meeting, 2022
EGFR mutation/ALKtranslocated	NCT02366143 (IMpower150)	Atezolizumab + Bevacizumab + Carboplatino + Paclitaxel	Mark A. Socinski, NEJM, 2018Nogami N, J Thorac Oncol 2022
EGFR amplification	NCT03944772 (ORCHARD)	Necitumumab + Osimertinib	
Lineage plasticity- Small cell lung cancer(SCLC)- Squamous cellcarcinomas (SCCs)		Carboplatino + EtoposideHistology based approach	Marcoux N, J Clin Oncol 2019Marcoux N, J Clin Oncol 2019
None acquiredresistance mechanismdetected	NCT03944772 (ORCHARD)NCT04765059 (COMPEL) NCT04001777NCT04676477NCT05153408 (HARMONY) NCT05017025NCT04285671NCT04517526NCT04486833NCT05498389	Durvalumab + Carboplatin + PemetrexedNecitumumab + OsimertinibPlatinum-based doublet + OsimertinibPelcitoclax + OsimertinibPatritumumab deruxtecan + OsimertinibBLU-701 vs BLU-701 + Osimertinib or + Platinum-basedDoubletAurora A Kinase Inhibitor LY3295668 + OsimertinibNecituzumab + Trastuzumab + OsimertinibPlatinum-based Doublet + Bevacizumab + DurvalumabReqorsa + OsimertinibEMB-01 + Osimertinib	

treatment with osimertinib and savolotinib are encouraging. Efficacy endpoints were analysed in the overall population defined by IHC 50% and/or FISH5+, and in subgroups including patients defined by exploratory higher cut-off levels: 3+ staining in ≥90% tumour cells (IHC90+) and MET copy number ≥10 (FISH10+). Activity was particularly promising in the latter subgroup of patients, representing 56% of the enrolled patients and 34% of the screened population and demonstrating an ORR of 49% with median DOR of 9.3 months and median PFS of 7.1 months (Ahn et al., 2022a). A phase III randomized trial is currently comparing the efficacy of savolitinib with osimertinib *versus* platinum-based chemotherapy in patients progressing to first-line Osimertinib; primary endpoint is PFS (Lu et al., 2022).

In addition to savolitinib, preliminary clinical data have been presented concerning another reversibile MET inhibitor, tepotinib. Following the results of the INSIGHT trial investigating the combination of tepotinib plus gefitinib in patients progressing to gefitinib with *MET* overexpression or amplification (ORR of 67% and mPFS 16.6 months) (Wu et al., 2020), the phase II INSIGHT 2 trial is now evaluating osimertinib plus tepotinib post osimertinib progression in *MET* amplified patients: *MET* amplification was centrally detected by FISH (MET gain copy number ≥5 and/or MET/CEP7 ≥2) in tissue biopsy and/or by NGS (MET gain copy number ≥2.3) in liquid biopsy. Among the pre-screened population who had an evaluable MET test (451 patients), 175 (38,8%) were defined as *MET* amplified and 122 patients received treatment. Preliminary data presented at ASCO 2023 showed, for the FISH positive patients, an ORR of 43,9% and a median DOR of 9.7 months; mPFS was 5,4 months and mOS was not reached. For the population with an NGS liquid biopsy positive test, ORR was 51.6%; mDOR was 5.6 months; mPFS 4.6 months and mOS was not reached (Tan et al., 2023). For INSIGHT 2 trials a specific arm evaluated tepotinib monotherapy after osimertinib progression, with an ORR of only 8.3% (Mazieres et al., 2022).

Furthermore, capmatinib, a reversible MET inhibitor, is under evaluation in the ongoing GEOMETRY-E trial, a phase III trial that randomize *MET* amplified, osimertinib progressed patients to osimertinib plus capmatinib versus platinum-pemetrexed based doublet chemotherapy. The completion date is expected for the end of the year (Wu et al., 2022).

Another common off-target mechanism to osimertinib resistance is associated with hyperactivation of *HER2* and therefore strategies including its inhibition have been suggested. Preclinical data showed the potential efficacy of targeting HER2 with the combination of osimertinib and trastuzumab emtansine (TDM1) in NSCLC *EGFR*-mutated cell lines to prevent or delay the appearance of resistance (La Monica et al., 2017). Several trials designed to test the efficacy of anti-HER2 drugs in association with osimertinib are now ongoing. TRAEMOS trial is a single-arm phase II study that evaluates the association between Osimertinib and TDM1 in *EGFR*-mutated, *HER2*-amplified patients (NCT03784599). Preliminary results in 27 patients showed ORR of 7% and clinical benefit rate of 35% after 13.5 months of follow up. Median PFS was 2.8 months (Jebbink et al., 2023). Moreover, CAPTUR trial is an ongoing basket trial that assign, for each mutation found in a set of solid tumors, a specific targeted agent. In particular, in arm 11 *HER2* amplificated tumors (including osimertinib-resistant patients) receive trastuzumab-pertuzumab (NCT03297606).

Molecularly driven combination treatment strategies have been explored also in patients with different acquired resistance mechanisms, even though tailored approaches show clear methological difficulaties due to the relatively low incidence of each molecular alteration. Main information concerning the possibility to target *ALK*, *RET* or *BRAF* acquired co-alterations derives from case reports, reporting about associations of alectinib or crizotinib with osimertinib in case of *ALK* rearrangement, dabrafenib-trametinib with osimertinib in the presence of *BRAF* mutation, BLU-667 plus osimertinib for *RET* rearrangements development at progression (Ding et al., 2020; Batra et al., 2020; Xie et al., 2021; Schrock et al., 2018).

In this field, from a biological point of view, trials designed to investigate in parallel multiple targeted strategies on the basis of molecular characterization performed in re-biopsy appear to be the most promising ones. On the other hand, the number of patients enrolled in each treatment arm is crucial in order to demonstrate the activity of a new treatment strategy. Some basket trials, like TATTON, CAPTUR and ORCHARD, are designed according to this concept and are likely to provide a considerable amount of information concerning acquired resistance mechanisms (NCT03944772, (De Langen et al., 2022; Oxnard et al., 2020). In particular, phase II ORCHARD trial is an ongoing trial with the aim of evaluating several possible resistance mechanisms after progression to first-line osimertinib, thus evaluating a second line option based on the resistance mechanism identified (NCT03944772, (De Langen et al., 2022). Patients are divided into three groups based on their molecular profiling from a post-progression tumor biopsy. Group A includes patients with protocol-determined biomarkers of resistance and treated with novel combination targeted therapies; group B includes patients without a detectable protocol-determined biomarker, treated with non biomarker-selected therapies and group C (observational) includes patients with histologically transformed disease and/or a biomarker with an available therapy not investigated in ORCHARD trial. The primary endpoint is investigator-assessed objective ORR; secondary outcomes include PFS, DOR, OS and pharmacokinetics of each treatment module.

6.2. *Molecular un-selected therapeutic strategies*

Although biologically reasonable, customized approach has several limitations, due to the need for tissue rebiopsy at the time of progression, not always feasible, the waiting time for tissue rebiopsy and characterization, but also to technical difficulties in the identification and standardization of the target. Non customized approach has obvious feasibility advantages and can be reasonable given the supposed high prevalence of a single resistace mechanism. Secondarily, in a quote of patient no targeted resistance mechanisms can be found and a different approach should be studied.

6.2.1. *Un-selected therapeutic strategies based on the high MET alterations prevalence*

CHRYSALIS phase 1 trial evaluated the feasibility and preliminary antitumor activity of amivantamab (a bispecific antibody targeting *EGFR* and *MET*) monotherapy or in combination with lazertinib (an oral, irreversible third generation EGFR TKI). In the dose expansion part, amivantamab was administered to six different cohorts assigned on the basis of *EGFR* and/or *MET* mutations or amplifications and of previous therapy. Both *EGFR* mutated and non mutated patients were included. Results were particularly significant in *EGFR* ex20ins cohort, including patients previously treated with platinum based chemotherapy (Park et al., 2021b). The ORR was 19% and 36% in *EGFR*-mutated patients progressing to osimertinib and treated with amivantamab alone and in combination with lazertinib, with a median DOR of 5.9 and 9.6 months, respectively. The median PFS was 6.9 and 11.1 months for patients treated with amivantamab alone or the combination with lazertinib (Cho et al., 2020; Leighl et al., 2021).

CHRYSALIS 2 trial, a phase 1/1b trial of lazertinib monotherapy and in combination with amivantamab, focuses on *EGFR* mutated patients: cohort A in particular enrols patients progressed after osimertinib and platinum chemotherapy (not selected for molecular alterations). Preliminary data presented at IASLC 2022 showed an ORR of 36% in this arm (Shu et al., 2022).

MARIPOSA 2 (NCT04988295) is a phase III trial enrolling *EGFR* mutated patients after progression on osimertinib. Molecularly unselected patients are randomly assigned to one of three arms: pemetrexed and carboplatin, amivantamab plus pemetrexed and carboplatin, or the combination of amivantamab and lazertinib plus pemetrexed and carboplatin. Preliminary results demonstrated that amivantamab lazertinib

chemotherapy and the less toxic combination of amivantamab and chemotherapy are able to significantly improve both PFS and intracranial PFS with respect to chemotherapy (Passaro et al., 2024).

6.2.2. *Un-selected therapeutic strategies in patients without detectable resistance mechanisms*

A completely different approach is at the basis of the development of ADCs. In this context, the two main targets identified so far are *TROP2* and *HER3*.

Datopotomab deruxtecan (Dato-DXd) is an ADC that consists on a humanized anti-TROP2 mAb coupled with the topoisomerase I inhibitor deruxtecan by a cleavable tetrapeptide-based linker. The drug is designed to bind TROP2, a transmembrane protein highly expressed in a variety of epithelial cancers, and internalize into cancer cells. Then the cytotoxic drugs are released into the cytoplasm leading to the target cell death (Okajima et al., 2021).

TROPION-PanTumor01 trial investigated the feasibility of Dato-DXd in several solid tumors. The NSCLC cohort consisted in 159 patients, of which 34 had an actionable genomic alteration (85% were *EGFR* mutated), who had progressed to several lines of treatments. The ORR in this group was 35% and median DOR was 9.5 months. The most common AEs were nausea (62%) and stomatitis (56%) (Lee et al., 2022; Garon et al., 2021). Moreover, module 10 in Orchard trial (NCT03944772) is investigating the role of associating Osimertinib to Dato-DXd after first line Osimertinib progression (De Langen et al., 2022).

A potential co-target in patients developing acquired resistance to osimertinib is *HER3*. *In vivo* and *in vitro* studies showed that Osimertinib inhibition on *EGFR* leads to increased *HER3* expression in order to overcome the signalling blockade (Haikala et al., 2022; Yonesaka et al., 2022). Patritumumab deruxtecan, an ADC targeting HER3, has been investigated in a phase I study of *EGFR*-mutated patients previously treated with TKI but not selected for molecular resistance mechanisms: ORR of 39% and mPFS of 8.2 months were reported (Jänne et al., 2022).

Adding chemotherapy and/or antiangiogenics represents a potential different non-targeted strategy at progression to osimertinib.

Discordant results were obtained adding chemotherapy to standard 1st and 2nd-gen TKIs: in IMPRESS trial, no clinical benefit was found by adding chemotherapy to gefitinib after gefitinib progression (Mok et al., 2017b). On the other hand, experience with osimertinib and chemotherapy combination initially derived from case reports, that found this strategy to have an acceptable toxicity. Two retrospective analyses were conducted to explore the feasibility of the combination and its effectiveness (Yoshida et al., 2018; Neal et al., 2019). The ongoing phase III COMPEL trial (NCT04765059), designed to investigate the use of platinum–pemetrexed chemotherapy with continued osimertinib in patients who experience non-CNS progression on first-line osimertinib, will provide additional information.

Finally, preclinical rationale to the strategy of combining EGFR TKI with anti- vascular endothelial growth factor (VEGF) was based on the observation of an increase in VEGF in *EGFR* activated tumor models (Naumov et al., 2009). On the other side, while encouraging data come from first line association of first generation EGFR TKI plus anti-angiogenics, the association of osimertinib plus bevacizumab for T790M-positive patients failed to show PFS prolongation *versus* osimertinib alone (Akamatsu et al., 2021; Piccirillo et al., 2022; Planchard et al., 2018).

On the other side, combination of immune checkpoint inhibitors with other agents has been explored as a strategy to improve the efficacy of immunotherapy in *EGFR*-mutated NSCLC.

A major safety concern emerged in TATTON trial, that evaluated Osimertinib/durvalumab combination in pre-treated and naïve EGFR mutated patients. Interstitial Lung Disease (ILD) was identified in 38% of cases and five G3–4 ILDs were observed and led to drug discontinuation. This increased risk of toxicity was more relevant when Osimertinib was administered in combination with or after ICI treatment (Schoenfeld et al., 2019).

A possible second-line approach after osimertinib progression was explored in the IMpower 150 trial, that evaluated the combination of chemotherapy with atezolizumab and/or bevacizumab. The combination of chemotherapy, ICI and anti-angiogenic was found to improve PFS and OS in the subgroup of patients progressed to an EGFR-TKI when compared to anti-angiogenic plus chemotherapy combination (Socinski et al., 2021).

Following these results, a phase II trial (NCT04517526) is evaluating the efficacy and safety of platinum-based chemotherapy, bevacizumab, durvalumab and salvage stereotactic body radiotherapy for patients with *EGFR*-mutated NSCLC after the failure of first-line osimertinib.

The role chemotherapy-immunotherapy combination in EGFR-mutated, osimertinib-resistant patients has been further clarified by the phase III KEYNOTE 789 trial, which compared platinum doublet chemotherapy plus pembrolizumab vs platinum doublet alone. The results for the two primary endpoints, PFS and OS, did not reach the statistical significance (median PFS: 5,6 in the experimental arm vs 5,5 in the placebo arm [HR 0,80; p=0,0122]; median OS: 15,9 vs 14,7 months [HR 0,84; p=0,0362]), thus confirming immunotherapy as a mainly not effective in EGFR mutated patients (Yang et al., 2023).

On the other hand, innovative approaches to immunomodulation in EGFR-mutated NSCLC are also being explored: REGN7075 is a human bispecific antibody which links EGFR-and CD28-positive T cells, in order to activate T-cells against tumor antigens.The ongoing phase I/II trial is evaluating the safety, tolerability, pharmacokinetics and preliminary anti-tumor activity of REGN7075 alone and in combination with cemiplimab (anti-[PD]−1 antibody) in patients with advanced solid tumors (NCT04626635). Part 2 of the trial will be open also for TKI resistant EGFR mutated NSCLC. Primary endpoints are safety and tolerability of REGN7075 for part 1, and ORR for part 2. The study is ongoing and currently open to enrollment (Segal et al., 2023).

7. Conclusions

Nowadays the most common first line standard option for *EGFR* mutated aNSCLC patients is Osimertinib and the biggest challenge is to improve the duration of clinical benefit by overcoming acquired and, less frequently, primary resistance.

The study of molecular resistance mechanisms is commonly recognized as pivotal in order to increase biological knowledge, improve therapeutic perspectives in clinical practice and contribute to develop new therapeutic strategies. Anyway, from a clinical perspective, customized treatment approach on the basis of molecular characterization is challenging, taking into account the feasibility of tissue rebiopsy and complete genetic characterization in tissue, the technical limitations of liquid biopsy and potential heterogeneity of resistance mechanisms.

While patients developing acquired resistance to Osimertinib are encouraged, whenever possible, to participate in clinical trials to grant access to innovative therapeutic strategies, the organization of systematic molecular characterization of tissue and plasma at baseline and at the time of progression to Osimertinib in real-life setting could be of help in order to increase biological knowledge and maximize access to investigational drugs in clinical practice. Liquid biopsy has numerous advantages, beeing less invasive, more likely to allowmultiple genetic alterations' screening and therefore to provide informations about genetic heterogeneity (Bonanno et al., 2022). On the other hand, tissue rebiopsy is able to identify histological transformation and it is still more reliable for some molecular alterations such as gene fusions. For this reason, a combined approach is to be promoted.

We have recently opened a multicentre molecular profiling study, in order to provide thematic molecular characterization in tissue and plasma in patients developing acquired resistance to first-line Osimertinib (CESC IOV 2021–107-PU, promoted by the University of Padova, Italy) and we strongly believe that academic translational

studies, in parallel with intervetional studies, might help to move further steps forward in the path of improving outcome for advanced NSCLC patients.

CRediT authorship contribution statement

Laura Bonanno and Alessandra Ferro: Conceptualization, Methodology, Validation. **Laura Bonanno, Alessandra Ferro and Gian Marco Marinato:** Data curation, Invastigation, Writing- Original draft preparation, visualization. **Monica Marino and Cristiana Mulargiu:** Invastigation, Resouces, Visualization. **Laura Bonanno:** Writing-Reviewing, Editing, Supervision. **Valentina Guarneri and Giulia Pasello:** Supervision.

Declaration of Competing Interest

The authors declare no conflict of interest that could have influenced the submitted work.

References

Zhang, Y.L., Yuan, J.Q., Wang, K.F., Fu, X.H., Han, X.R., Threapleton, D., et al., 2016. The prevalence of EGFR mutation in patients with non-small cell lung cancer: a systematic review and meta-analysis. Oncotarget 7 (48), 78985–78993. Nov 29.

Rosell, R., Moran, T., Queralt, C., Porta, R., Cardenal, F., Camps, C., et al., 2009. Screening for epidermal growth factor receptor mutations in lung cancer. N. Engl. J. Med. 361 (10), 958–967. Sep 3.

Douillard, J.Y., Ostoros, G., Cobo, M., Ciuleanu, T., McCormack, R., Webster, A., et al., 2014. First-line gefitinib in Caucasian EGFR mutation-positive NSCLC patients: a phase-IV, open-label, single-arm study. Br. J. Cancer 110 (1), 55–62. Jan 21.

Mok, T.S., Wu, Y.L., Thongprasert, S., Yang, C.H., Chu, D.T., Saijo, N., et al., 2009. Gefitinib or carboplatin–paclitaxel in pulmonary adenocarcinoma. N. Engl. J. Med. 361 (10), 947–957. Sep 3.

Wu, Y.L., Zhou, C., Hu, C.P., Feng, J., Lu, S., Huang, Y., et al., 2014. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. Lancet Oncol. 15 (2), 213–222 (Feb).

Rosell, R., Carcereny, E., Gervais, R., Vergnenegre, A., Massuti, B., Felip, E., et al., 2012. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol. 13 (3), 239–246 (Mar).

Sequist, L.V., Yang, J.C.H., Yamamoto, N., O'Byrne, K., Hirsh, V., Mok, T., et al., 2013. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J. Clin. Oncol. 31 (27), 3327–3334. Sep 20.

Yu, H.A., Arcila, M.E., Rekhtman, N., Sima, C.S., Zakowski, M.F., Pao, W., et al., 2013. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI Therapy in 155 patients with EGFR-mutant lung cancers. Clin. Cancer Res. 19 (8), 2240–2247. Apr 15.

Mok, T.S., Wu, Y.L., Ahn, M.J., Garassino, M.C., Kim, H.R., Ramalingam, S.S., et al., 2017a. Osimertinib or platinum–pemetrexed in EGFR T790M–Positive Lung Cancer. N. Engl. J. Med. 376 (7), 629–640. Feb 16.

Soria, J.C., Ohe, Y., Vansteenkiste, J., Reungwetwattana, T., Chewaskulyong, B., Lee, K. H., et al., 2018. Osimertinib in untreated EGFR-mutated advanced non–small-cell lung cancer. N. Engl. J. Med. 378 (2), 113–125. Jan 11.

Anon, FDA approves osimertinib for first-line treatment of metastatic NSCLC with most common EGFR mutations [Internet]. 2018 [cited 2023 Jun 1]. Available from: ⟨https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-osimertinib-first-line-treatment-metastatic-nsclc-most-common-egfr-mutations⟩.

Le, X., Puri, S., Negrao, M.V., Nilsson, M.B., Robichaux, J., Boyle, T., et al., 2018. Landscape of EGFR -dependent and -independent resistance mechanisms to osimertinib and continuation therapy beyond progression in EGFR-Mutant NSCLC. Clin. Cancer Res. 24 (24), 6195–6203. Dec 15.

Gomez, D.R., Blumenschein, G.R., Lee, J.J., Hernandez, M., Ye, R., Camidge, D.R., et al., 2016. Local consolidative therapy versus maintenance therapy or observation for patients with oligometastatic non-small-cell lung cancer without progression after first-line systemic therapy: a multicentre, randomised, controlled, phase 2 study. Lancet Oncol. 17 (12), 1672–1682 (Dec).

Ramalingam, S.S., Vansteenkiste, J., Planchard, D., Cho, B.C., Gray, J.E., Ohe, Y., et al., 2020. Overall survival with osimertinib in untreated, EGFR-mutated advanced NSCLC. N. Engl. J. Med [Internet] 382 (1), 41–50. ⟨https://pubmed.ncbi.nlm.nih.gov/31751012/⟩. Jan 2 [cited 2024 Jan 17];.

Reungwetwattana, T., Nakagawa, K., Cho, B.C., Cobo, M., Cho, E.K., Bertolini, A., et al., 2018. CNS response to osimertinib versus standard epidermal growth factor receptor tyrosine kinase inhibitors in patients with untreated *EGFR* -mutated advanced non–small-cell lung cancer. J. Clin. Oncol. 36 (33), 3290–3297. Nov 20.

Vendrell, J.A., Quantin, X., Aussel, A., Solassol, I., Serre, I., Solassol, J., 2021. EGFR-dependent mechanisms of resistance to osimertinib determined by ctDNA NGS analysis identify patients with better outcome. Transl. Lung Cancer Res 10 (11), 4084–4094 (Dec).

Cho, B.C., Felip, E., Spira, A.I., Girard, N., Lee, J.S., Lee, S.H., et al., 2023. LBA14 Amivantamab plus lazertinib vs osimertinib as first-line treatment in patients with EGFR-mutated, advanced non-small cell lung cancer (NSCLC): Primary results from MARIPOSA, a phase III, global, randomized, controlled trial. Ann. Oncol. 34, S1306 (Oct).

Planchard, D., Jänne, P.A., Cheng, Y., Yang, J.C.H., Yanagitani, N., Kim, S.W., et al., 2023. Osimertinib with or without Chemotherapy in *EGFR* -Mutated Advanced NSCLC. N. Engl. J. Med. 389 (21), 1935–1948. Nov 23.

Jackman, D., Pao, W., Riely, G.J., Engelman, J.A., Kris, M.G., Jänne, P.A., et al., 2010. Clinical definition of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non–small-cell lung cancer. J. Clin. Oncol. 28 (2), 357–360. Jan 10.

Santoni-Rugiu, Melchior, Urbanska, Jakobsen, Stricker, Grauslund, et al., 2019. Intrinsic resistance to EGFR-tyrosine kinase inhibitors in EGFR-mutant non-small cell lung cancer: differences and similarities with acquired Resistance. Cancers (Basel) 11 (7), 923. Jul 1.

Wang, J., Wang, B., Chu, H., Yao, Y., 2016. Intrinsic resistance to EGFR tyrosine kinase inhibitors in advanced non-small-cell lung cancer with activating EGFR mutations. Onco Targets Ther. 3711 (Jun).

Leonetti, A., Sharma, S., Minari, R., Perego, P., Giovannetti, E., Tiseo, M., 2019. Resistance mechanisms to osimertinib in EGFR-mutated non-small cell lung cancer. Br. J. Cancer 121 (9), 725–737. Oct 29.

Park, J.Y., Jang, S.H., Lee, C.Y., Kim, T., Chung, S.J., Lee, Y.J., et al., 2022. Pretreatment neutrophil-to-lymphocyte ratio and smoking history as prognostic factors in advanced non–small cell lung cancer patients treated with osimertinib. Tube Respir. Dis. (Seoul.) 85 (2), 155–164. Apr 1.

Guo, Y., Song, J., Wang, Y., Huang, L., Sun, L., Zhao, J., et al., 2020. Concurrent genetic alterations and other biomarkers predict treatment efficacy of EGFR-TKIs in EGFR-mutant non-small cell lung cancer: a review. Front Oncol. 10. Dec 10.

Kim, Y., Lee, B., Shim, J.H., Lee, S.H., Park, W.Y., Choi, Y.L., et al., 2019a. Concurrent genetic alterations predict the progression to target therapy in EGFR-mutated advanced NSCLC. J. Thorac. Oncol. 14 (2), 193–202 (Feb).

Brown, H., Vansteenkiste, J., Nakagawa, K., Cobo, M., John, T., Barker, C., et al., 2020. Programmed cell death ligand 1 expression in untreated EGFR mutated advanced NSCLC and response to osimertinib versus comparator in FLAURA. J. Thorac. Oncol. 15 (1), 138–143 (Jan).

Peng, Z., Lin, H., Zhou, K., Deng, S., Mei, J., 2021. Predictive value of pretreatment PD-L1 expression in EGFR-mutant non-small cell lung cancer: a meta-analysis. World J. Surg. Oncol. 19 (1), 145. Dec 8.

Arcila, M.E., Nafa, K., Chaft, J.E., Rekhtman, N., Lau, C., Reva, B.A., et al., 2013. EGFR Exon 20 insertion mutations in lung adenocarcinomas: prevalence, molecular heterogeneity, and clinicopathologic characteristics. Mol. Cancer Ther. 12 (2), 220–229. Feb 1.

Robichaux, J.P., Le, X., Vijayan, R.S.K., Hicks, J.K., Heeke, S., Elamin, Y.Y., et al., 2021. Structure-based classification predicts drug response in EGFR-mutant NSCLC. Nature 597 (7878), 732–737. Sep 30.

Russo, A., Franchina, T., Ricciardi, G., Battaglia, A., Picciotto, M., Adamo, V., 2019. Heterogeneous responses to epidermal growth factor receptor (EGFR) Tyrosine Kinase Inhibitors (TKIs) in patients with uncommon EGFR mutations: new insights and future perspectives in this complex clinical scenario. Int J. Mol. Sci. 20 (6), 1431. Mar 21.

Yang, J.C.H., Sequist, L.V., Geater, S.L., Tsai, C.M., Mok, T.S.K., Schuler, M., et al., 2015. Clinical activity of afatinib in patients with advanced non-small-cell lung cancer harbouring uncommon EGFR mutations: a combined post-hoc analysis of LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6. Lancet Oncol. 16 (7), 830–838 (Jul).

Bar, J., Peled, N., Schokrpur, S., Dudnik, E., Wollner, M., Girard, N., et al., 2022. Uncommon EGFR mutations on osimertinib, real-life data (UNICORN study): Updated results, brain efficacy, and resistance mechanisms. J. Clin. Oncol. 40 (16_suppl), 9109. Jun 1.

Pizzutilo, E.G., Agostara, A.G., Oresti, S., Signorelli, D., Giannetta, L.G., Stabile, S., et al., 2022. EP08.02-046 activity of osimeRTInib in NSCLC with Uncommon EGFR mutations: retrospective observational multicenter study (ARTICUNO). J. Thorac. Oncol. 17 (9), S418–S419 (Sep).

Bar, J., Peled, N., Schokrpur, S., Wolner, M., Rotem, O., Girard, N., et al., 2023. UNcommon EGFR mutations: international case series on efficacy of osimertinib in real-life practice in first-line setting (UNICORN). J. Thorac. Oncol. 18 (2), 169–180 (Feb).

Miura, S., Tanaka, H., Misumi, T., Yoshioka, H., Kurata, T., Tokito, T., et al., 2023. LBA66 Afatinib versus chemotherapy for treatment-naïve non-small cell lung cancer with a sensitizing uncommon epidermal growth factor receptor mutation: A phase III study (ACHILLES/TORG1834). Ann. Oncol. 34, S1310–S1311 (Oct).

Yasuda, H., Park, E., Yun, C.H., Sng, N.J., Lucena-Araujo, A.R., Yeo, W.L., et al., 2013. Structural, biochemical, and clinical characterization of epidermal growth factor receptor (EGFR) Exon 20 insertion mutations in lung cancer. Sci. Transl. Med 5 (216). Dec 18.

van Veggel, B., Madeira R Santos, J.F.V., Hashemi, S.M.S., Paats, M.S., Monkhorst, K., Heideman, D.A.M., et al., 2020. Osimertinib treatment for patients with EGFR exon 20 mutation positive non-small cell lung cancer. Lung Cancer 141, 9–13 (Mar).

Yasuda, H., Ichihara, E., Sakakibara-Konishi, J., Zenke, Y., Takeuchi, S., Morise, M., et al., 2021. A phase I/II study of osimertinib in EGFR exon 20 insertion mutation-positive non-small cell lung cancer. Lung Cancer 162, 140–146 (Dec).

Meador, C.B., Sequist, L.V., Piotrowska, Z., 2021. Targeting EGFR Exon 20 Insertions in non–small cell lung cancer: recent advances and clinical updates. Cancer Discov. 11 (9), 2145–2157. Sep 1.

Hou, J., Li, H., Ma, S., He, Z., Yang, S., Hao, L., et al., 2022. EGFR exon 20 insertion mutations in advanced non-small-cell lung cancer: current status and perspectives. Biomark. Res 10 (1), 21. Dec 13.
Song, Z., Li, Y., Chen, S., Ying, S., Xu, S., Huang, J., et al., 2022. Efficacy and safety of pyrotinib in advanced lung adenocarcinoma with HER2 mutations: a multicenter, single-arm, phase II trial. BMC Med 20 (1), 42. Dec 1.
Piotrowska, Z., Yu, H.A., Yang, J.C.H., Koczywas, M., Smit, E.F., Tan, D.S.W., et al., 2021. Safety and activity of CLN-081 (TAS6417) in NSCLC with EGFR Exon 20 insertion mutations (Ins20). J. Clin. Oncol. 39 (15_suppl), 9077. May 20.
Park, K., Haura, E.B., Leighl, N.B., Mitchell, P., Shu, C.A., Girard, N., et al., 2021a. Amivantamab in EGFR Exon 20 insertion–mutated non–small-cell lung cancer progressing on platinum chemotherapy: initial results from the CHRYSALIS phase i study. J. Clin. Oncol. 39 (30), 3391–3402. Oct 20.
Zhou, C., Ramalingam, S.S., Kim, T.M., Kim, S.W., Yang, J.C.H., Riely, G.J., et al., 2021. Treatment outcomes and safety of mobocertinib in platinum-pretreated patients with EGFR Exon 20 insertion–positive metastatic non–Small Cell Lung Cancer. JAMA Oncol. 7 (12), e214761. Dec 16.
Zhou, C., Tang, K.J., Cho, B.C., Liu, B., Paz-Ares, L., Cheng, S., et al., 2023. Amivantamab plus chemotherapy in NSCLC with *EGFR* Exon 20 Insertions. N. Engl. J. Med. 389 (22), 2039–2051. Nov 30.
Blakely, C.M., Watkins, T.B.K., Wu, W., Gini, B., Chabon, J.J., McCoach, C.E., et al., 2017a. Evolution and clinical impact of co-occurring genetic alterations in advanced-stage EGFR-mutant lung cancers. Nat. Genet 49 (12), 1693–1704. Dec 6.
Skoulidis, F., Heymach, J.V., 2019. Co-occurring genomic alterations in non-small-cell lung cancer biology and therapy. Nat. Rev. Cancer 19 (9), 495–509. Sep 12.
Rachiglio, A., Fenizia, F., Piccirillo, M., Galetta, D., Crinò, L., Vincenzi, B., et al., 2019. The Presence of concomitant mutations affects the activity of EGFR tyrosine kinase inhibitors in EGFR-mutant non-small cell lung cancer (NSCLC) Patients. Cancers (Basel) 11 (3), 341. Mar 10.
Canale, M., Petracci, E., Delmonte, A., Bronte, G., Chiadini, E., Ludovini, V., et al., 2020. Concomitant TP53 mutation confers worse prognosis in EGFR-mutated non-small cell lung cancer patients treated with TKIs. J. Clin. Med 9 (4), 1047. Apr 7.
Kim, Y., Lee, B., Shim, J.H., Lee, S.H., Park, W.Y., Choi, Y.L., et al., 2019b. Concurrent genetic alterations predict the progression to target therapy in EGFR-mutated advanced NSCLC. J. Thorac. Oncol. 14 (2), 193–202 (Feb).
Oren, M., Rotter, V., 2010. Mutant p53 gain-of-function in cancer. Cold Spring Harb. Perspect. Biol. 2 (2), a001107. Feb 1.
Poeta, M.L., Manola, J., Goldwasser, M.A., Forastiere, A., Benoit, N., Califano, J.A., et al., 2007. TP53 mutations and survival in squamous-cell carcinoma of the head and neck. N. Engl. J. Med. 357 (25), 2552–2561. Dec 20.
Molina-Vila, M.A., Bertran-Alamillo, J., Gascó, A., Mayo-de-las-Casas, C., Sánchez-Ronco, M., Pujantell-Pastor, L., et al., 2014. Nondisruptive p53 mutations are associated with shorter survival in patients with advanced non–small cell lung cancer. Clin. Cancer Res. 20 (17), 4647–4659. Sep 1.
Gristina, V., La Mantia, M., Galvano, A., Cutaia, S., Barraco, N., Castiglia, M., et al., 2021. Non-small cell lung cancer harboring concurrent EGFR genomic alterations: a systematic review and critical appraisal of the double dilemma. J. Mol. Pathol. 2 (2), 173–196. Jun 4.
Guibert, N., Barlesi, F., Descourt, R., Léna, H., Besse, B., Beau-Faller, M., et al., 2017. Characteristics and outcomes of patients with lung cancer harboring multiple molecular alterations: results from the IFCT study biomarkers France. J. Thorac. Oncol. 12 (6), 963–973 (Jun).
Zhuang, X., Zhao, C., Li, J., Su, C., Chen, X., Ren, S., et al., 2019. Clinical features and therapeutic options in non-small cell lung cancer patients with concomitant mutations of EGFR, ALK, ROS1, KRAS or BRAF. Cancer Med 8 (6), 2858–2866. Jun 24.
Nardo, G., Carlet, J., Marra, L., Bonanno, L., Boscolo, A., Dal Maso, A., et al., 2021. Detection of low-frequency KRAS mutations in cfDNA from EGFR-mutated NSCLC patients after first-line EGFR tyrosine kinase inhibitors. Front Oncol. 10. Jan 15.
Wang, L., Hu, H., Pan, Y., Wang, R., Li, Y., Shen, L., et al., 2014. PIK3CA Mutations frequently coexist with EGFR/KRAS mutations in non-small cell lung cancer and suggest poor prognosis in EGFR/KRAS wildtype subgroup. PLoS One 9 (2), e88291. Feb 12.
Wang, F., Diao, X.Y., Zhang, X., Shao, Q., Feng, Y.F., An, X., et al., 2019a. Identification of genetic alterations associated with primary resistance to EGFR-TKIs in advanced non-small-cell lung cancer patients with EGFR sensitive mutations. Cancer Commun. 39 (1), 7 (Dec).
Lee, J.K., Lee, J., Kim, S., Kim, S., Youk, J., Park, S., et al., 2017a. Clonal History and genetic predictors of transformation into small-cell carcinomas from lung adenocarcinomas. J. Clin. Oncol. 35 (26), 3065–3074. Sep 10.
Blakely, C.M., Watkins, T.B.K., Wu, W., Gini, B., Chabon, J.J., McCoach, C.E., et al., 2017b. Evolution and clinical impact of co-occurring genetic alterations in advanced-stage EGFR-mutant lung cancers. Nat. Genet 49 (12), 1693–1704. Dec 6.
La Monica, S., Fumarola, C., Cretella, D., Bonelli, M., Minari, R., Cavazzoni, A., et al., 2020. Efficacy of the CDK4/6 dual inhibitor abemaciclib in EGFR-Mutated NSCLC cell lines with different resistance mechanisms to osimertinib. Cancers (Basel) 13 (1), 6. Dec 22.
Costa, D.B., Halmos, B., Kumar, A., Schumer, S.T., Huberman, M.S., Boggon, T.J., et al., 2007. BIM Mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. PLoS Med 4 (10), e315. Oct 30.
Lv, F., Sun, L., Yang, Q., Pan, Z., Zhang, Y., 2021. Prognostic value of BIM Deletion in EGFR-mutant NSCLC Patients Treated with EGFR-TKIs: a meta-analysis. Biomed. Res Int 2021, 1–13. Oct 13.
Morrison, M.E., Palenski, T.L., Jamali, N., Sheibani, N., Sorenson, C.M., 2013. Modulation of vascular cell function by bim expression. Int J. Cell Biol. 2013, 1–15.
Cardona, A.F., Ordóñez-Reyes, C., Ruiz-Patiño, A., Garcia-Robledo, J.E., Barron, L.Z., Recondo, G., et al., 2021. EGFR inhibitors plus bevacizumab are superior than EGFR inhibitors alone as first-line setting in advanced NSCLC With EGFR mutations and BIM deletion polymorphisms (BIM-CLICaP). JCO Precis Oncol. (5), 839–848 (Nov).
Liu, S., Zhou, J., Li, W., Sun, H., Zhang, Y., Yan, H., et al., 2020. Concomitant genetic alterations having greater impact on the clinical benefit of EGFR-TKIs in EGFR -mutant advanced NSCLC than BIM deletion polymorphism. Clin. Transl. Med 10 (1), 337–345. Mar 19.
Ortiz-Cuaran, Scheffler, S., Plenker, M., Dahmen llona, D., Scheel, A.H., Fernandez-Cuesta, L., et al., 2016. Heterogeneous mechanisms of primary and acquired resistance to third-generation EGFR inhibitors. Clin. Cancer Res. 22 (19), 4837–4847. Oct 1.
Yu, H.A., Suzawa, K., Jordan, E., Zehir, A., Ni, A., Kim, R., et al., 2018. Concurrent alterations in EGFR-mutant lung cancers associated with resistance to EGFR kinase inhibitors and characterization of mtor as a mediator of resistance. Clin. Cancer Res. 24 (13), 3108–3118. Jul 1.
Martinez-Marti, A., Felip, E., Matito, J., Mereu, E., Navarro, A., Cedrés, S., et al., 2017. Dual MET and ERBB inhibition overcomes intratumor plasticity in osimertinib-resistant-advanced non-small-cell lung cancer (NSCLC). Ann. Oncol. 28 (10), 2451–2457 (Oct).
Zhao, S., Li, X., Zhao, C., Jiang, T., Jia, Y., Shi, J., et al., 2019. Loss of T790M mutation is associated with early progression to osimertinib in Chinese patients with advanced NSCLC who are harboring EGFR T790M. Lung Cancer 128, 33–39 (Feb).
Yu, H.A., Tian, S.K., Drilon, A.E., Borsu, L., Riely, G.J., Arcila, M.E., et al., 2015. Acquired resistance of EGFR-mutant lung cancer to a T790M-specific EGFR inhibitor: emergence of a third mutation (C797S) in the EGFR tyrosine kinase domain. JAMA Oncol. 1 (7), 982. Oct 1.
Ahn, S., Hwang, S.H., Han, J., Choi, Y.L., Lee, S.H., Ahn, J.S., et al., 2016. Transformation to small cell lung cancer of pulmonary adenocarcinoma: clinicopathologic analysis of six cases. J. Pathol. Transl. Med 50 (4), 258–263. Jul 15.
Schmid, S., Li, J.J.N., Leighl, N.B., 2020. Mechanisms of osimertinib resistance and emerging treatment options. Lung Cancer 147, 123–129 (Sep).
Ramalingam, S.S., Yang, J.C.H., Lee, C.K., Kurata, T., Kim, D.W., John, T., et al., 2018. Osimertinib as first-line treatment of EGFR mutation–positive advanced non–small-cell lung cancer. J. Clin. Oncol. 36 (9), 841–849. Mar 20.
Chmielecki, J., Gray, J.E., Cheng, Y., Ohe, Y., Imamura, F., Cho, B.C., et al., 2023. Candidate mechanisms of acquired resistance to first-line osimertinib in EGFR-mutated advanced non-small cell lung cancer. Nat. Commun. 14 (1), 1070. Feb 27.
Cardona, A.F., Ruiz-Patiño, A., Recondo, G., Martín, C., Raez, L., Samtani, S., et al., 2022. Mechanisms of resistance to first-line osimertinib in hispanic patients with EGFR mutant non-small cell lung cancer (FRESTON-CLICaP). Clin. Lung Cancer 23 (6), 522–531 (Sep).
Akli, A., Girard, N., Fallet, V., Rousseau-Bussac, G., Gounant, V., Friard, S., et al., 2022. Histomolecular resistance mechanisms to first-line osimertinib in egfr-mutated advanced non-small cell lung cancer: a multicentric retrospective French study. Target Oncol. 17 (6), 675–682. Nov 21.
Ahn, M. j, De Marinis, F., Bonanno, L., Cho, B.C., Kim, T.M., Cheng, S., et al., 2022a. EP08.02-140 MET biomarker-based preliminary efficacy analysis in SAVANNAH: savolitinib+osimertinib in EGFRm NSCLC post-osimertinib. J. Thorac. Oncol. 17 (9), S469–S470 (Sep).
Mazieres, J., Kim, T.M., Lim, B.K., Wislez, M., Dooms, C., Finocchiaro, G., et al., 2022. LBA52 Tepotinib + osimertinib for EGFRm NSCLC with MET amplification (METamp) after progression on first-line (1L) osimertinib: Initial results from the INSIGHT 2 study. Ann. Oncol. 33, S1419–S1420 (Sep).
Choudhury, N.J., Marra, A., Sui, J.S.Y., Flynn, J., Yang, S.R., Falcon, C.J., et al., 2023. Molecular biomarkers of disease outcomes and mechanisms of acquired resistance to first-line osimertinib in advanced EGFR-mutant lung cancers. J. Thorac. Oncol. 18 (4), 463–475 (Apr).
Schoenfeld, A.J., Chan, J.M., Kubota, D., Sato, H., Rizvi, H., Daneshbod, Y., et al., 2020. Tumor analyses reveal squamous transformation and off-target alterations as early resistance mechanisms to first-line osimertinib in egfr-mutant lung cancer. Clin. Cancer Res. 26 (11), 2654–2663. Jun 1.
Yu, Z., Boggon, T.J., Kobayashi, S., Jin, C., Ma, P.C., Dowlati, A., et al., 2007. Resistance to an irreversible epidermal growth factor receptor (EGFR) Inhibitor in EGFR-mutant lung cancer reveals novel treatment strategies. Cancer Res 67 (21), 10417–10427. Nov 1.
Passaro, A., Jänne, P.A., Mok, T., Peters, S., 2021. Overcoming therapy resistance in EGFR-mutant lung cancer. Nat. Cancer 2 (4), 377–391. Apr 15.
Papadimitrakopoulou, V.A., Mok, T.S., Han, J.Y., Ahn, M.J., Delmonte, A., Ramalingam, S.S., et al., 2020. Osimertinib versus platinum–pemetrexed for patients with EGFR T790M advanced NSCLC and progression on a prior EGFR-tyrosine kinase inhibitor: AURA3 overall survival analysis. Ann. Oncol. 31 (11), 1536–1544 (Nov).
Yang, Z., Yang, N., Ou, Q., Xiang, Y., Jiang, T., Wu, X., et al., 2018. Investigating novel resistance mechanisms to third-generation egfr tyrosine kinase inhibitor osimertinib in non–small cell lung cancer patients. Clin. Cancer Res. 24 (13), 3097–3107. Jul 1.
Oxnard, G.R., Hu, Y., Mileham, K.F., Husain, H., Costa, D.B., Tracy, P., et al., 2018. Assessment of resistance mechanisms and clinical implications in patients with EGFR T790M–positive lung cancer and acquired resistance to osimertinib. JAMA Oncol. 4 (11), 1527. Nov 1.
Lazzari, C., Gregorc, V., Santarpia, M., 2019. Impact of clinical features of epidermal growth factor receptor (EGFR)-mutated non-small cell lung cancer (NSCLC) patients on osimertinib efficacy. J. Thorac. Dis. 11 (11), 4400–4403 (Nov).
Tumbrink, H.L., Heimsoeth, A., Sos, M.L., 2021. The next tier of EGFR resistance mutations in lung cancer. Oncogene 40 (1), 1–11. Jan 7.
Fassunke, J., Müller, F., Keul, M., Michels, S., Dammert, M.A., Schmitt, A., et al., 2018. Overcoming EGFR G724S-mediated osimertinib resistance through unique binding

characteristics of second-generation EGFR inhibitors. Nat. Commun. 9 (1), 4655. Nov 7.
Zhang, Q., Zhang, X.C., Yang, J.J., Yang, Z.F., Bai, Y., Su, J., et al., 2018. EGFR L792H and G796R: two novel mutations mediating resistance to the third-generation EGFR tyrosine kinase inhibitor osimertinib. J. Thorac. Oncol. 13 (9), 1415–1421 (Sep).
Ou, S.H.I., Cui, J., Schrock, A.B., Goldberg, M.E., Zhu, V.W., Albacker, L., et al., 2017. Emergence of novel and dominant acquired EGFR solvent-front mutations at Gly796 (G796S/R) together with C797S/G and L792F/H mutations in one EGFR (L858R/T790M) NSCLC patient who progressed on osimertinib. Lung Cancer 108, 228–231 (Jun).
Dong, R.F., Zhu, M.L., Liu, M.M., Xu, Y.T., Yuan, L.L., Bian, J., et al., 2021. EGFR mutation mediates resistance to EGFR tyrosine kinase inhibitors in NSCLC: From molecular mechanisms to clinical research. Pharm. Res 167, 105583 (May).
Chen, K., Zhou, F., Shen, W., Jiang, T., Wu, X., Tong, X., et al., 2017. Novel Mutations on EGFR Leu792 potentially correlate to acquired resistance to osimertinib in advanced NSCLC. J. Thorac. Oncol. 12 (6), e65–e68 (Jun).
Fairclough, S.R., Kiedrowski, L.A., Lin, J.J., Zelichov, O., Tarcic, G., Stinchcombe, T.E., et al., 2019. Identification of osimertinib-resistant EGFR L792 mutations by cfDNA sequencing: oncogenic activity assessment and prevalence in large cfDNA cohort. Exp. Hematol. Oncol. 8 (1), 24. Dec 11.
Bersanelli, M., Minari, R., Bordi, P., Gnetti, L., Bozzetti, C., Squadrilli, A., et al., 2016. L718Q mutation as new mechanism of acquired resistance to AZD9291 in EGFR -mutated NSCLC. J. Thorac. Oncol. 11 (10), e121–e123 (Oct).
Ramalingam, S.S., Cheng, Y., Zhou, C., Ohe, Y., Imamura, F., Cho, B.C., et al., 2018 Oct. Mechanisms of acquired resistance to first-line osimertinib: Preliminary data from the phase III FLAURA study. Ann. Oncol. 29, viii740.
Shen, Q., Qu, J., Chen, Z., Zhou, J., 2021. Case Report: dacomitinib overcomes osimertinib resistance in NSCLC patient harboring L718Q mutation: a case report. Front Oncol. 11. Dec 2.
Yang, X., Huang, C., Chen, R., Zhao, J., 2020. Resolving resistance to osimertinib therapy with afatinib in an NSCLC patient with EGFR L718Q mutation. Clin. Lung Cancer 21 (4), e258–e260 (Jul).
Coleman, N., Hong, L., Zhang, J., Heymach, J., Hong, D., Le, X., 2021. Beyond epidermal growth factor receptor: MET amplification as a general resistance driver to targeted therapy in oncogene-driven non-small-cell lung cancer. ESMO Open 6 (6), 100319 (Dec).
Reita, D., Pabst, L., Pencreach, E., Guérin, E., Dano, L., Rimelen, V., et al., 2021. Molecular mechanism of EGFR-TKI resistance in EGFR-mutated non-small cell lung cancer: application to biological diagnostic and monitoring. Cancers (Basel) 13 (19), 4926. Sep 30.
Ahn, M. j, De Marinis, F., Bonanno, L., Cho, B.C., Kim, T.M., Cheng, S., et al., 2022b. EP08.02-140 MET biomarker-based preliminary efficacy analysis in SAVANNAH: savolitinib+osimertinib in EGFRm NSCLC Post-Osimertinib. J. Thorac. Oncol. 17 (9), S469–S470 (Sep).
Kawakami, H., Okamoto, I., Okamoto, W., Tanizaki, J., Nakagawa, K., Nishio, K., 2014. Targeting MET amplification as a new oncogenic driver. Cancers (Basel) 6 (3), 1540–1552. Jul 22.
Guo, R., Berry, L.D., Aisner, D.L., Sheren, J., Boyle, T., Bunn, P.A., et al., 2019. MET IHC Is a Poor Screen for MET amplification or MET Exon 14 mutations in lung adenocarcinomas: data from a tri-institutional cohort of the lung cancer mutation consortium. J. Thorac. Oncol. 14 (9), 1666–1671 (Sep).
Schubart, C., Stöhr, R., Tögel, L., Fuchs, F., Sirbu, H., Seitz, G., et al., 2021. MET Amplification in non-small cell lung cancer (NSCLC)—a consecutive evaluation using next-generation sequencing (NGS) in a real-world setting. Cancers (Basel) 13 (19), 5023. Oct 7.
Awad, M.M., Oxnard, G.R., Jackman, D.M., Savukoski, D.O., Hall, D., Shivdasani, P., et al., 2016. MET Exon 14 mutations in non–small-cell lung cancer are associated with advanced age and stage-dependent met genomic amplification and c-met overexpression. J. Clin. Oncol. 34 (7), 721–730. Mar 1.
Suzawa, K., Offin, M., Schoenfeld, A.J., Plodkowski, A.J., Odintsov, I., Lu, D., et al., 2019. Acquired MET Exon 14 Alteration drives secondary resistance to epidermal growth factor receptor tyrosine kinase inhibitor in egfr-mutated lung cancer. JCO Precis Oncol. (3), 1–8 (Dec).
Li, W.F., Kang, J., Zhang, X.C., Jian, S., Chen, H., Wang, Z., et al., 2017. Coexistence of MET exon 14 mutations with EGFR mutations in non-small cell lung cancer. J. Clin. Oncol. 35 (15_suppl), e20636. May 20.
Hsu, C.C., Liao, B.C., Liao, W.Y., Markovets, A., Stetson, D., Thress, K., et al., 2020. Exon 16–Skipping HER2 as a novel mechanism of osimertinib resistance in EGFR L858R/T790M–positive non–small cell lung cancer. J. Thorac. Oncol. 15 (1), 50–61 (Jan).
Michels, S., Heydt, C., van Veggel, B., Deschler-Baier, B., Pardo, N., Monkhorst, K., et al., 2019. Genomic profiling identifies outcome-relevant mechanisms of innate and acquired resistance to third-generation epidermal growth factor receptor tyrosine kinase inhibitor therapy in lung cancer. JCO Precis Oncol. (3), 1–14 (Dec).
Hondelink, L.M., Jebbink, M., von der Thüsen, J.H., Cohen, D., Dubbink, H.J., Paats, M. S., et al., 2021. Real-world approach for molecular analysis of acquired EGFR tyrosine kinase inhibitor resistance mechanisms in NSCLC. JTO Clin. Res Rep. 2 (12), 100252 (Dec).
Piotrowska, Z., Isozaki, H., Lennerz, J.K., Gainor, J.F., Lennes, I.T., Zhu, V.W., et al., 2018. Landscape of acquired resistance to osimertinib in EGFR-mutant NSCLC and clinical validation of combined EGFR and RET inhibition with osimertinib and BLU-667 for acquired RET Fusion. Cancer Discov. 8 (12), 1529–1539. Dec 1.
Belli, C., Penault-Llorca, F., Ladanyi, M., Normanno, N., Scoazec, J.Y., Lacroix, L., et al., 2021. ESMO recommendations on the standard methods to detect RET fusions and mutations in daily practice and clinical research. Ann. Oncol. 32 (3), 337–350 (Mar).
Bruno, R., Fontanini, G., 2020. Next generation sequencing for gene fusion analysis in lung cancer: a literature review. Diagnostics 10 (8), 521. Jul 27.
Rosenbaum, D.M., Rasmussen, S.G.F., Kobilka, B.K., 2009. The structure and function of G-protein-coupled receptors. Nature 459 (7245), 356–363. May 20.
Moody, T.W., Ramos-Alvarez, I., Jensen, R.T., 2023. Peptide G-protein-coupled receptors and erbb receptor tyrosine kinases in cancer. Biol. (Basel) 12 (7), 957. Jul 4.
Zhang, X.W., Li, L., Hu, W.Q., Hu, M.N., Tao, Y., Hu, H., et al., 2022. Neurokinin-1 receptor promotes non-small cell lung cancer progression through transactivation of EGFR. Cell Death Dis. 13 (1), 41. Jan 10.
Kuzumaki, N., Suzuki, A., Narita, M., Hosoya, T., Nagasawa, A., Imai, S., et al., 2012. Multiple analyses of g-protein coupled receptor (GPCR) Expression in the development of gefitinib-resistance in transforming non-small-cell lung cancer. PLoS One 7 (10), e44368. Oct 29.
Normanno, N., Tejpar, S., Morgillo, F., De Luca, A., Van Cutsem, E., Ciardiello, F., 2009. Implications for KRAS status and EGFR-targeted therapies in metastatic CRC. Nat. Rev. Clin. Oncol. 6 (9), 519–527. Sep 28.
Zhao, J., Lin, G., Zhuo, M., Fan, Z., Miao, L., Chen, L., et al., 2020. Next-generation sequencing based mutation profiling reveals heterogeneity of clinical response and resistance to osimertinib. Lung Cancer 141, 114–118 (Mar).
Aboubakar Nana, F., Ocak, S., 2021. Targeting BRAF Activation as Acquired resistance mechanism to EGFR tyrosine kinase inhibitors in EGFR-mutant non-small-cell lung cancer. Pharmaceutics 13 (9), 1478. Sep 15.
Leontiadou, H., Galdadas, I., Athanasiou, C., Cournia, Z., 2018. Insights into the mechanism of the PIK3CA E545K activating mutation using MD simulations. Sci. Rep. 8 (1), 15544. Oct 19.
Kim, T.M., Song, A., Kim, D.W., Kim, S., Ahn, Y.O., Keam, B., et al., 2015. Mechanisms of acquired resistance to AZD9291. J. Thorac. Oncol. 10 (12), 1736–1744 (Dec).
Bordi, P., Del Re, M., Minari, R., Rofi, E., Buti, S., Restante, G., et al., 2019. From the beginning to resistance: study of plasma monitoring and resistance mechanisms in a cohort of patients treated with osimertinib for advanced T790M-positive NSCLC. Lung Cancer 131, 78–85 (May).
Yang, X., Xia, Y., Xu, L., Liang, L., Zhuo, M., Wu, M., et al., 2021. Efficacy and safety of combination treatment with apatinib and osimertinib after osimertinib resistance in epidermal growth factor receptor-mutant non-small cell lung carcinoma—a retrospective analysis of a multicenter Clinical Study. Front Mol. Biosci. 8. May 5.
Niederst, M.J., Sequist, L.V., Poirier, J.T., Mermel, C.H., Lockerman, E.L., Garcia, A.R., et al., 2015. RB loss in resistant EGFR mutant lung adenocarcinomas that transform to small-cell lung cancer. Nat. Commun. 6 (1), 6377. Mar 11.
Sequist, L.V., Waltman, B.A., Dias-Santagata, D., Digumarthy, S., Turke, A.B., Fidias, P., et al., 2011. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci. Transl. Med 3 (75). Mar 23.
Marcoux, N., Gettinger, S.N., O'Kane, G., Arbour, K.C., Neal, J.W., Husain, H., et al., 2019. EGFR-mutant adenocarcinomas that transform to small-cell lung cancer and other neuroendocrine carcinomas: clinical outcomes. J. Clin. Oncol. 37 (4), 278–285. Feb 1.
Vendrell, J.A., Quantin, X., Serre, I., Solassol, J., 2020. Combination of tissue and liquid biopsy molecular profiling to detect transformation to small cell lung carcinoma during osimertinib treatment. Ther. Adv. Med Oncol. 12, 175883592097419.
Pizzutilo, E.G., Pedrani, M., Amatu, A., Ruggieri, L., Lauricella, C., Veronese, S.M., et al., 2021. Liquid biopsy for small cell lung cancer either de novo or transformed: systematic review of different applications and meta-analysis. Cancers (Basel) 13 (9), 2265. May 8.
Bonanno, L., Reale, M.L., Ferro, A., Righi, L., Indraccolo, S., Listì, A., et al., 2021. 1811P Molecular characterization of epidermal growth factor receptor-mutated (EGFR-m) non-small cell lung cancer (NSCLC) undergoing histological transformation. Ann. Oncol. 32, S1230–S1231 (Sep).
Shaurova, T., Zhang, L., Goodrich, D.W., Hershberger, P.A., 2020. Understanding lineage plasticity as a path to targeted therapy failure in EGFR-mutant non-small cell lung cancer. Front Genet 11. Mar 27.
Lee, J.K., Lee, J., Kim, S., Kim, S., Youk, J., Park, S., et al., 2017b. Clonal History and genetic predictors of transformation into small-cell carcinomas from lung adenocarcinomas. J. Clin. Oncol. 35 (26), 3065–3074. Sep 10.
Roca, E., Gurizzan, C., Amoroso, V., Vermi, W., Ferrari, V., Berruti, A., 2017. Outcome of patients with lung adenocarcinoma with transformation to small-cell lung cancer following tyrosine kinase inhibitors treatment: A systematic review and pooled analysis. Cancer Treat. Rev. 59, 117–122 (Sep).
Izumi, H., Yamasaki, A., Ueda, Y., Sumikawa, T., Maeta, H., Nakamoto, S., et al., 2018. Squamous cell carcinoma transformation from EGFR-mutated lung adenocarcinoma: a case report and literature review. Clin. Lung Cancer 19 (1), e63–e66 (Jan).
Moriya, R., Hokari, S., Shibata, S., Koizumi, T., Tetsuka, T., Ito, K., et al., 2017. Histological transformation to large cell neuroendocrine carcinoma from lung adenocarcinoma harboring an EGFR mutation: an autopsy case report. Intern. Med. 56 (15), 2013–2017.
Park, S., Shim, J.H., Lee, B., Cho, I., Park, W.Y., Kim, Y., et al., 2019. Paired genomic analysis of squamous cell carcinoma transformed from EGFR-mutated lung adenocarcinoma. Lung Cancer 134, 7–15 (Aug).
Pathak, R., Villaflor, V.M., 2021. Histologic transformation in EGFR-mutant lung adenocarcinomas: mechanisms and therapeutic implications. Cancers (Basel) 13 (18), 4641. Sep 16.
Han, X., Li, F., Fang, Z., Gao, Y., Li, F., Fang, R., et al., 2014. Transdifferentiation of lung adenocarcinoma in mice with Lkb1 deficiency to squamous cell carcinoma. Nat. Commun. 5 (1), 3261. Feb 17.
La Monica, S., Minari, R., Cretella, D., Flammini, L., Fumarola, C., Bonelli, M., et al., 2019. Third generation EGFR inhibitor osimertinib combined with pemetrexed or cisplatin exerts long-lasting anti-tumor effect in EGFR-mutated pre-clinical models of NSCLC. J. Exp. Clin. Cancer Res. 38 (1), 222. Dec 28.
Nakamura, A., Inoue, A., Morita, S., Hosomi, Y., Kato, T., Fukuhara, T., et al., 2018. Phase III study comparing gefitinib monotherapy (G) to combination therapy with

gefitinib, carboplatin, and pemetrexed (GCP) for untreated patients (pts) with advanced non-small cell lung cancer (NSCLC) with EGFR mutations (NEJ009). J. Clin. Oncol. 36 (15_suppl), 9005. May 20.
Hosomi, Y., Morita, S., Sugawara, S., Kato, T., Fukuhara, T., Gemma, A., et al., 2020. Gefitinib alone versus gefitinib plus chemotherapy for non–small-cell lung cancer with mutated epidermal growth factor receptor: NEJ009 Study. J. Clin. Oncol. 38 (2), 115–123. Jan 10.
Asahina, H., Tanaka, K., Morita, S., Maemondo, M., Seike, M., Okamoto, I., et al., 2021. A Phase II study of osimertinib combined with platinum plus pemetrexed in patients with EGFR-mutated advanced non–small-cell lung cancer: the opal study (NEJ032C/LOGIK1801). Clin. Lung Cancer 22 (2), 147–151 (Mar).
Planchard, D., Feng, P.H., Karaseva, N., Kim, S.W., Kim, T.M., Lee, C.K., et al., 2021. Osimertinib plus platinum–pemetrexed in newly diagnosed epidermal growth factor receptor mutation-positive advanced/metastatic non-small-cell lung cancer: safety run-in results from the FLAURA2 study. ESMO Open 6 (5), 100271 (Oct).
CHEVALLIER, M., TSANTOULIS, P., ADDEO, A., FRIEDLAENDER, A., 2020. Influence of concurrent mutations on overall survival in EGFR-mutated non-small cell lung cancer. Cancer Genom. - Proteom. 17 (5), 597–603. Aug 28.
Canale, M., Petracci, E., Delmonte, A., Chiadini, E., Dazzi, C., Papi, M., et al., 2017. Impact of TP53 mutations on outcome in EGFR-mutated patients treated with first-line tyrosine kinase inhibitors. Clin. Cancer Res. 23 (9), 2195–2202. May 1.
Shi, P., Oh, Y.T., Zhang, G., Yao, W., Yue, P., Li, Y., et al., 2016. Met gene amplification and protein hyperactivation is a mechanism of resistance to both first and third generation EGFR inhibitors in lung cancer treatment. Cancer Lett. 380 (2), 494–504 (Oct).
Peng, K., Su, J., Xie, Z., Wang, H., Fang, M., Li, W., et al., 2022. Clinical outcomes of EGFR+/METamp+ vs. EGFR+/METamp- untreated patients with advanced non-small cell lung cancer. Thorac. Cancer 13 (11), 1619–1630. Jun 18.
Li, A., Chen, H.J., Yang, J.J., 2023. Design and Rationale for a Phase II, randomized, open-label, two-cohort multicenter interventional study of osimertinib with or without savolitinib in de novo MET aberrant, EGFR-mutant patients with advanced non-small-cell lung cancer: the flowers trial. Clin. Lung Cancer 24 (1), 82–88 (Jan).
Cho, B.C., Felip, E., Hayashi, H., Thomas, M., Lu, S., Besse, B., et al., 2022. MARIPOSA: phase 3 study of first-line amivantamab + lazertinib versus osimertinib in EGFR-mutant non-small-cell lung cancer. Future Oncol. 18 (6), 639–647 (Feb).
Nakagawa, K., Garon, E.B., Seto, T., Nishio, M., Ponce Aix, S., Paz-Ares, L., et al., 2019. Ramucirumab plus erlotinib in patients with untreated, EGFR-mutated, advanced non-small-cell lung cancer (RELAY): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 20 (12), 1655–1669 (Dec).
Yamamoto, N., Seto, T., Nishio, M., Goto, K., Yamamoto, N., Okamoto, I., et al., 2021. Erlotinib plus bevacizumab vs erlotinib monotherapy as first-line treatment for advanced EGFR mutation-positive non-squamous non-small-cell lung cancer: Survival follow-up results of the randomized JO25567 study. Lung Cancer 151, 20–24 (Jan).
Maemondo, M., Fukuhara, T., Saito, H., Furuya, N., Watanabe, K., Sugawara, S., et al., 2020. NEJ026: Final overall survival analysis of bevacizumab plus erlotinib treatment for NSCLC patients harboring activating EGFR-mutations. J. Clin. Oncol. 38 (15_suppl), 9506. May 20.
Yu, H.A., Schoenfeld, A.J., Makhnin, A., Kim, R., Rizvi, H., Tsui, D., et al., 2020. Effect of osimertinib and bevacizumab on progression-free survival for patients with metastatic EGFR-mutant lung cancers. JAMA Oncol. 6 (7), 1048. Jul 1.
Kenmotsu, H., Wakuda, K., Mori, K., Kato, T., Sugawara, S., Kirita, K., et al., 2022. Randomized phase 2 study of osimertinib plus bevacizumab versus osimertinib for untreated patients with nonsquamous nsclc harboring EGFR mutations: WJOG9717L study. J. Thorac. Oncol. 17 (9), 1098–1108 (Sep).
Lisberg, A., Cummings, A., Goldman, J.W., Bornazyan, K., Reese, N., Wang, T., et al., 2018 Aug. A phase II study of pembrolizumab in egfr-mutant, PD-L1+, tyrosine kinase inhibitor naïve patients with advanced NSCLC. J. Thorac. Oncol. 13 (8), 1138–1145.
Attili, I., Passaro, A., Corvaja, C., Trillo Aliaga, P., Del Signore, E., Spitaleri, G., et al., 2023. Immune checkpoint inhibitors in EGFR-mutant non-small cell lung cancer: A systematic review. Cancer Treat. Rev. 119, 102602 (Sep).
Yang, J.C.H., Lee, D.H., Lee, J.S., Fan, Y., de Marinis, F., Okamoto, I., et al., 2023. Pemetrexed and platinum with or without pembrolizumab for tyrosine kinase inhibitor (TKI)-resistant, *EGFR* -mutant, metastatic nonsquamous NSCLC: Phase 3 KEYNOTE-789 study. J. Clin. Oncol. 41 (17_suppl), LBA9000. Jun 10.
To, K.K.W., Fong, W., Cho, W.C.S., 2021. Immunotherapy in treating EGFR-mutant lung cancer: current challenges and new strategies. Front Oncol. 11. May 25.
Di Noia, V., D'Aveni, A., D'Argento, E., Rossi, S., Ghirardelli, P., Bortolotti, L., et al., 2021. Treating disease progression with osimertinib in EGFR-mutated non-small-cell lung cancer: novel targeted agents and combination strategies. ESMO Open 6 (6), 100280 (Dec).
Lorenzi, M., Ferro, A., Cecere, F., Scattolin, D., Del Conte, A., Follador, A., et al., 2022. First-line osimertinib in patients with egfr-mutant advanced non-small cell lung cancer: outcome and safety in the real world: FLOWER study. Oncologist 27 (2), 87–e115. Mar 4.
De Mattos-Arruda, L., Mayor, R., Ng, C.K.Y., Weigelt, B., Martínez-Ricarte, F., Torrejon, D., et al., 2015. Cerebrospinal fluid-derived circulating tumour DNA better represents the genomic alterations of brain tumours than plasma. Nat. Commun. 6 (1), 8839. Nov 10.
Suh, J.H., Kotecha, R., Chao, S.T., Ahluwalia, M.S., Sahgal, A., Chang, E.L., 2020. Current approaches to the management of brain metastases. Nat. Rev. Clin. Oncol. 17 (5), 279–299. May 20.
Chaft, J.E., Oxnard, G.R., Sima, C.S., Kris, M.G., Miller, V.A., Riely, G.J., 2011. Disease flare after tyrosine kinase inhibitor discontinuation in patients with EGFR-mutant lung cancer and acquired resistance to erlotinib or gefitinib: implications for clinical trial design. Clin. Cancer Res. 17 (19), 6298–6303. Oct 1.
Chmielecki, J., Foo, J., Oxnard, G.R., Hutchinson, K., Ohashi, K., Somwar, R., et al., 2011. Optimization of dosing for EGFR-mutant non–small cell lung cancer with evolutionary cancer modeling. Sci. Transl. Med 3 (90). Jul 6.
Popat, S., Ahn, M.J., Ekman, S., Leighl, N.B., Ramalingam, S.S., Reungwetwattana, T., et al., 2023. Osimertinib for EGFR-mutant non-small-cell lung cancer central nervous system metastases: current evidence and future perspectives on therapeutic strategies. Target Oncol. 18 (1), 9–24. Jan 18.
Uchibori, K., Inase, N., Araki, M., Kamada, M., Sato, S., Okuno, Y., et al., 2017. Brigatinib combined with anti-EGFR antibody overcomes osimertinib resistance in EGFR-mutated non-small-cell lung cancer. Nat. Commun. 8 (1), 14768. Mar 13.
Zhao, J., Zou, M., Lv, J., Han, Y., Wang, G., Wang, G., 2018. Effective treatment of pulmonary adenocarcinoma harboring triple EGFR mutations of L858R, T790M, and cis-C797S by osimertinib, bevacizumab, and brigatinib combination therapy: a case report. Onco Targets Ther. Volume 11, 5545–5550 (Sep).
Wang, X., Zhou, L., Yin, J.C., Wu, X., Shao, Y.W., Gao, B., 2019b. Lung adenocarcinoma harboring EGFR 19del/C797S/T790M triple mutations responds to brigatinib and anti-EGFR antibody combination therapy. J. Thorac. Oncol. 14 (5), e85–e88 (May).
Lin, C.Y., Huang, K.Y., Lin, Y.C., Yang, S.C., Chung, W.C., Chang, Y.L., et al., 2021. Vorinostat combined with brigatinib overcomes acquired resistance in EGFR-C797S-mutated lung cancer. Cancer Lett. 508, 76–91 (Jun).
Wang, Yang, Y., Zhang, N., Li Li, Y., Han, R., Zhu, M., et al., 2020. Effective Treatment of lung adenocarcinoma harboring EGFR-activating mutation, T790M, and cis-C797S triple mutations by brigatinib and cetuximab combination therapy. J. Thorac. Oncol. 15 (8), 1369–1375 (Aug).
Koulouris, A., Tsagkaris, C., Corriero, A.C., Metro, G., Mountzios, G., 2022. Resistance to TKIs in EGFR-mutated non-small cell lung cancer: from mechanisms to new therapeutic strategies. Cancers 14 (14), 3337. Jul 8.
Beyett, To, T.S., Heppner DE, C., Rana, J.K., Schmoker, A.M., Jang, J., et al., 2022. Molecular basis for cooperative binding and synergy of ATP-site and allosteric EGFR inhibitors. Nat. Commun. 13 (1), 2530. May 9.
Jia, Y., Yun, C.H., Park, E., Ercan, D., Manuia, M., Juarez, J., et al., 2016. Overcoming EGFR(T790M) and EGFR(C797S) resistance with mutant-selective allosteric inhibitors. Nature 534 (7605), 129–132. Jun 25.
To, C., Jang, J., Chen, T., Park, E., Mushajiang, M., De Clercq, D.J.H., et al., 2019. Single and dual targeting of mutant EGFR with an allosteric inhibitor. Cancer Discov. 9 (7), 926–943. Jul 1.
Tavera, L., Schalm, S., Campbell, J., Guo, J., Medendorp, C., Chen, M., et al., 2022. Antitumor activity of BLU-945 and BLU-701 as single agents and in combination in EGFR L858R-driven models of NSCLC. : AACR Annu. Meet. N. Orleans.
Spira, A.I., Spigel, D.R., Camidge, D.R., De Langen, A., Kim, T.M., Goto, K., et al., 2022. A phase 1/2 study of the highly selective EGFR inhibitor, BLU-701, in patients with EGFR-mutant non–small cell lung cancer (NSCLC). J. Clin. Oncol. 40 (16_suppl), TPS9142. Jun 1.
Riess, J.W., De Langen, J.A., Piotrowska, Z., Goldberg, S.B., Goldman, J.W., Okamoto, I., et al., 2022. 329P ORCHARD: osimertinib + necitumumab in patients (pts) with advanced NSCLC whose disease progressed on first-line (1L) osimertinib. Ann. Oncol. 33, S1571–S1572 (Nov).
Bertoli, E., De Carlo, E., Del Conte, A., Stanzione, B., Revelant, A., Fassetta, K., et al., 2022. Acquired resistance to osimertinib in EGFR-mutated non-small cell lung cancer: how do we overcome it? Int J. Mol. Sci. 23 (13), 6936. Jun 22.
Deng, L., Kiedrowski, L.A., Ravera, E., Cheng, H., Halmos, B., 2018. Response to dual crizotinib and osimertinib treatment in a lung cancer patient with MET amplification detected by liquid biopsy who acquired secondary resistance to EGFR tyrosine kinase inhibition. J. Thorac. Oncol. 13 (9), e169–e172 (Sep).
Zhu, V.W., Schrock, A.B., Ali, S.M., Ou, S.H.I., 2019. Differential response to a combination of full-dose osimertinib and crizotinib in a patient with EGFR-mutant non-small cell lung cancer and emergent MET amplification. Lung Cancer.: Targets Ther. Volume 10, 21–26 (Mar).
Sequist, L.V., Han, J.Y., Ahn, M.J., Cho, B.C., Yu, H., Kim, S.W., et al., 2020. Osimertinib plus savolitinib in patients with EGFR mutation-positive, MET-amplified, non-small-cell lung cancer after progression on EGFR tyrosine kinase inhibitors: interim results from a multicentre, open-label, phase 1b study. Lancet Oncol. 21 (3), 373–386 (Mar).
Yu, H.A., Ambrose, H., Baik, C., Cho, B.C., Cocco, E., Goldberg, S.B., et al., 2021. 1239P ORCHARD osimertinib + savolitinib interim analysis: a biomarker-directed phase II platform study in patients (pts) with advanced non-small cell lung cancer (NSCLC) whose disease has progressed on first-line (1L) osimertinib. Ann. Oncol. 32, S978–S979 (Sep).
Santarpia, M., Massafra, M., Gebbia, V., D'Aquino, A., Garipoli, C., Altavilla, G., et al., 2021. A narrative review of MET inhibitors in non-small cell lung cancer with MET exon 14 skipping mutations. Transl. Lung Cancer Res 10 (3), 1536–1556 (Mar).
Lu, S., Xu, W., Telaranta-Keerie, A., Jia, N., Hartmaier, R., 2022. EP08.02-138 SAFFRON: Ph3 Savolitinib + osimertinib vs chemotherapy in EGFRm NSCLC with MET overexpression/amplification post-osimertinib. J. Thorac. Oncol. 17 (9), S468–S469 (Sep).
Wu, Y.L., Cheng, Y., Zhou, J., Lu, S., Zhang, Y., Zhao, J., et al., 2020. Tepotinib plus gefitinib in patients with EGFR-mutant non-small-cell lung cancer with MET overexpression or MET amplification and acquired resistance to previous EGFR inhibitor (INSIGHT study): an open-label, phase 1b/2, multicentre, randomised trial. Lancet. Respir. Med 8 (11), 1132–1143 (Nov).
Tan, D.S.W., Kim, T.M., Guarneri, V., VOON, P.J., Lim, B.K., Wislez, M., et al., 2023. Tepotinib + osimertinib for EGFR mutant (EGFRm) NSCLC with MET amplification (MET amp) after first-line (1L) osimertinib. J. Clin. Oncol. 41 (16_suppl), 9021. Jun 1.

Wu, Y.L., Han, J.Y., Kato, T., Barlesi, F., Garon, E.B., Cappuzzo, F., et al., 2022. Capmatinib plus osimertinib versus platinum-pemetrexed doublet chemotherapy as second-line therapy in patients with stage IIIb/IIIc or IV EGFR-mutant, T790M-negative NSCLC harboring MET amplification. J. Clin. Oncol. 40 (16_suppl), TPS9153. Jun 1.
La Monica, S., Cretella, D., Bonelli, M., Fumarola, C., Cavazzoni, A., Digiacomo, G., et al., 2017. Trastuzumab emtansine delays and overcomes resistance to the third-generation EGFR-TKI osimertinib in NSCLC EGFR mutated cell lines. J. Exp. Clin. Cancer Res. 36 (1), 174. Dec 4.
Jebbink, M., de Langen, A.J., Monkhorst, K., Boelens, M.C., van den Broek, D., van der Noort, V., et al., 2023. Trastuzumab-Emtansine and Osimertinib Combination Therapy to Target HER2 Bypass Track Resistance in EGFR Mutation-Positive NSCLC. JTO Clin. Res Rep. 4 (4), 100481 (Apr).
Ding, H., Zhuang, Z., Xie, J., Huang, H., Tao, Z., Liu, Z., 2020. Durable Clinical response of advanced lung adenocarcinoma harboring EGFR-19del/T790M/BRAFV600E mutations after treating with osimertinib and dabrafenib plus trametinib: a case report. Onco Targets Ther. Volume 13, 7933–7939 (Aug).
Batra, U., Sharma, M., Amrith, B.P., Mehta, A., Jain, P., 2020. EML4-ALK Fusion as a resistance mechanism to osimertinib and its successful management with osimertinib and alectinib: case report and review of the literature. Clin. Lung Cancer 21 (6), e597–e600 (Nov).
Xie, Z., Gu, Y., Xie, X., Lin, X., Ouyang, M., Qin, Y., et al., 2021. Lung Adenocarcinoma harboring concomitant EGFR mutations and BRAF V600E responds to a combination of osimertinib and vemurafenib to overcome osimertinib resistance. Clin. Lung Cancer 22 (3), e390–e394 (May).
Schrock, A.B., Zhu, V.W., Hsieh, W.S., Madison, R., Creelan, B., Silberberg, J., et al., 2018. Receptor tyrosine kinase fusions and BRAF kinase fusions are rare but actionable resistance mechanisms to EGFR tyrosine kinase inhibitors. J. Thorac. Oncol. 13 (9), 1312–1323 (Sep).
De Langen, J., Cho, B.C., Piotrowska, Z., Le, X., Goldberg, S.B., Goldman, J.W., et al., 2022. 1188TiP ORCHARD platform study: osimertinib + datopotamab deruxtecan (Dato-DXd) cohort in patients (pts) with advanced NSCLC (aNSCLC) who have progressed on first-line (1L) osimertinib. Ann. Oncol. 33, S1091–S1092 (Sep).
Oxnard, G.R., Yang, J.C.H., Yu, H., Kim, S.W., Saka, H., Horn, L., et al., 2020. TATTON: a multi-arm, phase Ib trial of osimertinib combined with selumetinib, savolitinib, or durvalumab in EGFR-mutant lung cancer. Ann. Oncol. 31 (4), 507–516 (Apr).
Park, K., Haura, E.B., Leighl, N.B., Mitchell, P., Shu, C.A., Girard, N., et al., 2021b. Amivantamab in EGFR Exon 20 insertion–mutated non–small-cell lung cancer progressing on platinum chemotherapy: initial results from the CHRYSALIS phase i study. J. Clin. Oncol. 39 (30), 3391–3402. Oct 20.
Cho, B.C., Lee, K.H., Cho, E.K., Kim, D.W., Lee, J.S., Han, J.Y., et al., 2020. 1258O Amivantamab (JNJ-61186372), an EGFR-MET bispecific antibody, in combination with lazertinib, a 3rd-generation tyrosine kinase inhibitor (TKI), in advanced EGFR NSCLC. Ann. Oncol. 31, S813 (Sep).
Leighl, N.B., Shu, C.A., Minchom, A., Felip, E., Cousin, S., Cho, B.C., et al., 2021. 1192MO Amivantamab monotherapy and in combination with lazertinib in post-osimertinib EGFR-mutant NSCLC: Analysis from the CHRYSALIS study. Ann. Oncol. 32, S951–S952 (Sep).
Shu, C.A., Goto, K., Ohe, Y., Besse, B., Lee, S.H., Wang, Y., et al., 2022. Amivantamab and lazertinib in patients with EGFR-mutant non–small cell lung (NSCLC) after progression on osimertinib and platinum-based chemotherapy: Updated results from CHRYSALIS-2. J. Clin. Oncol. 40 (16_suppl), 9006. Jun 1.
Passaro, A., Wang, J., Wang, Y., Lee, S.H., Melosky, B., Shih, J.Y., et al., 2024. Amivantamab plus chemotherapy with and without lazertinib in EGFR-mutant advanced NSCLC after disease progression on osimertinib: primary results from the phase III MARIPOSA-2 study. Ann. Oncol. 35 (1), 77–90 (Jan).
Okajima, D., Yasuda, S., Maejima, T., Karibe, T., Sakurai, K., Aida, T., et al., 2021. Datopotamab deruxtecan, a novel TROP2-directed antibody–drug conjugate, demonstrates potent antitumor activity by efficient drug delivery to tumor cells. Mol. Cancer Ther. 20 (12), 2329–2340. Dec 1.
Lee, J., Piotrowska, Z., Soo, R., Cho, B.C., Lim, S.M., 2022. Combatting acquired resistance to osimertinib in EGFR-mutant lung cancer. Ther. Adv. Med Oncol. 14, 17588359221144100.
Garon, E.B., Johnson, M.L., Lisberg, A.E., Spira, A., Yamamoto, N., Heist, R.S., et al., 2021. Efficacy of datopotamab deruxtecan (Dato-DXd) in patients (pts) with advanced/metastatic (adv/met) non-small cell lung cancer (NSCLC) and actionable genomic alterations (AGAs): Preliminary results from the phase I TROPION-PanTumor01 study. Ann. Oncol. 32 (5). Sep 19.
Haikala, H.M., Lopez, T., Köhler, J., Eser, P.O., Xu, M., Zeng, Q., et al., 2022. EGFR inhibition enhances the cellular uptake and antitumor-activity of the HER3 antibody–drug conjugate HER3–DXd. Cancer Res 82 (1), 130–141. Jan 1.
Yonesaka, K., Tanizaki, J., Maenishi, O., Haratani, K., Kawakami, H., Tanaka, K., et al., 2022. HER3 augmentation via blockade of EGFR/AKT signaling enhances anticancer activity of HER3-targeting patritumab deruxtecan in EGFR-mutated non–small cell lung cancer. Clin. Cancer Res. 28 (2), 390–403. Jan 15.
Jänne, P.A., Baik, C., Su, W.C., Johnson, M.L., Hayashi, H., Nishio, M., et al., 2022. Efficacy and safety of patritumab deruxtecan (HER3-DXd) in EGFR inhibitor–resistant, EGFR-mutated non–small cell lung cancer. Cancer Discov. 12 (1), 74–89. Jan 1.
Mok, T.S.K., Kim, S.W., Wu, Y.L., Nakagawa, K., Yang, J.J., Ahn, M.J., et al., 2017b. Gefitinib plus chemotherapy versus chemotherapy in epidermal growth factor receptor mutation–positive non–small-cell lung cancer resistant to first-line gefitinib (IMPRESS): overall survival and biomarker analyses. J. Clin. Oncol. 35 (36), 4027–4034. Dec 20.
Yoshida, H., Ooi, M., Kim, Y.H., 2018. Successful treatment with osimertinib and chemotherapy in a non–small cell lung cancer patient with EGFR mutation and meningeal carcinomatosis. J. Thorac. Oncol. 13 (11), e219–e220 (Nov).
Neal, J.W., Hausrath, D., Wakelee, H.A., Cunanan, K., Liu, S.V., Banwait, M., et al., 2019. Osimertinib with chemotherapy for EGFR-mutant NSCLC at progression: Safety profile and survival analysis. J. Clin. Oncol. 37 (15_suppl), 9083. May 20.
Naumov, G.N., Nilsson, M.B., Cascone, T., Briggs, A., Straume, O., Akslen, L.A., et al., 2009. Combined vascular endothelial growth factor receptor and epidermal growth factor receptor (EGFR) blockade inhibits tumor growth in xenograft models of egfr inhibitor resistance. Clin. Cancer Res. 15 (10), 3484–3494. May 15.
Akamatsu, H., Toi, Y., Hayashi, H., Fujimoto, D., Tachihara, M., Furuya, N., et al., 2021. Efficacy of osimertinib plus bevacizumab vs osimertinib in patients with EGFR T790M–mutated non–small cell lung cancer previously treated with epidermal growth factor receptor–tyrosine kinase inhibitor. JAMA Oncol. 7 (3), 386. Mar 1.
Piccirillo, M.C., Bonanno, L., Garassino, M.C., Esposito, G., Dazzi, C., Cavanna, L., et al., 2022. Addition of bevacizumab to erlotinib as first-line treatment of patients with EGFR-mutated advanced nonsquamous NSCLC: the beverly multicenter randomized phase 3 trial. J. Thorac. Oncol. 17 (9), 1086–1097 (Sep).
Planchard, D., Yu, H.A., Yang, J.C.H., Lee, K.H., Garrido Lopez, P., Park, K., et al., 2018. Efficacy and safety results of ramucirumab in combination with osimertinib in advanced T790M-positive EGFR-mutant NSCLC. J. Clin. Oncol. 36 (15_suppl), 9053. May 20.
Schoenfeld, A.J., Arbour, K.C., Rizvi, H., Iqbal, A.N., Gadgeel, S.M., Girshman, J., et al., 2019. Severe immune-related adverse events are common with sequential PD-(L)1 blockade and osimertinib. Ann. Oncol. 30 (5), 839–844 (May).
Socinski, M.A., Nishio, M., Jotte, R.M., Cappuzzo, F., Orlandi, F., Stroyakovskiy, D., et al., 2021. IMpower150 final overall survival analyses for atezolizumab plus bevacizumab and chemotherapy in first-line metastatic nonsquamous NSCLC. J. Thorac. Oncol. 16 (11), 1909–1924 (Nov).
Segal, N.H., Pelster, M., Girda, E., Fong, L., Olszanski, A.J., Han, H., et al., 2023. A phase 1/2 study of REGN7075 (EGFRxCD28 costimulatory bispecific antibody) in combination with cemiplimab (anti–PD-1) in patients with advanced solid tumors: Trial-in-progress update. J. Clin. Oncol. 41 (4_suppl), TPS277. Feb 1.
Bonanno, L., Dal Maso, A., Pavan, A., Zulato, E., Calvetti, L., Pasello, G., et al., 2022. Liquid biopsy and non-small cell lung cancer: are we looking at the tip of the iceberg? Br. J. Cancer 127 (3), 383–393. Aug 1.

Alessandra Ferro is a Medical Oncologist at the Istituto Oncologico Veneto IOV - IRCCS in Padova (Italy). In November 2021, she specialized with full marks in Medical Oncology at the Department of Surgery, Oncology and Gastroenterology, University of Padova with a thesis work on stage III non-small cell lung cancer (NSCLC) "The Unmet Needs in Stage III Non-Small Cell Lung Cancer: Evolution of Multidisciplinary Approach and Predictors of Treatment Outcome and Prognosis". Currently, Dr. Ferro is a Medical Oncologist focused in thoracic malignancies' care at the Division of Oncology 2 of the Istituto Oncologico Veneto, a research and care institute. She is a member of the IOV Thoracic Oncology team and IOV - Azienda Ospedale Università Padova Thoracic Oncology Multidisciplinary team. As regards the research activity, since 2018 Dr. Ferro has been involved in thoracic cancer clinical and translational research and she has experience as sub-investigator of several clinical trial programs. Lung cancer is the main topic of her research activity, with particular reference to new treatment strategies development, assessment/monitoring of predictive and prognostic biomarkers and real-world studies. Dr. Ferro is published in some peer-review journals with about 50 publications among original articles and abstracts/posters.

Gian Marco Marinato is a Medical Oncologist. He specialized with full marks in 2023 at the University of Padova and now he has a permanent position as an Oncologist. During his residency he developed specific interest in thoracic oncology and was actively involved in clinical activity, clinical trials and multidisciplinary management of thoracic malignancies.

Cristiana Mulargiu is a Medical Oncology resident at the Department of Surgery, Oncology and Gastroenterology, University of Padova. In March 2019 she graduated in Medicine e Surgery and, since november 2019, She works at IOV - IRCCS in Padova (Italy), focused in thoracic malignancies at the Division of Oncology 2 of the Istituto Oncologico Veneto. Since 2021 Dr. Mulargiu has collaboratore within thoracic cancer clinical and translational research and she has experience as sub-investigator of several clinical trial programs.

Monica Marino is a resident in Medical Oncology at the Department of Surgery, Oncology and Gastroenterology of the University of Padua. Since 2021, she is committed to medical treatment and clinical research in the field of solid tumors, mainly focusing on breast cancer. She is involved as sub-investigator in several phase II and III clinical trials on breast cancer. Currently she is working at the department of medical oncology receiving training in clinical practice.

Giulia Pasello Giulia Pasello (MD, PhD) is Associate Professor in Oncology at the Department of Surgery, Oncology and Gastroenterology, University of Padova, and Medical Oncologist at the Istituto Oncologico Veneto IRCCS in Padova (Italy). She received Residency in Oncology in 2010 with full marks with a translational thesis work on Malignant Pleural Mesothelioma (MPM) "Induction of senescence markers after neo-adjuvant chemotherapy of malignant pleural mesothelioma and association with clinical outcome: an exploratory analysis". This work was conducted at the molecular oncology laboratories of the University of Zurich where she spent the last 6 months of residency school. Dr. Pasello received a PhD in Experimental, Medical and Clinical Sciences of the University of

Padova in 2014, with a research project on neoangiogenic biomarkers and therapeutic targets in malignant pleural mesothelioma. In 2019, Since 2010 she has coordinated several translational projects on MPM and lung cancer, which are the main topic of her clinical and translational research activity, with particular reference to new treatment targets discovery, innovative drugs development, and assesment/monitoring of inflammatory tissue and circulating predictive and prognostic biomarkers. Dr. Pasello is extensively published in peer-review journals with more than 100 papers and some books chapter and has delivered several talks in Italian and International conferences. She additionally has been a member of the scientific committee of national and international conferences on thoracic oncology. Currently, she is associate editor of Frontiers in Oncology and Pulmonology. She is currently assistant professor in Oncology at the Department of Surgery, Oncology and Gastroenterology of University of Padova Dr. Pasello has been recipient of national and international research grants for translational studies on thoracic cancers.

Valentina Guarneri, MD, PhD, is Full Professor of Oncology at the University of Padua, Head of the Division of Oncology 2 at the Istituto Oncologico Veneto, and head of the Specialization School in medical Oncology at the University of Padova. She completed her fellowship in Oncology in 2003, and she obtained her PhD Degree in Clinical and Experimental Oncology in 2007. In 2005 she was appointed Assistant Professor of Oncology at the University of Modena and Reggio Emilia, where she also chaired the Breast Unit. In 2005, she also completed a 6-month-research experience at the Department of Breast Medical Oncology, UT. MD Anderson Cancer Center, Houston, Texas, where in 2009 she was Visiting Assistant Professor. In 2014 she was appointed Associate Professor of Oncology at the University of Padua. Prof Guarneri's research interest is mainly focused on clinical and translational research for breast cancer patients. She has published more than 200 papers in peer-reviewed journals.

Bonanno Laura, MD, is Medical Oncologist at the Division of Medical Oncology 2, Istituto Oncologico Veneto in Padova. She is mainly involved in the management of thoracic malignancies, actively working in multidisciplinary dedicated team and clinical research projects in the field. In 2006 she graduated with full marks and in 2011 she achieved her Residency in Medical Oncology with full marks at the University of Padova. During her residency, she has also worked at Dexeus University Institute of Barcelona, under the supervision of Dr. Rosell, involved in a translational research project in lung cancer. She is now involved as principal investigator and coinvestigator in more than 50 clinical trials, both spontaneous and good clinical practice, concerning the treatment of lung cancer, malignant pleural mesothelioma and thymic tumors. She has achieved also molecular biology expertise and she is currently coordinating translational research projects in lung cancer. She is author or co-author of more than 80 full papers published in peer-reviewed journals in the field of translational research and thoracic oncology. She is currently member of ASCO, European Society of Medical Oncology (ESMO), Italian Association of Medical Oncology (AIOM), International Association for the Study of Lung Cancer (IALSC) and European Thoracic Oncology Platform (ETOP).