Understanding EGFR heterogeneity in lung cancer

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ABSTRACT

The advances in understanding the inherited biological mechanisms of non-small cell lung cancer harbouring epidermal growth factor receptor (EGFR) mutations led to a significant improvement in the outcomes of patients treated with EGFR tyrosine kinase inhibitors. Despite these clinically impressive results, clinical results are not always uniform, suggesting the need for deepening the molecular heterogeneity of this molecularly defined subgroup of patients beyond the clinical and biological surface. The availability of tissue and blood-based tumour genotyping allows us to improve the understanding of molecular and genetic intratumor heterogeneity, driving the measurement of clonal evaluation in patients with lung cancer carrying EGFR mutations. Genetic diversification, clonal expansion and selection are highly variable patterns of genetic diversity, resulting in different biological entities, also a prerequisite for Darwinian selection and therapeutic failure. Such emerging pieces of evidence on the genetic diversity, including adaptive and immunomodulated aspects, provide further evidence for the role of the tumour microenvironment (TME) in drug-resistance and immune-mediated mechanisms. Matching in daily clinical practice, the detailed genomic profile of lung cancer disease and tracking the clonal evolution could be the way to individualise the further target treatments in patients with other gene mutations.16 Interestingly, the presence of multiple nodules is related survival (PFS) in different molecular subsets, including the EGFR-mutated subtype.2–6

The importance of the epidermal growth factor receptor (EGFR) gene molecular assessment for EGFR TKI administration was raised over a decade ago. As a matter of fact, the efficacy of these novel drugs is subordinated to the identification of sensitising mutations in the EGFR gene.7 From an epidemiological point of view, EGFR mutations range from 10% to 15% in Caucasian patients and up to 50% in East-Asian patients, and are identified more frequently in patients with adenocarcinoma, who are female and who have never smoked or are former smokers.8 9 For this reason, the College of American Pathologists, the International Association for the Study of Lung Cancer, the Association for Molecular Pathology, the National Comprehensive Cancer Network and the American Society of Clinical Oncology guidelines identified several genes to that necessarily require testing in patients with advanced NSCLC, including EGFR.10–12 Noteworthy, despite the clinical efficacy of EGFR TKIs in the vast majority of patients with advanced-stage NSCLC harbouring a sensitising EGFR mutation, is that a non-negligible percentage of patients displayed mixed responses or progressive disease.13 Furthermore, the intratumor heterogeneous distribution of EGFR mutations has been shown to be involved as a resistance mechanism during TKI treatments.14 As a consequence, only cells harbouring EGFR mutations will respond to TKI action, with the remaining tumour cells, insensitive to the treatment, responsible for disease persistence and ultimately progression15 (figure 1). In this setting, the EGFR sensitive mutant tumour cells may coexist with other subclonal tumour cells with either different EGFR genetic alterations or with other gene mutations.16 Interestingly, the presence of multiple nodules is related survival (PFS) in different molecular subsets, including the EGFR-mutated subtype.2–6

INTRODUCTION

Lung cancer is the main cause of death for cancer worldwide.1 In the last decades, many efforts have been spent in order to improve the overall survival (OS) and quality of life of patients with advanced-stage non-small cell lung cancer (NSCLC). In particular, significant results were obtained from several clinical trials that investigated the adoption of tyrosine kinase inhibitors (TKIs) instead of chemotherapy, improving significantly overall response rates (RRs) and progression-free

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to a higher rate of heterogeneity.\textsuperscript{13} Besides, mutational heterogeneity is present in a non-negligible percentage of patients with advanced-stage NSCLC between the primary tumour and its metastases.\textsuperscript{17} Driver mutations (e.g., EGFR) are early events in tumour development and for this reason are homogenously existing in all tumour cells, whereas other alterations may arise during cancer progression and adaptation.\textsuperscript{17,16} Of note, a wide range of mutations can affect EGFR with different responsiveness to the different target treatments.\textsuperscript{17} In addition, EGFR mutations may coexist with other alterations in the same gene or other different genes.\textsuperscript{20,21}

Patients harbouring EGFR sensitive mutations, who receive EGFR TKIs in the metastatic setting, usually achieved a significant disease response with prolonged survival.

Although the effectiveness of TKIs is confirmed by a multitude of preclinical and clinical studies, not all patients equally benefit from these target treatments,\textsuperscript{22} mainly confirming three clusters within the treated EGFR population, characterised by different prognosis subgroups: poor prognosis, characterised with limited survival and fast progression (OS <12 months); good prognosis, overlapping survival of pivotal randomized clinical trials (RCTs) (OS 24–30 months); excellent prognosis, characterised by doubled or tripled survival, compared with standards (OS more than 36 months). This survival heterogeneity appears closely linked to the tumour heterogeneity, through the increasing role of co-occurring mutations and immune-microenvironment, identifying new potential prognostic and predictive biomarkers in EGFR-positive disease. In this review, we overview the tumour heterogeneity of NSCLC harbouring EGFR mutations, from tumour clonality to co-occurring mutations, through the complex role of the tumour microenvironment (TME).

**EGFR TUMOUR CLONALITY**

The concept of clonal evolution of tumour cell population was reported for the first time as early as 1976.\textsuperscript{23} In this theory, neoplastic cells take origin from a single progenitor that subsequently acquires, under selective pressure, different genomic alterations.\textsuperscript{23} For this reason, these genomic alterations, which occur in the early phases of cancer development and give an advantage in cancer growth, are identified in all neoplastic cells.\textsuperscript{23,24} To date, lung adenocarcinoma seems to originate from a multistep progression, from atypical adenomatous hyperplasia to adenocarcinoma in situ, and finally invasive adenocarcinoma.\textsuperscript{25} In this evolution, EGFR driver alterations are acquired in the early step of cancer progression and can be identified in the vast majority of neoplastic cancer cells. Driver mutations, such as those in the EGFR, have been shown to be significantly more often truncal events compared with mutations in non-driver genes that are usually branch mutations.\textsuperscript{26} As a consequence, the heterogeneous distribution of EGFR mutations in lung adenocarcinomas is extremely rare, as demonstrated in a seminal study of Yatabe et al.\textsuperscript{15} Of note, similar results were obtained by Sun et al.\textsuperscript{27} In this study, identical EGFR mutations were identified in different areas of tumours featuring mixed histology.\textsuperscript{27} In the experience of Mattsson et al, even if three different histological areas were selected for molecular analysis, the same EGFR molecular status emerged.\textsuperscript{28} In order to evaluate the spatial distribution of EGFR and Kirsten Rat Sarcoma Viral Oncogene Homolog mutations, Dietz et al analysed central tumour sections (5 mm×5 mm segments) of lung mutated

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**Figure 1.** Clonal evolution through epidermal growth factor receptor-targeted therapy. (A) Intratumor heterogeneity based on multiregion sequencing. (B) Linear cancer evolution through subclonal selection. (C) Cell progression after tyrosine kinase inhibitors showing different genomic patterns of selection through different lines of therapy.
Biodiversity of EGFR Mutations: Driver, Passenger and Co-Occurring Mutations

As far as EGFR mutations are concerned, the vast majority is represented by in-frame deletions involving exon 19 (about 45%) and exon 21 p.L858R (about 40%).40 Of note, these mutations lie in the tyrosine kinase domain of EGFR protein and are targetable by TKIs. As early as 2004, Lynch et al and Paez et al discovered for the first time the driver role of EGFR mutations in patients with NSCLC.74 The authors reported for the first time that mutations involving the tyrosine kinase domain of EGFR protein might predict responsiveness to the first generation TKI gefitinib.7 The remaining (10%-15%) ‘uncommon’ EGFR mutations are still under investigation for their ability to predict response or resistance to specific EGFR TKIs.19 In this setting, a broad range of different alterations, covering exons 18–21, should be correctly classified in order to administrate the best treatment choice.19 Exon 18 mutations rarely occur (about 3% and 4%) in patients with advanced-stage NSCLC and limited literature focused the attention on their function.82–84 However, most frequently, the alterations within exon 18 lie in codons 719 and 709.55 Collectively, these mutations seem to be more sensitive to second-generation EGFR TKIs followed by third-generation, and then first-generation inhibitors (primary resistance or low responsiveness).19 In addition to the frequent classical deletions (comprising up to 30 alterations) investigated in the different clinical trials,46 exon 19 harbours many other less investigated deletions.47 Of note, exon 19 deletions may interest the entire exon (codons 746–761) and, in a non-negligible percentage of cases (>50%), may be associated with additional insertions (indels).48 Despite a high RR to all TKIs, it would be preferred to administrate the third-generation TKIs.19 Exon 20 harbours a heterogeneous group of mutations (point mutations, duplications, insertions).49 The resistance mutation p.T790M represents the most common EGFR exon 20 point mutation. This latter occurs in 50%-60% of patients with advanced-stage NSCLC with acquired resistance to first-generation or second-generation TKIs, but it is sensitive to the third-generation TKI osimertinib.5 50 51 Noteworthy, the prevalence of this alteration in treatment naive patients is quite low with (about 2%),52 and has been associated with inherited susceptibility to lung cancer.53–54

Exon 20 is also involved in other resistance mechanisms. Thress et al reported for the first time the EGFR exon 20 p.C797S resistance point mutation after treatment with osimertinib.55 Conversely, EGFR exon 20 insertions represent 4%-12% of all EGFR mutations,56 whereas less frequent point mutations are identified in codon 768 (p.S768I; about 1%).57 Nevertheless, in both cases, responsiveness to afatinib or osimertinib was reported.19 As far as exon 21 is concerned, the second most frequent mutated codon is 861 (p.L861Q; 1% and 2%).58 The spectrum of the response of this alteration is similar to that seen in exon 20 insertions and p.S768I.19 When considering EGFR mutations, it pivotal to distinguish between mutations able to confer an advantage in tumour growth and development (so-called ‘driver mutations’) and the other mutations that can arise in cancer cells without pathogenic features (so-called ‘passenger mutations’).39 In patients with NSCLC, tobacco habits may induce the highest rate of mutations. Of note, a high percentage of these alterations are passenger mutations, useful to identify a mutational signature characteristic of tobacco smoking.60 In particular, it was evidenced a higher percentage of signature 4 (C>A mutations) and 5 (C>Tand T>C mutations) in lung cancer associated with smoking history.60 To this end, several efforts have been spent in order to classify these alterations correctly. In this setting, computational analysis may be helpful to define driver and passenger

Adenocarcinomas.29 Of note, driver mutations were identified in 462 (98.9%) out of 467 tumour segments with different allelic frequencies (range: 0.04–19.36).39 Sun et al evidenced the higher presence of EGFR mutations in cancer cells, performing a cytological fine needle aspiration (FNA) approach.30 A higher concordance (91.7%) was reached when the FNA molecular approach was compared with the histological one.30 Despite these pieces of evidence, it is currently known that tumours are characterised by distinct subclones with several genomic alterations.31 Intratumoral heterogeneity was reported in different cancer types, including NSCLC, as a consequence of genetic and epigenetic alterations derived from genomic and chromosomal instability and different patterns of clonal evolution over space and time.32,33 In fact, despite driver mutations (eg, EGFR) occurring early in tumour growth and development and consequently homogenously distributing within the tumour, other alterations may arise during cancer progression and adaptation.17 Single-cell analysis may provide insight into the occurrence of intratumoral heterogeneity of EGFR mutations.44 In cases in which both EGFR-mutated and non-mutated neoplastic cells are present, response to TKIs may be of low intensity.14 When different neoplastic cells are discovered within the same lesion, only tumour cells harbouring EGFR sensitising mutations display responsiveness to TKI treatments. Conversely, the remaining non-mutated neoplastic cells, which are not sensitive to the target treatment, without the selective pressure may replace the decaying cells.15 In this setting, subclonal tumour cells without EGFR sensitising mutations may coexist EGFR sensitive mutant tumour cells.16 Interestingly, the higher rate was evidenced when multiple nodules affect the same patient.13 As far as mutational heterogeneity is concerned, metastases feature different genomic alterations with respect to the primary site in a non-negligible percentage of patients with advanced-stage NSCLC.37 Liquid biopsy may overcome the limitation of spatial heterogeneity in lung cancer.35,36 Each single tumour cell actively or passively sheds nucleic acids into the bloodstream.37 This evidence may be relevant, in particular when resistance mutations arise.38,39
mutations. In particular, the analysis of amino acid residues through the protein in both wild-type and mutant proteins and the analysis of tridimensional structure should be taking into account. Anoosha et al underlined that leucine and glycine substitutions in helix and strand are more frequently associated with driver mutations, whereas charged residues arginine and glutamic acid are more frequently associated with coil-buried and coil-exposed mutants, respectively. Finally, EGFR mutations can be associated with each other or with other genetic alterations which may be present. EGFR multiple mutations account for about 25% of patients with EGFR mutations. In the vast majority of cases, classical sensitising mutations are associated with additional rare alterations. In these cases, second-generation and third-generation TKIs may play a pivotal therapeutic role. Interestingly, EGFR mutations may be associated with other gene alterations, in particular in tumor protein P53 (TP53). Yu et al identified in pretreated EGFR-mutated samples co-occurring mutations in TP53, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), catenin beta 1 and retinoblastoma 1 (RB1). The authors underlined a shorter time to progression on EGFR TKI when a TP53 mutation was evidenced. In another study, Chen et al identified co-occurring mutations in TP53, RB1, PIK3CA, FA tumour suppressor homolog 1 (FAT1), or ATP-binding cassette subfamily B member 1 (ABCB1), mitogen-activated protein kinase kinase 2. Another gene that may occur in association with EGFR is represented by AT-rich interaction domain IA. Despite its clinical role is not completely explain, the authors hypothesised that this association might limit targeted therapy response. Recent findings showed that co-mutations can occur in several druggable genes, as well, including MET deregulation, BRAF mutations, HER2 amplification and gene fusions. Despite the encouraging results from dual TKI inhibition with EGFR TKIs. Interestingly, a recent work by Hastings et al showed that EGFR L85R and G719 tumours have higher TMB compared with EGFR del19 tumours, consistently with the finding of worse outcome with ICIs of EGFR del19 tumours compared with EGFR WT.

Conversely, PD-L1 expression shows an opposite behaviour than TMB in EGFR-mutant NSCLC, as it is frequently highly expressed in oncogene-addicted tumours, both at preclinical and clinical level. This finding is apparently in contrast with the data showing an increase in response to immunotherapy with the increasing levels of PD-L1. Indeed, PD-L1 expression in EGFR-mutant cells is the result of signalling pathways that are activated downstream of the receptor tyrosine kinase (RTK). Phosphoinositide 3-kinase/AKT pathway, as well as mitogen-activated protein kinase and signal transducer and activator of transcription 3 (STAT3) through Src and Src-homology region 2 domain-containing phosphatase-2, can induce upregulation of PD-L1. EGFR inhibition with EGFR TKIs decreases PD-L1 levels, which are restored at the occurrence of TKI resistance. Since EGFR TKI resistance is commonly mediated by the activation of other RTKs and downstream mediators, a profound role of cross-talking pathways and signalling molecules as immune modulators are emerging and attempts to combine TKIs and immunotherapy are currently ongoing. The immune features of EGFR-mutant cells are also strongly dependent on the TME. EGFR-mutant tumours
show a complex interaction with the TME, leading to an increase in T regulatory cells (T regs), decrease in tumour infiltrating lymphocytes and downregulation of major histocompatibility complex.83

IFNg, secreted by immune infiltrating cells, modulates STAT3/STAT1 balance through Janus kinase and, consequently, mediates PD-L1 expression. Moreover, through the activation of cyclin-dependent kinase 5, it also inhibits the activity of PD-L1 repressors.84 85 In EGFR-mutant cells, where the activity of STAT3 is crucial for survival, the ability of IFNg of modulating STAT3 plays an essential role in immune modulation.

Also, VEGF is essential in EGFR-mutant NSCLC, not only because of its well-established role in EGFR–VEGF cross-talk, alteration affecting peritumoral and intratumoral vascularisation and consequently drug delivery impairment and EGFR TKI resistance.86 Indeed, VEGF is responsible for intratumoral, clonal, immune and clinical scenario. The described mechanisms are thus at the basis of the dynamic immune modulation are thus at the basis of the dynamic immune modulating drugs.

The complex mechanisms of EGFR-mediated immune modulation are thus at the basis of the dynamic immune heterogeneity in EGFR-mutant NSCLC, which reflects the influence of EGFR-activating status in different moments of the lung cancer disease, EGFR TKI-naïve, TKI treatment, TKI resistance and subsequent treatments.

CONCLUSIONS

Several distinct features contribute to heterogeneity in EGFR-positive lung tumours. Tumour clonality affects intratumoral heterogeneity, whereas the biodiversity of EGFR mutations and the presence of co-mutations have an impact also on immune heterogeneity and clinical heterogeneity. Indeed, specific subtypes of EGFR mutations determine different patterns of response to EGFR TKIs, ranging from high and prolonged sensitivity to minimal or no benefit. The presence of co-occurring mutations can reduce EGFR TKI activity driving earlier resistance to EGFR inhibition sustained by the selection of resistant cell clones. On the other hand, the presence of co-mutations may increase TMB, therefore influencing tumour immunogenicity and subsequent potential efficacy of immune-modulating drugs.

In parallel, TME is dynamically influenced by EGFR signalling pathways. Consequently, it may substantially differ at different tumour sites, not only due to intrinsic organ-specific features but also as a reflection of EGFR tumour clonality across metastatic sites.

Current clinical standard of care in EGFR-mutant lung cancer is barely able to face this complex biological and clinical scenario. The described mechanisms responsible for intratumoral, clonal, immune and clinical heterogeneity are not easy to assess in a comprehensive and dynamic study. In our view, a multilevel diagnostic approach based on both tissue and blood next-generation sequencing should always be considered, when available, to obtain a more comprehensive snapshot of EGFR-mutant disease. The application of such a systematic and dynamic approach including repeated tissue and liquid biopsies at disease progression could be a highly effective bench-to-bedside method, with the potentiality to better select treatments according to specific features and correlates of EGFR heterogeneity.

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REFERENCES

1 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019;69:7–34.
2 Mok TS, Wu Y-L, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonaryadenocarcinoma. N Engl J Med 2009;361:947–57.
3 Rosell R, Carcereny E, Gervais R, et al. 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 2012;13:339–46.
4 Sequist LV, Yang J-C, Yamamoto N, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J Clin Oncol 2013;31:3327–34.
5 Mok TS, Wu Y-L, Ahn M-J, et al. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. N Engl J Med 2017;376:629–40.
Soria J-C, Ohe Y, Vansteenkiste J, et al. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. N Engl J Med 2018;378:113–25.

Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129–39.

D’Angelo SP, Pietanza MC, Johnson ML, et al. Incidence of EGFR exon 19 deletions and L858R in tumor specimens from men and cigarette smokers with lung adenocarcinomas. J Clin Oncol 2011;29:2060–7.

Midha A, Dearden S, McCormack R. Egfr mutation incidence in non-small-cell lung cancer of adenocarcinoma histology: a systematic review and global map by ethnicity (mutMap). Am J Cancer Research 2015;5:2892–911.

Lindeman NI, Cagle PT, Aisner DL, et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the College of American pathologists, the International association for the study of lung cancer, and the association for molecular pathology. Arch Pathol Lab Med 2011;135:226–45.

Kalemkerian GP, Narula N, Kennedy EB, et al. Molecular testing guideline for the selection of patients with lung cancer for treatment with targeted tyrosine kinase inhibitors: American Society of Clinical oncology endorsement of the College of American Pathologists International association for the study of lung Cancer/Association for molecular pathology clinical practice guideline update. JCO 2018;36:911–9.

Ettinger DS, Aisner DL, Wood DE, David SE, Dara LA, Douglas EW, et al. NCCN guidelines insights: non-small cell lung cancer, version 5.2018. J Natl Compr Canc Netw 2018;16:807–21.

Chen Z-Y, Zhong W-Z, Zhang X-C, et al. Egfr mutation heterogeneity and the mixed response to EGFR tyrosine kinase inhibitors of lung adenocarcinomas. Oncologist 2012;17:978–85.

Taniguchi K, Okami J, Kodama K, et al. Intratumor heterogeneity of epidermal growth factor receptor mutations in lung cancer and its correlation to the response to gefitinib. Cancer Sci 2008;99:929–35.

Yatabe Y, Matsu k0, Mitsumoto T. Heterogeneous distribution of EGFR mutations is extremely rare in lung adenocarcinoma. J Clin Oncol 2011;29:2972–7.

Kohsaka S, Petronczki M, Solca F, et al. Tumor clonality and resistance mechanisms in EGFR mutation-positive non-small-cell lung cancer; implications for therapeutic sequencing. Future Oncol 2015;11:1037–50.

Gridelli C, Rossi A, Carbone DP, et al. Non-small-cell lung cancer, Nat Rev Dis Primers 2015;1:15009.

Del Re M, Arrigoni E, Restante G, et al. Intratumor heterogeneity of EGFR mutations in non-small-cell lung cancer: focus on clinical pharmacology and mechanisms of resistance. Pharmacogenomics 2019;20:1255–7.

McDermott U, Sharma SV, Settleman J. High-Throughput lung cancer cell line screening for genotype-correlated sensitivity to an EGFR kinase inhibitor. Methods Enzymol 2008;438:331–41.

Paez JG, Jänne PA, Lee JC, et al. Egfr mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497–500.

Wu J-Y, Yu C-J, Chang Y-C, et al. Effectiveness of tyrosine kinase inhibitors on "uncommon" epidermal growth factor receptor mutations of unknown clinical significance in non-small-cell lung cancer. Clin Cancer Res 2011;17:3812–21.

Beau-Faller M, Prim N, Ruppert A-M, et al. Rare EGFR exon 18 and exon 20 mutations in non-small-cell lung cancer on 1017 patients: a multicentre observational study by the French EMERIC-IFCT network. Ann Oncol 2014;25:126–31.

Kobayashi Y, Togashi Y, Yatabe Y, et al. Egfr exon 18 mutations in lung cancer: molecular predictors of augmented sensitivity to afatinib or neratinib as compared with first- or third-generation TKIs. Clin Cancer Res 2015;21:5305–13.

Massarelli E, Johnson HM, Erickson HS, et al. Uncommon epidermal growth factor receptor mutations in non-small cell lung cancer and their mechanisms of EGFR tyrosine kinase inhibitors sensitivity and resistance. Lung Cancer 2013;80:23–41.

Tian Y, Zhao J, Ren P, et al. Different subtypes of EGFR exon19 mutation can affect prognosis of patients with non-small cell lung adenocarcinoma. PLoS One 2018;13:e0201682.

Chung K-P, Wu S-J, Wu J-Y, et al. Clinical outcomes in non-small-cell lung cancers harboring different exon 19 deletions in EGFR. Clin Cancer Res 2012;18:3470–7.

Su J, Zhong W, Zhang X, et al. Molecular characteristics and clinical outcomes of EGFR exon 19 indel subtypes to EGFR TKIs in NSCLC patients. Oncotarget 2017;8:111246–57.

Vyas S, Huang PH, Tan SH, et al. EGFR exon 20 insertion mutations in non-small cell lung cancer. Signal Transduct Target Ther 2019;4:5.

Westover D, Zuzaga-zeltz J, Cho BC, et al. Mechanisms of acquired resistance to first- and second-generation EGFR tyrosine kinase inhibitors. Ann Oncol 2018;29:10–19.

Reale G, Giovannetti E, Hong DS, et al. Novel therapeutic strategies for patients with NSCLC who do not respond to treatment with EGFR inhibitors. Cancer Treat Rev 2014;40:990–1004.

Kosaka T, Yatabe Y, Endoh H, et al. Mutations of the epidermal growth factor receptor gene in lung cancer: high incidence of rare and specific EGFR mutations in Japanese patients. Cancer Res 2004;64:8919–23.

Bell DW, Gore I, Okimoto RA, et al. Inherited susceptibility to lung cancer may be associated with the T790M drug resistance mutation in EGFR. Nat Genet 2005;37:1315–6.

Osmond GR, Miller VA, Robson ME, et al. Screening for germline EGFR T790M mutations through lung cancer genotyping. J Thorac Oncol 2012;7:1049–52.
Acquired EGFR C797S mutations in non-small cell lung cancer: new evidence and treatment. *Transl Lung Cancer Res* 2019;8:302–16.

Greenman C, Stephens P, Smith R, et al. Patterns of somatic mutation in human cancer genomes. *Nature* 2007;446:153–8.

Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. *Nature* 2013;500:415–21.

Staaf J, Jönsson G, Jönsson M, et al. Immunotherapy biomarker in oncogene-addicted non-small cell lung cancer: new evidence and treatment. *Transl Lung Cancer Res* 2019;8:302–16.

Lee CK, Man J, Lord S, et al. Checkpoint inhibitors in metastatic EGFR-mutated non-small-cell lung cancer—a meta-analysis. *J Thorac Oncol* 2017;12:403–7.

Lee CK, Man J, Lord S, et al. Checkpoint inhibitors in metastatic EGFR-mutated non-small-cell lung cancer—a meta-analysis. *J Thorac Oncol* 2017;12:403–7.

Sequist LV, Han J-Y, Ahn M-J, et al. 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* 2020;21:373–86.

Garassino MC, Cho B-C, Kim J-H, et al. Durvalumab as third-line or later treatment for advanced non-small-cell lung cancer (CheckMate 817): an open-label, single-arm, phase 2 study. *Lancet Oncol* 2018;19:521–36.

Lee CK, Man J, Lord S, et al. Checkpoint inhibitors in metastatic EGFR-mutated non-small-cell lung cancer—a meta-analysis. *J Thorac Oncol* 2017;12:403–7.

Sequist LV, Han J-Y, Ahn M-J, et al. 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* 2020;21:373–86.

Garassino MC, Cho B-C, Kim J-H, et al. Durvalumab as third-line or later treatment for advanced non-small-cell lung cancer (CheckMate 817): an open-label, single-arm, phase 2 study. *Lancet Oncol* 2018;19:521–36.

Lee CK, Man J, Lord S, et al. Checkpoint inhibitors in metastatic EGFR-mutated non-small-cell lung cancer—a meta-analysis. *J Thorac Oncol* 2017;12:403–7.

Sequist LV, Han J-Y, Ahn M-J, et al. 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* 2020;21:373–86.

Garassino MC, Cho B-C, Kim J-H, et al. Durvalumab as third-line or later treatment for advanced non-small-cell lung cancer (CheckMate 817): an open-label, single-arm, phase 2 study. *Lancet Oncol* 2018;19:521–36.

Lee CK, Man J, Lord S, et al. Checkpoint inhibitors in metastatic EGFR-mutated non-small-cell lung cancer—a meta-analysis. *J Thorac Oncol* 2017;12:403–7.