Introduction

Cancer remains a major cause of mortality throughout the world despite extraordinary efforts over numerous decades to develop effective therapeutic interventions. Importantly, however, our understanding of the impact of genetic, epigenetic, and Darwinian adaptive evolutionary processes that concurrently impact cellular adaptation and the development of malignancies has dramatically improved (1). Hanahan and Weinberg initially proposed six “hallmarks of cancer” that enable tumor growth and metastatic dissemination which have been updated to include additional hallmarks as our understanding of cancer has improved (2). These characteristics can be acquired longitudinally in different sequences and by different mechanisms including mutations in DNA during replication, in repair machinery, and by exposure to mutagens. Hence, malignancies are a heterogeneous group of aberrant cells with dysregulation of a set of core pathways (3). Non-small cell lung cancer (NSCLC) has become a model for understanding the intricacy of how complex genomic alterations, pathway dysregulations and external selective pressures drive tumorigenesis, evolution, and provide targets for therapeutic intervention. Here, we review the basic principles of cancer evolution as has been demonstrated in NSCLC as well as our understanding of how epidermal growth factor receptor (EGFR) oncogene driven NSCLC evolves.

The genetic basis of lung cancer

Early studies in human cancer genomes were limited to evaluating the sequential somatic mutations in specific oncogenes and tumor-suppressor genes (4). Studies.
analyzing lung cancer development, identified loss of heterozygosity at chromosomal regions that encode tumor-suppressor genes at 3p21.3 (RASSF1A), 3p14.2 (FHIT), 9p21 (p16), and 17p13 (p53) as early events during the development of NSCLC (5). Further, the mutational landscape in the kinomes of multiple cancers, including lung, colon, and breast tumors among others, was analyzed and demonstrated that the majority of somatic mutations in cancer are “passenger” mutations that do not contribute to oncogenesis (6-12). When expanded to genome-wide sequencing, studies similarly demonstrated that tumors harbor thousands of genetic and epigenetic alterations that are not present in germline DNA of which only a very small fraction are oncogenic “driver genes” (13). These oncogenic driver genes function through a limited number of routes that regulate growth and survival pathways critical for oncogenesis (14,15). This is true for multiple cancer subtypes; however, lung cancer has become a paradigm of the power of utilizing targeted therapy to block these oncogenic pathways with the identification of EGFR, and other, activating mutations. The targetability of activating mutations in EGFR with tyrosine kinase inhibitor (TKI) was first demonstrated in 2004 (16-18). This was followed by the identification of rearrangements of the anaplastic lymphoma kinase (ALK) gene, which are uniquely sensitive to ALK TKIs and affect approximately 6% of NSCLC (19,20). In short succession, multiple other oncogenic drivers have been identified, most of which have targeted therapies that are commercially available or in clinical trial. These include more immediately targetable activating mutations such as BRAF (21,22), ROSI (23,24), neurotrophic receptor tyrosine kinase (NTRK) gene fusion (25,26), mesenchymal-epithelial transition factor (MET) amplification (27), human epidermal growth factor receptor 2 (HER2/ERBB2) (28-30), and translocations in RET (31-35); as well as those for which targeted therapy development has been more challenging such as Kirsten rat sarcoma virus oncogene homolog (KRAS) (36-38), and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PI3CA) (39).

Previously, lung cancer had been thought to develop primarily due to carcinogen exposure from tobacco smoking (40). However, 25% of all lung cancer cases worldwide occur in never-smokers, representing 15% of lung cancers in men and 53% in women (40). Studies comparing the genomic landscape of NSCLC in never-smokers versus smokers have found several significant differences among these: (I) 10-fold higher mutation frequencies observed in smokers; (II) different mutation spectrum between smokers (C-G → A:T) and never-smokers (C-G → T:A); and (III) distinctive sets of mutations identified in never-smokers (EGFR mutations and ROSI and ALK fusions) and smokers (KRAS, TP53, BRAF, JAK2, and JAK3 and mismatch repair gene mutations) (41). Among these mutations, oncogenic mutations in the EGFR kinase domain occur early in the development of NSCLC in never-smokers, and KRAS mutations occur early in the development of smoking-related NSCLC (42,43). The discovery of these genomic alterations, pathway dysregulations, and external selective pressures that drive NSCLC tumorigenesis and evolution have become a model for understanding cancer development and the development of therapeutic targets.

**Oncogenic EGFR mutations in lung cancer**

Based on two large comprehensive analyses of lung cancer, the Lung Cancer Mutation Consortium (LCMC) and the Cancer Genome Atlas (TCGA), it is known that almost 62% of patients with NSCLC harbor an oncogenic driver mutation (LCMC: 622/1,007; TCGA: 143/230) (44-46). Among those with oncogenic alterations, only approximately half have a therapeutically targetable lesion (44-46). These showed that KRAS is the most common oncogenic mutation occurring in 25–32% of patients (44-46). Somatic activating mutations in EGFR are the second most common driver mutation, occurring in 11–15% of all lung cancer patients (44-47). EGFR mutations occur with increased frequency in women, never smokers, and those of East Asian ethnicity, affecting 30–50% of patients with NSCLC in East Asia (48).

EGFR mutations can occur anywhere within the tyrosine kinase domain, however those associated with responses to TKI therapy are observed in exons 18–21. *In vitro* studies have identified 21 EGFR activating mutations, among 7,216 possible randomly mutated EGFR single-nucleotide variants (49). Clinically, the most common EGFR alterations are exon 19 deletions and the exon 21 L858R substitutions, which account for approximately 90% of EGFR mutations (50). Less common EGFR activating mutations include exon 20 insertions and exon 18-point mutations, which represent 1–17% and 3–4% of all EGFR mutations, respectively (50-58). These alterations lead to activation of the EGFR receptor and subsequent downstream activation of Ras/Raf/RAF-MAPK, PI3K-AKT-mTOR, and JAK-STAT signaling pathways, which induce cellular proliferation, apoptosis, angiogenesis, invasion, and metastasis (42,59-62). Patients with EGFR-mutant NSCLC often respond to first-
generation non-covalent EGFR TKI drugs such as gefitinib and erlotinib (16-18), but usually develop drug resistance within 9–12 months (63,64). Third generation TKIs such as osimertinib have a response rate of ~80% with an 18.9-month progression free survival (65). Importantly, the efficacy of EGFR TKIs varies among specific alterations, EGFR exon 19 deletions and EGFR L858R mutations in exon 21 show high rates of EGFR TKIs response versus EGFR exon 20 insertions which are associated with poor response to EGFR TKIs (53,66-68). Hence, comprehensive molecular genetic testing has become vital to the management of advanced NSCLC since the recognition of the predictive benefit of specific targeted agents varies among different oncogenic-driven tumors.

Lung cancer heterogeneity and evolution

One of the critical components to understanding tumor evolution is to recognize the impact of tumor heterogeneity. Several large-scale data monitoring projects in different tumor subtypes have characterized and tracked intra-tumoral heterogeneity. A landmark study which demonstrated the importance of tumor heterogeneity was Gerlinger and colleagues’ work with metastatic renal cell cancer in which they analyzed tumor tissue from primary and metastatic disease sites within the same patient (69). They found that 63% to 69% of all somatic mutations identified in one sample, were not found in geographically separate samples from the same patient, this demonstrated branched evolutionary tumor growth. In addition, and conversely, they specifically analyzed tumor-suppressor genes, which showed distinct inactivating mutations within a single tumor, revealing convergent phenotypic evolution (69). Their work also revealed that heterogeneity was present in all levels of analysis: genome, transcriptome, and proteome. In lung cancer, multiple groups have attempted to characterize heterogeneity and evolution. Among these, Imielinski and colleagues performed exome and genome sequencing of 183 lung adenocarcinoma tumors and identified novel oncogenic mutations in U2AF1, RBM10 and ARID1A as well novel activating in-frame fusions of EGFR (70). Similarly, the Cancer Genome Atlas molecularly profiled 230 resected lung adenocarcinomas. They identified alterations in NFI, MET, ERBB2 and RIT1 in 13% of cases and showed these alterations were enriched in samples that otherwise lacked an activated oncogene (45). These findings suggested previously unrecognized driver roles for these genetic alterations, highlighting the diversity among tumors and expanding the range of possible targetable alterations.

The Lung Tracking Cancer Evolution through Therapy (TRACERx) program is a translational longitudinal study aimed at understanding the mechanisms of cancer evolution by analyzing intratumoral heterogeneity and tracking its evolution from time of diagnosis to relapse. In their initial analysis with multi-region whole-exome sequencing of 100 early-stage NSCLC patients, they found that a median of 30% of somatic mutations and 48% of copy-number alterations are subclonal, highlighting that genomic-instability processes are ongoing during tumor development (71,72). Also, they observed substantial variation in intratumoral heterogeneity, reporting a wide range of number of subclonal mutations and percentage of the genome (0.06% to 81%) affected by subclonal copy-number alterations (72). When comparing histologic subtypes, squamous-cell carcinomas carried significantly more clonal mutations than adenocarcinomas (P=0.003), potentially reflecting differences in smoking history (72). In adenocarcinomas, higher clonal and subclonal mutational burden was also observed in smokers. Tumor stage also correlated with the proportion of subclonal copy-number alterations in this group. Within this group, high copy-number heterogeneity was associated with an increased risk of recurrence or death (HR =4.9; P=4.4×10^-5), suggesting those patients represent a high-risk group which may require closer monitoring (72). The timeline of genetic alterations was also evaluated, leading to demonstration of early genome doubling and ongoing chromosomal instability as drivers of parallel evolution (72). Driver alterations as EGFR, MET, and BRAF were almost exclusively clonal and occurred early in evolution, compared to heterogeneous subclonal driver alterations in genes as PIK3CA and NFI which occurred later and were found in more than 75% of the tumors (72). These findings highlight the limitations of single diagnostic biopsies in accurately capturing intratumor heterogeneity and evolution.

In a follow-up analysis of Lung TRACERx, the authors explored allele-specific HLA loss and immune escape in the same cohort of patients, HLA loss was present in 40% of early-stage NSCLCs (73). HLA loss is an evolutionarily selected immune escape mechanism that is subject to strong microenvironmental selection pressures later in tumor evolution. It is associated with higher subclonal neoantigen burden, increased nonsynonymous mutations, APOBEC-mediated mutagenesis, upregulation of cytolytic activity, and PD-L1 positivity. Among those with stage IV disease,
Heterogeneity, evolution, and resistance of EGFR-mutant lung cancer

EGFR-mutant NSCLC has become a paradigm for understanding the complexity of how genetic alterations evolve over time and under treatment pressures leading to therapeutic resistance. Resistance to targeted therapies such as EGFR TKIs can develop by multiple mechanisms including: intrinsic resistance, adaptive resistance, and acquired resistance (74). Some tumors exhibit intrinsic resistance to treatment, such as those with EGFR TKIs can develop by multiple mechanisms. Among several recent studies to address these questions, Blakely and colleagues performed genomic analysis of 1,122 ctDNA samples from patients with advanced-stage EGFR-mutant NSCLC at different time points during treatment, compared to 1,008 EGFR-negative advanced-stage NSCLC samples (84). Canonical EGFR mutations commonly co-occurred with subclonal oncogenic driver alterations in PIK3CA, BRAF, MET, MYC, CDK6, AR, and CTNNB1 (84). To further understand the evolution of resistance in EGFR-mutant tumors they analyzed changes in ctDNA under EGFR TKI therapy. Those with progressive disease on first- and second-line TKI had an increased number of detectable somatic alterations which increased with each subsequent line of therapy, regardless of age, sex, or tobacco exposure (84). EGFR T790M and EGFR C797S cases were further analyzed, and demonstrated increased co-occurring genetic alterations and more frequent alterations in cell-cycle and WNT-pathway genes (84). Furthermore, cell-cycle-gene aberrations in CDK4 or CDK6 were associated with shorter progression-free survival (PFS) and overall survival (OS) to EGFR TKIs (84). In summary, they demonstrated that the number of somatic mutations increases during treatment and co-occurring genetic alterations may function as co-drivers of tumor progression and drug resistance leading to increased tumor genomic complexity and genetic diversity that facilitates further tumor evolution, adaptation, and resistance to therapy (84).

Piotrowska and colleagues analyzed tumor samples and circulating tumor DNA (ctDNA) in 12 EGFR T790M positive NSCLC patients who developed resistance to rociletinib (a 3rd generation EGFR TKI). By examining the heterogeneity of tumors and mechanisms of resistance, they observed small cell lung cancer transformation, acquired EGFR amplifications, and, half of tumors acquired T790M-negative NSCLC patients who developed resistance to rocciletinib (a 3rd generation EGFR TKI). By examining the heterogeneity of tumors and mechanisms of resistance, they observed small cell lung cancer transformation, acquired EGFR amplifications, and, half of tumors acquired T790M-wild-type clones as mechanisms of rocciletinib resistance (85). One biopsy showed coexisting T790M-wild-type and T790M-positive clones prior to initiation of therapy. These and other findings demonstrated that tumors are composed of a mixture of cancer clones with different mechanisms of survival, some of which emerge under selective pressures (85). Separately, this group described the longitudinal molecular changes that occurred in a single tumor with an EGFR exon 19 deletion and TP53 V173L metastatic lung adenocarcinoma from diagnosis and through therapy (86). This patient developed small cell transformation during erlotinib therapy, acquiring additional mutations in PIK3CA, ERBB3, and FBXW7. ctDNA profiling after progression on EGFR TKI revealed emergence of EGFR T790M for which osimertinib was initiated. Subsequent ctDNA analysis revealed that the EGFR T790M mutation became undetectable, but there were increases in PIK3CA and EGFR del19 mutations, and development of new ERBB2, PIK3CA, c-MYC, and
FGFR1 amplifications (86). These cases highlighted the role of ctDNA in understanding the complexities of clonal evolution in acquired resistance, and illustrate how resistant subclones fluctuate in response to therapy.

The mechanisms of resistance to third-generation TKIs, such as nazartinib and osimertinib, among tumors harboring EGFR T790M were also described by Piotrowska and colleagues in a report analyzing the molecular profile of 2 patients with EGFR T790M treated with nazartinib (87). In one case, they observed the emergence of a new BRAF V600E subclone with concurrent decline of the EGFR T790M mutant allele fraction suggesting effective inhibition of the dominant EGFR T790M subclone and development of a new mechanism of resistance (87). At disease progression, they observed the emergence of a new EGFR T790M/C797S subclone in addition to the previously identified BRAF V600E-EGFR T790-wild-type subclone (87). This highlights the heterogeneity of EGFR-dependent mechanisms of resistance and coexistence of multiple subclones which emerge at different points under treatment pressure. Similar findings were reported by Le and colleagues who analyzed a cohort of 118 patients with EGFR T790M mutations, 95% had progressed on at least one prior TKI and subsequently developed resistance to osimertinib (82). Molecular profiling showed that acquisition of EGFR C797S and L792 mutations were the most common EGFR-dependent resistance mechanisms and were observed exclusively in those who preserved an EGFR T790M mutation (82).

Other groups also evaluated how tumors evolve under the selective pressures of osimertinib. They identified known mechanisms of acquired resistance previously associated with resistance to first-generation EGFR TKIs such as small cell transformation, MET amplifications, PIK3CA mutations, and BRAF mutations, as well as novel mechanisms specific to third-generation EGFR TKIs such as new mutations, fusions, and/or loss of EGFR T790M (83,88). Third-generation TKIs bind the EGFR C797 location, mutations in this locus within the EGFR gene, confer acquired resistance in 7–20% of patients (88,89-91). Further preclinical and clinical studies have identified that the allelic configuration (cis versus trans) of co-occurring EGFR C797S and EGFR T790M mutations may have therapeutic implications, with those in trans configuration having responses to combination of first- and third-generation EGFR TKIs (92,93). Other mechanisms of osimertinib resistance include RET, FGFR3, and BRAF fusions, as well as mutations in KRAS Q61K and EGFR G796D and MET amplifications (83,94). As previously shown by Blakely and colleagues, alterations of cell-cycle genes had prognostic implications and were associated with shorter PFS. Ultimately, these studies illustrate the critical role of heterogeneity in cancer growth and resistance, as well as, the therapeutic challenges of the available targeted-therapies in controlling tumor growth while tumor cells undergo dynamic evolution under treatment pressure leading to the survival of selected oncogenic subclones that drive resistance.

As noted above, alternate resistance mechanisms include activation of complementary signaling pathways, concurrent alterations to other oncogenic genes (as described above), transformation to small cell histology (95), and epithelial to mesenchymal transition (EMT). An elegant example of the activation of alternative signaling pathways was demonstrated in recent work by Shah and colleagues who identified Aurora kinase A (AURKA) as a mediator of non-genetic acquired resistance to third-generation TKIs (96). Persistent EGFR inhibition leads to activation of AURKA by its co-activator TPX2, this activation is maintained in drug-tolerant cells and those with acquired resistance (96). AURKA mitigates drug-induced apoptosis and contributes to pathways associated with resistance to EGFR inhibition, including NF-κB, extracellular-signal-regulated kinase (ERK), and EMT (96). In addition, based on preclinical studies the combination of EGFR TKIs and Aurora kinase inhibitors suppresses this adaptive resistance mechanism, and enhance the initial response to EGFR inhibitor thus, forestall acquired resistance (96). Our evolving understanding of heterogeneity and evolution of resistance in advanced NSCLC highlights the importance of developing strategies to prevent and identify earlier mechanisms of resistance as well as clinical trial strategies that can overcome multiple concomitant resistance mechanisms.

Epigenetics of NSCLC and EGFR resistance

Epigenetic dysregulation has been identified as a critical factor in tumorigenicity and heterogeneity, and understanding mechanisms of resistance (97-99). Commonly recognized epigenetic mechanisms that can promote or inhibit tumor cell growth include DNA methylation, histone or chromatin modifications, and dysregulation of miRNAs. In NSCLC, resistant cancer cells have been shown to develop after aberrant promoter methylation of CDKN2A (100), MLH1 and MSH2 (101), APC (102), RARB (103), and MGMT (104)
under therapy. Resistance acquired by drug exposure may be reversed after prolonged drug withdrawal or with epigenetic therapy, as histone deacetylase (HDAC) inhibitors and DNA methyltransferase inhibitors (DNMTi), which may re-sensitize NSCLC cells to targeted therapy or chemotherapy (99). HDAC inhibitors have been shown to upregulate tumor suppressor genes involved in apoptotic pathways, as TRAIL and DR5, and inhibit the expression of pro-survival genes as BCL2 (105-110). In pre-clinical models and early clinical trials, HDAC inhibitors, such as panobinostat, in combination with EGFR TKIs have shown some signal for overcoming resistance to EGFR TKI resistance (111-116). Interestingly, HDAC inhibitors can also increase immune activation by upregulating MHC I and II expression (105). This has led to multiple trials assessing the safety of HDAC inhibitors in combination with immune checkpoint inhibitors [ClinicalTrials.gov identifier: NCT02437136 (117), NCT02954991 (118), NCT03233724 (119), NCT02638090 (120), NCT03590054 (121), NCT02635061 (122), NCT02805660 (123)].

The epithelial-to-mesenchymal transition (EMT) is another example of a complex adaptive, epigenetic process in which transcription factors such as TGF-β induce the conversion of cells from epithelial to mesenchymal state, resulting in increased capacity for cell invasion, migration and drug resistance (124-129). In NSCLC, this is a well-explored mechanism of resistance to apoptotic signaling triggered by cisplatin and acquired resistance to EGFR, ALK and PI3K inhibitors (130-135). Resistant EGFR-mutant NSCLC cells have shown loss of epithelial cell junction proteins such as E-cadherin and TTF-1 and elevation in mesenchymal markers as vimentin, ZEB1, and CD44 (136-141). EMT and reduction in E-cadherin has been described in 20–25% of EGFR TKI resistant cases that lack secondary mutations (95,131,142-144).

miRNAs are another type of epigenetic alterations that can regulate EMT, response and resistance to chemotherapy, radiotherapy, and targeted therapies as EGFR TKIs (145-148). Among these, miR-21 has been shown to induce gefitinib resistance by suppressing PTEN and activating ALK and ERK (149,150). In contrast, upregulation of miR-133b has shown improved outcomes in NSCLC patients treated with erlotinib in the second- or third-line setting (151). Preclinical studies have suggested a therapeutic potential of miRNAs by synergistically sensitizing both EGFR wild-type and mutant NSCLC cells to TKIs (152,153). Few phase I clinical trials have aimed to evaluate miRNAs with varying degrees of tolerability (ClinicalTrials.gov Identifier: NCT02369198; NCT01829971) (154-156). Ultimately, the role of epigenetically directed treatments in constraining NSCLC tumor evolution and development of resistance must be further studied to identify the best therapeutic strategies for clinical implementation.

Immunotherapy and EGFR-mutant NSCLC treatment response and evolution

Immunotherapy (IO) has radically changed the treatment paradigm for patients with stage III and IV NSCLC, providing significant therapeutic benefit to many patients when compared with classical chemotherapy regimens (157-162). However, clinical studies in patients with EGFR-mutant tumors and other targetable oncogenic activating alterations have shown limited benefit with IO and lower response rates when compared to those without oncogenic activating alterations (163). For example, a meta-analysis assessing the role of IO in second line treatment included a subgroup of 186 EGFR-mutant patients with advanced NSCLC and showed no improvement in overall survival with single agent IO when compared chemotherapy, docetaxel (HR =1.05, 95% CI: 0.70–1.55) (164). Similar results were observed in TKI-naïve EGFR-mutant NSCLC patients, in which a phase II trial of single agent pembrolizumab was closed early due to futility (165). Notably, in the majority (70%) of these EGFR-mutant patients, tumor PD-L1 expression was high (PD-L1 ≥50%), suggesting that PD-L1 is not a predictive biomarker in patients with EGFR activating mutations (165).

Pre-clinical studies have suggested that high PD-L1 expression in this population is driven by the EGFR activating mutation and inhibition of EGFR activation by EGFR TKIs reduces PD-L1 expression (166,167). Based on these observations clinical studies aimed to assess the potential synergistic effects of combination of IO and EGFR TKIs in NSCLC therapy. However, multiple clinical trials demonstrated significant increase in grade ≥3 toxicities with combination of IO and EGFR- or ALK-directed TKIs, as well as decreased efficacy when compared to TKI monotherapy (168-174).

Interestingly, a subgroup analysis of the IMPOWER 150 trial including patients with advanced EGFR- or ALK-altered NSCLC who had progressed on TKI therapy showed improved overall survival with combination of chemotherapy, bevacizumab (anti-VEGF), and atezolizumab (anti-PD-L1) when compared to chemotherapy and bevacizumab alone (HR =0.59; 95% CI: 0.37–0.94) (175).
This observation is provocative, given the lack of clinical benefit observed on prior trials assessing the role of IO as 1st line and 2nd line therapy of advanced-stage EGFR-mutant NSCLC (163,165). Further studies are warranted to identify the subgroup of patients who are most likely to benefit from this quadruple combination therapy.

There are multiple ongoing efforts to further define the population of NSCLC patients who benefit from IO, these studies aim to characterize the immune compartment of patient tumors and its interactions with the tumor microenvironment. In a study evaluating EGFR-mutant NSCLC patients who progressed during EGFR TKI therapy and who were T790M negative, PD-L1 expression ≥1% and high density of tumor infiltrating lymphocytes were associated with longer progression free survival with subsequent IO (176). Further studies, have identified that TMB is lower among those with EGFR, ROS-1, or ALK oncogene when compared to wild-type tumors, possibly explaining the lack of benefit observed with IO in oncogene-driven tumors (177-179).

In addition, existing or de novo somatic alterations acquired during EGFR TKI exposure can improve response to IO, such as those impacting MHC functionality and neoantigen presentation, but have not been reported in the existing data (73,180).

Studies analyzing the interactions between tumors and the immune system aim to find factors and pathways that promote immune-escape and tumor growth, as well as identify alternate targets with potential clinical impact. An example of a gene with this potential is the Human Endogenous Retrovirus-H Long Terminal Repeat-Associating Protein 2 gene (HHLA2), which encodes for protein ligand HHLA2 found on the surface of monocytes and is a member of the B7 ligand family that demonstrates T-cell co-inhibitory properties (181). HHLA2 was found to be widely expressed in lung cancer and, importantly, it is highly expressed in EGFR-mutant NSCLC when compared to other lung cancer subtypes (182). Further efforts to identify the role of IO and novel checkpoint inhibitor targets in EGFR-mutant as well as another oncogene-driven NSCLC are needed.

**Conclusions**

Significant discoveries on our understanding of cancer cell growth, progression, and acquisition of drug-resistance highlight the complexity of the genetic, metabolic, environmental, and evolutionary processes that concurrently shape lung cancer evolution. While these discoveries have allowed for the development of targeted therapeutic interventions, they have also highlighted the existence of dynamic evolutionary changes leading to cellular adaptation, and activation of bypass signaling pathways that fuel cancer progression. In NSCLC, understanding of the clonality of EGFR-mutant tumors with co-occurring mutations impacts responses to treatments and how these evolve in response to different therapeutic agents is vital to guide treatment selection and their sequence. Multifaceted analysis of the changing molecular features of the pathways driving NSCLC growth at baseline and throughout the course of therapy is required to take into account tumor evolution and development of drug resistance. As additional therapeutic targets are identified and novel therapies are developed, reliable and accessible tools are needed to monitor and capture tumor heterogeneity and its evolution over time.

**Acknowledgments**

**Funding:** None.

**Footnote**

**Provenance and Peer Review:** This article was commissioned by the Guest Editors (Trever G. Bivona) for the series “Mechanisms of Resistance to EGFR-targeted Therapy” published in *Journal of Thoracic Disease*. The article was sent for external peer review organized by the Guest Editor and the editorial office.

**Conflicts of Interest:** Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jtd.2019.08.31). The series “Mechanisms of Resistance to EGFR-targeted Therapy” was commissioned by the editorial office without any funding or sponsorship. AIV reports grants from ASCO & Niarchos Foundation, stocks from Portola Pharmaceuticals, Corbus Pharmaceuticals, Midatech, BioNTech SE, and Moderna, and an immediate family member is a contractor for Johnson & Johnson Innovation, outside the submitted work. CEM reports grants and personal fees from Novartis, grants from Revolution Medicines, personal fees from Genentech, personal fees from Guardant Health, outside the submitted work. The other authors have no other conflicts of interest to declare.

**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related
to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

**Open Access Statement:** This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: https://creativecommons.org/licenses/by-nc-nd/4.0/.

**References**

1. Stratton MR. Exploring the genomes of cancer cells: progress and promise. Science 2011;331:1553-8.
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-74.
3. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nat Med 2004;10:789-99.
4. Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. N Engl J Med 1988;319:525-32.
5. Wistuba II, Mao L, Gazzard AF. Smoking molecular damage in bronchial epithelium. Oncogene 2002;21:7298-306.
6. Bardelli A, Parsons DW, Silliman N, et al. Mutational analysis of the tyrosine kinase in colorectal cancers. Science 2003;300:949.
7. Bignell G, Smith R, Hunter C, et al. Sequence analysis of the protein kinase gene family in human testicular germ-cell tumors of adolescents and adults. Genes Chromosomes Cancer 2006;45:42-6.
8. Greenman C, Stephens P, Smith R, et al. Patterns of somatic mutation in human cancer genomes. Nature 2007;446:153-8.
9. Hunter C, Smith R, Cahill DP, et al. A hypermutation phenotype and somatic MSH6 mutations in recurrent human malignant gliomas after alkylator chemotherapy. Cancer Res 2006;66:3987-91.
10. Stephens P, Edkins S, Davies H, et al. A screen of the complete protein kinase gene family identifies diverse patterns of somatic mutations in human breast cancer. Nat Genet 2005;37:590-2.
11. Weir BA, Woo MS, Getz G, et al. Characterizing the cancer genome in lung adenocarcinoma. Nature 2007;450:893-8.
12. Davies H, Hunter C, Smith R, et al. Somatic mutations of the protein kinase gene family in human lung cancer. Cancer Res 2005;65:7591-5.
13. Vogelstein B, Kinzler KW. The Path to Cancer --Three Strikes and You're Out. N Engl J Med 2015;373:1895-8.
14. Ding L, Getz G, Wheeler DA, et al. Somatic mutations affect key pathways in lung adenocarcinoma. Nature 2008;455:1069-75.
15. Kan Z, Jaiswal BS, Stinson J, et al. Diverse somatic mutation patterns and pathway alterations in human cancers. Nature 2010;466:869-73.
16. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497-500.
17. Pao W, Miller V, Zakowski M, et al. EGFR receptor gene mutations are common in lung cancers from “never smokers” and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 2004;101:13306-11.
18. 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.
19. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363:1693-703.
20. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007;448:561-6.
21. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature 2002;417:949-54.
22. Brose MS, Volpe P, Feldman M, et al. BRAF and RAS mutations in human lung cancer and melanoma. Cancer Res 2002;62:6997-7000.
23. Rikova K, Guo A, Zeng Q, et al. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell 2007;131:1190-203.
24. Bergethon K, Shaw AT, Ou SH, et al. ROS1 rearrangements define a unique molecular class of lung cancers. J Clin Oncol 2012;30:863-70.
25. Vaishnavi A, Capelletti M, Le AT, et al. Oncogenic and drug-sensitive NTRK1 rearrangements in lung cancer. Nat Med 2013;19:1469-72.
26. Cocco E, Scaltriti M, Drilon A. NTRK fusion-positive cancers and TRK inhibitor therapy. Nat Rev Clin Oncol 2018;15:731-47.
27. Go H, Jeon YK, Park HJ, et al. High MET gene copy number leads to shorter survival in patients with non-small cell lung cancer. J Thorac Oncol 2010;5:305-13.
28. Cappuzzo F, Varella-Garcia M, Shigematsu H, et al.
Increased HER2 gene copy number is associated with response to gefitinib therapy in epidermal growth factor receptor-positive non-small-cell lung cancer patients. J Clin Oncol 2005;23:5007-18.
29. Shigematsu H, Takahashi T, Nomura M, et al. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. Cancer Res 2005;65:1642-6.
30. Hotta K, Aoe K, Kozuki T, et al. A Phase II Study of Trastuzumab Emtansine in HER2-Positive Non-Small Cell Lung Cancer. J Thorac Oncol 2018;13:273-9.
31. Ju YS, Lee WC, Shin JY, et al. A transforming KIF5B and RET gene fusion in lung adenocarcinoma revealed from whole-genome and transcriptome sequencing. Genome Res 2012;22:436-45.
32. Lipson D, Capelletti M, Yelensky R, et al. Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies. Nat Med 2012;18:382-4.
33. Wang R, Hu H, Pan Y, et al. RET fusions define a unique molecular and clinicopathologic subtype of non-small-cell lung cancer. J Clin Oncol 2012;30:4352-9.
34. Li GG, Somwar R, Joseph J, et al. Antitumor Activity of RXDX-105 in Multiple Cancer Types with RET Rearrangements or Mutations. Clin Cancer Res 2017;23:2981-90.
35. Drilon A, Fu S, Patel MR, et al. A Phase I/Ib Trial of the VEGFR-Sparing Multikinase RET Inhibitor RXDX-105. Cancer Discov 2019;9:384-95.
36. Tam IY, Chung LP, Suen WS, et al. Distinct epidermal growth factor receptor and KRAS mutation patterns in non-small cell lung cancer patients with different tobacco exposure and clinicopathologic features. Clin Cancer Res 2006;12:1647-53.
37. Zaman A, Bivona TG. Emerging application of genomics-guided therapeutics in personalized lung cancer treatment. Ann Transl Med 2018;6:160.
38. Nichols RJ, Haderk F, Stahlhut C, et al. RAS nucleotide cycling underlies the SHP2 phosphatase dependence of mutant BRAF-, NF1- and RAS-driven cancers. Nat Cell Biol 2018;20:1064-73.
39. Misale S, Fatherree JP, Cortez E, et al. KRAS G12C NSCLC Models Are Sensitive to Direct Targeting of KRAS in Combination with PI3K Inhibition. Clin Cancer Res 2019;25:796-807.
40. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87-108.
41. Govindan R, Ding L, Griffith M, et al. Genomic landscape of non-small cell lung cancer in smokers and never-smokers. Cell 2012;150:1121-34.
42. Tang X, Shigematsu H, Bekele BN, et al. EGFR tyrosine kinase domain mutations are detected in histologically normal respiratory epithelium in lung cancer patients. Cancer Res 2005;65:7568-72.
43. Westra WH. Early glandular neoplasia of the lung. Respir Res 2000;1:163-9.
44. Cancer Genome Atlas Research N. Comprehensive genomic characterization of squamous cell lung cancers. Nature 2012;489:519-25.
45. Cancer Genome Atlas Research N. Comprehensive molecular profiling of lung adenocarcinoma. Nature 2014;511:543-50.
46. Sholl LM, Aisner DL, Varella-Garcia M, et al. Multi-institutional Oncogenic Driver Mutation Analysis in Lung Adenocarcinoma: The Lung Cancer Mutation Consortium Experience. J Thorac Oncol 2015;10:768-77.
47. Rosell R, Moran T, Queralt C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 2009;361:958-67.
48. Sequist LV, Bell DW, Lynch TJ, et al. Molecular predictors of response to epidermal growth factor receptor antagonists in non-small-cell lung cancer. J Clin Oncol 2007;25:587-95.
49. Chakroborty D, Kurppa KJ, Patero I, et al. An unbiased in vitro screen for activating epidermal growth factor receptor mutations. J Biol Chem 2019;294:9377-89.
50. Mitsudomi T, Yatabe Y. Epidermal growth factor receptor in relation to tumor development: EGFR gene and cancer. FEBS J 2010;277:301-8.
51. O’Kane GM, Bradbury PA, Feld R, et al. Uncommon EGFR mutations in advanced non-small cell lung cancer. Lung Cancer 2017;109:137-44.
52. Beau-Faller M, Prim N, Ruppert AM, et al. Rare EGFR exon 18 and exon 20 mutations in non-small-cell lung cancer on 10 117 patients: a multicentre observational study by the French ERMETIC-IFCT network. Ann Oncol 2014;25:126-31.
53. Wu JY, Wu SG, Yang CH, et al. Lung cancer with epidermal growth factor receptor exon 20 mutations is associated with poor gefitinib treatment response. Clin Cancer Res 2008;14:4877-82.
54. Arcila ME, Nafa K, Chaft JE, et al. EGFR exon 20 insertion mutations in lung adenocarcinomas: prevalence, molecular heterogeneity, and clinicopathologic characteristics. Mol Cancer Ther 2013;12:220-9.
55. Massarelli E, Johnson FM, 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
2905

56. Oxnard GR, Lo PC, Nishino M, et al. Natural history and molecular characteristics of lung cancers harboring EGFR exon 20 insertions. J Thorac Oncol 2013;8:179-84.

57. Yeh P, Chen H, Andrews J, et al. DNA-Mutation Inventory to Refine and Enhance Cancer Treatment (DIRECT): a catalog of clinically relevant cancer mutations to enable genome-directed anticancer therapy. Clin Cancer Res 2013;19:1894-901.

58. Wu JY, Yu CJ, Chang YC, 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.

59. Scaldati M, Baselga J. The epidermal growth factor receptor pathway: a model for targeted therapy. Clin Cancer Res 2006;12:5268-72.

60. Weihsa Z, Tsan R, Huang WC, et al. Survival of cancer cells is maintained by EGFR independent of its kinase activity. Cancer Cell 2008;13:385-93.

61. Sun S, Schiller JH, Gazdar AF. Lung cancer in never smokers—a different disease. Nat Rev Cancer 2007;7:778-90.

62. Sato M, Shames DS, Gazdar AF, et al. A translational view of the molecular pathogenesis of lung cancer. J Thorac Oncol 2007;2:327-43.

63. Pao W, Miller VA, Politi KA, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2005;2:e73.

64. Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med 2005;352:786-92.

65. Soria JC, Ohe Y, Vansteenkiste J, et al. Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. N Engl J Med 2018;378:2109-21.

66. Kim JY, Lee WJ, Park HY, et al. Differential MicroRNA Expression between EGFR T790M and L858R Mutated Lung Cancer. J Pathol Transl Med 2018;52:275-82.

67. Yang JC, Ahn MJ, Kim DW, et al. Osimertinib in Pretreated T790M-Positive Advanced Non-Small-Cell Lung Cancer: AURA Study Phase II Extension Component. J Clin Oncol 2017;35:1288-96.

68. Yun CH, Mengwasser KE, Toms AV, et al. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. Proc Natl Acad Sci U S A 2008;105:2070-5.

69. Lee X, Puri S, Negrao MV, et al. Landscape of EGFR-Dependent and -Independent Resistance Mechanisms to Osimertinib and Continuation Therapy Beyond Progression in EGFR-Mutant NSCLC. Clin Cancer Res 2013;80:235-41.
83. Oxnard GR, Hu Y, Mileham KE, et al. Assessment of Resistance Mechanisms and Clinical Implications in Patients With EGFR T790M-Positive Lung Cancer and Acquired Resistance to Osimertinib. JAMA Oncol 2018;4:1527-34.

84. Blakely CM, Watkins TBK, Wu W, et al. Evolution and clinical impact of co-occurring genetic alterations in advanced-stage EGFR-mutant lung cancers. Nat Genet 2017;49:1693-704.

85. Piotrowska Z, Niederst MJ, Karlovich CA, et al. Heterogeneity Underlies the Emergence of EGFR T790M Wild-Type Clones Following Treatment of T790M-Positive Cancers with a Third-Generation EGFR Inhibitor. Cancer Discov 2015;5:713-22.

86. Mooradian MJ, Piotrowska Z, Drapkin BJ, et al. Clonal Evolution and the Role of Serial Liquid Biopsies in a Case of Small-Cell Lung Cancer-Transformed EGFR Mutant Non-Small-Cell Lung Cancer. JCO Precis Oncol 2017. doi: 10.1200/PO.17.00123.

87. Piotrowska Z, Hazar-Rethinam M, Rizzo C, et al. Heterogeneity and Coexistence of T790M and T790 Wild-Type Resistant Subclones Drive Mixed Response to Third-Generation Epidermal Growth Factor Receptor Inhibitors in Lung Cancer. JCO Precis Oncol 2018;2018.

88. Yu HA, Tian SK, Drilon AE, et al. 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 2015;1:982-4.

89. Papadimitrakopoulou VA, Wu YL, Han JY, et al. Analysis of resistance mechanisms to osimertinib in patients with EGFR T790M advanced NSCLC from the AURA3 study. Ann Oncol 2018;29:LBA51.

90. Ramalingam SS, Cheng Y, Zhou C, et al. Mechanisms of acquired resistance to first-line osimertinib: Preliminary data from the phase III FLAURA study. Ann Oncol 2018;29:LBA50.

91. Piotrowska Z, Nagy R, Faireclough S, et al. Characterizing the Genomic Landscape of EGFR C797S in Lung Cancer Using ctDNA Next-Generation Sequencing. J Thorac Oncol 2017;12:S1767.

92. Niederst MJ, Hu H, Mulvey HE, et al. The Allelic Context of the C797S Mutation Acquired upon Treatment with Third-Generation EGFR Inhibitors Impacts Sensitivity to Subsequent Treatment Strategies. Clin Cancer Res 2015;21:3924-33.

93. Zheng D, Hu M, Bai Y, et al. EGFR G796D mutation mediates resistance to osimertinib. Oncotarget 2017;8:49671-9.

94. Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 2011;3:75ra26.

95. Shah KN, Bhatt R, Rotow J, et al. Aurora kinase A drives the evolution of resistance to third-generation EGFR inhibitors in lung cancer. Nat Med 2019;25:111-8.

96. Chen C, He M, Zhu Y, et al. Five critical elements to ensure the precision medicine. Cancer Metastasis Rev 2015;34:313-8.

97. Schiffmann I, Greve G, Jung M, et al. Epigenetic therapy approaches in non-small cell lung cancer: Update and perspectives. Epigenetics 2016;11:858-70.

98. Easwaran H, Tsai HC, Baylin SB. Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance. Mol Cell 2014;5:716-27.

99. Virmani AK, Rath I, Zochbauer-Muller S, et al. Promoter hypermethylation of DNA repair genes MLH1 and MSH2 in adenocarcinomas and squamous cell carcinomas of the lung. Rev Port Pneumol 2014;20:20-30.

100. Do H, Wong NC, Murone C, et al. A critical reassessment of DNA repair gene promoter methylation in non-small cell lung carcinoma. Sci Rep 2014;4:4186.

101. Ansari J, Shackelford RE, El-Osta H. Epigenetics in non-small cell lung cancer: from basics to therapeutics. Transl Lung Cancer Res 2016;5:155-71.

102. Miyanaga A, Gemma A, Noro R, et al. Antitumor activity of histone deacetylase inhibitors in non-small cell lung cancer cells: development of a molecular predictive model. Mol Cancer Ther 2008;7:1923-30.

103. Sambucetti LC, Fischer DD, Zabludoff S, et al. Histone deacetylase inhibition selectively alters the activity and expression of cell cycle proteins leading to specific
chromatin acetylation and antiproliferative effects. J Biol Chem 1999;274:34940-7.
108. Kim MS, Kwon HJ, Lee YM, et al. Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. Nat Med 2001;7:437-43.
109. Butler LM, Zhou X, Xu WS, et al. The histone deacetylase inhibitor SAHA arrests cancer cell growth, up-regulates thioroxygen-binding protein-2, and down-regulates thioroxygen. Proc Natl Acad Sci U S A 2002;99:11700-5.
110. Yu X, Guo ZS, Marcu MG, et al. Modulation of p53, ErbB1, ErbB2, and Raf-1 expression in lung cancer cells by depsipeptide FR901228. J Natl Cancer Inst 2002;94:504-13.
111. Chen MC, Chen CH, Wang JC, et al. The HDAC inhibitor, MPT0E028, enhances erlotinib-induced cell death in EGFR-TKI-resistant NSCLC cells. Cell Death Dis 2013;4:e810.
112. Greve G, Schiiffmann I, Pfeifer D, et al. The pan-HDAC inhibitor panobinostat acts as a sensitizer for erlotinib activity in EGFR-mutated and -wildtype non-small cell lung cancer cells. BMC Cancer 2015;15:947.
113. Smith DL, Acquaviva J, Sequeira M, et al. The HSP90 inhibitor ganetespib potentiates the antitumor activity of EGFR tyrosine kinase inhibition in mutant and wild-type non-small cell lung cancer. Target Oncol 2015;10:235-45.
114. Tanimoto A, Takeuchi S, Arai S, et al. Histone Deacetylase 3 Inhibition Overcomes BIM Deletion Polymorphism-Mediated Osimertinib Resistance in EGFR-Mutant Lung Cancer. Clin Cancer Res 2017;23:3139-49.
115. Zhang W, Peyton M, Xie Y, et al. Histone deacetylase inhibitor romidepsin enhances antitumor activity of erlotinib in non-small cell lung cancer. J Thorac Oncol 2009;4:161-6.
116. Sharma SV, Lee DY, Li B, et al. Chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell 2010;141:69-80.
117. Ph1b/2 Dose Escalation Study of Enzontostat With Pembrolizumab in NSCLC With Expansion Cohorts in NSCLC, Melanoma, and Colorectal Cancer. Available online: https://ClinicalTrials.gov/show/NCT02437136
118. Phase 2 Study of Glesatinib, Sitravatinib or Mocetinostat in Combination With Nivolumab in Non-Small Cell Lung Cancer. Available online: https://ClinicalTrials.gov/show/NCT02954991
119. The Immune Checkpoint Inhibitor Pembrolizumab in Combination With Oral Decitabine and Tetracyhydrodouridine as First-Line Therapy for Inoperable, Locally Advanced or Metastatic Non-small Cell Lung Cancer. Available online: https://ClinicalTrials.gov/show/NCT03233724
120. Pembrol and Vorinostat for Patients With Stage IV Non-small Cell Lung Cancer (NSCLC). Available online: https://ClinicalTrials.gov/show/NCT02638090
121. A Phase Ib Dose Escalation/Expansion Study of Abexinostat in Combination With Pembrolizumab in Patients With Advanced Solid Tumor Malignancies. Available online: https://ClinicalTrials.gov/show/NCT03590054
122. Selective HDAC6 Inhibitor ACY 241 in Combination With Nivolumab in Patients With Unresectable Non Small Cell Lung Cancer. Available online: https://ClinicalTrials.gov/show/NCT02635061
123. Phase 1/2 Study of Mocetinostat and Durvalumab in Patients With Advanced Solid Tumors and NSCLC. Available online: https://ClinicalTrials.gov/show/NCT02805660
124. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest 2009;119:1420-8.
125. Legras A, Pecuchet N, Imbeaud S, et al. Epithelial-to-Mesenchymal Transition and MicroRNAs in Lung Cancer. Cancers (Basel) 2017. doi: 10.3390/cancers9080101.
126. Fischer KR, Durrans A, Lee S, et al. Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. Nature 2015;527:472-6.
127. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 2014;15:178-96.
128. Mani SA, Guo W, Liao MJ, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008;133:704-15.
129. Witta SE, Gemmill RM, Hirsch FR, et al. Restoring E-cadherin expression increases sensitivity to epidermal growth factor receptor inhibitors in lung cancer cell lines. Cancer Res 2006;66:944-50.
130. Chung JH, Rho JK, Xu X, et al. Clinical and molecular evidences of epithelial to mesenchymal transition in acquired resistance to EGFR-TKIs. Lung Cancer 2011;73:176-82.
131. Suda K, Tomizawa K, Fujii M, et al. Epithelial to mesenchymal transition in an epidermal growth factor receptor-mutant lung cancer cell line with acquired resistance to erlotinib. J Thorac Oncol 2011;6:1152-61.
132. Uramoto H, Iwata T, Onitsuka T, et al. Epithelial-mesenchymal transition in EGFR-TKI acquired resistant lung adenocarcinoma. Anticancer Res 2010;30:2513-7.
133. Wu DW, Lee MC, Hsu NY, et al. FHT loss confers cisplatin resistance in lung cancer via the AKT/NF-kappaB/Slug-mediated PUMA reduction. Oncogene
134. Gower A, Hsu WH, Hsu ST, et al. EMT is associated with, but does not drive resistance to ALK inhibitors among EML4-ALK non-small cell lung cancer. Mol Oncol 2016;10:601-9.

135. Sakuma Y. Epithelial-to-mesenchymal transition and its role in EGFR-mutant lung adenocarcinoma and idiopathic pulmonary fibrosis. Pathol Int 2017;67:379-88.

136. Ding XM. MicroRNAs: regulators of cancer metastasis and epithelial-mesenchymal transition (EMT). Chin J Cancer 2014;33:140-7.

137. Sakuma Y, Matsukuma S, Nakamura Y, et al. Enhanced autophagy is required for survival in EGFR-independent EGFR-mutant lung adenocarcinoma cells. Lab Invest 2013;93:1137-46.

138. Sakuma Y, Nishikiori H, Hirai S, et al. Prolyl isomerase Pin1 promotes survival in EGFR-mutant lung adenocarcinoma cells with an epithelial-mesenchymal transition phenotype. Lab Invest 2016;96:391-8.

139. Tam WL, Weinberg RA. The epigenetics of epithelial-mesenchymal plasticity in cancer. Nat Med 2013;19:1438-49.

140. Kidd ME, Shumaker DK, Ridge KM. The role of vimentin intermediate filaments in the progression of lung cancer. Am J Respir Cell Mol Biol 2014;50:1-6.

141. Richardson AM, Havel LS, Koyen AE, et al. Vimentin Is Required for Lung Adenocarcinoma Metastasis via Heterotypic Tumor Cell-Cancer-Associated Fibroblast Interactions during Collective Invasion. Clin Cancer Res 2018;24:420-32.

142. Zhou J, Wang J, Zeng Y, et al. Implication of epithelial-mesenchymal transition in IGF1R-induced resistance to EGFR-TKIs in advanced non-small cell lung cancer. Oncotarget 2015;6:44332-45.

143. Fan C, Miao Y, Zhang X, et al. Btbd7 contributes to reduced E-cadherin expression and predicts poor prognosis in non-small cell lung cancer. BMC Cancer 2014;14:704.

144. Zhang Z, Lee JC, Lin L, et al. Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer. Nat Genet 2012;44:852-60.

145. Lu J, Zhan Y, Feng J, et al. MicroRNAs associated with therapy of non-small cell lung cancer. Int J Biol Sci 2018;14:390-7.

146. Markou A, Sourvinou I, Vorkas PA, et al. Clinical evaluation of microRNA expression profiling in non small cell lung cancer. Lung Cancer 2013;81:388-96.

147. Price C, Chen J. MicroRNAs in Cancer Biology and Therapy: Current Status and Perspectives. Genes Dis 2014;1:53-63.

148. Watt K, Newsted D, Voorand E, et al. MicroRNA-206 suppresses TGF-beta signalling to limit tumor growth and metastasis in lung adenocarcinoma. Cell Signal 2018;50:25-36.

149. Garofalo M, Romano G, Di Leva G, et al. EGFR and MET receptor tyrosine kinase-altered microRNA expression induces tumorigenesis and gefitinib resistance in lung cancers. Nat Med 2011;18:74-82.

150. Shen H, Zhu F, Liu J, et al. Alteration in Mir-21/PTEN expression modulates gefitinib resistance in non-small cell lung cancer. PLoS One 2014;9:e103305.

151. Bisagni A, Pagano M, Maramotti S, et al. Higher expression of miR-133b is associated with better efficacy of erlotinib as the second or third line in non-small cell lung cancer patients. PLoS One 2018;13:e0196350.

152. Zhao J, Kelm K, Kelm K, et al. Synergy between next generation EGFR tyrosine kinase inhibitors and miR-34a in the inhibition of non-small cell lung cancer. Lung Cancer 2017;108:96-102.

153. Zhao J, Kelm K, Bader AG. In-depth analysis shows synergy between erlotinib and miR-34a. PLoS One 2014;9:e89105.

154. Beg MS, Brenner AJ, Sachdev J, et al. Phase I study of MRX34, a liposomal miR-34a mimic, administered twice weekly in patients with advanced solid tumors. Invest New Drugs 2017;35:180-8.

155. MesomiR 1: A Phase I Study of TaromiRs as 2nd or 3rd Line Treatment for Patients With Recurrent MPM and NSCLC. Available online: https://ClinicalTrials.gov/show/NCT02369198

156. van Zandwijk N, Pavlakis N, Kao SC, et al. Safety and activity of microRNA-loaded minicells in patients with recurrent malignant pleural mesothelioma: a first-in-man, phase 1, open-label, dose-escalation study. Lancet Oncol 2017;18:1386-96.

157. Antonia SJ, Villegas A, Daniel D, et al. Durvalumab after Chemoradiotherapy in Stage III Non-Small-Cell Lung Cancer. N Engl J Med 2017;377:1919-29.

158. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. N Engl J Med 2015;373:1267-39.

159. Brahmer J, Reckamp KL, Baas P, et al. Pembrolizumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. N Engl J Med 2016;375:1823-33.

160. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive,
advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet 2016;387:1540-50.

162. Rittmeyer A, Barlesi F, Waterkamp D, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. Lancet 2017;389:255-65.

163. Gainor JF, Shaw AT, Sequist LV, et al. EGFR Mutations and ALK Rearrangements Are Associated with Low Response Rates to PD-1 Pathway Blockade in Non-Small Cell Lung Cancer: A Retrospective Analysis. Clin Cancer Res 2016;22:4585-93.

164. 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.

165. Lisberg A, Cummings A, Goldman JW, et al. A Phase II Study of Pembrolizumab in EGFR-Mutant, PD-L1+, Tyrosine Kinase Inhibitor Naive Patients With Advanced NSCLC. J Thorac Oncol 2018;13:1138-45.

166. Chen N, Fang W, Zhan J, et al. Upregulation of PD-L1 by EGFR Activation Mediates the Immune Escape in EGFR-Driven NSCLC: Implication for Optional Immune Targeted Therapy for NSCLC Patients with EGFR Mutation. J Thorac Oncol 2015;10:910-23.

167. Akbay EA, Koyama S, Carretero J, et al. Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. Cancer Discov 2013;3:1355-63.

168. Ahn MJ, Yang J, Yu H, et al. Osimertinib combined with durvalumab in EGFR-mutant non-small cell lung cancer: Results from the TATTON phase Ib trial. J Thorac Oncol 2016;11:S115.

169. Rudin C, Cervantes A, Dowlati A, et al. Long-Term Safety and Clinical Activity Results from a Phase Ib Study of Erlotinib Plus Atezolizumab in Advanced NSCLC. J Thorac Oncol 2018;13:S407.

170. Gibbons DL, Chow LQ, Kim DW, et al. Efficacy, safety and tolerability of MEDI4736 (durvalumab [D]), a human IgG1 anti-programmed cell death-ligand-1 (PD-L1) antibody, combined with gefitinib (G): A phase I expansion in TKI-naive patients (pts) with EGFR mutant NSCLC. J Thorac Oncol 2016;11:S79.

171. Yang JC, Gadgeel SM, Sequist LV, et al. Pembrolizumab in Combination With Erlotinib or Gefitinib as First-Line Therapy for Advanced NSCLC With Sensitizing EGFR Mutation. J Thorac Oncol 2019;14:553-9.

172. Spigel DR, Reynolds C, Waterhouse D, et al. Phase 1/2 Study of the Safety and Tolerability of Nivolumab Plus Crizotinib for the First-Line Treatment of Anaplastic Lymphoma Kinase Translocation - Positive Advanced Non-Small Cell Lung Cancer (CheckMate 370). J Thorac Oncol 2018;13:682-8.

173. Felip E, Braud FGD, Maur M, et al. Ceritinib plus nivolumab (NIVO) in patients (pts) with anaplastic lymphoma kinase positive (ALK+) advanced non-small cell lung cancer (NSCLC). J Clin Oncol 2017;35:abstr 2502.

174. Kim DW, Gadgeel SM, Gettinger SN, et al. Safety and clinical activity results from a phase Ib study of alectinib plus atezolizumab in ALK+ advanced NSCLC (aNSCLC). J Clin Oncol 2018;36:abstr 9009.

175. Socinski MA, Jotte RM, Cappuzzo F, et al. Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. N Engl J Med 2018;378:2288-301.

176. Haratani K, Hayashi H, Tanaka T, et al. Tumor immune microenvironment and nivolumab efficacy in EGFR mutation-positive non-small-cell lung cancer based on T790M status after disease progression during EGFR-TKI treatment. Ann Oncol 2017;28:1532-9.

177. Hellmann MD, Ciuleanu TE, Pluzanski A, et al. Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden. N Engl J Med 2018;378:2093-104.

178. Gandara DR, Paul SM, Kowanetz M, et al. Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. Nat Med 2018;24:1441-8.

179. Hatakeyama K, Nagashima T, Urakami K, et al. Tumor mutational burden analysis of 2,000 Japanese cancer genomes using whole exome and targeted gene panel sequencing. Biomed Res 2018;39:159-67.

180. McGranahan N, Furness AJ, Rosenthal R, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science 2016;351:1463-9.

181. Zhao R, Chinai JM, Buhl S, et al. HHLA2 is a member of the B7 family and inhibits human CD4 and CD8 T-cell function. Proc Natl Acad Sci U S A 2013;110:9879-84.

182. Cheng H, Janakiram M, Borczuk A, et al. HHLA2, a New Immune Checkpoint Member of the B7 Family, Is Widely Expressed in Human Lung Cancer and Associated with EGFR Mutational Status. Clin Cancer Res 2017;23:825-32.