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LUNG CANCER (R MUDAD, SECTION EDITOR)

Consensus on Molecular Testing in Lung Cancer

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Abstract

Purpose of Review Molecular testing for lung cancer has evolved dramatically over the last decade, driven primarily by the rapid development of targeted therapies. Initial testing was intended to make appropriate therapeutic choices with primary single gene testing and has evolved into larger sensitive and specific panels to evaluate multiple genes.

Recent Findings The wide array of technologies and an increasing number of targeted therapies have resulted in increasingly complex management algorithms. In this article, we review the current guidelines, briefly discuss individual targets, and introduce some of the complexities associated with genomic testing.

Summary We generally recommend next generation sequencing (NGS) panel testing when available and discuss other reasonable alternatives. Circulating tumor assays are commonly utilized, particularly when tissue is unavailable for genomic testing.

Keywords Non-small cell lung cancer · Molecular testing · EGFR · KRAS · Liquid biopsy · Next generation sequencing

Introduction

The landscape of molecular testing in lung cancer has evolved rapidly over the last decade with the advent of newer molecular biology techniques and effective targeted therapy. This has had significant therapeutic and prognostic implications especially in non-small cell lung cancer (NSCLC). Adenocarcinoma has been the greatest beneficiary of this progress.

The development of drugs targeting genes with activating mutations has made molecular testing of lung cancer quintessential. However, as the discovery of targets and development of therapies continue to evolve, there is sometimes controversy about the relativeness of different genes. We outline current genomic testing technologies and guidelines with a brief overview of the most relevant, circulating tumor DNA testing, and brief discussion of the challenges of creating guidelines. We generally prefer comprehensive NGS testing when feasible and discuss reasonable alternatives.

This article is part of the Topical Collection on *Lung Cancer*

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Genetics

Several genes, including EGFR, ALK, ROS1, KRAS, and BRAF, aid in primary management at the time of diagnosis. Several other genes, including MET, RET, NTRK1, HER2 and PIK3CA, also have significant relevance. Importantly, the mutational profile, as well as clonal burden of these genes, evolves with oncologic evolution, making a clear understanding of genomic relevance crucial to the decision-making process.

The genomics of lung cancer in an individual patient, and the understanding of a tumor's mutational spectrum, are highly dependent on the technology used to test it (Table 1).

Over the past several years, a large spectrum of technologies has become available for molecular testing with the most pertinent being real-time PCR, digital PCR, and next-generation sequencing (NGS). Several other technologies, including but not limited to BEAMing, CAPPseq, and ARMS PCR, are also utilized.

Real-time PCR is the most widely utilized test on account of its relative ease of implementation and is generally extremely efficient for single gene tests where only limited mutations need to be tested. It generally has excellent sensitivity and specificity. However, sensitivity at < 1% is generally hard to obtain without compromising specificity.

Digital PCR is a well-established technology which is entering main stream clinical diagnostics. It generally offers less breadth of testing compared to real-time PCR but offers

significantly enhanced sensitivity while maintaining specificity, which makes it an ideal tool for applications such as liquid biopsies for specific targeted genomic testing. While real-time PCR is based on relative linear statistics, digital PCR is based on Poisson statistics, enabling absolute quantification rather than the traditional relative quantification used in real-time PCR.

Massively parallel sequencing, more commonly known as next-generation sequencing (NGS), is a technology that has revolutionized molecular testing in cancers. NGS has enabled panel-based testing and allowed optimal utilization of scarce tumor tissue. In addition to this, NGS accomplishes simultaneous testing of common SNPs, Indels, and complex mutations along with translocations. Moreover, it allows scanning of much larger regions of an increased number of genes and enables rare, as well as novel, mutation detection. As we enter an era of ever-growing numbers of targetable drugs, the utilization of NGS and large-scale tumor profiling is already proving to be crucial in affecting current management and research.

It is noted that current FDA-approved tests are limited to real-time PCR and next-generation sequencing-based tests with no digital PCR tests yet FDA-approved.

Current Guidelines

Recently updated guidelines for molecular testing in lung cancer by a consensus statement from IASLC, CAP, and AMP recommend mandatory initial testing for EGFR, ALK, and ROS1 [1]. Although BRAF testing was not included in the "must-test" genes, due to lack of sufficient data at the time of submission for publication, an acknowledgment was included indicating this would likely be part of future recommendations. Per this statement, RET and MET testing are specifically recommended in a setting of a clinical trial or as part of a larger NGS panel. The guidelines recommend testing specifically adenocarcinoma histology and are not relevant to pure squamous histology. NGS panels are discussed to allow broader testing of various genes.

Compared to the IASLC, CAP, and AMP guidelines, the NCCN guidelines have some minor differences [2]. The primary genes recommended include EGFR and ALK (category 1) and ROS1 and BRAF (category 2A). Squamous cell NSCLCs were included in recommendations for EGFR and ALK testing in never smoker patients and those with small biopsy specimens. NCCN guidelines also recommend testing in the setting of mixed histology with a non-squamous component. The overarching statement, however, has been that broad molecular profiling should be performed when possible.

EGFR

The *EGFR* gene provides instructions for making a receptor protein called the epidermal growth factor receptor, which spans the cell membrane so that one end of the protein remains inside the cell and the other projects from the outer surface. Epidermal growth factor receptor binds to at least seven different ligands, which activates the receptor complex, triggering pathways within the cell that promote cell proliferation [3].

EGFR activates at least four significant pathways including RAS-RAF-MEK-ERK, PI3 kinase-AKT, PLCgamma-PKC, and STATs modules. It may also have an activating effect on NF-kappa-B signaling [4].

Clinical testing guidelines are most impacted by the treatment implications. Clinically relevant EGFR mutations have primarily been documented in exons 18, 19, 20, and 21 which involve, or are extremely close to, the regions involving cytoplasmic binding with ATP. These mutations are seen in approximately 10–40% of patients depending on their ethnicity, sex, and smoking history.

Most EGFR mutations including the G719X mutation, exon 19 deletions/insertions, L858R, and L861Q are EGFR sensitizing to tyrosine kinase inhibitors. However, some EGFR mutations, such as exon 20 insertions and the T790M mutation, have been associated with resistance to the first and second generation tyrosine kinase inhibitors. Interestingly, A763_Y764insFQEA, a specific mutation in exon 20 of EGFR, is an exception and has been associated with TKI sensitivity [5].

The T790M EGFR mutation occurs in about 50–70% of those with progression and resistance to first or second generation EGFR TKIs [6, 7, 8•, 9]. Osimertinib, a third generation EGFR TKI, was initially approved in the setting of a T790M EGFR mutation based on data demonstrating significantly longer duration of progression-free survival (PFS) and response rate vs platin-pemetrexed [10•]. Recently, first-line osimertinib for any sensitizing EGFR-mutant lung cancer demonstrated impressive PFS results vs standard first generation EGFR-directed therapy, leading to a recommendation for first-line treatment in some guidelines [11•]. Importantly, C797S mutation results in resistance to osimertinib, much like T790M correlates with resistance to the first and second generation EGFR TKIs [12]. However, there have been reports of C797S mutations that are sensitive to the first generation EGFR TKIs [13].

EGFR testing is crucial at diagnosis of NSCLC, particularly in the setting of metastatic non-squamous histology. Patients with a detected sensitizing mutation should be treated with targeted therapy. No matter which therapy is initially given, testing should again be performed at the time of progression. This cannot be emphasized enough as there are still multiple patients not getting this standard of care test [14, 15].

Current testing for anti EGFR therapies is available with various technologies. Current drugs which are FDA-approved for EGFR-mutated NSCLC are gefitinib, erlotinib, afatinib, and osimertinib with multiple companion diagnostics approved. It is important to note that FDA approval as companion diagnostics is generally limited to the USA but several other well-validated tests are also available. Although IHC testing was initially used for determining eligibility for TKI therapy, the last IASLC, CAP, and AMP guidelines now strongly advise against using IHC and instead recommend molecular testing. Refer to Table 2 for details of companion diagnostics which are currently FDA-approved.

Anaplastic Lymphoma Kinase

Around 3–7% of NSCLCs harbor fusions involving the kinase domain of the anaplastic lymphoma kinase (ALK) gene. The most common partner genes are EML4 with the others being KIF5B and TFG. There are other partners that are seen in a small percentage. The active portion in these fusion combinations is the kinase domain of the ALK gene).

For patients with ALK fusions, initial accelerated FDA approval was granted to crizotinib in 2011 based on results from two single-arm studies demonstrating an ORR of 50–61% and median duration of response of 42–48 weeks [16, 17]. More recently, alectinib was FDA-approved as first-line therapy for advanced ALK-positive NSCLC after demonstrating significant improvement in PFS vs crizotinib [18].

There are various ALK variants that can impact sensitivity and resistance to ALK-directed therapies [19]. G1202R in the TKI domain of ALK has been noted to cause resistance to the first and second generation TKIs. The C1156Y mutation also causes resistance. Other mutations are noted in lesser frequencies, but most mutations are sensitive to lorlatinib. There are reports of patients being treated sequentially with various ALK inhibitors based on the ALK mutation variations at times of progression [20]. The growing understanding of different ALK inhibitor sensitivities reinforces the importance of testing for these TKI domain mutations at progression.

ALK testing was initially performed by IHC testing with an IHC assay currently FDA-approved for the same. Recently, break-apart FISH probes, which are FDA-approved, have become more reliable and widely used. Even more recently, molecular tests like FoundationOne have become FDA-approved for companion diagnostics for ALK rearrangements. Several novel molecular chemistries primarily involving next-generation sequencing continue to evolve to help identify ALK rearrangements even from low input material. Importantly, standard testing such as FISH or standard translocation testing does not detect resistance mutations. Sequencing or targeted mutation testing is essential to detect the ALK fusion resistance mutation.

KRAS

The *KRAS* gene provides instructions for making a protein called K-Ras, part of a signaling pathway known as the RAS/MAPK pathway. The protein relays signals from outside the cell to the cell's nucleus. These signals instruct the cell to proliferate and differentiate.

Table 1 Comparison of commonly used technologies

	NGS	Digital PCR	Real-time PCR
Principle	High throughput, massively parallel or deep sequencing	Absolute quantification based on specific taqman probes, partitioning into several wells/droplets	Relative quantification based on specific taqman probes
Turn Around Time	More	Less	Less
Cost/sample*	High	Low	Low
Subclone determination	Yes, panel dependent	Possible	No
Novel mutation detection	Yes	No	No
Target	Simultaneously screen many targets	Targets specific region	Targets specific region
Specificity	High specificity	High specificity	High specificity
Sensitivity	Extremely sensitive	Extremely sensitive	Sensitive

*Cost per gene is generally equivalent to real-time or digital PCR. NGS tests for liquid biopsies and tissue biopsies are significantly different in design, implementation, and analysis

Table 2 FDA-approved tests for Molecular testing in NSCLC

VYSIS ALK break-apart FISH probe kit	ALK rearrangements	Xalkori (crizotinib)
Cobas EGFR Mutation Test V2	EGFR exon 19 deletions and EGFR exon 21 L858R alterations	TARCEVA (erlotinib)
	EGFR exon 20 T790M mutations	TAGRISSO (osimertinib)
Cobas EGFR Mutation Test V2 - liquid biopsy	EGFR exon 19 deletions and EGFR exon 21 L858R alterations	TARCEVA (erlotinib)
	EGFR exon 20 T790M mutations	TAGRISSO (osimertinib)
Therascreen EGFR RGQ PCR kit	EGFR exon 19 deletions and EGFR exon 21 L858R alterations	Gilotrif (afatinib)
		Iressa (gefitinib)
Oncomine Dx Target Test	BRAF V600E	TAFINLAR (dabrafenib) in combination with MEKINIST(trametinib)
	ROS1 fusions	XALKORI (crizotinib)
	EGFR L858R and Exon 19 deletions	IRESSA (gefitinib)
FoundationOne Cdx	EGFR exon 19 deletions and EGFR exon 21 L858R alterations	Iressa (gefitinib), Tarceva (erlotinib), Gilotrif (afatinib),
	EGFR exon 20 T790M mutations	TAGRISSO (osimertinib)
	BRAF V600E	Tafinlar (dabrafenib) in combination with Mekinist (tramatinib)
	ALK Rearrangements	Alecensa (alectinib), Xalkori (crizotinib), Zykadia (ceritinib)
VENTANA ALK (D5F3) Cdx Assay	ALK expression4	Ceritinib

*Although these are the FDA-approved tests, there are various other validated tests consistent with standard of care

The *KRAS* gene is in the Ras family of oncogenes, which also includes two other genes: *HRAS* and *NRAS*. These proteins play important roles in cell division, differentiation, and apoptosis [21]. The KRAS mutations in codons 12, 13, and 61 have been associated with several cancers.

KRAS mutations do not have any clinically targetable therapy that is yet available. At this time, KRAS is most useful to capture for the purpose of enrollment on clinical trials specifically targeting this population of patients. Also, generally speaking, when a KRAS mutation is detected, it significantly decreases the likelihood of a sensitizing mutation, preventing further unnecessary testing.

ROS1

ROS1 rearrangements are found in approximately 1–2% of NSCLC. ROS1 encodes an orphan receptor tyrosine kinase (RTK) that plays a role in epithelial cell differentiation and regionalization of the proximal epididymal epithelium. It may activate several downstream signaling pathways related to cell differentiation, proliferation, growth, and survival, including the PI3 kinase-mTOR signaling pathway. It mediates the phosphorylation of PTPN11, an activator of this pathway, along with phosphorylation and activation of the transcription factor STAT3 to control anchorage-independent cell growth.

Crizotinib demonstrated an ORR of 72% in ROS1 rearranged cancer and was granted expanded approval in 2016, making detection of ROS1 rearrangements critical when it is present [22]. Currently, ROS1 translocation testing is primarily available through FISH testing. The only FDA-approved companion diagnostic for ROS1 is the Oncomine Dx Target Test, which is a next-generation sequencing-based best. Other drugs that are active in ROS1 rearranged NSCLC include ceritinib and brigatinib.

MET

This gene encodes a member of the receptor tyrosine kinase family of proteins and the product of the proto-oncogene MET. The encoded preproprotein is proteolytically processed to generate alpha and beta subunits that are linked via disulfide bonds to form the mature receptor. Binding of its ligand, hepatocyte growth factor induces dimerization and activation of the receptor, which plays a role in cellular survival, embryogenesis, and cellular migration and invasion. Amplification and overexpression of this gene are associated with multiple human cancers [23]. MET amplification and exon 14 skipping mutations are present in about 3–4% and 2–4%, respectively of NSCLCs [24].

Crizotinib, a drug used in ALK- and ROS1-mutated lung cancers, also has activity against tumors with MET mutations. MET mutations and amplification have been documented as resistance mutations in patients with progressed or recurrent NSCLC [25]. Although MET appears to have significant clinical impact as a driver mutation in lung cancer, with possible targeted therapies, there is no FDA approval for testing

purposes as of yet. MET-driven lung cancers can have MET exon 14 skipping mutations, as well as MET amplifications, making it important to verify the individual scope of a test in picking up both. In targeted testing, MET amplifications and exon 14 skipping mutations are generally detected in separate tests. However, several NGS-based panel tests are comprehensive and will detect mutations and amplification.

BRAF

The *BRAF* gene provides instructions for making a protein that helps transmit chemical signals from outside the cell to the cell's nucleus. This protein is part of a signaling pathway known as the RAS/MAPK pathway, which regulates proliferation, differentiation, and migration of cells as well as apoptosis [26].

BRAF mutations are found in 1–4% of NSCLC. Unlike melanoma where BRAF mutations are primarily found at codon 600, in lung cancer, codons 469 and 549 are also commonly found. The combination of dabrafenib and trametinib is approved for only the specific BRAF V600E mutation in stage IV NSCLC. Interestingly, non-V600E BRAF mutations may coincide with KRAS mutations, while BRAF V600E mutations are considered mutually exclusive of EGFR, ALK, and KRAS alterations [27].

BRAF V600E as a companion diagnostic in the setting of metastatic melanoma, prior to treatment with combination vemurafenib and dabrafenib, is currently FDA-approved for only the FoundationOne Cdx and the Oncomine Target Test, but there are various other available platforms on which this test can be performed. Recent FDA approval of therapy for BRAF V600E makes inclusion of BRAF in genomics testing of non-squamous NSCLC a mandatory, standard of care and practice.

ERBB2 (HER2)

The ERBB2 gene has been studied in extreme detail in breast cancer. However, only 2–3% of patients with NSCLC have been found to have ERBB2 mutations, which includes amplification as well as exon 20 insertions. There are no approved therapies associated with this gene in NSCLC, but there are various clinical trials that make testing relevant for patients with access to centers with these trials.

RET

The RET protein spans the cell membrane, allowing it to interact with specific factors outside the cell. When molecules that stimulate growth and development (growth factors) attach to the RET protein, a complex cascade of chemical reactions inside the cell is triggered, leading to actions such as cell division or maturation.

RET currently does not have any FDA-approved therapies in NSCLC, but there are multiple ongoing clinical trials, making testing particularly important in patients with access to centers enrolling patients.

Other Genes

Several genes including MEK1, FGFR 1-4, NTRK1-3, NRG1, RIT1, NF1, PIK3CA, AKT1, NRAS, MTOR, TSC1, TSC2, KIT, PDGFRA, and DDR2 have been described as emerging molecular markers in the current IASLC guidelines as potential future targets.

Limitations of Current Testing and Guidelines

There is an array of various technologies currently available for genomic testing, making it difficult to provide specific detailed guidelines about the technology. It is important for each provider to understand the limitations of available testing. Current guidelines aim to provide guidance without limiting options and therefore address only the most basic molecular testing in lung cancer and lack consensus on the type of test to be ordered. In addition, there is no enforceable guideline for a lung cancer panel to include specific regions of genes to ensure complete coverage of clinically relevant mutations.

With the advent of tumor mutational burden, hotspot mutation testing may miss out on relevant information, and this is an evolving aspect of testing with ongoing studies.

The development and proliferation of subclones in cancers is now well appreciated, but the classification of these clones is subject to the nature of the test. With several tests, such as real-time PCR, there is little scope to determine the percentage of the clone carrying the targetable mutation, and a cutoff point with particular clinical relevance is not well defined. For example, detection of T790M on a first generation TKI prior to radiographic progression is of debatable clinical utility. At the same time, when radiographic progression is noted, even low-level detection of T790M is likely useful.

In addition to the limitation of the technologies in themselves, a large hurdle in effective testing today is the extensive number of options available where every test has its own nuance thus making appropriate test selection even more important. In order to ensure complete molecular profiling, even if just for the primary genes, appropriate tests have to be used. As an example, most EGFR mutation panels by PCR do not assess kinase domain duplication. Similarly, ALK FISH cannot assess tyrosine kinase domains for patients who develop first-line resistance.

As a result, interpreting a test is based on understanding the scope of the test. Testing guidelines will ultimately need to address the minimum requirements of assays for adequate standard of care testing and mandate disclosure regarding the genomic regions tested and the type of mutations tested. As noted above in discussions for MET and ALK testing, IHC- or FISH-based assays tend to be limited. As larger panel-based testing continues to evolve, panels which can detect amplifications, translocations, single, and multiple nucleotide variation as well as complex indels will continue to become more standard. As more targets become addressed with approved therapies, the need for broader testing will grow, leading to larger panels of testing to conserve scarce tissue and allow comprehensive profiling.

Liquid Biopsy

Cell-free DNA (cfDNA) is a term that describes DNA detected by means other than tissue biopsy and is most commonly collected by evaluation of blood. A critical aspect of testing is the differentiation of circulating tumor DNA (ctDNA) from non-cancer DNA. This is one of the most active areas of lung cancer research with significant progress being made each year in the ability to detect cancer DNA. Current consensus guidelines leave consideration for liquid biopsy when there is not enough tissue for appropriate testing. Although there are various technologies being utilized, these generally demonstrate good specificity and sensitivity. Mutation detection in liquid biopsies is dependent on tumor burden and tumor tissue death, which makes detection of an alteration reliable but the absence of detection not definitive. If there is high suspicion of an alteration despite a negative liquid biopsy result, tissue biopsy is indicated.

This technology may ultimately be utilized for more than just initial diagnostics. Data from liquid biopsy may impact the understanding of lung cancer in terms of clonal evolution [28] and may eventually have a role in the setting of treatment with checkpoint inhibitors [29]. There are reports of patients having positive tests in liquid biopsies for targetable mutations with negative results in solid tumor biopsies [30].

The cobas EGFR mutation test is currently the only test that is FDA-approved among liquid biopsies for T790M testing in EGFR for osimertinib. However, several next-generation sequencing, BEAMing, ARMS PCR, and digital PCR-based tests are also available, which have significantly enhanced sensitivity and specificity compared to conventional real-time PCR tests. Liquid biopsy for fusion and rearrangement-based testing remains limited in availability and lacks true validation compared to SNV and indel. Liquid biopsies have become standard, particularly when tissue is not available.

Conclusion

We generally prefer NGS-based panel testing for adequately testing many genomic alterations with less tissue than is required for individual testing. Another reasonable alternative is a comprehensive profile of all recommended genes using appropriate testing methodologies such as PCR and FISH at initial diagnosis on all patients with metastatic non-squamous NSCLC. A broad panel testing strategy is particularly supported in the academic setting where clinical trial enrollment is common. At a minimum, standard of care should include testing EGFR, ALK, ROS1, BRAF V600E, and MET. We include KRAS mutation testing, despite the lack of targeted therapy, as a presence of a KRAS mutation is a strong indicator of the lack of a known target as well as a qualifying factor for clinical trial enrollment. We recommend re-evaluation of genomics at progression on targeted therapies, particularly in the setting of EGFR and ALK-mutated neoplasms. It is strongly recommended that at the time of re-evaluation on initial progression, comprehensive NGS panel testing be performed given the multitude of resistance mechanisms. Circulating tumor DNA is reliable with good specificity and is particularly indicated when tissue is not available for genomic testing, but a negative test does not rule out the presence of a genomic alteration.

Compliance with Ethical Standards

Conflict of Interest Jacobs Sands declares financial connection as consulting/advisory board at Foundation Medicine, Merck, Incyte, Celgene, Astra-Zeneca, and Trovagene, outside the submitted work. Parth Shah reports no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- Of importance

1. Lindeman NI, et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors. J Thorac Oncol. 2018;Volume 13(Issue 3):323–58. https://doi.org/10.1016/j.jtho.2017.12.001. **This publication details clinical trial data which supports current consensus guidelines and also provides an outlook for testing in the coming years.**
2. NCCN Clinical Practice Guidelines in Oncology https://www.nccn.org/professionals/physician_gls/pdf/nscl_blocks.pdf [Accessed 11th Mar 2018].
3. Genetics Home Reference, US National Library of Medicine https://ghr.nlm.nih.gov/gene/EGFR [cited 11th Mar 2018].
4. Universal Protein Resource. https://www.uniprot.org/uniprot/P00533 [Accessed cited 11th Mar 2018].

5. Yasuda H, Park E, Yun C-H, Sng NJ, Lucena-Araujo AR, Yeo WL, et al. Structural, biochemical and clinical characterization of epidermal growth factor receptor (EGFR) exon 20 insertion mutations in lung cancer. Sci Transl Med. 2013;5(216): 216ra177. https://doi.org/10.1126/scitranslmed.3007205.
6. Wu S-G, Liu Y-N, Tsai M-F, Chang YL, Yu CJ, Yang PC, et al. The mechanism of acquired resistance to irreversible EGFR tyrosine kinase inhibitor-afatinib in lung adenocarcinoma patients. Oncotarget. 2016;7(11):12404–13. https://doi.org/10.18632/oncotarget.7189.
7. Tanaka K, Nosaki K, Otsubo K, Azuma K, Sakata S, Ouchi H, et al. Acquisition of the T790M resistance mutation during afatinib treatment in EGFR tyrosine kinase inhibitor-naïve patients with non-small cell lung cancer harboring *EGFR* mutations. Oncotarget. 2017;8(40):68123–30. https://doi.org/10.18632/oncotarget.19243.
8.• Yu HA, Arcila ME, Rekhtman N, Sima CS, Zakowski MF, Pao W, et al. 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: Off J Am Assoc Cancer Res. 2013;19(8):2240–7. https://doi.org/10.1158/1078-0432.CCR-12-2246. **This study explores the mechanisms of evolving genomic-based resistance to initial treatment with erlotinib and gefitinib, establishing that T790M mutations are one of the most common reasons for resistance to initial therapy.**
9. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med. 2011;3(75):75ra26. https://doi.org/10.1126/scitranslmed.3002003.
10.• Mok TS, Wu YL, Ahn MJ, Garassino MC, Kim HR, Ramalingam SS, et al. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. N Engl J Med. 2017;376(7):629–40. https://doi.org/10.1056/NEJMoa1612674. **This study compares the efficacy of osimertinib versus standard platinum-pemetrexed therapy in T790M-positive lung cancer and demonstrated significant benefit in progression-free survival as well as reduced occurence of adverse reactions in the osimertinib arm.**
11.• Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, et al. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. N Engl J Med. 2018;378(2):113–25. https://doi.org/10.1056/NEJMoa1713137. **This study compared osimertinib to standard first generation EGFR TKIs in EGFR mutation-positive (exon 19 deletion or L858R) NSCLC patients. This study demonstrated a significant increase in progression-free survival accompanied by improved tolerability in the osimertinib arm.**
12. Wang S, Tsui ST, Liu C, Song Y, Liu D. EGFR C797S mutation mediates resistance to third-generation inhibitors in T790M-positive non-small cell lung cancer. J Hematol Oncol. 2016;9(1): 59. https://doi.org/10.1186/s13045-016-0290-1.
13. Niederst MJ, Hu H, Mulvey HE, Lockerman EL, Garcia AR, Piotrowska Z, 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: Off J Am Assoc Cancer Res. 2015;21(17):3924–33. https://doi.org/10.1158/1078-0432.CCR-15-0560.
14. Ruggiero JE, Rughani J, Neiman J, Swanson S, Revol C, Green RJ. Real-world concordance of clinical practice with ASCO and NCCN guidelines for EGFR/ALK testing in aNSCLC. J Clin Oncol. 2017;35(8_suppl):212. https://doi.org/10.1200/JCO.2017.35.8_suppl.212.
15. Enewold L, Thomas A. Real-world patterns of *EGFR* testing and treatment with erlotinib for non-small cell lung cancer in the United States. PLoS One. 2016;11(6):e0156728. https://doi.org/10.1371/journal.pone.0156728.
16. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med. 2010;363(18):1693–703.
17. Camidge DR, Bang Y-J, Kwak EL, Iafrate AJ, Varella-Garcia M, Fox SB, et al. Activity and safety of crizotinib in patients with *ALK*-positive non-small-cell lung cancer: updated results from a phase 1 study. The lancet oncology. 2012;13(10):1011–9. https://doi.org/10.1016/S1470-2045(12)70344-3.
18. Peters S, Camidge DR, Shaw AT, Gadgeel S, Ahn JS, Kim DW, et al. Alectinib versus crizotinib in untreated ALK-positive non-small-cell lung cancer. N Engl J Med. 2017;377(9):829–38. https://doi.org/10.1056/NEJMoa1704795.
19. Lin JJ, Zhu VW, Yoda S, et al. Impact of EML4-ALK variant on resistance mechanisms and clinical outcomes in ALK-positive lung cancer. J Clin Oncol. 2018; JCO2017762294. https://doi.org/10.1200/JCO.2017.76.2294.
20. Shaw AT, Friboulet L, Leshchiner I, Gainor JF, Bergqvist S, Brooun A, et al. Resensitization to crizotinib by the lorlatinib ALK resistance mutation L1198F. N Engl J Med. 2016;374(1):54–61. https://doi.org/10.1056/NEJMoa1508887.
21. Genetics Home Reference, US National Library of Medicine https://ghr.nlm.nih.gov/gene/KRAS [Accessed 11th Mar 2016].
22. Shaw AT, Solomon BJ. Crizotinib in ROS1-rearranged non-small-cell lung cancer. N Engl J Med. 2015;372(7):683–4. https://doi.org/10.1056/NEJMc1415359.
23. Genetics Home Reference, US National Library of Medicine https://ghr.nlm.nih.gov/gene/MET [Accessed 11th Mar 2018].
24. My Cancer Genome Database. Solomon, B. 2015. MET Amplification in Non-Small Cell Lung Cancer. My Cancer Genome https://www.mycancergenome.org/content/disease/lung-cancer/met/59/ [Accessed 11th Mar 2018].
25. Drilon AE, Ross Camidge D, Ignatius Ou S-H, Clark JW, Socinski MA, Weiss J, et al. J Clin Oncol. 2016;34(15_suppl):108.
26. Genetics Home Reference, US National Library of Medicine https://ghr.nlm.nih.gov/gene/BRAF [Accessed 11th Mar 2018].
27. Kinno T, Tsuta K, Shiraishi K, Mizukami T, Suzuki M, Yoshida A, et al. Clinicopathological features of nonsmall cell lung carcinomas with BRAF mutations. Ann Oncol. 2014;25(1):138–42. https://doi.org/10.1093/annonc/mdt495.
28. Blakely CM, Watkins TBK, Wu W, Gini B, Chabon JJ, McCoach CE, et al. Evolution and clinical impact of co-occurring genetic alterations in advanced-stage EGFR-mutant lung cancers. Nat Genet. 2017;49(12):1693–704. https://doi.org/10.1038/ng.3990.
29. Khagi Y, Goodman AM, Daniels GA, Patel SP, Sacco AG, Randall JM, et al. Hypermutated circulating tumor DNA: correlation with response to checkpoint inhibitor-based immunotherapy. Clin Cancer Res. 2017;23(19):5729–36. https://doi.org/10.1158/1078-0432.
30. Besse B, Remon J, Lacroix L, Mezquita L, Jovelet C, Howarth K, et al. J Clin Oncol. 2017;35(15_suppl):11540.