ΕΝΩΣΗ ΠΝΕΥΜΟΝΟΛΟΓΩΝ ΕΛΛΑΔΑΣ

ΕΤΗΣΙΟ ΣΥΝΕΔΡΙΟ

30 Μαΐου - 2 Ιουνίου 2019

Αθήνα, *Ξενοδοχείο* Royal Olympic

ΣΤΡΟΓΓΥΛΗ ΤΡΑΠΕΖΑ: ΚΑΡΚΙΝΟΣ ΠΝΕΥΜΟΝΑ

Μοριακός έλεγχος στο μη μικροκυτταρικό καρκίνο πνεύμονα

Η περίπτωση του EGFR

Δρ. Φωτεινή Παπαγεωργίου

Βιολόγος, Diagnostics Manager AstraZeneca

Conflict Of Interest

Fotini Papageorgiou holds a PhD in Molecular Biology from the Biology Department of University of Athens and graduated from the Biology Department of the same University. From 1998-2002, she participated in research projects with collaborating parties the Hellenique Pasteur Institute, the Regional Oncological Hospital "Agios Savvas", and the University of Ioannina.

Currently, she is Diagnostics Manager in Oncology Business Unit of AstraZeneca Hellas, being indirectly involved with the diagnostic procedures to be applied and the parameters that these procedures must meet for the selection and implementation of personalized oncology therapies, existing or under development.

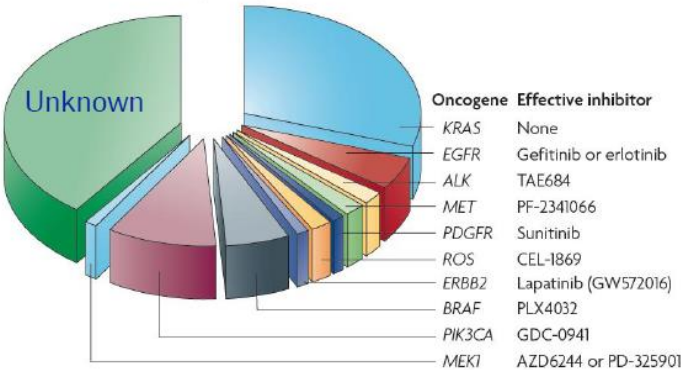
In this Congress participates as speaker pro bono by the above scientific and professional experience.

Advances in science and technology

Driver Mutations in NSCLC 2000

- **Treatment: Platinum doublet chemotherapy**
- **Response rate: 10-20%**

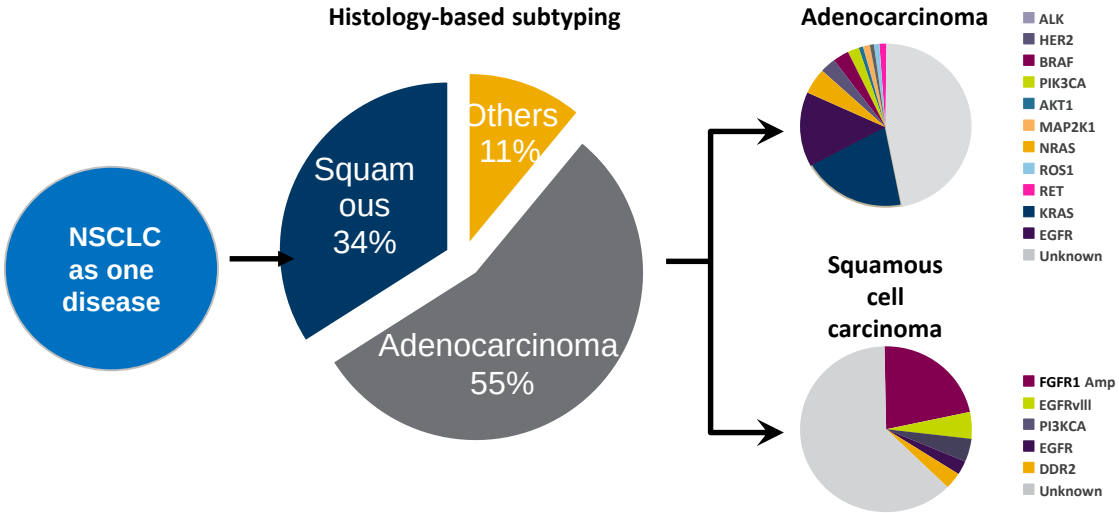
Driver Mutations in NSCLC 2012



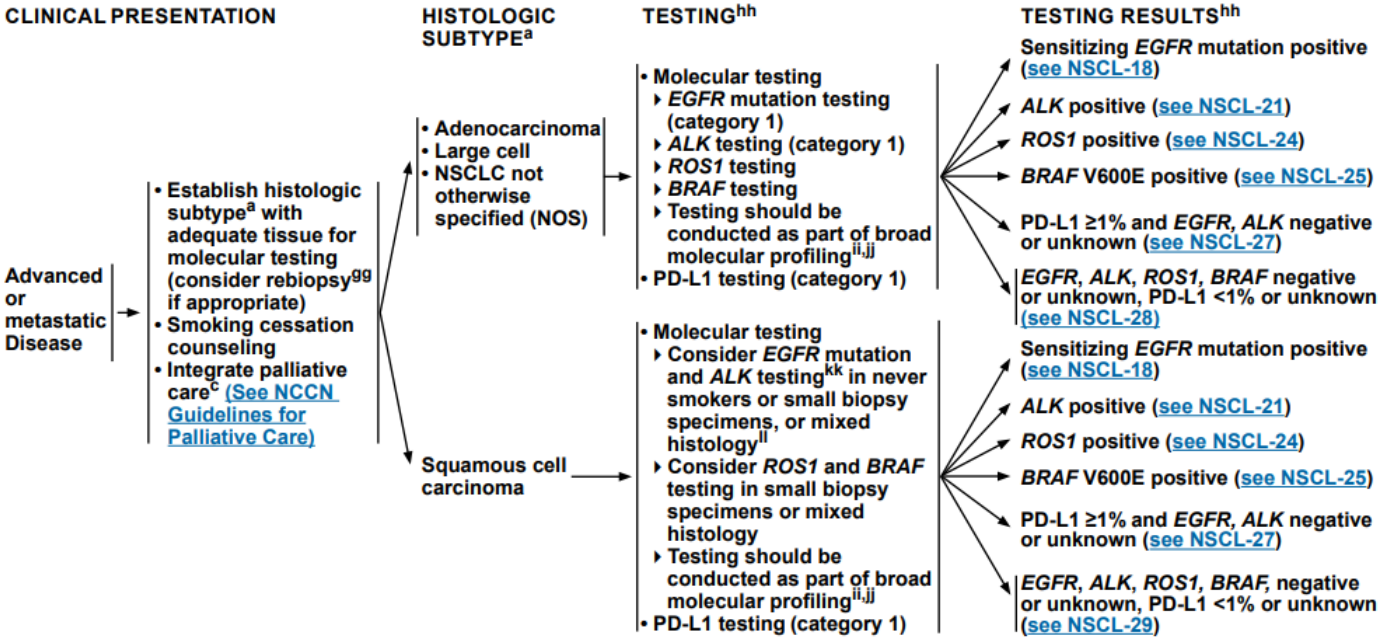
- **Treatment: Targeted therapies matched to molecular defects (e.g. EGFRmut, ALK, MET)**
- **Response rate: 50-70%**

Image adapted from Sharma et al., Nature Rev Ca. Vol 10, pg 241-253, 2010

Lung cancer classification has evolved from a single disease to histological and molecular subtyping



Li T, et al. *J Clin Oncol*. 2013;31(8):1039-1049.


CLINICAL PRESENTATION
HISTOLOGIC SUBTYPE[a]
TESTING[hh]
TESTING RESULTS[hh]
Advanced or metastatic Disease
• Establish histologic subtype[a] with adequate tissue for molecular testing (consider rebiopsy[gg] if appropriate)
• Smoking cessation counseling
• Integrate palliative care[c] (See NCCN Guidelines for Palliative Care)
• Adenocarcinoma
• Large cell
• NSCLC not otherwise specified (NOS)
Squamous cell carcinoma
• Molecular testing
▸ EGFR mutation testing (category 1)
▸ ALK testing (category 1)
▸ ROS1 testing
▸ BRAF testing
▸ Testing should be conducted as part of broad molecular profiling[ii,jj]
• PD-L1 testing (category 1)
• Molecular testing
▸ Consider EGFR mutation and ALK testing[kk] in never smokers or small biopsy specimens, or mixed histology[ll]
▸ Consider ROS1 and BRAF testing in small biopsy specimens or mixed histology
▸ Testing should be conducted as part of broad molecular profiling[ii,jj]
• PD-L1 testing (category 1)
Sensitizing EGFR mutation positive (see NSCL-18)
ALK positive (see NSCL-21)
ROS1 positive (see NSCL-24)
BRAF V600E positive (see NSCL-25)
PD-L1 ≥1% and EGFR, ALK negative or unknown (see NSCL-27)
EGFR, ALK, ROS1, BRAF negative or unknown, PD-L1 <1% or unknown (see NSCL-28)
Sensitizing EGFR mutation positive (see NSCL-18)
ALK positive (see NSCL-21)
ROS1 positive (see NSCL-24)
BRAF V600E positive (see NSCL-25)
PD-L1 ≥1% and EGFR, ALK negative or unknown (see NSCL-27)
EGFR, ALK, ROS1, BRAF, negative or unknown, PD-L1 <1% or unknown (see NSCL-29)

ESMO Guidelines for biomarkers testing in NSCLC

Table 1. A personalised medicine synopsis table for metastatic NSCLC

Biomarker	Method	Use	LoE, GoR
EGFR mutation	Any appropriate, validated method, subject to external quality assurance	To select those patients with *EGFR*-sensitising mutations most likely to respond to EGFR TKI therapy	I, A
ALK rearrangement	Any appropriate, validated method, subject to external quality assurance. FISH is the historical standard but IHC is now becoming the primary therapy-determining test, provided the method is validated against FISH or some other orthogonal test approach. NGS is an emerging technology	To select those patients with *ALK* gene rearrangements most likely to respond to ALK TKI therapy	I, A
ROS1 rearrangement	FISH is the trial-validated standard. IHC may be used to select patients for confirmatory FISH testing but currently lacks specificity. NGS is an emerging technology. External quality assurance is essential	To select those patients with *ROS1* gene rearrangements most likely to respond to ROS1 TKI therapy	II, A
BRAF mutation	Any appropriate, validated method, subject to external quality assurance	To select those patients with *BRAF V600*-sensitising mutations most likely to respond to BRAF inhibitor, with or without MEK inhibitor therapy	II, A
PD-L1 expression	IHC to identify PD-L1 expression at the appropriate level and on the appropriate cell population(s) as determined by the intended drug and line of therapy. Only specific trial assays are validated. Internal and external quality assurance are essential	To enrich for those patients more likely to benefit from anti-PD-1 or anti-PD-L1 therapy. For pembrolizumab, testing is a companion diagnostic for nivolumab and atezolizumab, testing is complementary	I, A

ESMO

Annals of Oncology 29 (Supplement 4): iv192–iv237, 2018
doi:10.1093/annonc/mdy275
Published online 3 October 2018; updated 26 January 2019

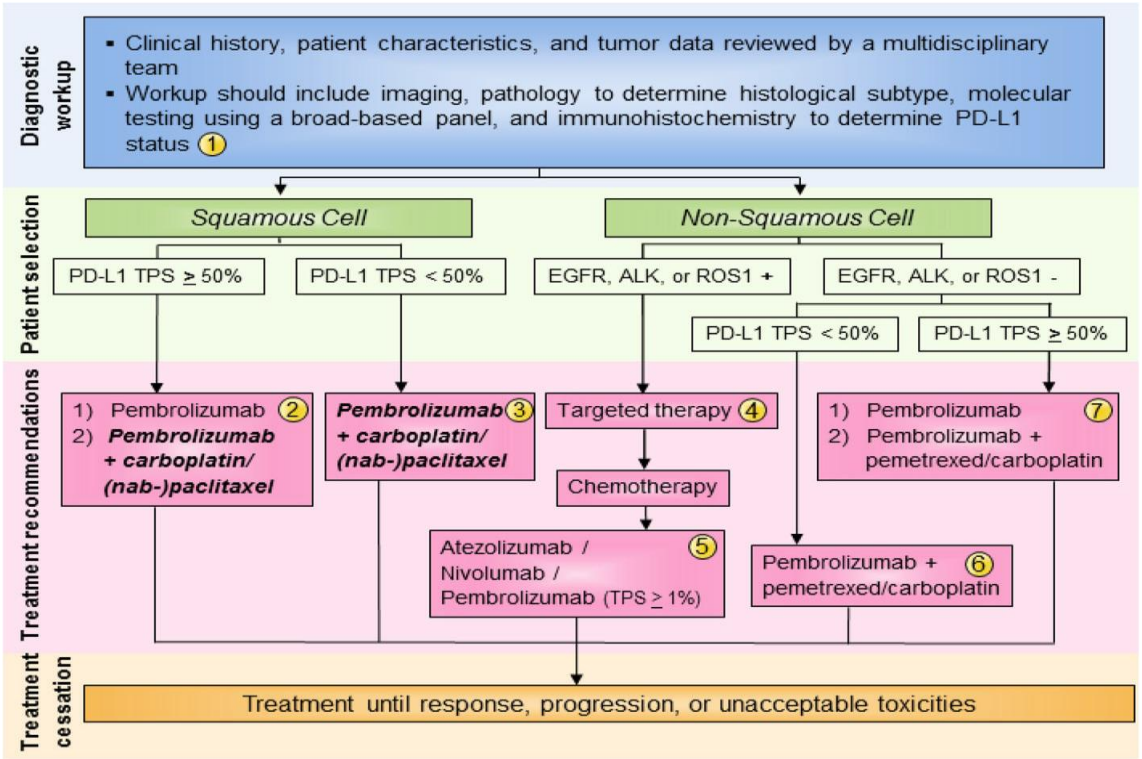
D. Planchard et. al

CLINICAL PRACTICE GUIDELINES

POSITION ARTICLE AND GUIDELINES Open Access

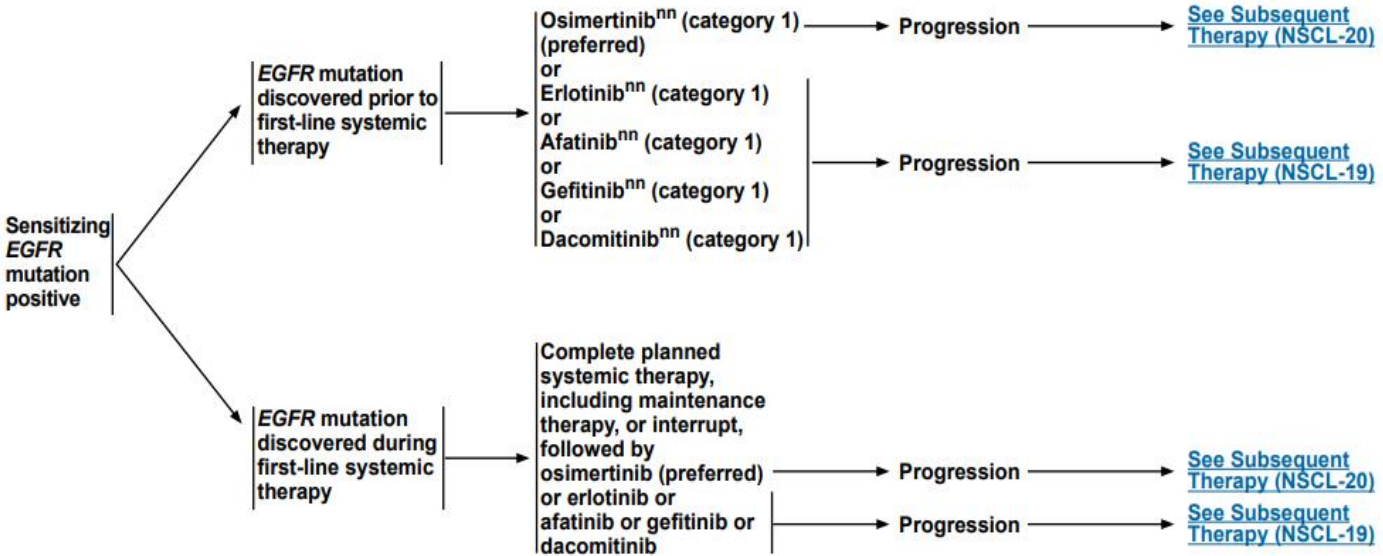
The Society for Immunotherapy of Cancer consensus statement on immunotherapy for the treatment of non-small cell lung cancer (NSCLC)

Brahmer et al. Journal for ImmunoTherapy of Cancer (2018) 6:75
https://doi.org/10.1186/s40425-018-0382-2



SENSITIZING EGFR MUTATION POSITIVE[hh]

FIRST-LINE THERAPY[mm]



Several types of sample can be used for EGFR mutation testing

1. Tumour biopsy samples

Preferred sample type for all genetic mutation testing in advanced NSCLC[1–3]

2. Cytology samples

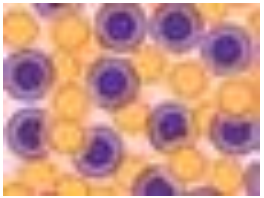
Sample quality and tumour cell content may be lower than biopsy samples[4–9]

1. Pirker R et al. J Thorac Oncol 2010;5:1706–1713;
2. Marchetti A and Normanno N. Patholgica 2010;102:119–122;
3. Eberhard D et al. J Clin Oncol 2008;26:983–994;
4. Kimura H et al. Br J Cancer 2006;95:95:1390–1395;
5. Oshita F et al. Br J Cancer 2006;95:1070–1075;
6. Molina-Vila M et al. J Thorac Oncol 2008;3:1224–1235;
7. Smouse J et al. Cancer Cytopathol 2009;117:67–72;
8. Van Ejik R et al. PLoS One 2011;6:e177791;
9. Rekhtman N et al. J Thorac Oncol 2011;6:451–458

Technical challenges linked to FFPET *

% cancer cells

Every testing method should need to define minimum cancer cells content required for testing



% clones bearing EGFR mutations (heterogeneity)

Amongst cancer cells not all bearing mutations.

% DNA that can be amplified and analyzed via testing methods

Degraded DNA /necrotic areas

% inhibitors

Presence of inhibitors (eg microorganisms, buccal etc)

* Formalin Fixed Paraffin Embeeded Tissue

The Journal of Molecular Diagnostics, Vol. 20, No. ■, ■ 2018

Neal I. Lindeman,* Philip T. Cagle,† Dara L. Aisner,‡ Maria E. Arcila,§ Mary Beth Beasley,¶ Eric Bernicker,‖ Carol Colasacco,** Sanja Dacic,†† Fred R. Hirsch,‡‡ Keith Kerr,§§ David J. Kwiatkowski,¶¶ Marc Ladanyi,‖‖ Jan A. Nowak,*** Lynette Sholl,* Robyn Temple-Smolkin,††† Benjamin Solomon,‡‡‡ Lesley H. Souter, Erik Thunnissen,§§§ Ming S. Tsao,¶¶¶ Christina B. Ventura,** Murry W. Wynes,‖‖‖ and Yasushi Yatabe****

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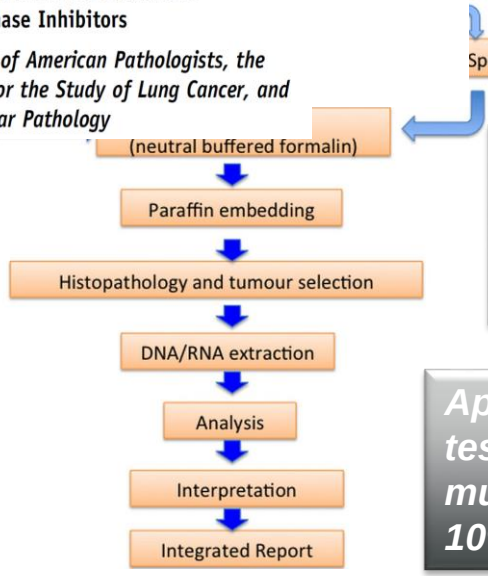
SPECIAL ARTICLE

Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors

Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology



Pathologists to determine the adequacy of EGFR testing by assessing tumor cells content

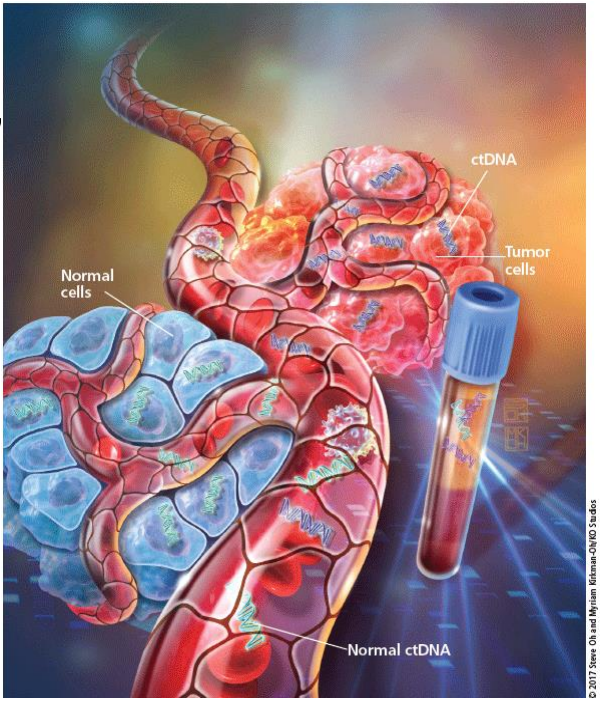
Application of sensitive tests that are able to detect mutations in as little as 10% tumor cells

Ian A Cree et al. J Clin Pathol doi:10.1136/jclinpath-2014-202404

JCP

CtDNA Advantages

- In the absence of suitable or sufficient tissue biopsy, allows molecular analysis
- demonstrate resistance to targeted therapy
- non invasive
- useful in cases of inter- & intra-heterogeneity
- Several clinical applications
- Fast results



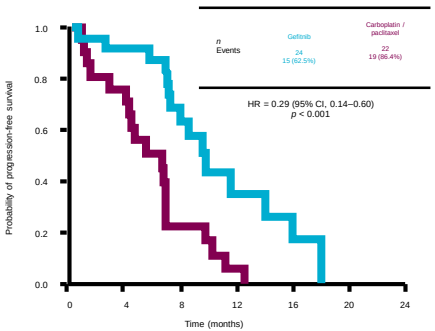
CtDNA Challenges

- Highly fragmented
- Half –life ~ 2 hrs
- may be very rare (<0.01%)
- difficult to detect in certain cancers such as those localized in the central nervous system
- Shedding in bloodstream is unclear

Diaz & Bardelli, J Clin Oncol 32, 579-586, 2014

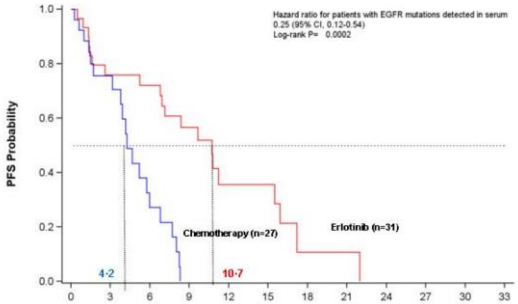
Clinical Relevance of ctDNA in NSCLC

IPASS



Goto *et al.*, J Thorac Oncol, 2012

EURTAC



Rosell et al, Lancet Oncol, 2012

LIMITED NO OF BLOOD SAMPLES

IFUM study: Comparison of *EGFR* mutation frequency in evaluable tumour and evaluable plasma samples

Tumour vs plasma 1 ctDNA samples by *EGFR* mutation status (screened patients evaluable for both samples, n=652)			
	Tumour *EGFR* mutation status, n*		
	Positive	Negative	Total
Plasma 1 *EGFR* mutation status, n			
Positive	69	1	70
Negative	36	546	582
Total	105	547	652

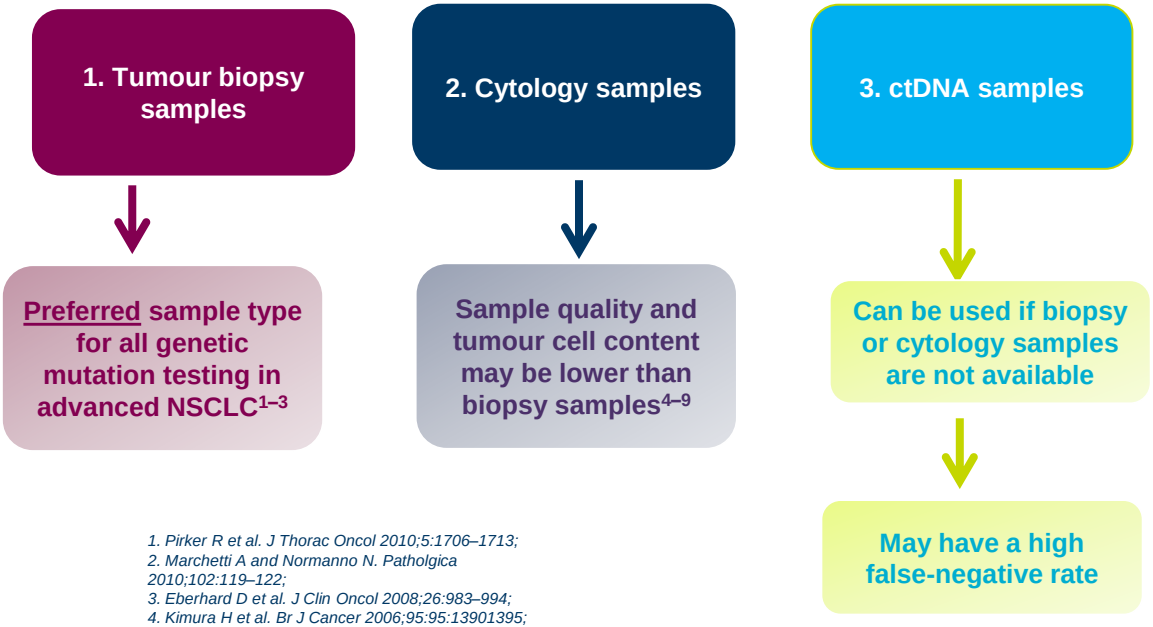
	n	Rate, % (95% CI)
Concordance	652	94.3 (92.3, 96.0)
Sensitivity	105	65.7 (55.8, 74.7)
Specificity	547	99.8 (99.0, 100.0)
PPV	70	98.6 (92.3, 100.0)
NPV	582	93.8 (91.5, 95.6)

Sep 2014

1st EMA update for IRESSA label ctDNA use in case of tissue unavailability

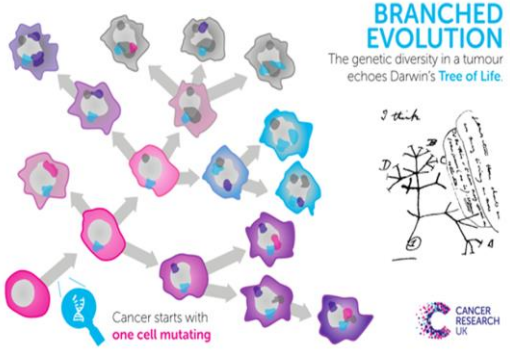
PLASMA IS THE PREFFERED MATERIAL

Several types of sample can be used for mutation EGFRtesting

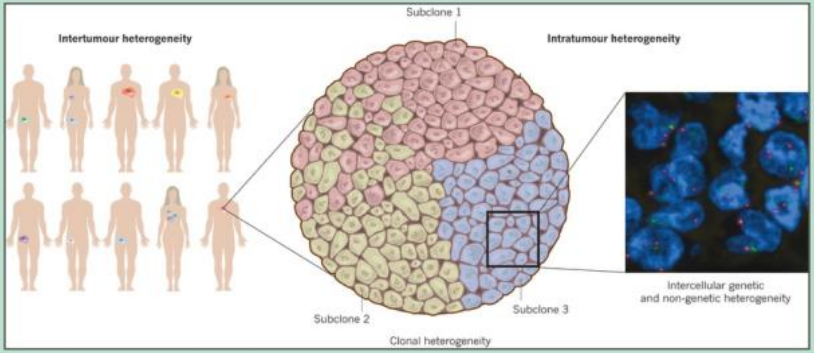


1. Pirker R et al. J Thorac Oncol 2010;5:1706–1713;
2. Marchetti A and Normanno N. Patholgica 2010;102:119–122;
3. Eberhard D et al. J Clin Oncol 2008;26:983–994;
4. Kimura H et al. Br J Cancer 2006;95:95:13901395;
5. Oshita F et al. Br J Cancer 2006;95:1070–1075;
6. Molina-Vila M et al. J Thorac Oncol 2008;3:1224–1235;
7. Smouse J et al. Cancer Cytopathol 2009;117:67-72;
8. Van Ejik R et al. PLoS One 2011;6:e177791;
9. Rekhtman N et al. J Thorac Oncol 2011;6:451-458

Each ~~cancer~~ tumor is different



Tumor Heterogeneity: You Can Miss the Mutation, Even When It's Somewhere in the Tumor



Burrell, Nature 2013

Mechanisms of resistance to first/ second generation EGFR TKIs in NSCLC

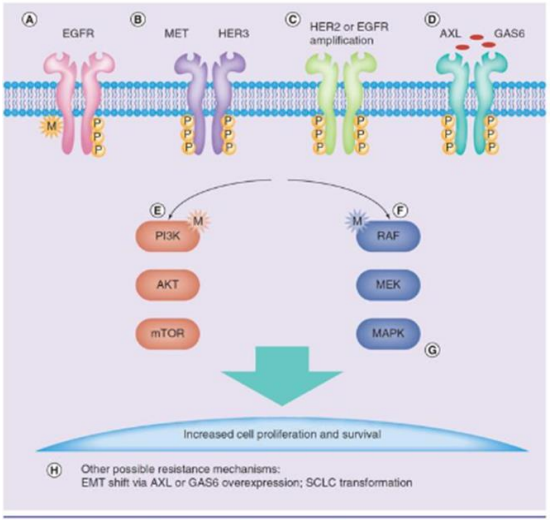


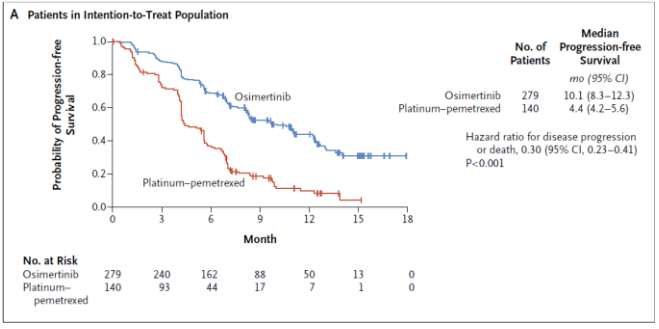
Table 1. Main mechanisms involved in acquired resistance to EGF receptor-tyrosine kinase inhibitors.

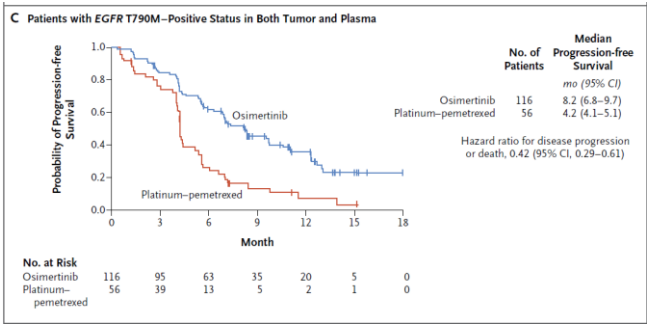
Molecular alteration	Frequency (%)†
T790M mutation	~50
MET amplification	5–20
EGFR amplification	8‡
HER2 amplification	5–13
MAPK1 amplification	4.8
PIK3CA mutations	5
BRAF mutations	1
AXL overexpression	20
GAS6 overexpression	25
EMT	1–2
SCLC transformation	5–14

†Frequencies are derived from different studies [5,9,22,37–41].
‡*EGFR* amplification + T790M mutation [37].
EMT: Epithelial-to-mesenchymal transition; SCLC: Small-cell lung carcinoma.

Fenizia *Future Oncology* 2015

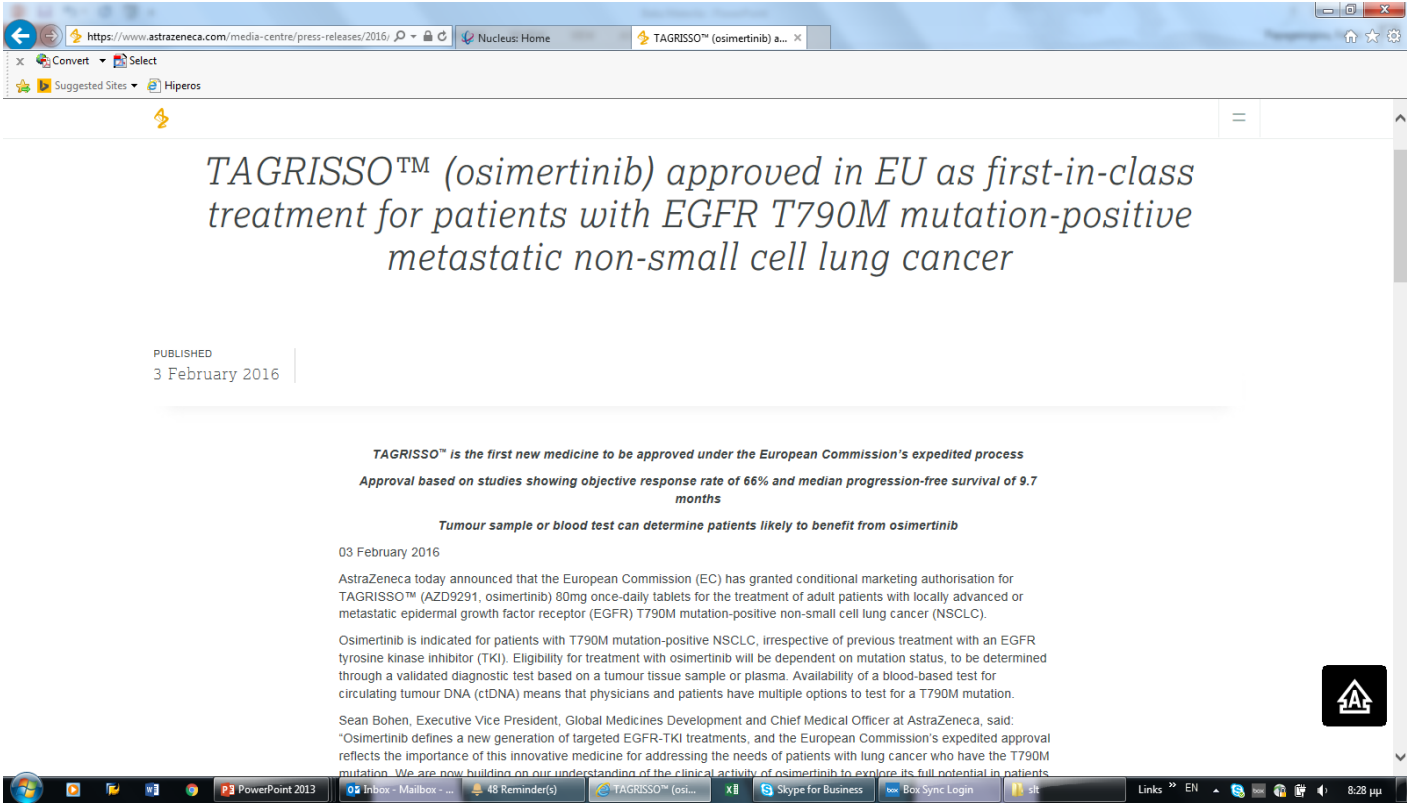
Clinical Relevance of ctDNA in NSCLC with a T790M mutation





Mok et al. 2016

The NEW ENGLAND JOURNAL of MEDICINE


https://www.astrazeneca.com/media-centre/press-releases/2016/
Nucleus: Home
TAGRISSO™ (osimertinib) a...
Convert
Select
Suggested Sites
Hiperos
TAGRISSO™ (osimertinib) approved in EU as first-in-class treatment for patients with EGFR T790M mutation-positive metastatic non-small cell lung cancer
PUBLISHED
3 February 2016
TAGRISSO™ is the first new medicine to be approved under the European Commission's expedited process
Approval based on studies showing objective response rate of 66% and median progression-free survival of 9.7 months
Tumour sample or blood test can determine patients likely to benefit from osimertinib
03 February 2016
AstraZeneca today announced that the European Commission (EC) has granted conditional marketing authorisation for TAGRISSO™ (AZD9291, osimertinib) 80mg once-daily tablets for the treatment of adult patients with locally advanced or metastatic epidermal growth factor receptor (EGFR) T790M mutation-positive non-small cell lung cancer (NSCLC).
Osimertinib is indicated for patients with T790M mutation-positive NSCLC, irrespective of previous treatment with an EGFR tyrosine kinase inhibitor (TKI). Eligibility for treatment with osimertinib will be dependent on mutation status, to be determined through a validated diagnostic test based on a tumour tissue sample or plasma. Availability of a blood-based test for circulating tumour DNA (ctDNA) means that physicians and patients have multiple options to test for a T790M mutation.
Sean Bohen, Executive Vice President, Global Medicines Development and Chief Medical Officer at AstraZeneca, said:
"Osimertinib defines a new generation of targeted EGFR-TKI treatments, and the European Commission's expedited approval reflects the importance of this innovative medicine for addressing the needs of patients with lung cancer who have the T790M
PowerPoint 2013
Inbox - Mailbox - ...
48 Reminder(s)
TAGRISSO™ (osi...
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Overview of plasma analyses of ctDNA in AURA trials

Across the AURA trials, plasma was collected for analyses to determine whether genotyping of plasma ctDNA could identify patients who gain clinical benefit from osimertinib

	Phase III study: AURA3[1]	Phase II studies: AURA extension and AURA2[2]	Phase I study: AURA[3]
Treatment/dosing	Osimertinib 80 mg QD vs platinum pemetrexed	Osimertinib 80 mg QD	Osimertinib dose-escalation and dose-expansion cohorts (20–240 mg QD)
Tissue T790M status	T790M-positive	T790M-positive	T790M-positive and -negative cases
Analysis	Pre-planned analysis; plasma collected contemporaneous with tissue and tested retrospectively	Pre-planned for regulatory submission	Exploratory post hoc analysis
Plasma assay	cobas®	cobas®	BEAMing
Method of comparison	cobas® FFPE tissue	NGS	ddPCR or cobas® FFPE tissue
Number of patients	399 (n=399 T790M positive by tissue test; n=184 plasma T790M positive; n=175 T790M plasma negative; n=40 missing/invalid)	873 (n=401 AURA extension; n=472 in AURA2)	216

BEAMing, beads, emulsion, amplification, and magnetics; ctDNA, circulating tumor deoxyribonucleic acid; ddPCR, droplet digital polymerase chain reaction; NGS, next-generation sequencing, QD, once daily.

1. Wu Y-L, et al. Presented at: IASLC 17th World Conference on Lung Cancer; December 4-7, 2016; Vienna, Austria. Abs MA08.03. 2. Jenkins S, et al. Presentation at ELCC 2016. 3. Oxnard GR, et al. *J Clin Oncol*. 2016;34(28):3375-3382.

Plasma ctDNA analysis for detection of the EGFR T790M mutation in patients with advanced non-small cell lung cancer

Suzanne Jenkins,[a] James C-H Yang,[b] Suresh S Ramalingam,[c] Karen Yu,[d] Sabina Patel,[e] Susie Weston,[a] Rachel Hodge,[e] Mireille Cantarini,[a] Pasi A Jänne,[f] Tetsuya Mitsudomi,[g] Glenwood D Goss[h]

Table 2. Percent agreement of the cobas plasma test with the cobas tissue test as a reference method for the detection of EGFR T790M, L858R and exon 19 deletion

	Percent Agreement (95% CI)								
	T790M			L858R			Exon 19 Deletion		
	AURA Extension (n=397)		Pooled	AURA2 (n=460)	Pooled AURA extension and AURA2 (n=857)		AURA Extension (n=397)	AURA2 (n=460)	Pooled AURA extension and AURA2 (n=857)
PPA	64 (57–71)			76 (67–84)	76 (69–82)		88 (81–93)	83 (77–88)	85 (81–89)
NPA	[a]	80 (		98 (95–99)	98 (96–99)		98 (92–100)	98 (94–100)	98 (95–100)
OPA	65 (58–71)	66 (		90 (86–93)	91 (88–93)		91 (86–94)	89 (86–93)	90 (87–92)

T790M PPA for those receiving osimertinib at ≥ 3rd line

[a] Not calculated due to low number of samples (total <20).
PPA, positive percent agreement (sensitivity); NPA, negative percent agreement (specificity); OPA, overall percent agreement (concordance).

J Thor Oncology April 2017

EGFR mutation detection i
cross-platform comparisoı
clinical development of AZ

Personalized Medicine and Imaging

Clinical Cancer Research

Assessment of *EGFR* Mutation Status in Matched Plasma and Tumor Tissue of NSCLC Patients from a Phase I Study of Rociletinib (CO-1686)

EGFR Mutation	Disease classification	Patients with mutation[a]	Subset with mutation in plasma	Percentage	*P*[b]
Activating mutations	M1a/M0	18	7	39%	
	M1b	55	52	95%	<0.001
T790M	M1a/M0	15	4	27%	
	M1b	49	47	96%	<0.001

[a]Includes patients with an *EGFR* mutation detected in tissue only, plasma only, or both tissue and plasma.

[b]Fisher exact test used for comparisons.

Thress *et al*., Lung Cancer 90 (2015)

Carlovitch *et al*, Clin Cancer Res; 22(10) May 15, 2016

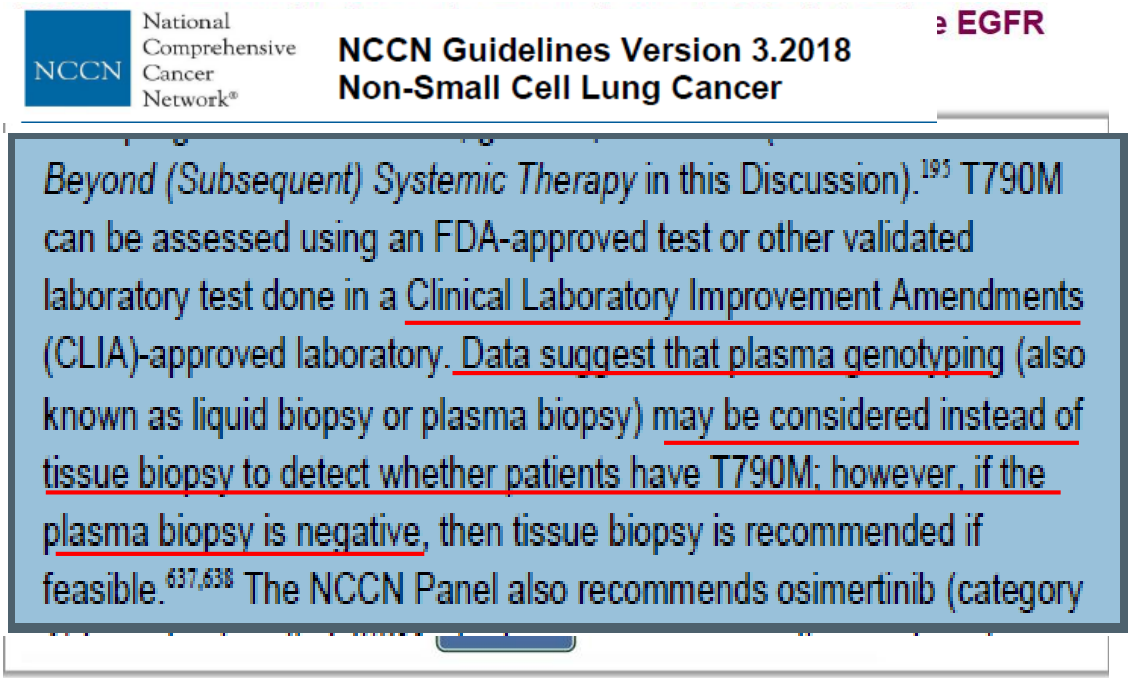
AURA3: T790M mutation is detected in plasma of ~50% of patients with T790M in tumor tissue

- Patients with tissue sample available at screening (n=756)

Plasma ctDNA test results, n	Tissue T790M positive (n=399)	Tissue Exon 19 deletion positive (n=427)	Tissue L858R positive (n=253)
Plasma positive	184	273	139
Plasma negative	175	60	67
No plasma test / invalid	37 / 3	91 / 3	47 / 0
Percent agreement using tissue test as reference, % (95% CI)*	**T790M**	**Exon 19 deletion**	**L858R**
Positive percent agreement (sensitivity)	**51 (46, 57)**	82 (77, 86)	68 (61, 74)
Negative percent agreement (specificity)	**77 (71, 83)**	98 (96, 100)	99 (98, 100)
Overall concordance	61 (57, 65)	89 (86, 91)	88 (85, 90)

Data cut-off April 15, 2016.
*Percent agreement of the **cobas®** plasma test with the **cobas®** tissue test. Positive percent agreement and negative percent agreement are used here as measures of test sensitivity and specificity, respectively, and calculated with invalid results excluded.
Wu YL, et al. *J Thorac Oncol*. 2017; 12(S1

Plasma ctDNA T790M mutation at TKI-progression as a first screening



National Comprehensive Cancer Network®

NCCN Guidelines Version 3.2018
Non-Small Cell Lung Cancer

e EGFR

Beyond (Subsequent) Systemic Therapy in this Discussion).[195] T790M can be assessed using an FDA-approved test or other validated laboratory test done in a Clinical Laboratory Improvement Amendments (CLIA)-approved laboratory. Data suggest that plasma genotyping (also known as liquid biopsy or plasma biopsy) may be considered instead of tissue biopsy to detect whether patients have T790M; however, if the plasma biopsy is negative, then tissue biopsy is recommended if feasible.[637,638] The NCCN Panel also recommends osimertinib (category

1. Novello S, et al. Annals of Oncology. 2016 Sep 1;27(suppl 5):v1-27.

Molecular Testing Guideline for the Selection of Patients With Lung Cancer for Treatment With Targeted Tyrosine Kinase Inhibitors: American Society of Clinical Oncology Endorsement of the College of American Pathologists/ International Association for the Study of Lung Cancer/ Association for Molecular Pathology Clinical Practice Guideline Update

Gregory P. Kalemkerian, Navneet Narula, Erin B. Kennedy, William A. Biermann, Jessica Donington, Natasha B. Leighl, Madelyn Lew, James Pantelas, Suresh S. Ramalingam, Martin Reck, Anjali Saqi, Michael Simoff, Navneet Singh, and Baskaran Sundaram

VOLUME 36 · NUMBER 9 · MARCH 20, 2018

JOURNAL OF CLINICAL ONCOLOGY | ASCO SPECIAL ARTICLE

Key Question 5: What is the role of testing for circulating cell-free DNA (cfDNA) for patients with lung cancer?

15. No Recommendation: There is currently insufficient evidence to support the use of cfDNA molecular methods for the diagnosis of primary lung adenocarcinoma.
16. Recommendation: In some clinical settings in which tissue is limited and/or insufficient for molecular testing, physicians may use a cfDNA assay to identify EGFR mutations.
17. Expert Consensus Opinion: Physicians may use cfDNA methods to identify *EGFR* T790M mutations in patients with lung adenocarcinoma who have progression or secondary clinical resistance to EGFR-targeted TKIs; testing of the tumor sample is recommended if the plasma result is negative.
18. No Recommendation: There is currently insufficient evidence to support the use of circulating tumor cell molecular analysis for the diagnosis of primary lung adenocarcinoma, the identification of *EGFR* or other mutations, or the identification of *EGFR* T790M mutations at the time of EGFR TKI resistance.

Different technologies for EGFR analysis

Approach	Method	Technology	LoD	Advantages	Disadvantages
Candidate-gene analysis	qPCR*	PNA clamp-PCR‡ (REF. 104)	0.1%	• Rapid • High sensitivity • Suitable for the detection of specific point mutations, copy-number variations, short indels, and gene fusions • No bioinformatic analysis • Cost-effective	• Only enables monitoring of known mutations
		LNA/DNA-PCR‡ (REF. 208)	0.1%		
		ARMS[209]	0.05–0.1%		
		COLD-PCR‡ (REF. 210)	0.1–0.01%		
	Digital PCR	BEAMing[211]	0.01%		
		ddPCR[212–214]	0.001%		
	NA	InPlex§ (REFS 133,215,216)	<0.01%		
		Endpoint PCR¶ (REF. 156)	<0.0001%		
Deep-sequencing	Targeted	AmpliSeq[217]	>2%	• Does not require any prior knowledge of the molecular alteration	• Longer time needed to obtain, process and analyse results than that needed for candidate-gene analysis • Bioinformatic expertise required • Expensive
		TAm-Seq[218]	>2%		
		SAFE-SeqS[219]	0.1%		
		Guardant360 digital sequencing test[197]	<0.1%		
		CAPP-Seq[134]	0.01%		
		iDES[220]	<0.01%		
		PARE[221]	0.001%		
	WES (nontargeted)	NA	>1–3%		
	WGS (nontargeted)	Digital karyotyping[221–223]	0.001%		
		PARE[221,224,225]	0.001%		

ARMS, amplification refractory mutation system; BEAMing, beads, emulsion, amplification, magnetics; CAPP-Seq, cancer personalized profiling by deep sequencing; COLD-PCR, complete enrichment coamplification at lower denaturation temperature PCR; ctDNA, circulating cell-free tumour DNA; ddPCR, droplet digital PCR; EMA, European Medicines Agency; FDA, US Food and Drug Administration; iDES, integrated digital error suppression; LNA/DNA-PCR, locked nucleic acids/DNA chimera PCR; LoD, limit of detection; NA, not applicable; PARE, parallel analysis of RNA ends; PNA clamp-PCR, peptide nucleic acids clamp PCR; qPCR, quantitative PCR; SAFE-SeqS, safe-sequencing system; TAm-Seq, tagged-amplicon deep sequencing; WES, whole-exome sequencing; WGS, whole-genome sequencing. *The therascreen EGFR RGQ PCR Kit and cobas EGFR Mutation Test v2 are qPCR assays approved by the EMA and FDA, respectively, for the analysis of plasma ctDNA for *EGFR* mutations that determine eligibility of patients with non-small-cell lung cancer for treatment with EGFR tyrosine-kinase inhibitors. ‡Semiquantitative technologies. §InPlex allele-specific blocker qPCR involves the construction of original and specific PCR primers. ¶Endpoint PCR incorporates an increased number of cycles, which enables an amplification plateau to be reached.

G. Siragneva *et al.*,
Published online 2 Mar 2017

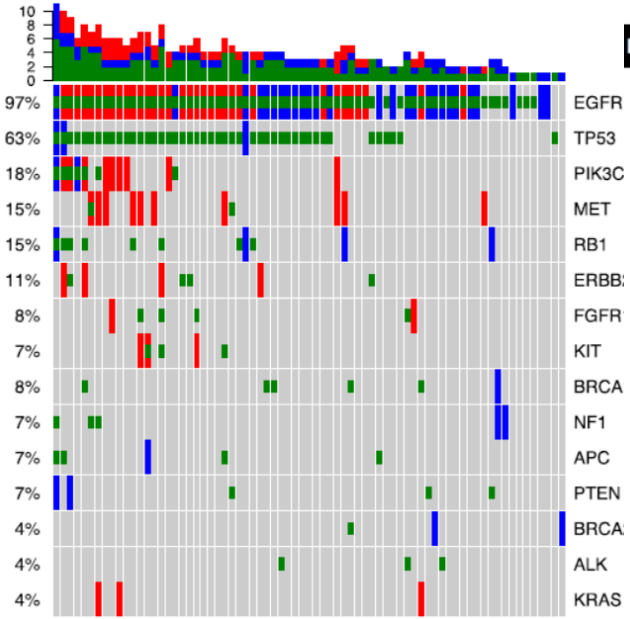
NATURE REVIEWS | CLINICAL ONCOLOGY

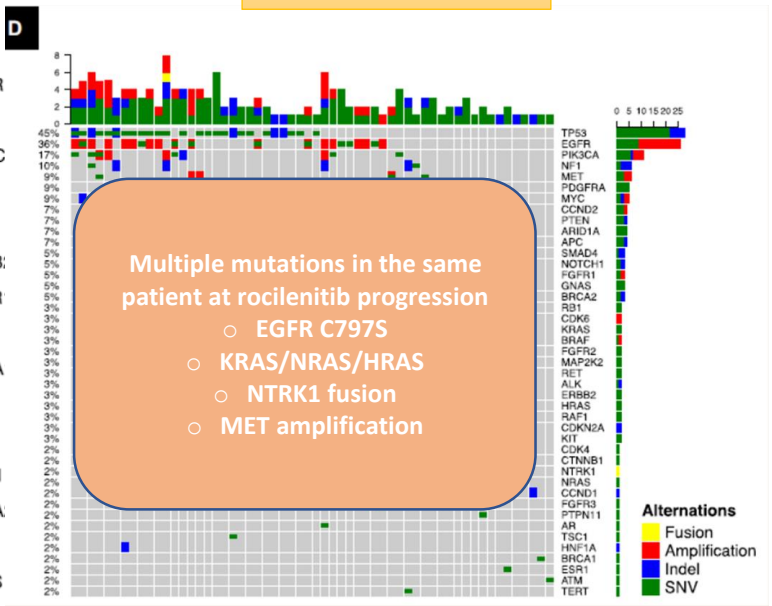
Cell-Free DNA Next-Generation Sequencing Prediction of Response and Resistance to Third-Generation *EGFR* Inhibitor

Elena Helman,[1] Minh Nguyen,[2] Chris A. Karlovich,[3] Darrin Despain,[2] A. Karin Choquette,[4] Alexander I. Spira,[4] Helena A. Yu,[5] D. Ross Camidge,[6] Thomas C. Harding,[2] Richard B. Lanman,[1] Andrew D. Simmons[2]

Clinical Lung Cancer 2018

77 patients with T790M





Multiple mutations in the same patient at rocilenitib progression

- **EGFR C797S**
- **KRAS/NRAS/HRAS**
- **NTRK1 fusion**
- **MET amplification**

Association Between Plasma Genotyping and Outcomes of Treatment With Osimertinib (AZD9291) in Advanced Non–Small-Cell Lung Cancer

Geoffrey R. Oxnard, Kenneth S. Thress, Ryan S. Alden, Rachael Lawrance, Cloud P. Paweletz, Mireille Cantarini, James Chih-Hsin Yang, J. Carl Barrett, and Pasi A. Jänne

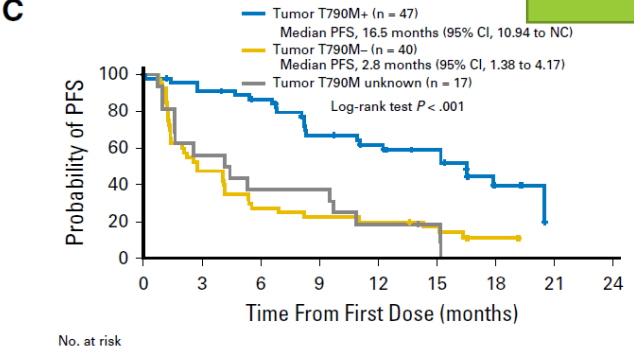
ddPCR

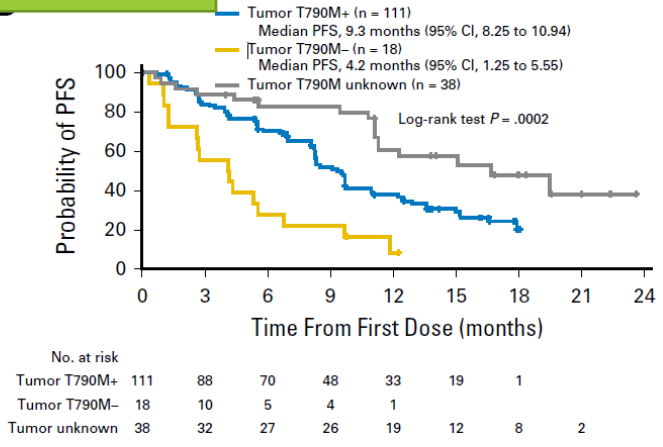
Tumor genotyping was used as an additional biomarker

Negative Plasma Results

Positive Plasma Results

C





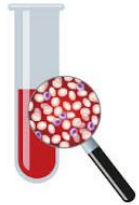
VOLUME 34 · NUMBER 28 · OCTOBER 1, 2016

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

The tissue samples generally available for molecular testing in lung cancer are very limited

Current Testing Paradigm = Sequential Testing

- ✓ tests biomarkers in order of their frequency
- ✓ potentially more economical approach
- ✓ availability of commercially available, validated and/or FDA approved tests
- ✓ Reimbursement restrictions
- ✓ Drug availability
- ✗ as more biomarker testing is required, testing becomes more challenging to deliver (eg EGFR, ALK, ROS1, BRAF, PDL-1…)
- ✗ Different testing requirements (eg molecular, IHC, FISH..)
- ✗ High Turn Around Time (TAT)



ctDNA testing:

- ✓ EGFR activating mutations in lack of tissue at diagnosis of NSCLC [1]
- ✓ T790M mutation at progression as a first screening [2,3,4]

1. Douillard *et al. British J of Cancer, 2014*
2. Mok *et al, N Engl J Med*, 2016
3. *Novello et al. Annals of Oncol, 2016*
4. *NCCN guidelines , NSCLC v6.2018*

The tissue samples generally available for molecular testing in lung cancer are very limited

Biomarkers results used for selecting appropriate therapy should be:

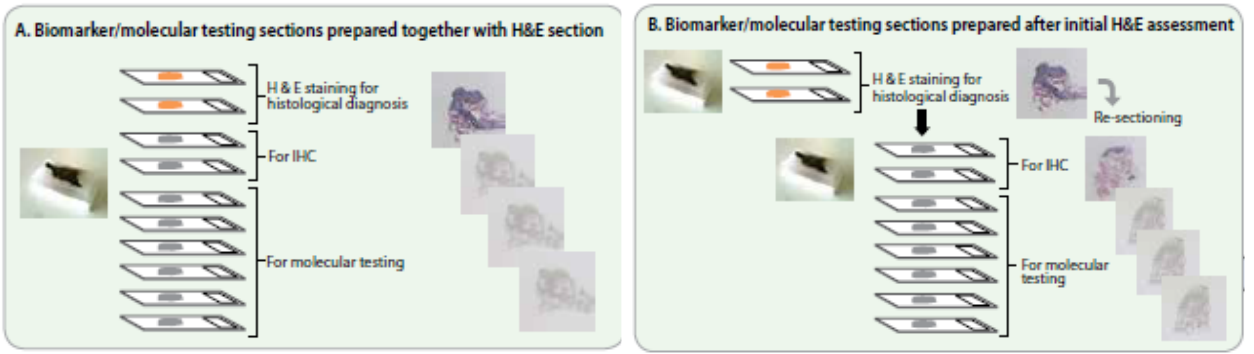
FAST

ACCURATE

REPRODUCIBLE

Given that the tissue samples generally available for molecular testing in lung cancer are very limited, it is imperative to take steps to preserve tissue

Block cutting strategies to limit tissue waste [1,2]



Guidelines for Tissue Management are needed in Greece

1. Kerr KM, Lopez-Rios Ann Oncol. 2016 2. IASLC Atlas of PD-L1 Testing 2017

ΕΝΩΣΗ ΠΝΕΥΜΟΝΟΛΟΓΩΝ ΕΛΛΑΔΑΣ

ΕΤΗΣΙΟ ΣΥΝΕΔΡΙΟ

30 Μαΐου - 2 Ιουνίου 2019

Αθήνα, *Ξενοδοχείο* Royal Olympic

ΣΑΣ ΕΥΧΑΡΙΣΤΩ ΓΙΑ ΤΗΝ ΠΡΟΣΟΧΗ ΣΑΣ