Mechanisms of Resistance to Targeted Therapy

Educational Session: Liquid Biopsies in Lung Cancer
September 25, 2018

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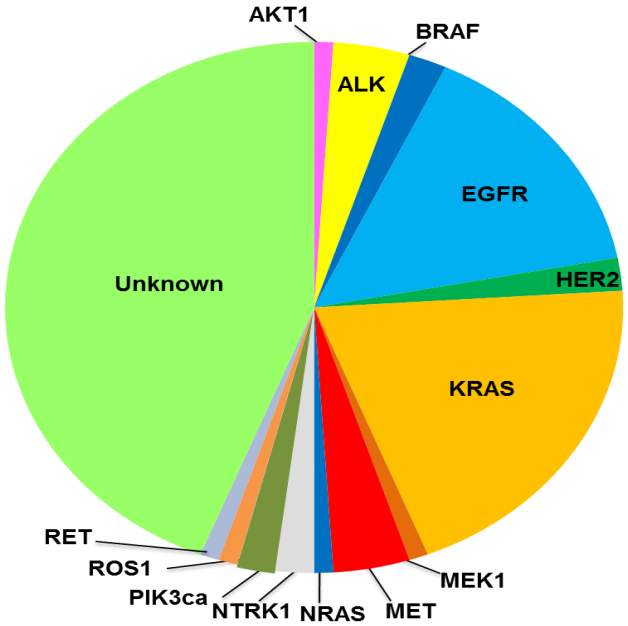
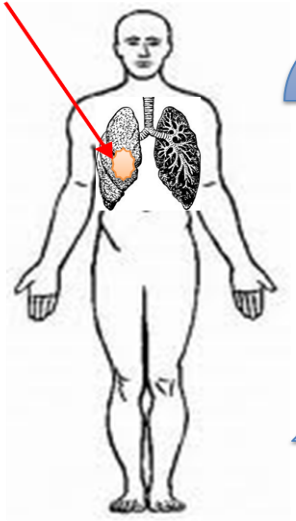
2018 - Molecular Subsets of Lung Adenocarcinoma Defined by 'Driver' Mutations



Frequency of driver mutations in NSCLC	
AKT1	1%
ALK	3-7%
BRAF	1-3%
EGFR	10-35%
HER2	2-4%
KRAS	15-25%
MEK1	1%
MET	~4%
NRAS	1%
NTRK1	~3%
PIK3CA	1-3%
RET	1-2%
ROS1	1-2%

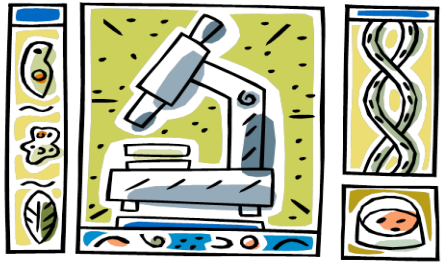
Understanding and Overcoming Acquired Resistance to Targeted Therapies

Site of enlarging tumor on therapy



Rebiopsy

It is critical to biopsy progressing lesions to identify the mechanism of resistance and to direct the patient towards appropriate therapy!



Molecular analysis of the resistant tumor and cell culture models of TKI resistance

Possible strategies to treat progressive disease

Clinical application of "liquid biopsies" to study and treat acquired resistance to targeted therapies in lung cancer

- First studies of acquired resistance to TKIs in lung cancer were all done with tissue based studies.
- With increasing frequency, blood based studies ("liquid biopsies") are being utilized to monitor treatment response and progression (development of acquired resistance).
- Molecular profiling of tissue and / or blood at the time of disease progression can lead to the identification of genomic drivers of acquired resistance (e.g., *EGFR* T790M, *EGFR* C797S, *MET* amplification, etc.).
- To date, the best example of the clinical utilization of "liquid biopsies" to detect acquired resistance is EGFR T790M → covered very thoroughly by Dr. Tsao in his lecture.
- *We will discuss emerging applications of blood-based studies to detect mechanisms of acquired resistance.*

Acquired resistance to the EGFR mutant-selective TKI, Osimertinib

Christine M. Lovly, MD, PhD Vanderbilt Ingram Cancer Center Nashville, TN USA

First line osimertinib

FLAURA DOUBLE-BLIND STUDY DESIGN

Patients with locally advanced or metastatic NSCLC

Key inclusion criteria
- ≥18 years*
- WHO performance status 0 / 1
- Exon 19 deletion / L858R (enrolment by local‡ or central‡ EGFR testing)
- No prior systemic anti-cancer / EGFR-TKI therapy
- Stable CNS metastases allowed

Stratification by mutation status (Exon 19 deletion / L858R) **and race** (Asian / non-Asian)

Osimertinib (80 mg p.o. qd) (n=279)

Randomised 1:1

EGFR-TKI SoC§: **Gefitinib** (250 mg p.o. qd) or **Erlotinib** (150 mg p.o. qd) (n=277)

RECIST 1.1 assessment every 6 weeks¶ until objective progressive disease

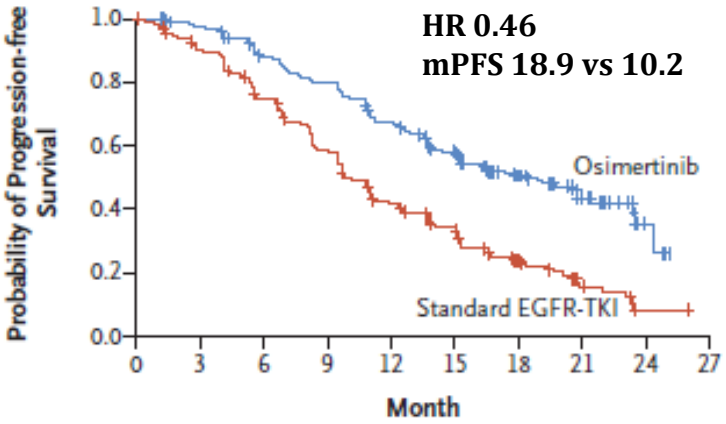
Crossover was allowed for patients in the **SoC** arm, who could receive open-label osimertinib upon central confirmation of progression and T790M positivity

Endpoints
- **Primary endpoint:** PFS based on investigator assessment (according to RECIST 1.1)
  - The study had a 90% power to detect a hazard ratio of 0.71 (representing an improvement in median PFS from 10 months to 14.1 months) at a two-sided alpha-level of 5%
- **Secondary endpoints:** objective response rate, duration of response, disease control rate, depth of response, overall survival, patient reported outcomes, safety

Soria NEJM 2018, Ramalingam ESMO 2017

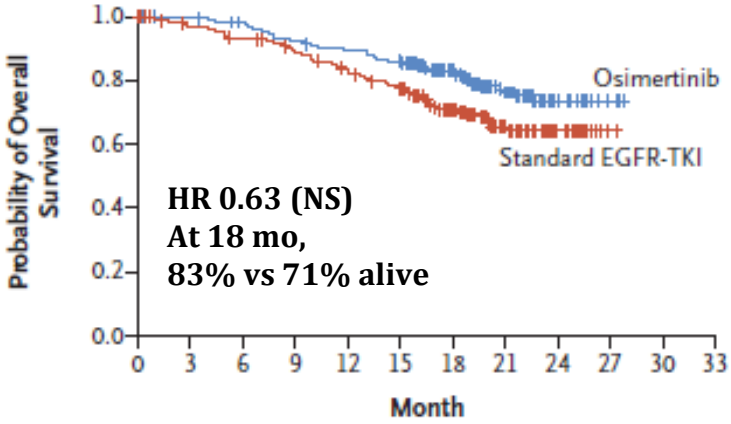
First line osimertinib

Progression-free survival



HR 0.46
mPFS 18.9 vs 10.2

Overall survival



HR 0.63 (NS)
At 18 mo,
83% vs 71% alive

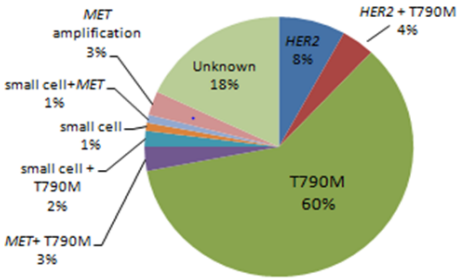
Soria NEJM 2018, Ramalingam ESMO 2017

What is known about resistance to first line osimertinib?

EGFR TKI
Erlotinib
Afatinib
Gefitinib

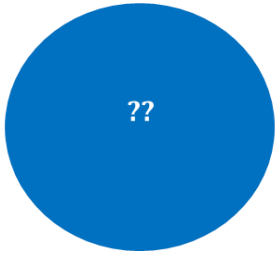
→ **Acquired Resistance** →

EGFR TKI
Osimertinib



→ **Acquired Resistance #2**

EGFR TKI
Osimertinib →



Acquired *EGFR* mutations
Acquired alterations
Tumor heterogeneity
Histologic transformation

Adapted from Hu Y ASCO 2018

What have blood based studies taught us about osimertinib resistance?

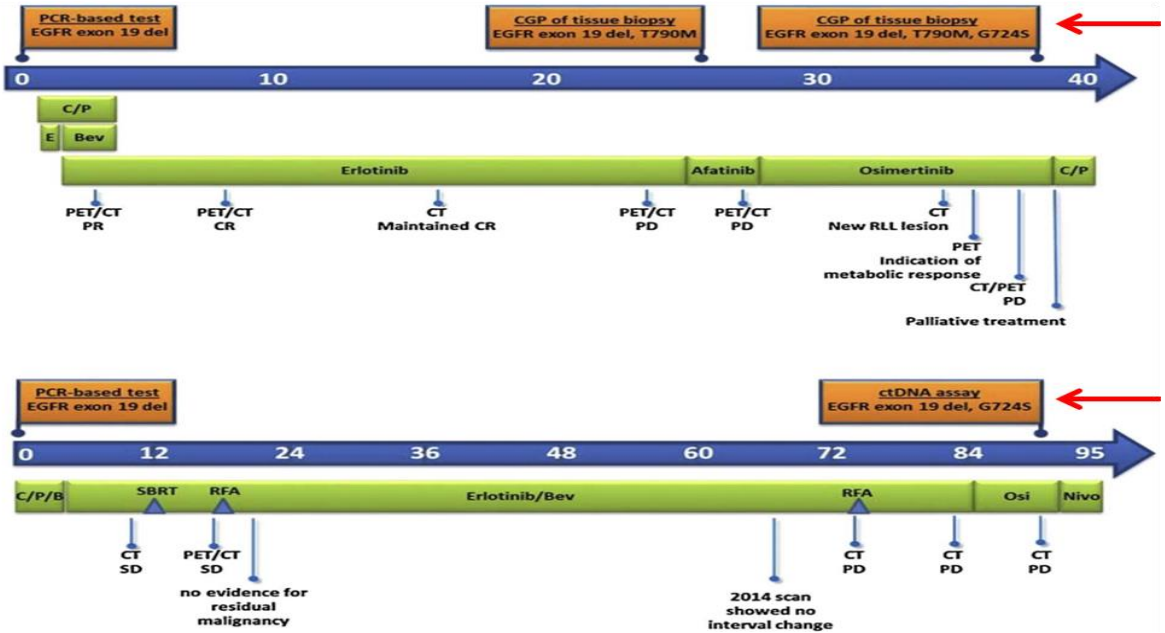
- Novel EGFR kinase domain mutations which arise at the time of acquired resistance to osimertinib.

✓ EGFR G796S/R, C797S/R, L792F/H, G724S

- Novel 'bypass tracks' that emerge at the time of acquired resistance to osimertinib.

✓ Acquired kinase fusions.

EGFR G724S has been reported in patients with acquired resistance to osimertinib by tissue (top) and blood (bottom) based studies

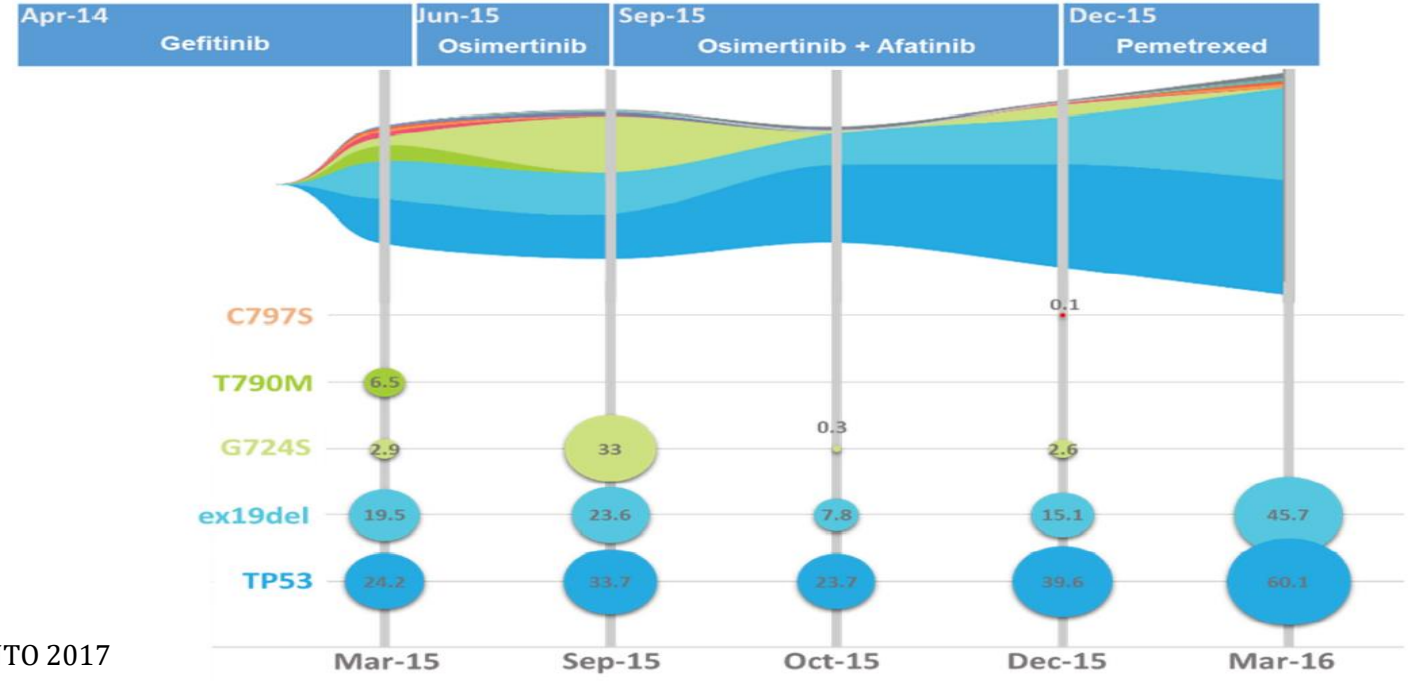


EGFR ex19del MAF: 10%
EGFR G724S MAF: 5%

EGFR ex19del MAF: 1.9%
EGFR G724S MAF: 0.7%

Oztan et al., Lung Cancer 2017

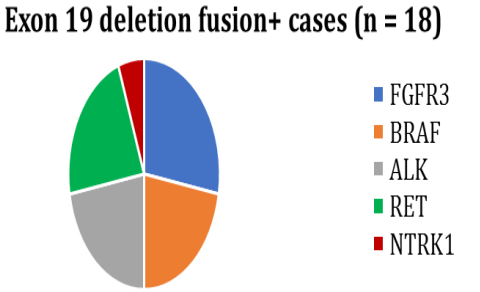
Case report of combination osimertinib plus afatinib overcoming resistance in a patient with EGFR ex19del-G724S

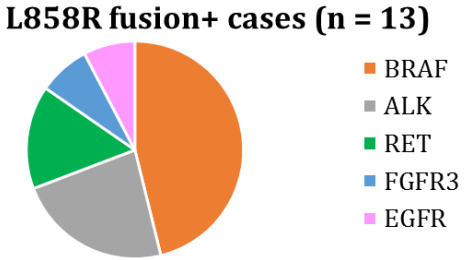


Peled et al., JTO 2017

RTK fusions as acquired resistance mechanisms to EGFR TKIs

- In 31 post-EGFR TKI NSCLCs samples a kinase fusion (*ALK*, *RET*, *FGFR3*, *EGFR*, *NTRK1*, *BRAF*) was identified, including 12 paired pre-treatment samples).
- The post-TKI tissue samples lacked other known resistance mechanisms, but retained the original driver mutation. T790M was typically lost post-osimertinib in the fusion+ sample
- Kinase fusions account for acquired resistance to EGFR TKIs in >1% of cases
- Clinical data suggests that combination therapy targeting EGFR + the acquired fusion is necessary, but more investigation is needed to optimizing dosing and ensure access in the trial setting.

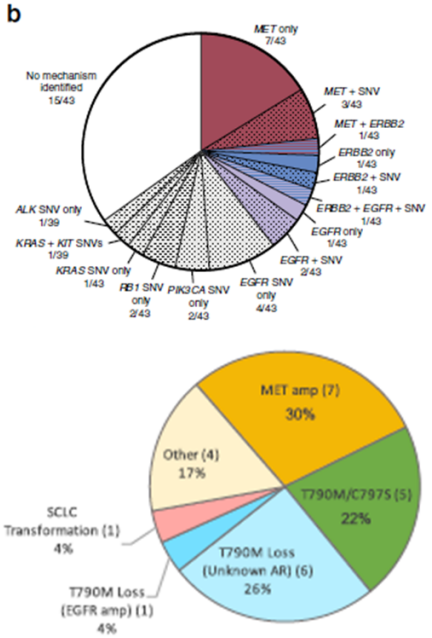




Other mechanisms of acquired resistance

Mutations identified	Where identified	Therapy
MET amplification	Tissue, plasma	MET inhibitor
HER2 amplification	Tissue, plasma	HER2 inhibitor
BRAF V600E	Tissue	BRAF/MEK inhibitor
PIK3CA	Plasma	PIK3CA inhibitor
KRAS mutation/amp	Plasma	--
EGFR amplification	Tissue, plasma	EGFR antibody
RB1 loss, p53 loss	Plasma	--
Small cell transformation	Tissue	Chemotherapy
Loss of EGFR T790M	Tissue, plasma	1st/2nd gen EGFR TKI

All these alterations can occur concurrently with EGFR so pre-treatment tissue/plasma is key to identify acquired alterations.



Ramalingam JCO 2017, Ho JTO 2016, Ou Lung Cancer 2016, Planchard Ann Onc 2015, Chabon Nat Comm 2016, Piotrowska PASCO 2017, Yang CCR 2018, Ou JCO 2017

Adapted from Yu H ASCO 2018

Acquired resistance to the ALK TKIs

Landscape of ALK inhibitors in clinical use

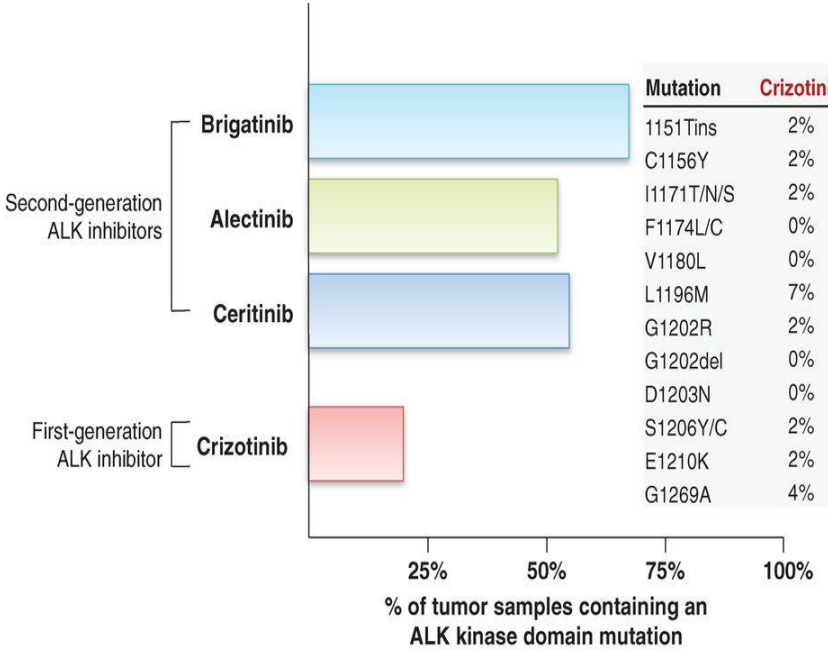
ALK TKI		ADDITIONAL TARGETS	STATUS
1st generation	Crizotinib	MET, ROS1	▪ FDA-approved (11/2013)
2nd generation	Alectinib	RET, LTK	▪ FDA approved, post crizotinib (12/2015) ▪ FDA apprroved, first line (11/2017)
	Brigatinib	Mutant EGFR, ROS1	▪ FDA accelerated approval, post crizotinib (4/2017)
	Ceritinib	IGF-R1, IR, ROS1	▪ FDA-approved, post crizotinib (4/2014) ▪ FDA-approved, first line (5/2017)
	Ensartinib	MET, ABL, AXL	Investigational
	Entrectinib	NTRKs, ROS1	Investigational
3rd generation	Lorlatinib	ROS1	▪ FDA breakthrough-therapy designation, in patients who have received 1 or more ALK inhibitors (4/2017)

Hematol Oncol
2) Roskoski R 2017 Pharmacological Research, and
3) the USA FDAhttps://www.fda.gov/default.htm

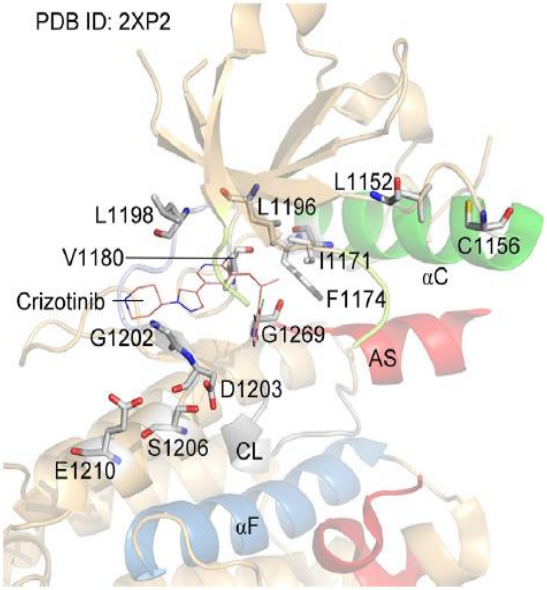
What have blood based studies taught us about osimertinib resistance?

- Complexity and heterogeneity of ALK kinase domain mutations which confer drug resistance.
- Histological transformation to SCLC.

What do we know about resistance to 'next generation' ALK TKIs?

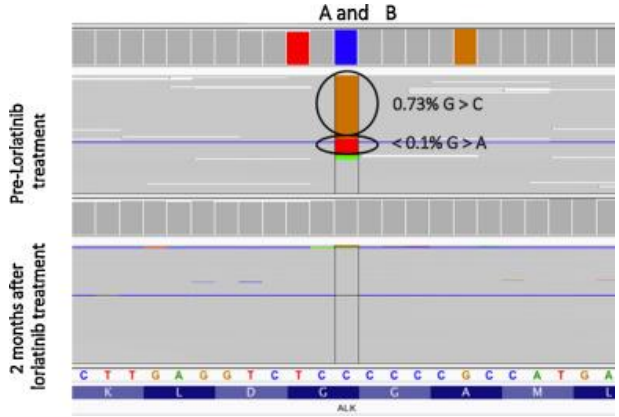


Mutation	Crizotinib	Ceritinib	Alectinib	Brigatinib
1151Tins	2%	0%	0%	0%
C1156Y	2%	8%	0%	0%
I1171T/N/S	2%	4%	12%	0%
F1174L/C	0%	17%	0%	0%
V1180L	0%	4%	6%	0%
L1196M	7%	8%	6%	0%
G1202R	2%	21%	29%	43%
G1202del	0%	8%	0%	0%
D1203N	0%	4%	0%	14%
S1206Y/C	2%	0%	0%	14%
E1210K	2%	0%	0%	29%
G1269A	4%	0%	0%	0%

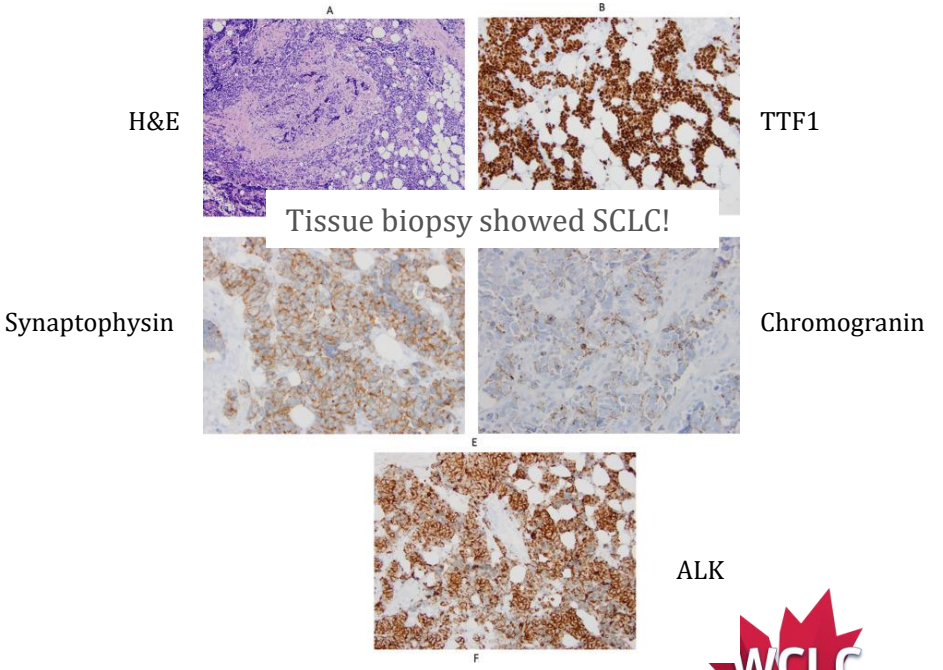


Huan Qiao, and Christine M. Lovly Cancer Discov 2016;6:1084-1086
Justin F. Gainor et al. Cancer Discov 2016;6:1084-1086
R. Roskoski Pharmacological Research 2017; 117: 343-356

Pitfall of solely relying on liquid re-biopsy?



NGS of ctDNA showing the presence of ALK G1202R mutations before (A) and disappearance of G1202R (B) after treatment with **lorlatinib**.



Ou, SI Lung Cancer 2017

Resistance to MET-Directed Targeted Therapy – lessons from tissue and blood

Drug Administered	MET alteration	Putative resistance mechanism	Notes
Crizotinib 8 mo of disease control	MET D1010H	**MET D1228N** (acquired second site mutation on tumor rebiopsy)	high total MET and phospho-MET IHC+ on post-PD biopsy
Crizotinib 13 mo of disease control	MET D1010H (MET Y1230C)	**MET Y1230C** (detected in ctDNA on PD)	
Crizotinib 8 mo of disease control	MET c.3028delG	**MET Y1230H** (acquired in tumor, MET amp + MET D1228N, Y1230H, Y1230S, and G1163R in plasma)	thereafter responded to Glesatinib
Savolitinib + Osimertinib 9 mo of disease control	MET amplification (+EGFR ex19 del)	**MET D1228V** (acquired second site mutation on tumor rebiopsy)	thereafter responded to Cabozantinib + Erlotinib

Mechanisms of acquired resistance to MET TKIs in MET exon 14mutant NSCLC

Presented Sunday, June 3, 2018. Mark M. Awad (Abst 9069)

-Secondary mutations in MET included H1094Y, G1163R, L1195F, L1195V, D1228N, Y1230H, and Y1230S.

-bypass track activation : amplification of wild-type KRAS, BRAF, and/or EGFR.

- acquired amplification of the mutated METex14 allele

Adapted from Planchard D ASCO 2018

Heist R et al, J Thoracic Oncol, 2016; Ou et al, J Thoracic Oncol, 2017; Bachall et al, Cancer Discov, 2017; Qi et al, Cancer Res, 2011; Tiedt et al, Cancer Res 2011; Engstrom et al, Clinical Cancer Res 20

Thoughts on integration of liquid biopsies into standard clinical practice

1. To obtain genomic profiling if a tissue biopsy is not safe / feasible.

2. To monitor disease burden in a longitudinal fashion (analogous to 'classic' tumor markers, like CEA).
   - Dynamic changes in ctDNA may predict disease response or progression prior to radiographic imaging.

3. To supplement radiographic studies.
   - Liquid biopsies may be helpful to clarify ambiguous radiographic findings.

➢ More research is needed

Take Home Messages

- Liquid biopsies are rapidly integrating into clinical care for routine purposes (e.g., detection of EGFR T790M) and exploratory analyses (e.g. to detect novel mechanisms of drug resistance).

- Liquid biopsies may better assess the heterogeneity of drug resistance compared to tissue biopsy.

- In the future, liquid biopsies may help us to monitor disease burden in a longitudinal fashion (analogous to 'classic' tumor markers, like CEA).
  → Dynamic changes in ctDNA may predict disease response or progression prior to radiographic imaging.
  → Additional research is needed.

- Liquid biopsies may miss non-genomic mechanisms of acquired resistance, such as SCLC transformation.

Liquid Biopsies in Lung Cancer

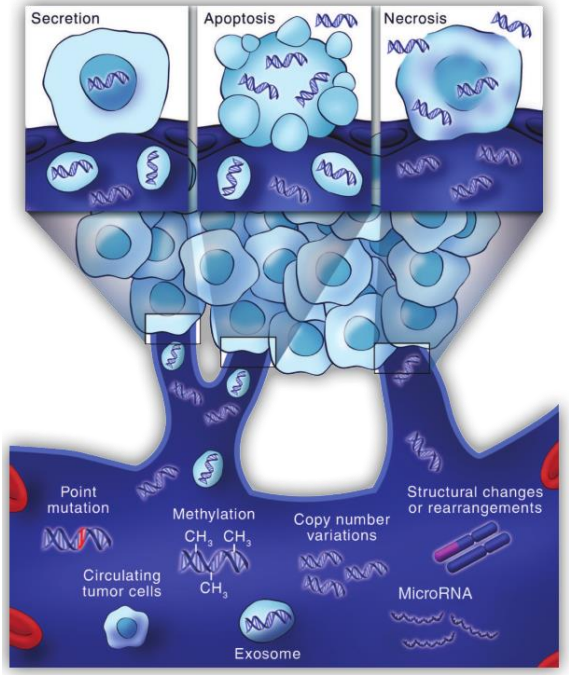
Ming S. Tsao, MD, FRCPC
Princess Margaret Cancer Centre
University of Toronto
Toronto, Canada

Liquid biopsy

Analysis of tumor cells or cell products in body fluids obtained by non or minimally invasive procedures to identify pathological processes

- Blood
- Urine
- Pleural/peritoneal fluid
- Cerebrospinal fluid (CSF)
- Bronchial washing/brushing

- Cell products: DNA, RNA, non-coding RNA, protein, exosome etc.
  - Circulating tumor cells (CTC)
  - Circulating tumor DNA (ctDNA) / Cell-free DNA (cfDNA)
- Tumour cell derived DNA is only ≤0.5% of total cell-free DNA (vs. ≥ 10% tissue analysis*)
- Must use methods that are more sensitive than those used for tissue analysis



Diaz & Bardelli. J Clin Oncol 2014;32:579-86

* 2018 CAP/IASLC/AMP

Pre-analytical factors for optimal ctDNA testing

Specimen type:

- **Plasma** (serum contain higher cfDNA from leukocyte lysis during clotting)

Blood collection:

- **K_2EDTA tube:** processed <6 hrs
- **Leukocyte stabilizing tube:** 48-7 days

Blood storage and transport:

- **Unprocessed:** room temperature
- **Plasma:** room temperature or -20C

Methods for detecting mutations in ctDNA

Assay	Detection sensitivity	Mutations identified	Cost
ARMS	1%	Known only	$
Cobas®	0.1–1%	Known only	$
ddPCR	0.05–0.1%	Known only	$
BEAMing	<0.1%	Known only	$$
NGS	0.02-0.2%	Known or unknown	$$-$$$

ARMS: amplification refractory mutation system; dd: digital droplet; BEAM: bead, emulsion, amplification, magnetics; NGS: next generation sequencing

Tan et al, J Thorac Oncol. 2016;11:946-63; Moding EJ, et al. Lung Cancer 2018;119:42-7

NEXT GENERATION SEQUENCING PLATFORMS

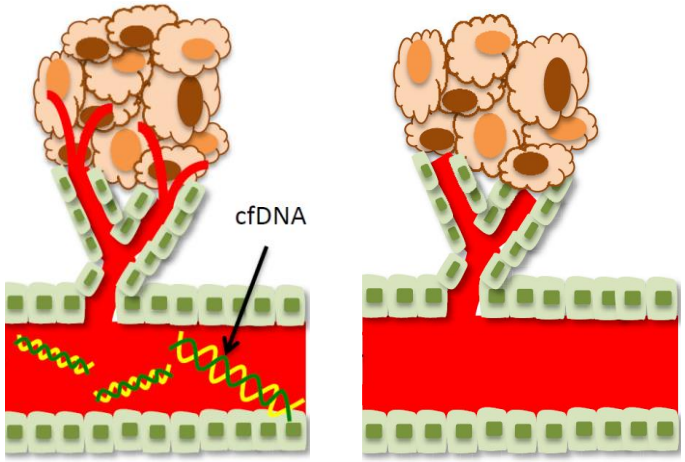
1. **Assay:** laboratory developed *vs.* commercial
2. **Commercial tests:** test panel *vs.* central CLIA-lab
3. **Coverage:** number of bases, genes, exons
4. **Enrichment technology:** multiplex PCR, Hybrid capture
5. **Limit of detection:** % mutant allele / wild type allele
6. **Sensitivity & specificity:** samples with known mutant allele frequency (MAF)
7. **Bioinformatics:** variant calling and error correction methods

Commercial Next Generation Sequencing Liquid Biopsy Tests

- Guardant 360 (Guardant Health)
- Foundation Medicine FACT (Foundation Medicine Inc)
- Oncomine™ Lung cfDNA Assay (ThermoFisher)
- Archer Reveal ctDNA (ARCHER diagnostics)
- Oncotype SEQ (Genomic Health)
- LiquidDx (MolecularMD)
- CancerIntercept™ Detect and CancerIntercept™ Monitor (Pathway Genomics)
- OptiSeq™ NGS Pan-Cancer Panel (DiCarta)
- PlasmaSELECT (Personal Genome Diagnostics)

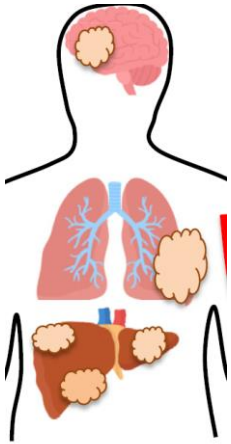
Factors that may influence sensitivity and specificity of ctDNA detection

Shedding vs. Non-shedding

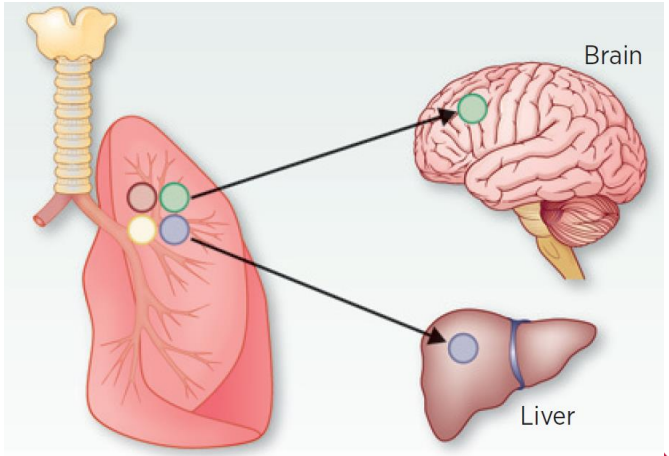


Sacher AG, et al. J Thorac Oncol 2017;12:1344-56

stage



Inter-metastatic heterogeneity



Jamal-Hanjani et al. CCR 2015;21:1258-66

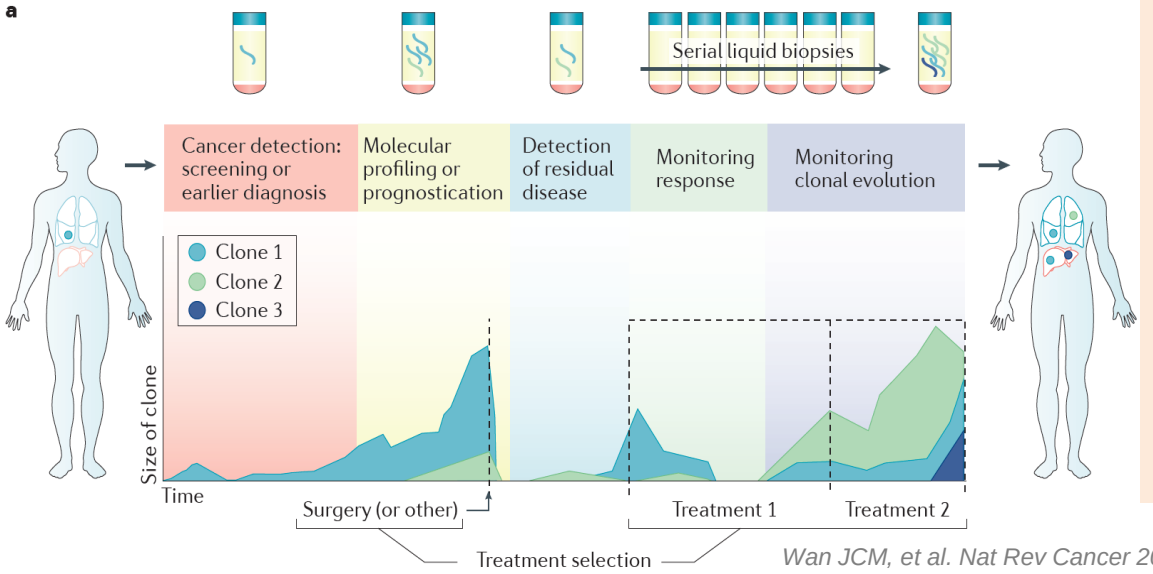
Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review

Jason D. Merker, Geoffrey R. Oxnard, Carolyn Compton, Maximilian Diehn, Patricia Hurley, Alexander J. Lazar, Neal Lindeman, Christina M. Lockwood, Alex J. Rai, Richard L. Schilsky, Apostolia M. Tsimberidou, Patricia Vasalos, Brooke L. Billman, Thomas K. Oliver, Suanna S. Bruinooge, Daniel F. Hayes, and Nicholas C. Turner

Analytical validity	the ability of a test to **accurately and reliably detect the variant(s) of interest** and includes measures of accuracy, sensitivity, specificity, and robustness.
Clinical validity	test may **accurately detect the presence or absence of a pathologic state** or **predict outcomes for groups of patients whose test results differ**.
Clinical utility	High levels of evidence exist to demonstrate that the **use of the test improves patient outcomes compared with not using it**.

J Clin Oncol 2018;36:1631-41

Liquid biopsies come of age: Towards implementation of circulating tumor DNA



Wan JCM, et al. Nat Rev Cancer 2017;17:223-8

POTENTIAL APPLICATION

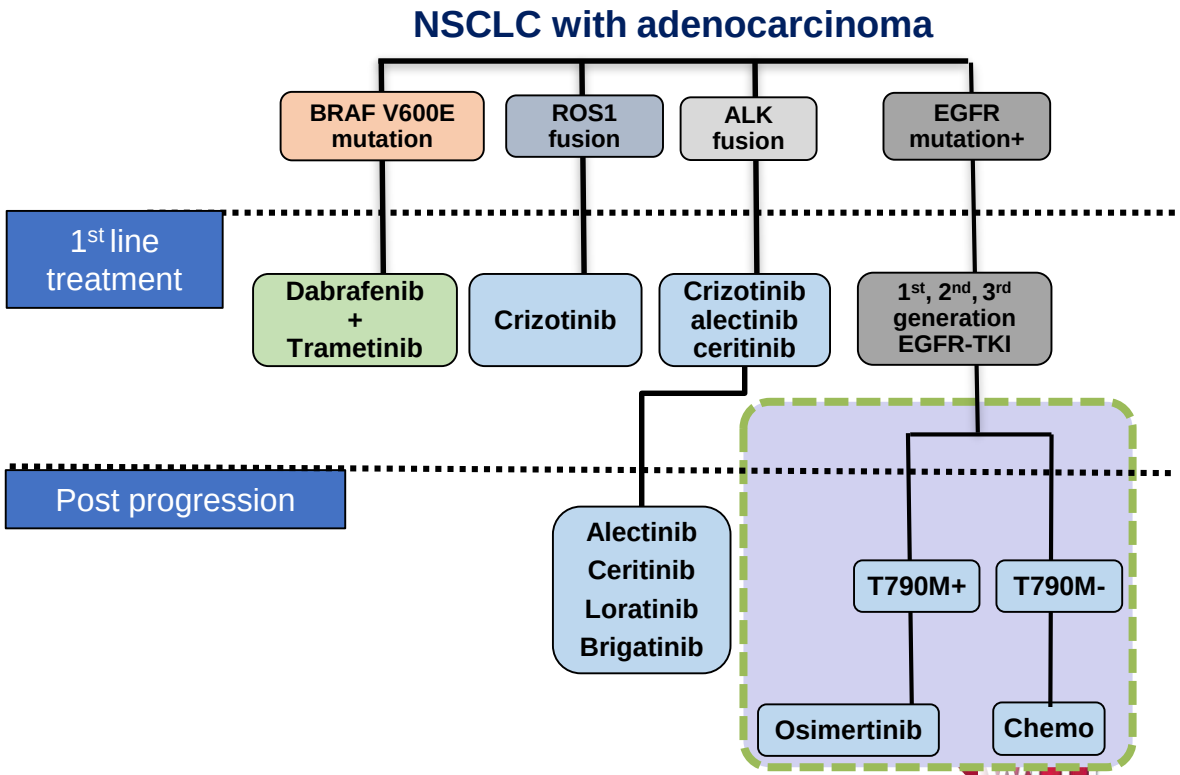
1. Cancer early detection
2. Monitoring of minimal residual disease, tumor dynamics and recurrence
3. Estimation of tumor and mutation burden and for prognostication
4. Identification of driver mutations for targeted therapy
5. Identification of resistant mutation (mechanism) and real time assessment of evolution of resistance
6. Evaluation of early treatment response

Evidence for clinical application

1. Identification of resistant mutation and driver mutations for targeted therapies
2. Cancer early detection and monitoring of post-surgery minimal residual disease and tumor recurrence
3. Estimation of tumor and mutation burden

Question

Is ctDNA testing clinically reliable to detect T790M resistant mutation in lung cancer patients with EGFR mutant tumors, who progress on 1st/2nd generation EGFR TKI?



Analytical validation of ctDNA assays for EGFR T790M mutation in Post-TKI patients

Study	# Matched samples	Methods	Sensitivity	PPV	Specificity	NPV
Ishii (2015)	18	ddPCR	82%	90%	86%	75%
Sacher (2016)	60	ddPCR	77%	77%	63%	63%
Takahama (2016)	41	ddPCR	65%	87%	70%	39%
Jenkins (2017)	540	Cobas	61%	90%	79%	38%
Thress (2015)	72	Cobas	73%	79%	67%	59%
Karlovich (2016)	95	Cobas	64%	95%	98%	84%
Thress (2015)	72	BEAMing	80%	77%	58%	64%
Oxnard (2016)	216	BEAMing	70%	86%	69%	46%
Reckamp (2016)	54	NGS	93%	90%	31%	95%

Sensitivity: probability that patient with T790M in tumor has T790M+ plasma result

PPV (+ve predictive value): chance that plasma T790M+ patient has T790M+ tumor

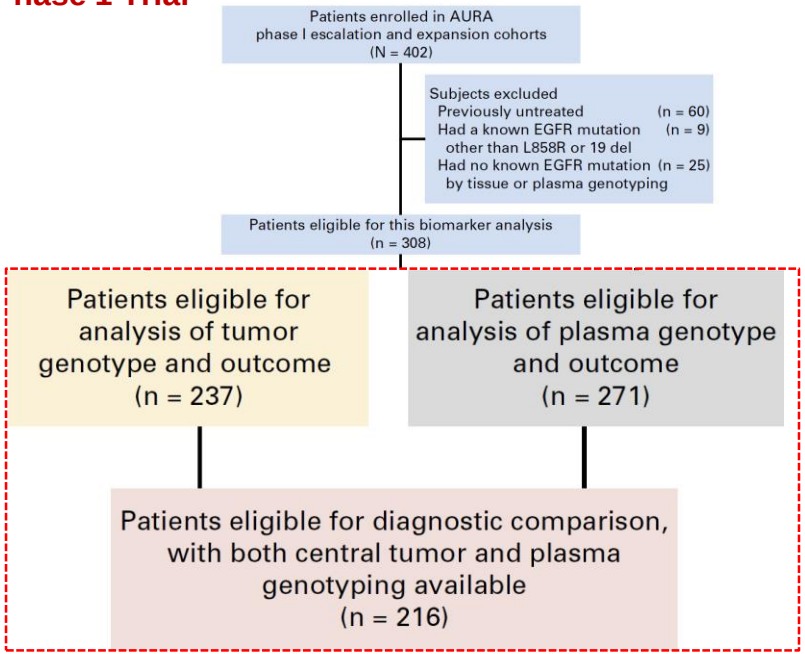
Specificity: probability that patient without T790M in tumor has T790M- plasma result

NPV (-ve predictive value): chance that plasma T790M- patient has T790M- tumor

Clinical validation

AURA Phase 1 Trial

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



ctDNA by BEAMing

Sensitivity of plasma testing:
- T790M: 70.3%

Liver metastasis (p=0.008):
- Yes: 94%
- No: 79%

Extrathoracic mets (p=0.06):
- Yes: 86%
- No: 75%

Oxnard GR, et al. J Clin Oncol 2016;34:3375-82

Clinical validation

Response rates for T790M patients identified by tumor biopsy and liquid biopsy are similar and higher

Tumor T790M	Plasma T790M	Patient number	ORR
+	NA	173	62%
-	NA	58	26%
NA	+	164	63%
NA	-	102	46%

But: ctDNA T790M –ve patients have higher response rate than tumor T790M –ve patients

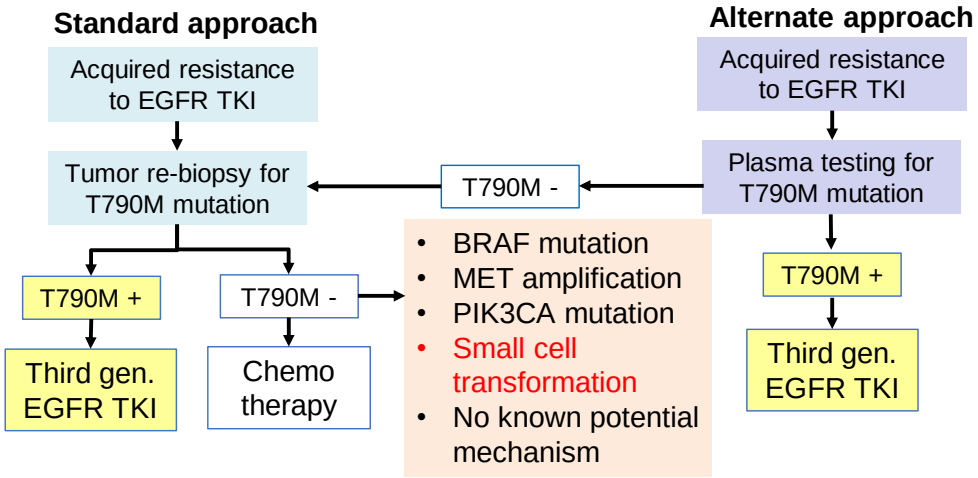
Median PFS survival for tumor T790M+ and plasma T790M+ patients are comparable.

Tumor T790M	Plasma T790M	Median PFS (m)	95% CI	Long rank P value
+	NA	9.7	8.3-12.5	<0.001
-	NA	3.4	2.1-4.3	
NA	+	9.7	8.3-11.1	0.188
NA	-	8.2	5.3-10.9	

Plasma T790M negative result cannot be excluded from Osimertinib treatment.

Oxnard GR, et al. J Clin Oncol 2016;34:3375-82

Clinical utility



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

This analysis has several practical limitations. Whereas it is a large analysis of a prospective dataset, **it was not a preplanned analysis**, in part because of the rapid evolution of plasma genotyping technologies. The **study population is also not fully representative of all patients with acquired EGFR-TKI resistance** given the intentional enrichment for T790M-positive patients. Lastly, **BEAMing was not performed under Clinical Laboratory Improvement Amendments conditions**; this analysis used an investigational assay that is identical to the commercially available BEAMing assay. **Prospective validation is needed to confirm the clinical benefit of osimertinib in patients with T790M-positive plasma genotyping.**

J Clin Oncol 2016;34:3375-3382.

2018 updated CAP/IASLC/AMP Guideline

Expert consensus opinions:

- Cell-free plasma DNA methods can be used to identify EGFR T790M mutations in patients who responded then progress on EGFR TKI; testing of tumor sample is recommended if the plasma result is negative

Lindeman NI, et al. J Thorac Oncol 2018; 13: 323-358

Analytical validation

Question:

Is ctDNA testing sufficiently reliable to Identify driver mutations (e.g. EGFR) for patients to receive targeted therapy?

Study	Trial	# samples	Methods	Mutation	Sensitivity	Specificity
Mok (2015)	FASTACT-2	96	Cobas	L858R+19del	**75%**	**96%**
Weber	-	96	Cobas	L858R+19del	**60%**	**96%**
Thress (2015)	AURA-1	38	Cobas	L858R/19del	**90% / 86%**	**100% / 100%**
Thress (2015)	AURA-1	72	Cobas	L858R/19del	**82% / 82%**	**97% / 97%**
Jenkins (2017)	AURA-1/2	226	Cobas	L858R+19del	**59% / 85%**	**100% / 99%**
Sacher (2016)	-	120	ddPCR	L858R/19del	**74% / 86%**	**100% / 100%**
Lee (2016)	-	58	ddPCR	L858R/19del	**71% / 77%**	**-**
Thress (2015)	AURA-1	38	ddPCR	19del	**90%**	**100%**
Jenkins	AURA-1/2	208	ddPCR	L858R/19del	**69% / 72%**	**98% / 100%**
Thress (2015)	AURA-1	38	BEAMing	L858R/19del	**100% / 93%**	**93% / 100%**
Thress (2015)	AURA-1	72	BEAMing	L858R/19del	**87% / 82%**	**97% / 97%**
Oxnard (2016)	AURA-1	216	BEAMing	L858R/19del	**86% / 82%**	**97% / 98%**

Adapted from Rolfo C, et al. J Thorac Oncol 2018

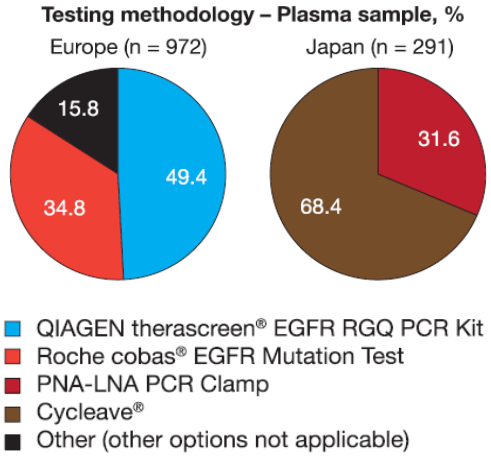
Clinical validity & utility study (prospective)

ctDNA Determination of *EGFR* Mutation Status in European and Japanese Patients with Advanced NSCLC: The ASSESS Study

Martin Reck, MD, PhD,[a] Koichi Hagiwara, MD, PhD,[b] Baohui Han, MD, PhD,[c] Sergei Tjulandin, MD, PhD,[d] Christian Grohé, MD,[e] Takashi Yokoi, MD, PhD,[f]

56 centres, 1311 patients enrolled, 1288 eligible

	Patients number	% Concordance rate (95% CI)	% Sensitivity (95% CI)	% Specificity (95% CI)
Overall	1162	89 (87.1 – 90.8)	46 (38.8 – 53.4)	97 (96.2 – 98.3)
Japan	281	81 (75.7 – 85.2)	40 (29.2 – 50.7)	99 (96.3 – 99.9)
Europe	881	92 (89.7 – 93.4)	51 (41.4 – 61.4)	97 (95.6 – 98.1)
Qiagen Therascreen	138	95 (89.8 – 97.9)	73 (49.8 – 89.3)	99 (95.3 – 100)
COBAS	23	96 (78.1 – 99.9)	75 (19.4 – 99.4)	100 (82.4 – 100)
Cycleave	190	85 (78.8 – 89.5)	51 (37.3 – 64.4)	99 (95.9 – 100)
PNA – LNA PCR clamp	91	84 (74.3 – 90.5)	52 (32.5 – 70.6)	98 (91.3 – 100)



J Thorac Oncol 2016;11:1682-83

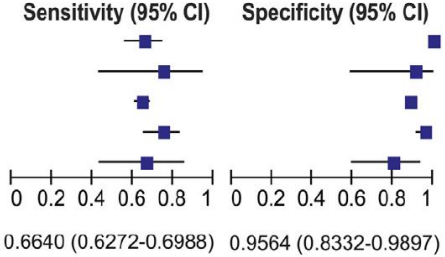
WCLC 2018

2018 updated CAP/IASLC/AMP Guideline

Question 5: What Is the Role of Testing for Circulating cfDNA for Lung Cancer Patients?

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.

Study	TP	FP	FN	TN	Detection System	Sensitivity (95% CI)	Specificity (95% CI)
Douillard 2014[242]	69	1	36	546	ARMs	0.66 [0.56, 0.75]	1.00 [0.99, 1.00]
Kukita 2013[234]	9	1	3	10	PNA/LNA clamp	0.75 [0.43, 0.95]	0.91 [0.59, 1.00]
Li 2014[243]	389	114	214	874	Multiple	0.65 [0.61, 0.68]	0.88 [0.86, 0.90]
Mok 2015[235]	72	6	24	136	allele-specific PCR	0.75 [0.65, 0.83]	0.96 [0.91, 0.98]
Oxnard 2014[232]	14	5	7	20	ddPCR	0.67 [0.43, 0.85]	0.80 [0.59, 0.93]



Lindeman NI, et al. J Thorac Oncol 2018; 13: 323-358

Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review

Jason D. Merker, Geoffrey R. Oxnard, Carolyn Compton, Maximilian Diehn, Patricia Hurley, Alexander J. Lazar,

Definitively establishing the clinical utility of ctDNA assays, as compared with a standard biopsy for tumor genotyping, is challenging, because prospective trial data are lacking. At present, one PCR-based ctDNA assay for the detection of EGFR variants in patients with NSCLC has received regulatory approval in the United States and Europe, and PCR-based ctDNA assays for EGFR in NSCLC and KRAS in colorectal cancer are available for commercial use in Europe. These assays have demonstrated clinical validity, but the clinical utility in this setting is based on retrospective analyses. Evidence demonstrated that, although positive EGFR testing results may effectively be used to guide therapy, undetected results should be confirmed with analysis of a tissue sample, if possible.

J Clin Oncol 2018;36:1631-41

OPINION

Early stage NSCLC — challenges to implementing ctDNA-based screening and MRD detection

Question

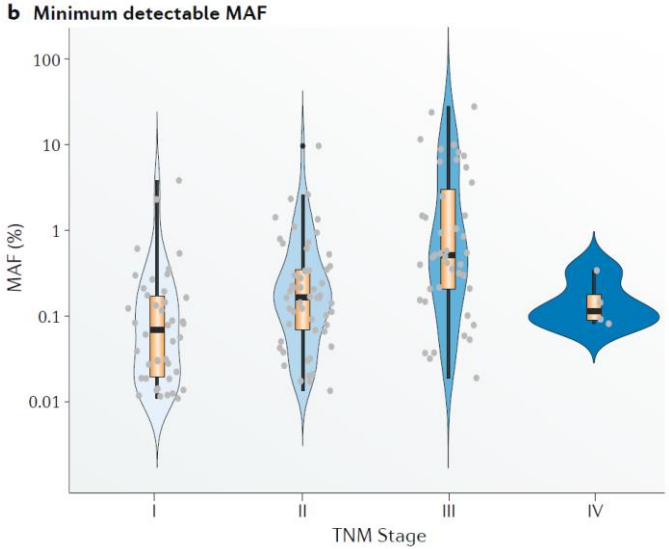
How reliable is ctDNA in detecting early stage lung cancer or minimal residual disease after complete surgical resection ?

Circulating tumor DNA for lung cancer detection

Technique (purpose)	Panel size (base pairs)	Enrichment technology	Stage I	Stage II	Stage III
CAPP-Seq (detection & MRD)	128 genes (188 kbp)	Hybridization	5/5 **(100%)**	4/6 **(67%)**	20/21 **(95%)**
TEC-Seq (detection)	58 genes (80.9 kbp)	Hybridization	13/29 **(45%)**	23/31 **(74%)**	4/5 **(80%)**
CancerSEEK (detection)	16 genes (4.6 kbp)	Multiplex PCR	2/46 **(4%)**	10/26 **(38%)**	11/31 **(35%)**
TRACERx (MRD)	18 patient-specific SNV (1.5 kbp)	Multiplex PCR	22/37 **(59%)**	16/23 **(70%)**	8/14 **(57%)**

MRD, minimal residual disease; SNV, single-nucleotide variant

Abbosh C et al. Nat Rev Clin Oncol. 2018;15:577-586.



MAF: mutant allelic frequency

Factors for ctDNA to detect stage I-II cancer or post-surgery minimal residual disease (MRD):

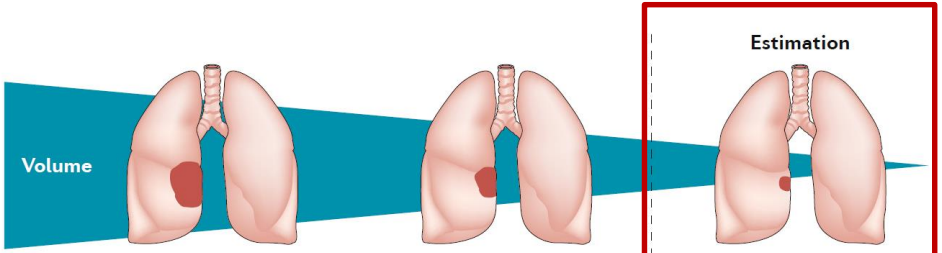
- Technique (sensitivity)
  - must be able to detect MAF <0.5% for detection and <0.1% for MRD (minimum detectable MAF: 0.01%)
  - need to use error minimizing algorithms
- More blood for small tumors
- Clonal hematopoiesis (e.g.,TP53, KRAS, JAK2 in 5-6% individuals >70%)
- Sensitivity may be histology dependent (SQC > ADC)
- Detection correlates with higher tumor proliferation, PET+, tumor necrosis, lymphovascular invasion

Abbosh C et al. Nat Rev Clin Oncol. 2018;15:577-586.

ctDNA use for screening and MRD:

- Stand alone ?
- Plus protein biomarkers ?
- Post low dose spiral CT imaging to increase specificity of cancer detection ?
- Adjuvant therapy in MRD+ patients to improve survival?
- Require further research to improve sensitivity and prospective trials on clinical utility of ctDNA assay in early stage lung cancer patients

Correlation between abundance of ctDNA , tumour volume, tumour diameter, and T stage.



MAF	1.4% (0.62–3.1%)	0.1% (0.06–0.18%)	0.008% (0.002–0.03%)
Nodule diameter	5.8 cm	2.6 cm	1.2 cm
Nodule volume	100 cm^3	10 cm^3	1 cm^3
T stage	T3	T1c	T1b

Nat Rev Clin Oncol. 2018 Sep;15(9):577-586.

2018 updated CAP/IASLC/AMP Guideline

Question 5: What Is the Role of Testing for Circulating cfDNA for Lung Cancer Patients?

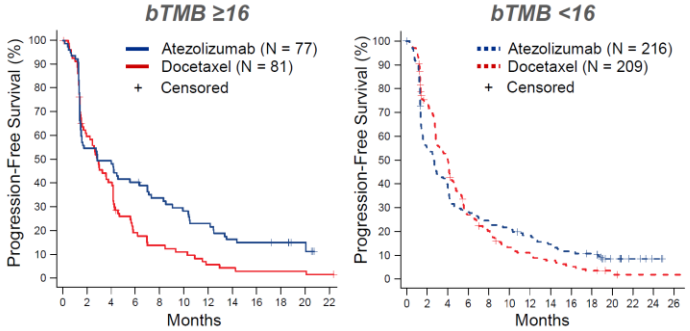
No Recommendation: There is currently insufficient evidence to support the use of circulating plasma cfDNA molecular methods for establishing a primary diagnosis of lung adenocarcinoma.

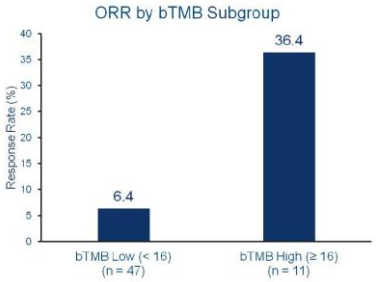
Lindeman NI, et al. J Thorac Oncol 2018; 13: 323-358

MADRID 2017 ESMO congress

BLOOD-BASED BIOMARKERS FOR CANCER IMMUNOTHERAPY: TUMOR MUTATIONAL BURDEN IN BLOOD (bTMB) IS ASSOCIATED WITH IMPROVED ATEZOLIZUMAB EFFICACY IN 2L+ NSCLC (POPLAR AND OAK)

David R. Gandara,[1] Marcin Kowanetz,[2] Tony Mok,[3] Achim Rittmeyer,[4] Louis Fehrenbacher,[5] David Fabrizio,[6] Geoff Otto,[6] Christine Malboeuf,[6] Daniel Lieber,[6] Sarah M. Paul,[2] Lukas Amler,[2] Todd Riehl,[2] Erica Schleifman,[2] Yan Li,[2] Craig Cummings,[2] Priti S. Hegde,[2] Wei Zou,[2] Alan Sandler,[2] Marcus Ballinger,[2] David S. Shames[2]





	bTMB High (n = 11)	bTMB Low (n = 47)
Median PFS	9.5 mo	2.8 mo
90% CI[a]	1.3, 9.5	1.7, 4.3
HR	0.51	
90% CI[a]	0.24, 1.08	
p value	0.1315	

In the bTMB high vs. low subgroups, the median PFS was 9.5 vs 2.8 months, respectively; HR = 0.51

ASCO 2018

Prospective Clinical Evaluation of Blood-Based Tumor Mutational Burden (bTMB) as a Predictive Biomarker for Atezolizumab in 1L NSCLC: Interim B-F1RST Results

Vamsidhar Velcheti,[1] Edward Kim,[2] Tarek Mekhail,[3] Christopher S.R. Dakhil,[4] Phillip Stella,[5] Vincent Shen,[6] Sylvia Hu,[6] Sarah M. Paul,[6] David S. Shames,[6] Cindy Yun,[6] See Phan,[6] Mark A. Socinski[3]

ASCO/CAP Joint Review

Table 3. Comparison of ctDNA Versus Tumor Tissue Testing

Consideration	ctDNA Assay	Tissue Assay
Logistics	Easy to draw Variable venipuncture risks Easy serial testing	Invasive, more challenging to obtain Variable biopsy risks Serial testing more difficult
Biology	Cannot directly correlate ctDNA results with histology or cellular phenotype More likely to represent whole tumor, but differential tumor cell turnover may bias representation	Can correlate with histology and cellular phenotype Represents one small tumor region
Pre-analytical	Easier to standardize across sites Requires special processing and handling unless using cell-stabilization tubes Limited data on confounding patient-related factors	More difficult to standardize across sites Uses existing, validated tissue processing and handling approaches
Clinical utility	Limited evidence for treatment selection in advanced cancer No evidence for other potential indications	Substantial evidence for treatment selection in multiple malignancies for early and advanced cancers

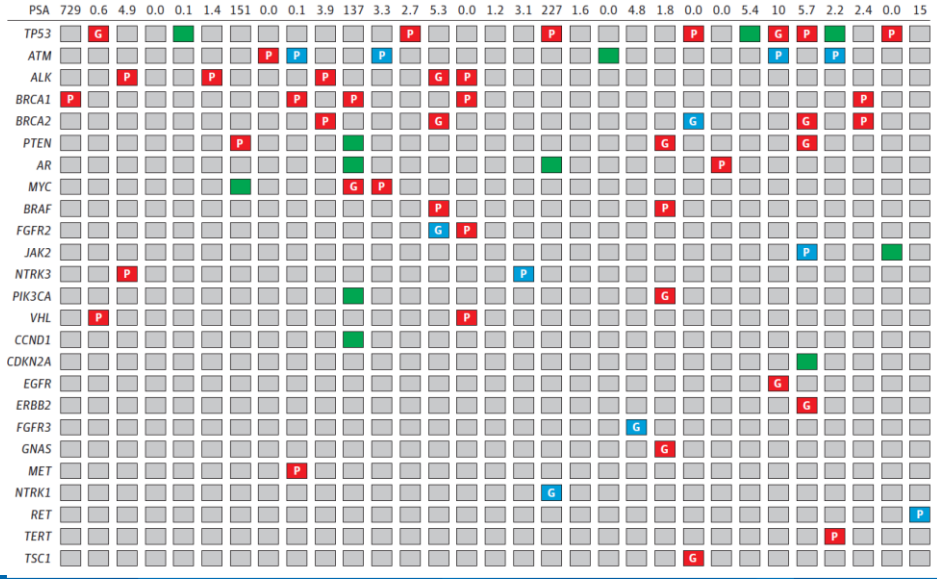
Abbreviation: ctDNA, circulating tumor DNA.

Merker JD, et al. J Clin Oncol 2018;36:1631-41

Patient-Paired Sample Congruence Between 2 Commercial Liquid Biopsy Tests

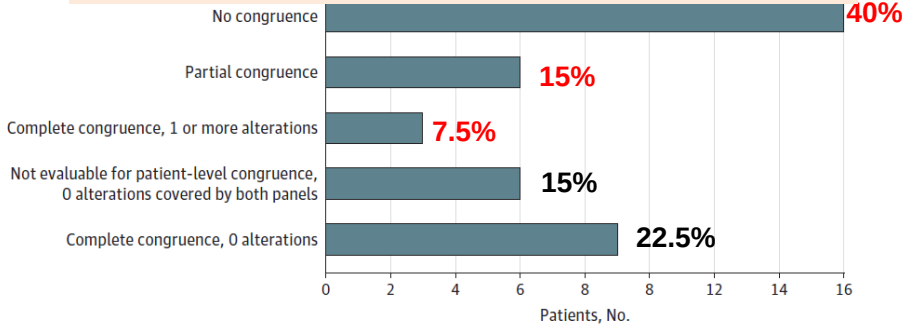
Torga G and Pienta KJ. JAMA Oncology 2018;4 (6):868-70

- **Identical alteration reported by both tests**
- **Alteration covered by both panels reported by only 1 cfDNA test**
- **No alterations reported**
- **Reported alteration with coverage by only one panel**



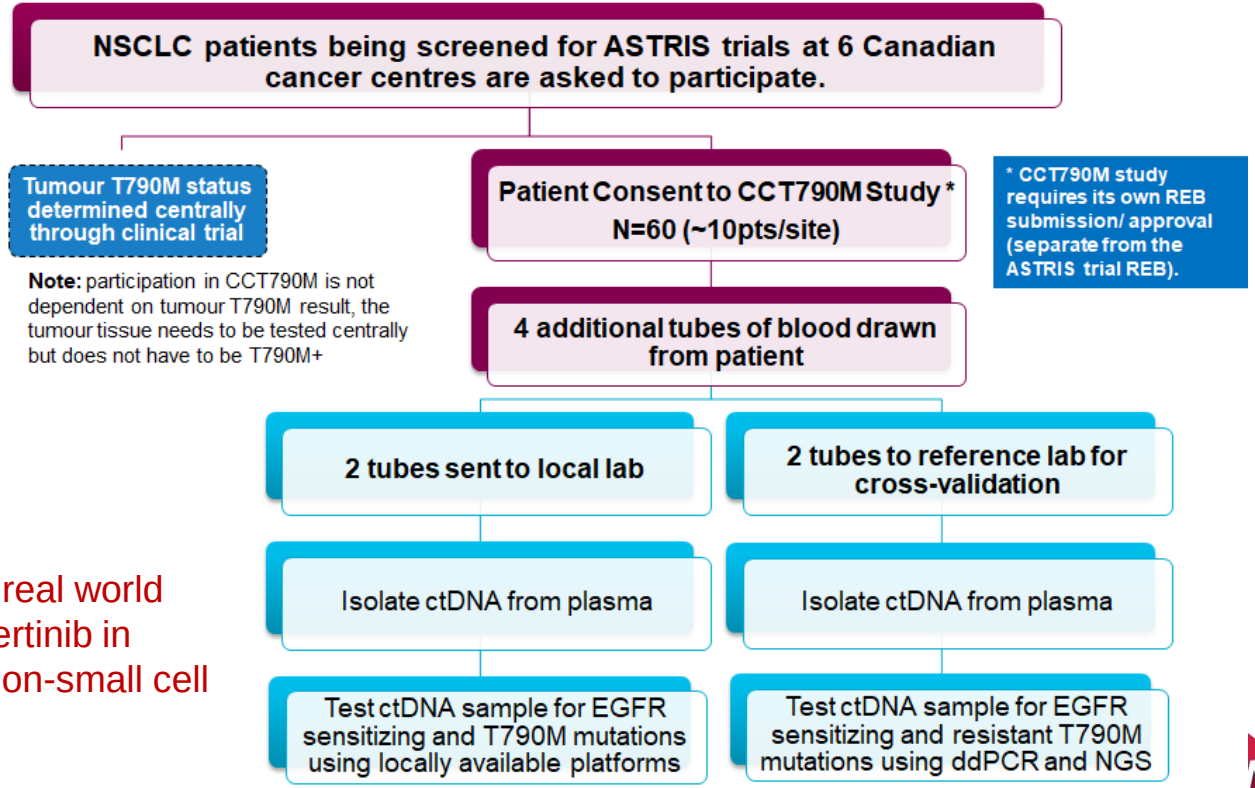
Sample	Paired plasma samples from 40 metastatic prostate cancer patients	
Tests	Guardant360	PlasmaSELECT
Panel	73 genes	64 genes
Exome coverage for each gene: Differ		

Congruence evaluated based on 42 genes covered by both platforms



Canadian multicentre Ct T790M (CCT790M) study

ASTRIS: A multicenter, real world treatment study of osimertinib in EGFR T790M positive non-small cell lung cancer



CCT790M study: Patient Demographics

Characteristics	N (%)
Median Age (range)	63 years (range 31-87)
Sex – Male	29 (46%)
Ethnicity	
• Asian	41 (67%)
• White	19 (30%)
• African	1
• Not reported	2
Smoking history	
• Never	38 (60%)
• Former	24 (38%)
• Current	1 (2%)
ECOG Performance Status	
• 0	7 (11%)
• 1	47 (75%)
• 2	8 (13%)
• 3	1 (1%)
Pathology - Adenocarcinoma	63 (100%)
Original sensitizing mutation	
• Exon 19 deletion	37 (59%)
• Exon 21 L858R	21 (33%)
• Not reported	5 (8%)
CNS metastasis	26 (41%)
Repeat Biopsy for T790M	
Successful	51 patients
Insufficient	8 biopsies in 6 patients
Not amenable for biopsy	6 patients
Validated out-of-country blood-based ctDNA assay as alternate	12
Biopsy complications	None
ctDNA draw complications	None

Tissue *versus* ctDNA Results

	Reference T790M results (tissue/blood)		
CtDNA (any lab)	**T790M+**	**T790M-**	Totals
T790M+*	24 (77.4%)	15 (46.9%)	39 (61.9%)
T790M-	7 (22.5%)	17 (53.1%)	24 (38.1%)
Totals	31	32	63

Interlaboratory concordance in detection of plasma T790M mutation

		Reference laboratory A		
		T790M +	**T790M -**	**T790M i**
Laboratory B, C & D (n=34)	**T790M +**	**17**	1	1
	T790M -	2	**11**	0
	T790M i	1	1	0

Tsao MS and Leighl N, et al. 2017 WCLC

TAKE HOME MESSAGE

1. Liquid biopsy is becoming an important complementary biomarker testing in cancer patient management
2. ctDNA testing should be “fit for purpose” as it impacts on the selection of assay including assay sensitivity, and result interpretation
3. Plasma T790M test can be used to identify patients who responded then progress on EGFR TKI to receive Osimertinib treatment, but negative test result should prompt tumor rebiopsy for definitive testing
4. Testing for plasma sensitizing *EGFR* mutations should only be reserved for patients whose diagnostic sample has been exhausted and repeat biopsy poses significant risk or delay. Negative results does not exclude the presence of mutation.
5. Plasma DNA testing has great promise in early stage lung cancer patients but requires more research to demonstrate its clinical validity and utility.