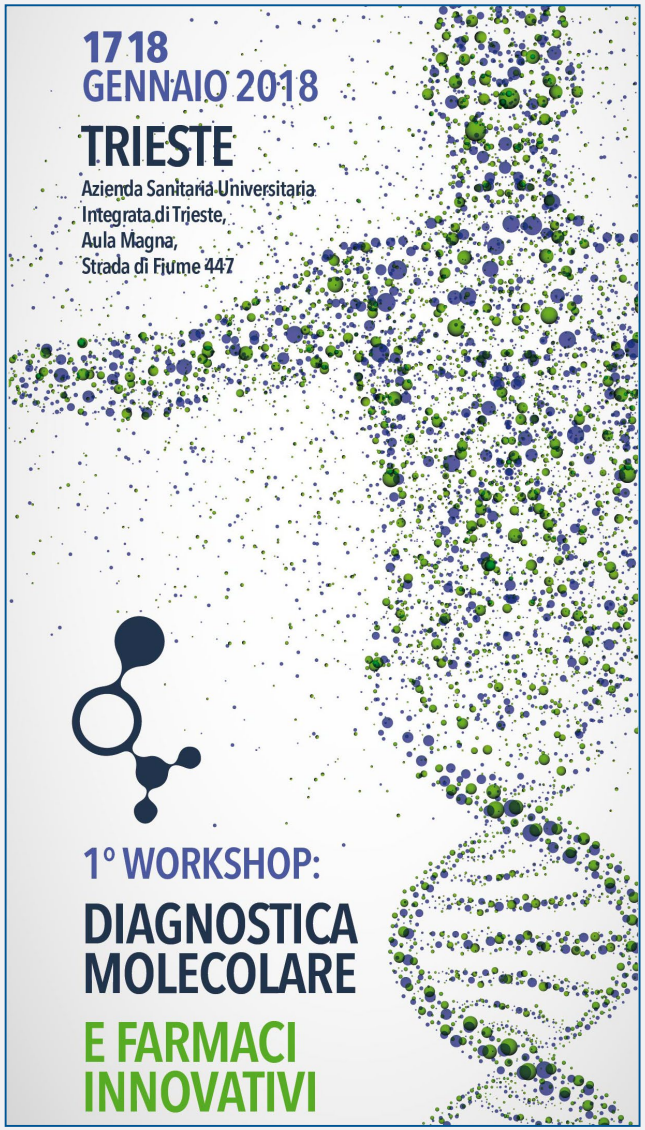
Sistema Socio Sanitario
Ospedale Niguarda
Regione Lombardia
CANCER CENTER

1° WORKSHOP:
DIAGNOSTICA MOLECOLARE
E FARMACI INNOVATIVI



Cell-free circulating DNA : Test diagnostico ? Test predittivo ?

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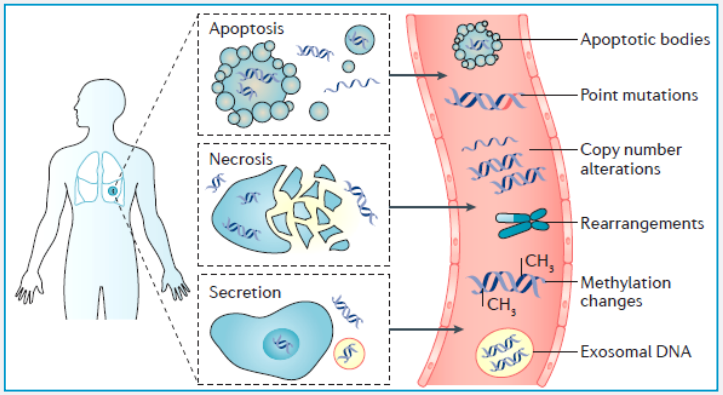
The Biological Basis of Liquid Biopsy

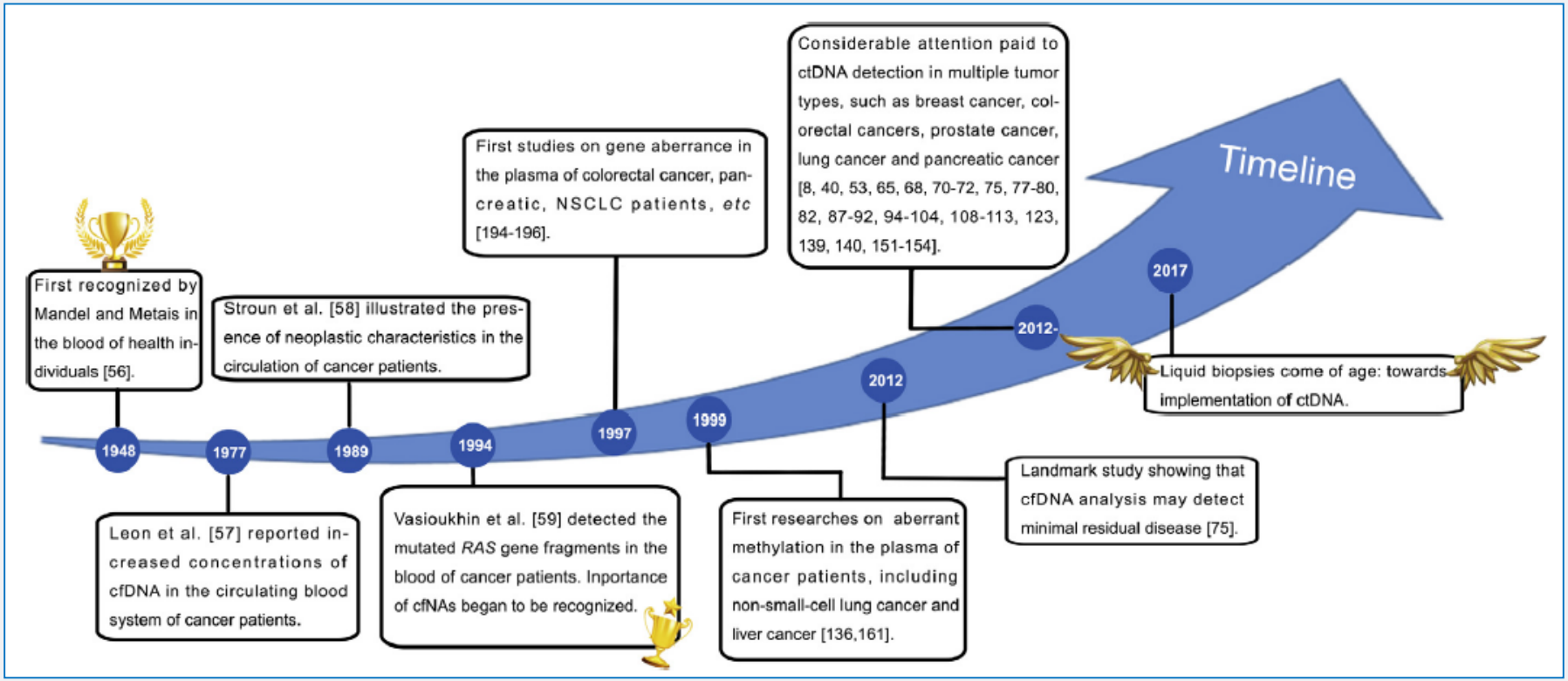


Bodily fluids (blood,urine, saliva, etc.)

1. Circulating Tumor Cells (CTCs)
2. **Cell-free circulating DNA (cf-DNA)**
3. **Cell-free circulating tumor DNA (ct-DNA)**
4. Small/Long noncoding RNAs
5. Exosomes
6. Tumor-Educated Platelets (TEPs)
   (interaction between blood platelets and tumor cells that alters the RNA profile of platelets and affects tumor growth)

- **Circulating tumor DNA (ctDNA) fragments are released by tumor cells into the bloodstream and contain, in principle, defects identical to the tumor cells they originate from.**
- **Molecular alterations, which can be detected in cell-free DNA (cfDNA), span the types of genomic alterations identified in tumors and include point mutations, rearrangements, and gene copy number variations.**
- **Free DNA** (although fragmented) **is quite stable in the circulation with a half-life between 16 minutes and 2.5 hours (”real-time” snapshot of disease burden).**
- **On the contrary, free RNA molecules do not generally survive in the bloodstream with the exception of microRNAs.**

Landmarks in the detection of ctDNAs in patients with different cancers



Methodologies for detecting circulating tumor DNA

Technology	Platform	Sensitivity (%)
Sanger sequencing	Many	10
Next-generation sequencing	Illumina, Life Technologies	2
TAm-Seq	Illumina	2
Quantitative-PCR	Cobas	2
ARMS-PCR	Many	0.1
Scorpion-PCR	Many	0.1
PNA-PCR	Many	0.1
Digital-PCR	Bio-Rad, Life Technologies	0.01
Droplet-PCR	BEAMing, Bio-Rad, Raindance	0.01
CAPP-Seq	Illumina	0.01

ARMS: Amplification refractory mutation testing; PCR: Polymerase-chain reaction; PNA: Peptide nucleic acid.

Clin. Invest. (Lond.) (2014)

Table 1. Continued

Platform	Study Design	Paired Samples, n	Mutation	Sensitivity, %	Specificity, %	Reference
cobas and BEAMing	Assessment of detection of *EGFR*-activating mutations and T790M mutations in plasma using both cobas and BEAMing assays compared with tumor tissue genotyping. Patients were enrolled in either an observational study of newly diagnosed or relapsed NSCLC, or the TIGER-X trial (phase 1 study of rociletinib in patients with previously treated *EGFR*-mutant NSCLC)	153		Positive % agreement	Negative % agreement	Karlovich et al. 2016[28]
			EGFR-activating mutations (cobas)	73% (62%-83%)	100% (86%-100%)	
			EGFR T790M (cobas)	64% (45%-80%)	98% (91%-100%)	
			EGFR-activating mutations (BEAMing PCR)	82% (70%-90%)	67% (9%-99%)	
			EGFR T790M (BEAMing PCR)	73% (58%-85%)	50% (26%-74%)	
cobas	Comparison of plasma genotyping for *EGFR* with tissue genotyping in patients enrolled on the FASTACT-2 study (intercalated erlotinib or placebo with gemcitabine-platinum followed by maintenance erlotinib or placebo in advanced NSCLC)	238	*EGFR* (all)	75%	96%	Mok et al. 2015[29]
			EGFR exon 19 del	82.5%	98.3%	
			EGFR L858R	62.2%	99%	
			EGFR G719x	50%	100%	
			EGFR L861Q	100%	100%	
NGS	Validation study comparing plasma genotyping utilizing a cancer panel with tissue genotyping in nonsmoker patients with NSCLC who were enrolled on the BioCAST/IFCT-1002 lung cancer study	68	12-amplicon panel (*EGFR*, *PI3KCA*, *BRAF*, *KRAS*, *ERBB2*)	58% (43%-71%)	87% (62%-96%)	Couraud et al. 2014[30]
NGS	Evaluation of an ultradeep NGS platform to capture alterations in cfDNA in a panel of 37 lung cancer-related genes (SNV, indels, fusions, and copy number gains). In a subset of patients with acquired resistance to targeted therapy, plasma NGS was able to capture *EGFR* T790M and additional somatic alterations	51	37-gene lung cancer panel	88%	Not available	Li et al. 2016[18]
		16	*EGFR* T790M	94% (concordance)	Not available	
NGS	Pilot study comparing plasma genotyping with an NGS panel against a reference standard of plasma ddPCR or tissue genotyping in patients with advanced NSCLC	48	62-driver and resistance mutations	77%	100%	Paweletz et. al. 2016[20]
			EGFR or *KRAS* mutations	79%	100%	
NGS	Analysis of *EGFR* mutations in urine and plasma collected from patients enrolled in the TIGER-X trial (phase 1/2 study of rociletinib in patients with previously treated *EGFR*-mutant NSCLC). This study utilized a short footprint mutation enrichment NGS assay compared with tissue genotyping, which was used as the reference standard	60	Plasma:			Reckamp et al. 2016[31]
			EGFR exon 19 del	87%	96%	
			EGFR L858R	100%	100%	
			EGFR T790M	93%	94%	
			Urine:			
			EGFR exon 19 del	67%	94%	
			EGFR L858R	75%	100%	
			EGFR T790M	72%	96%	

Table 1. Continued

Platform	Study Design	Paired Samples, n	Mutation	Sensitivity, %	Specificity, %	Reference
NGS (eTAm-Seq)	Detection of *EGFR* T790M mutation status by enhanced tagged amplicon sequencing in patients with *EGFR*-mutant advanced NSCLC who progressed while receiving first- or second-generation TKI therapy and were ineligible for a new tissue biopsy. Response to osimertinib in patients who were T790M positive by cfDNA analysis was assessed	48	*EGFR* T790M	50% (detection rate)	Not available	Remon et al. 2017[32]
PNA-mediated PCR	Validation study comparing detection of *EGFR* mutations in plasma and tissue from patients enrolled in the EURTAC study (platinum chemotherapy vs. erlotinib in metastatic *EGFR*-mutant NSCLC)	97	*EGFR* exon 19 del or L585R	78%	100%	Karachaliou et al. 2015[33]
Scorpion-ARMS	Detection of plasma *EGFR* mutations in patients with metastatic NSCLC who were receiving gefitinib monotherapy. Plasma genotyping was compared with tumor genotyping	42	*EGFR*-sensitizing mutations	85.7%	94.3%	Kimura et al. 2007[34]
Scorpion-ARMS	Detection of *EGFR* mutations in pretreatment plasma compared with tissue from patients enrolled in the IPASS study (gefitinib vs. carboplatin-paclitaxel for first-line treatment of advanced NSCLC)	86	*EGFR*-sensitizing mutations	43.1%	100%	Goto et al. 2012[35]
Scorpion-ARMS	Validation study comparing detection of *EGFR* mutations in plasma and tissue from patients with advanced *EGFR*-mutant NSCLC who were receiving first-line gefitinib on a single-arm clinical study	652	*EGFR* (all) *EGFR* exon 19 del *EGFR* L858R	65.7% (55.8%-74.7%) 67.6% (55.5%-78.2%) 61.8% (43.6%-77.8%)	99.8% (99.0%-100%) 100% (99.4%-100%) 99.8% (99.1%-100%)	Douillard et al. 2014[36]
Retrospectively Validated Assays (retrospective analysis of specimens from retrospectively identified cohorts)						
NGS (CAPP-Seq)	Evaluation utilizing the CAPP-Seq technology to detect alterations in plasma from patients across all stages of NSCLC	17	Customized panel by tumor type	85% (all stages) 50% (stage I) 100% (stage II-IV)	96% (all stages)	Newman et al. 2014[37]
cobas	Comparison of *EGFR* mutations in archived plasma and tissue from a cohort of patients with advanced NSCLC	196	*EGFR*-sensitizing mutations	60.7%	96.4	Weber et al. 2014[38]
Digital PCR	Comparison of plasma *EGFR* genotyping using digital PCR with tumor genotyping in a cohort of patients with advanced NSCLC	35	*EGFR* exon 19 del or L858R	92%	100%	Yung et al. 2009[39]
High-resolution melting analysis	Comparison of plasma *EGFR* genotyping using high-resolution melting analysis with tumor genotyping in a cohort of patients with NSCLC	24	*EGFR*-sensitizing mutations	91.67%	100%	Hu et al. 2012[40]
Mass spectrometry genotyping	Comparison of plasma *EGFR* genotyping using mass spectrometry with tumor genotyping in a cohort of patients with NSCLC	31	*EGFR* exon 19 del or L858R	38.9%	84.6%	Brevet et al. 2011[41]

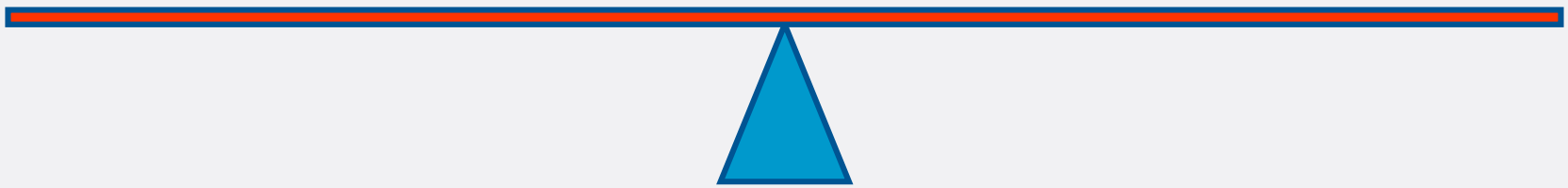
FDA, U.S. Food and Drug Administration; PCR, polymerase chain reaction; NGS, next-generation sequencing; DHPLC, denaturing high-performance liquid chromatography; ddPCR, digital droplet polymerase chain reaction; del, deletion; BEAMing, beads, emulsions, amplification and magnetics; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha gene; *ERBB2*, erb-b2 receptor tyrosine kinase 2 gene; cfDNA, cell-free DNA; SNV, single-nucleotide variation; eTAm-Seq, enhanced tagged-amplicon sequencing; PNA, peptide nucleic acid; ARMS, amplified refractory mutation system; CAPP-Seq, cancer personalized profiling by deep sequencing.

Tissue

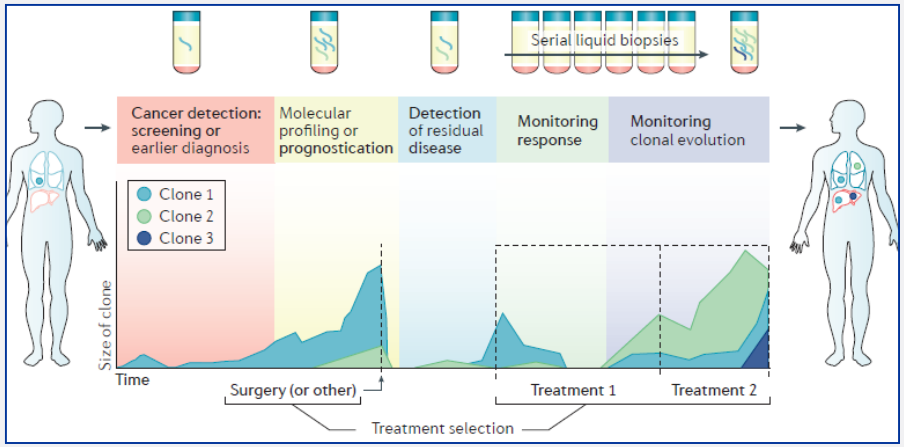
- Gold Standard
- **Diagnosis and subtyping**
- Detection of alterations in early stage of disease
- **Histologic transformation** (EMT, etc.)

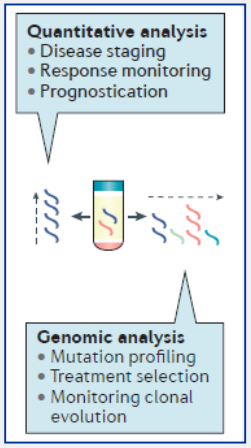
Liquid biopsy

- **Insufficient or no available tissue or cells**
  (20-30% of patients do not have accessible tissue)
- Tumor accessibility
- Poor performance status of patient
- Profiling of a “more comprehensively genomic landscape” of alterations
- Cost
- **Turn Around Time (TAT)**
- Source of fresh material
- Minimally invasive
- Possibility of repeating blood sampling and/or analysis
- **Dynamic monitoring of disease**
  (response, stability or progression)
- **Early identification of targetable resistance driver alterations**



Areas of application of ctDNA analysis





Cancer diagnosis :
- **earlier diagnosis of disease** (both in symptomatic and presymptomatic patients)

Prognosis and risk of relapse :
- **assessment of risk of progression**
- **identification of patients at high risk of relapse** (undertreatment vs overtreatment)

Treatment selection :
- **tools for molecular profiling of patients and treatment stratification**

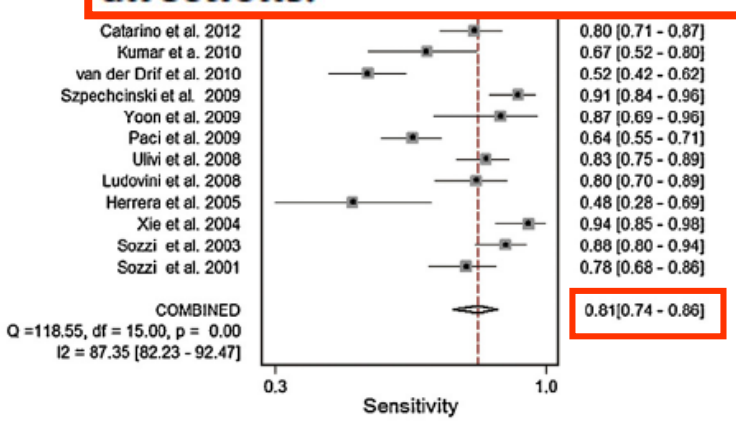
Monitoring disease burden :
- **treatment monitoring to identify response or progression** (the ideal monitoring assay should be repeatable serially over time with minimal risk to patient and should provide an accurate read-out of tumor burden)

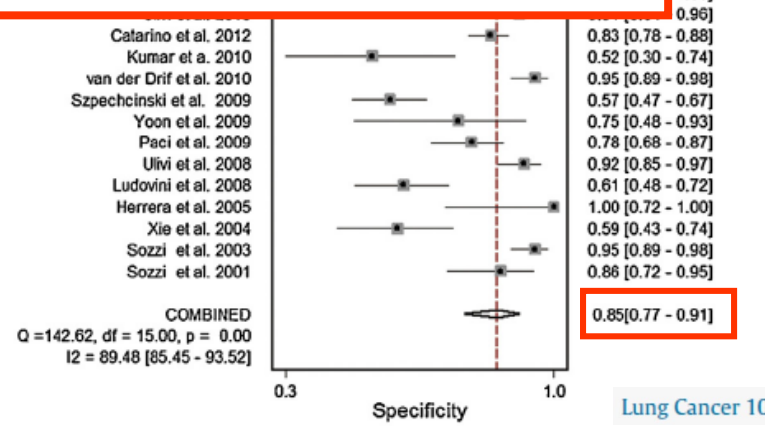
The diagnostic value of circulating cell free DNA quantification in non-small cell lung cancer: A systematic review with meta-analysis

Summary of outcomes for the included studies.

Authors	Year	Time of sample collection	Material	Test method	Reference gene	Cutoff value	AUC (95% CI)
Sozzi et al.	2001	Before surgery	Plasma	DNA DipStick	NA	25 ng/mL	0.84 (0.77, 0.90)
Sozzi et al.	2003	Before surgery	Plasma	Real-time PCR	hTERT	10 ng/mL	0.94 (0.91, 0.97)
Xie et al.	2004	NA	Plasma	PicoGreen	NA	21.9 ng/mL	0.86 (0.80, 0.91)
Herrera et al.	2005	Undergoing surgical treatment	Plasma	Real-time PCR	β actin	14 μg/L	0.63 (0.44, 0.82)
Ludovini et al.	2008	After surgical resection	Plasma	Real-time PCR	hTERT	3.25 ng/mL	0.82 (0.75, 0.88)
Ulivi et al.	2008	NA	Serum	Real-time PCR	NA	25 ng/mL	0.92 (0.88, 0.96)
Paci et al.	2009	After informed consent was obtained	Plasma	Real-time PCR	hTERT	4 ng/mL	0.79 (0.71, 0.83)
Yoon et al.	2009	After informed consent was obtained	Plasma	Real-time PCR	β actin	11 ng/mL	0.86 (0.81, 0.91)
Szpechcinski et al.	2009	Before treatment	Plasma	Real-time PCR	β actin	2.78 ng/mL	0.86 (0.67, 0.96)
van der Drif et al.	2010	Before surgery	Plasma	Real-time PCR	β actin	32 ng/mL	0.66 (0.53, 0.80)
Kumar et a.	2010	NA	Plasma	PicoGreen	NA	104.5 ng/mL	0.83 (0.77, 0.89)
Catarino et al.	2012	Before treatment	Plasma	Real-time PCR	hTERT	20 ng/mL	0.88 (0.84, 0.92)

In conclusion, the current evidence suggests that quantification of cfDNA may be a relatively promising and effective biomarker for discriminating NSCLC from healthy individuals. Combination of cfDNA quantification with other tumor markers would be the future directions.





The potential of liquid biopsies for the early detection of cancer

Table 1. Biological and technical differences for applying liquid biopsy technologies on precancers and earlier stages of neoplastic development versus advanced cancers

Parameter	Precancers/early stages	Advanced cancers
Size of lesion	Usually small ($< 1\ cm^3$)	Large ($\geq 1\ cm^3$)
Clinical signs	Usually none	Apparent
Detectable by imaging	Often not detectable	Yes
Biology of lesion	May range from favorable to unfavorable (refs. 44, 45)	Advanced cancers have in general unfavorable (sub)clones (ref. 6)
Presence of established other tumor markers (e.g., PSA, CEA, CA 125)	Uncertain (ref. 129)	Frequently available, but without high specificity/sensitivity; useful for disease monitoring (ref. 129)
Knowledge of genes to be targeted in liquid biopsy assays	Often unknown (refs. 78, 80)	Usually known or can be established from available tumor tissue (refs. 3, 35, 73)
Established driver genes	Often unknown (refs. 130, 131)	Usually known (refs. 3, 35)
Release of tumor DNA into the circulation	Uncertain (refs. 42, 80)	At stage III and IV disease close to 100% of patients (ref. 42)
Applicable plasma DNA technologies	Usually focused high-sensitivity assays (refs. 16–18)	Broad range of targeted and untargeted approaches (refs. 16–18)
Option of proximal sampling	Only if endangered tissue is known (refs. 16, 17)	In selected tumor entities, but frequently not necessary
Option to design personalized assays	Possible, provided that tissue is available (refs. 73, 84, 85)	Tissue is usually available, can be designed for truncal and branch mutations (refs. 73)
Expected VAF of somatic mutations in blood	Extremely low, if present at all (refs. 42, 80)	Frequently high (refs. 32, 42, 107, 132)
Tumor heterogeneity	Relatively low (refs. 6, 133)	High (refs. 6, 133)
Presence of potentially confounding mutations	In particular, persons with increased age may have acquired cancer-associated mutations without ever developing cancer (refs. 65, 71, 72)	Distinction between driver and passenger mutations needed for disease monitoring (ref. 35)
Presence of potentially confounding clones	Clonal expansion of non-tumorous tissue may mimic a malignant event (refs. 65, 67–70)	Likely that all metastatic sites are reflected in plasma DNA analysis (ref. 134)
Detection of SCNAs	Hard to detect due to low VAF at this disease stage (refs. 23, 27)	Often informative and may indicate evolution of novel clones (ref. 32)
Availability of established clinical guidelines	None	Emerging, e.g., EGFR mutation testing as blood-based companion diagnostic for patients with NSCLC

PSA prostate-specific antigen, *CEA* carcinoembryonic antigen, *CA 125* cancer antigen 125, *VAF* variant allelic frequency, *SCNAs* somatic copy number alterations, *NSCLC* non-small-cell lung cancer

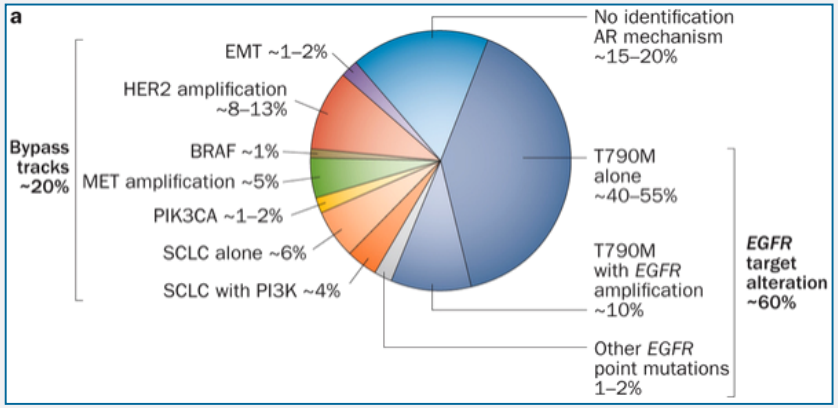
Categories of resistance mechanisms in patients at progression with EGFR-TK inhibitors :

1. **Secondary mutations in the EGFR gene**
   (**p.T790M**, p.L747S, p.D761Y,p.T854A)

2. **Activation of bypass pathways**
   (**HER2 ampl./mutations, MET ampl./mutations**, HGF overexpression, IGF-IR activation, VEGF-VEGFRs interaction, FGFRs activation, PDGF-PDGFRs interaction, AXL overexpression, excess secretion of IL-6)

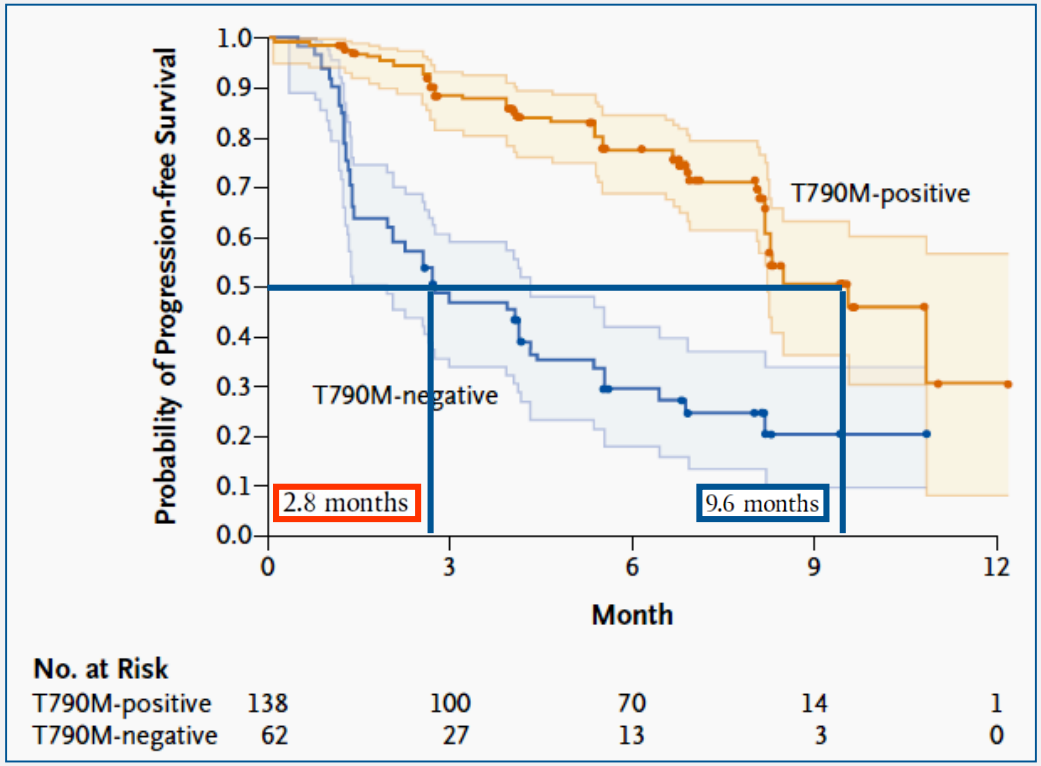
3. **Abnormal downstream pathways**
   (**KRAS, BRAF** and **PIK3CA mutations**, loss of PTEN, aberrant expression of NF1)

4. **Histologic transformation** (EMT, SCLC, SCC, ecc.)



AZD9291 in EGFR Inhibitor–Resistant Non–Small-Cell Lung Cancer

Pasi A. Jänne, M.D., Ph.D., James Chih-Hsin Yang, M.D., Ph.D., Dong-Wan Kim, M.D., Ph.D., David Planchard, M.D., Ph.D., Yuichiro Ohe, M.D., Suresh S. Ramalingam, M.D., Myung-Ju Ahn, M.D., Ph.D., Sang-We Kim, M.D., Ph.D., Wu-Chou Su, M.D., Leora Horn, M.D., Daniel Haggstrom, M.D., Enriqueta Felip, M.D., Ph.D., Joo-Hang Kim, M.D., Ph.D., Paul Frewer, M.Sc., Mireille Cantarini, M.D., Kathryn H. Brown, Ph.D., Paul A. Dickinson, Ph.D., Serban Ghiorghiu, M.D., and Malcolm Ranson, M.B., Ch.B., Ph.D.

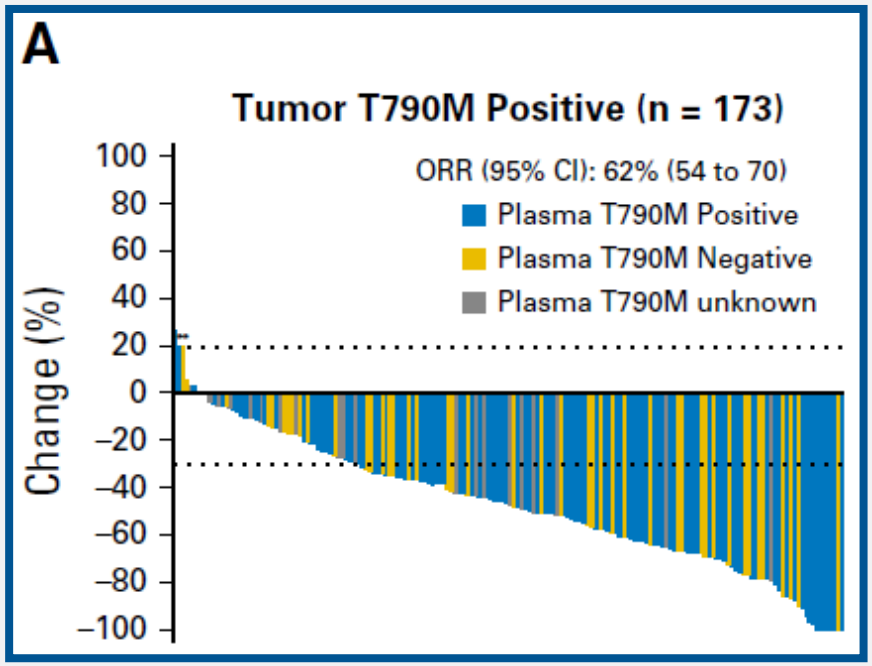


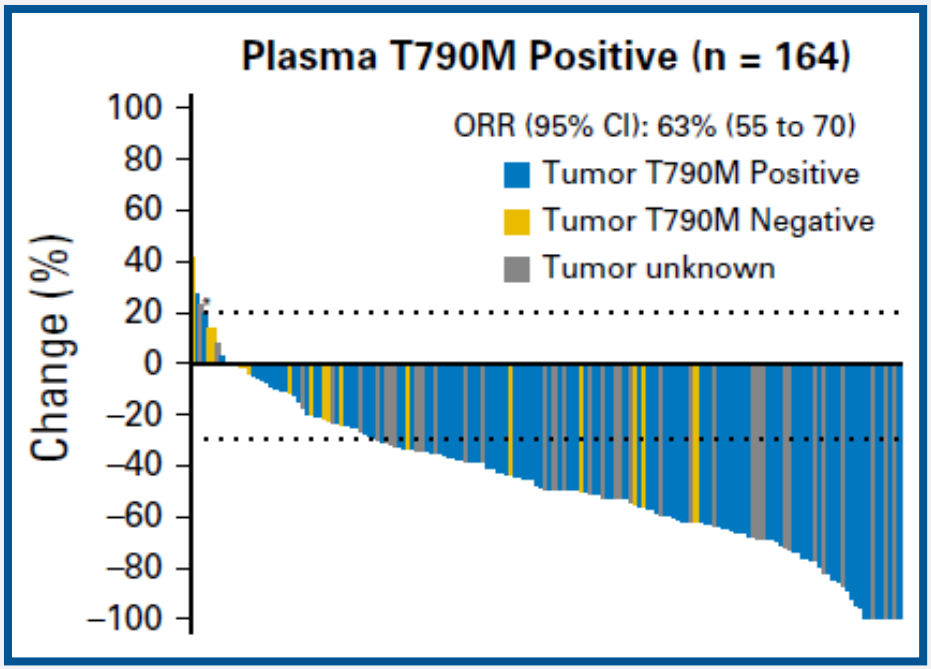
N Engl J Med 2015;372:1689-99.

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

JOURNAL OF CLINICAL ONCOLOGY VOLUME 34 · NUMBER 28 · OCTOBER 1, 2016





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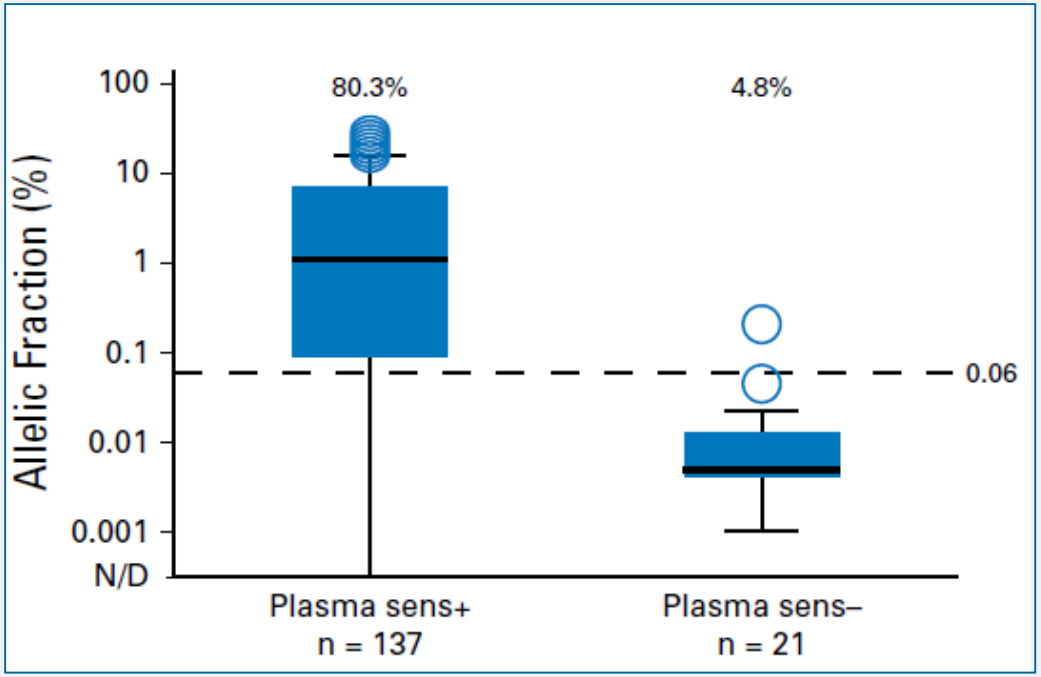

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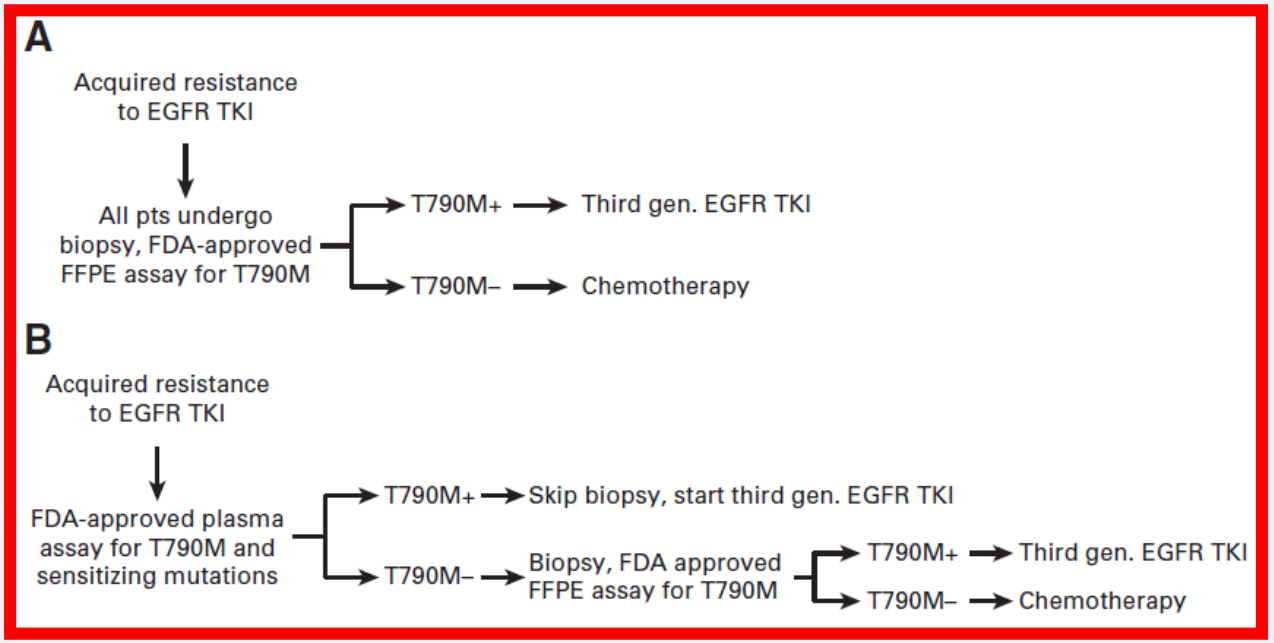
A proposed paradigm for use of plasma genotyping for epidermal growth factor receptor (EGFR) T790M



NEW ENGLAND JOURNAL of MEDICINE November 20, 2017.

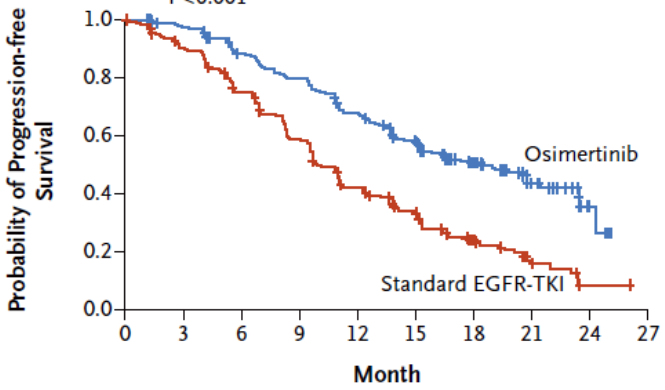
Osimertinib in Untreated *EGFR*-Mutated Advanced Non–Small-Cell Lung Cancer

J.-C. Soria, Y. Ohe, J. Vansteenkiste, T. Reungwetwattana, B. Chewaskulyong, K.H. Lee, A. Dechaphunkul, F. Imamura, N. Nogami, T. Kurata, I. Okamoto, C. Zhou, B.C. Cho, Y. Cheng, E.K. Cho, P.J. Voon, D. Planchard, W.-C. Su, J.E. Gray, S.-M. Lee, R. Hodge, M. Marotti, Y. Rukazenkov, and S.S. Ramalingam, for the FLAURA Investigators*

Progression-free Survival in Full Analysis Set

	No. of Patients	Median Progression-free Survival (95% CI)
		mo
Osimertinib	279	18.9 (15.2–21.4)
Standard EGFR-TKI	277	10.2 (9.6–11.1)

Hazard ratio for disease progression or death, 0.46 (95% CI, 0.37–0.57)
P<0.001



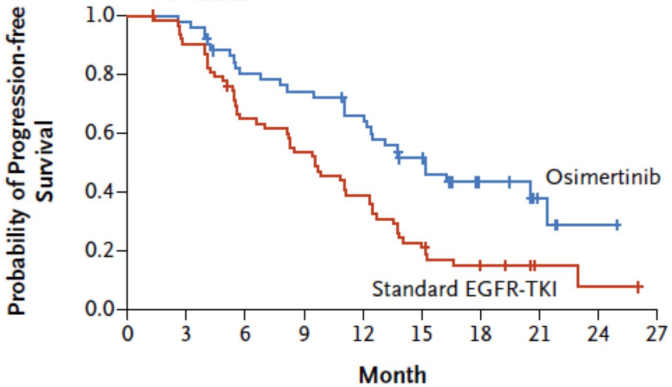
No. at Risk

Osimertinib	279	262	233	210	178	139	71	26	4	0
Standard EGFR-TKI	277	239	197	152	107	78	37	10	2	0

Progression-free Survival in Patients with CNS Metastases

	No. of Patients	Median Progression-free Survival (95% CI)
		mo
Osimertinib	53	15.2 (12.1–21.4)
Standard EGFR-TKI	63	9.6 (7.0–12.4)

Hazard ratio for disease progression or death, 0.47 (95% CI, 0.30–0.74)
P<0.001



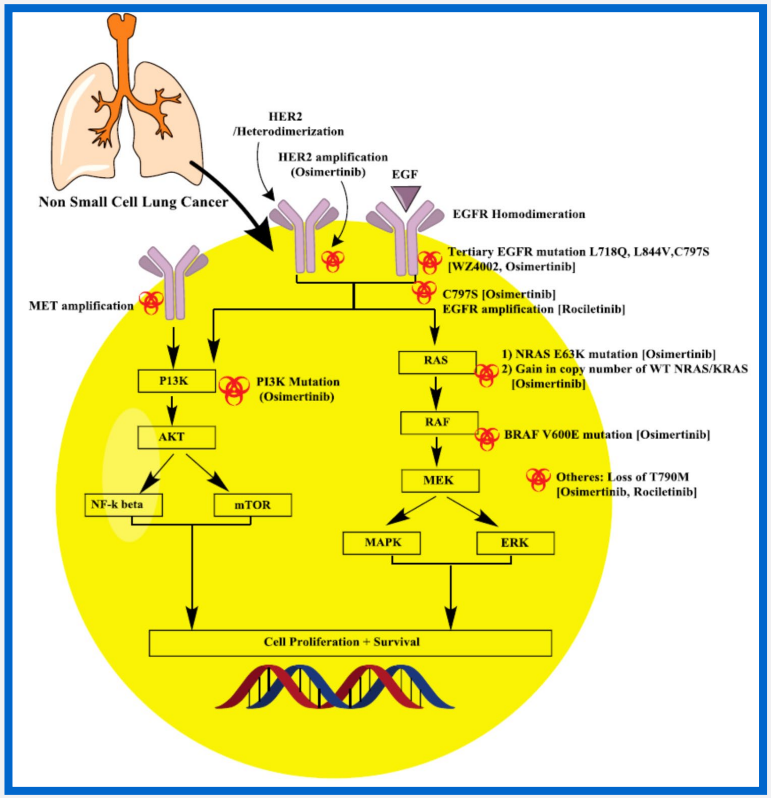
No. at Risk

Osimertinib	53	51	40	37	32	22	9	4	1	0
Standard EGFR-TKI	63	57	40	33	24	13	6	2	1	0

Mechanisms of resistance to 3rd-generation EGFR TKIs

Resistance Mechanism[23,29,56,60,62]	No. (%)
Total No. of patients	35
C797S/T790M	8 (23)[a]
T790M Maintained (no clear resistance mechanism identified)	12 (34)
Loss of T790M	10 (29)
MET Amp/T790-wt	1 (3)
ERBB2 (Her2) Amp/T790-wt	1 (3)
SCLC/T790-wt	3 (9)

JAMA Oncology July 2016



European Journal of Medicinal Chemistry (2017)

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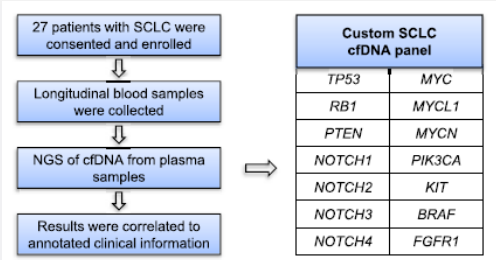
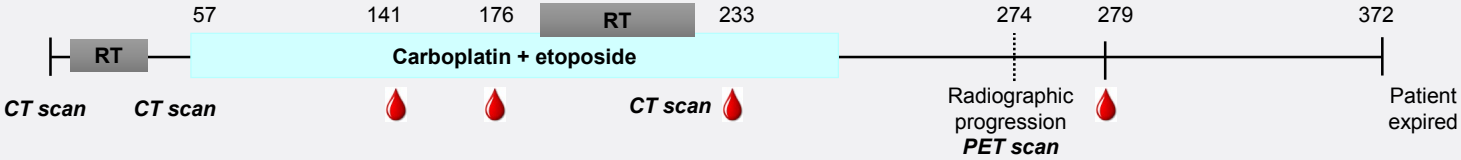
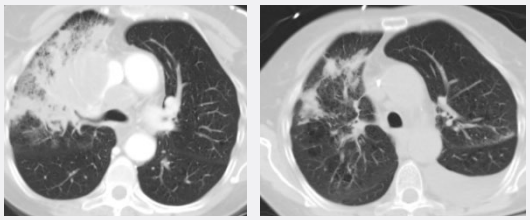
Longitudinal Cell-Free DNA Analysis in Patients with Small Cell Lung Cancer Reveals Dynamic Insights into Treatment Efficacy and Disease Relapse

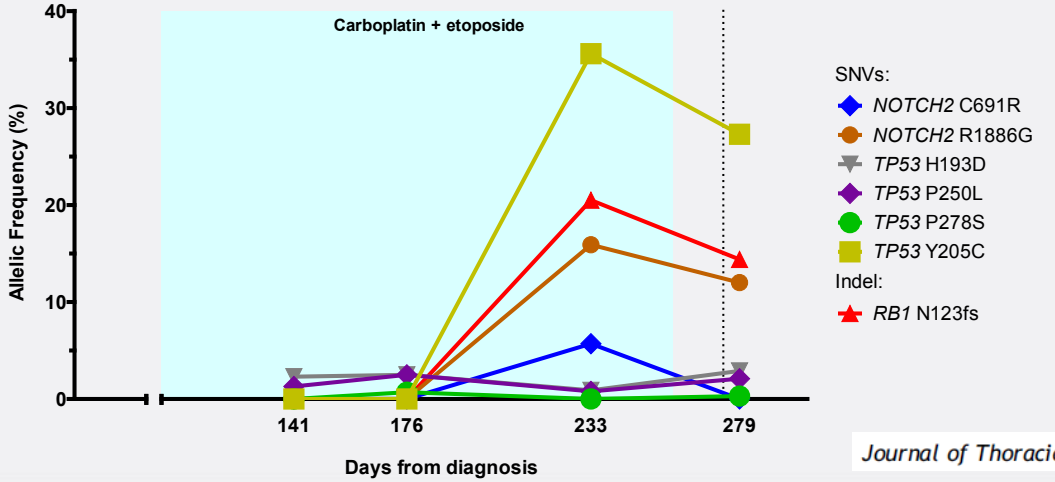
Karinna Almodovar, PhD,[a,*] Wade T. Iams, MD,[a,*] Catherine B. Meador, MD, PhD,[b] Zhiguo Zhao, MS,[c] Sally York, MD, PhD,[a,d] Leora Horn, MD,[a,d] Yingjun Yan, MS,[a] Jennifer Hernandez, BS,[e] Heidi Chen, PhD,[c] Yu Shyr, PhD,[c] Lee P. Lim, PhD,[e] Christopher K. Raymond, PhD,[e] Christine M. Lovly, MD, PhD[a,b,d,*]







Day 0 | Day 51 | Day 232 | Day 274



Journal of Thoracic Oncology Vol. 13 No. 1: 112-123

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1° WORKSHOP:
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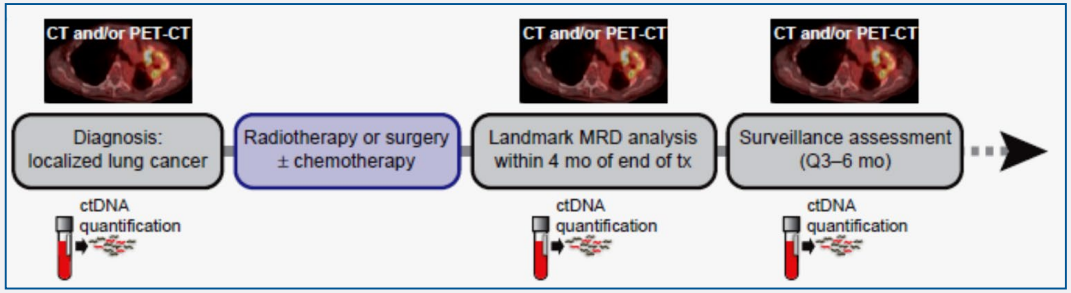
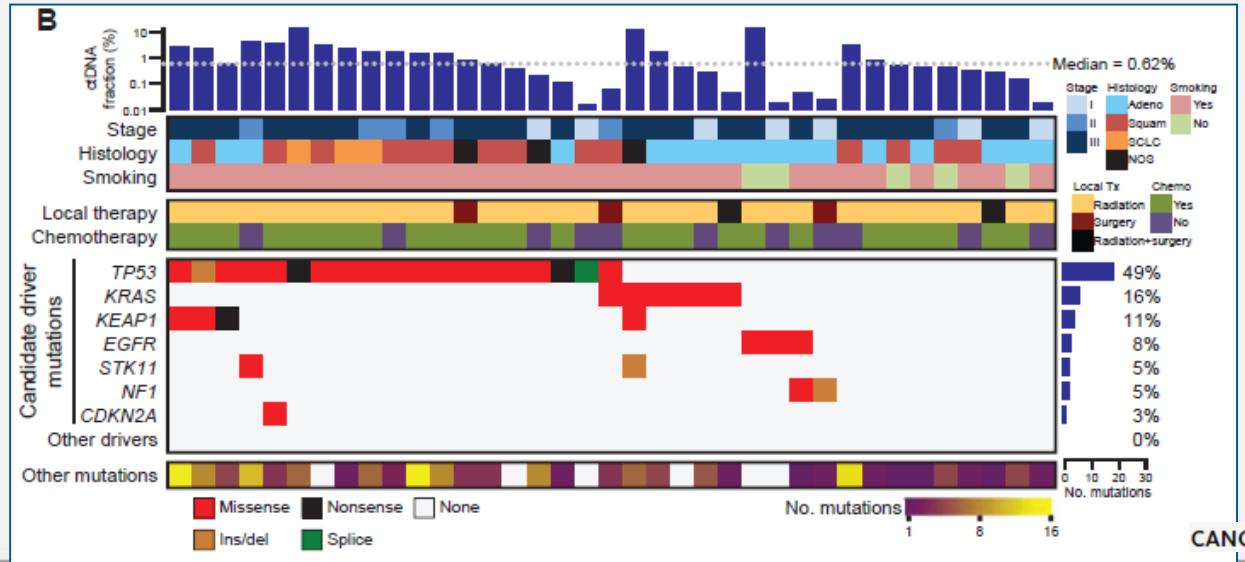
Early Detection of Molecular Residual Disease in Localized Lung Cancer by Circulating Tumor DNA Profiling

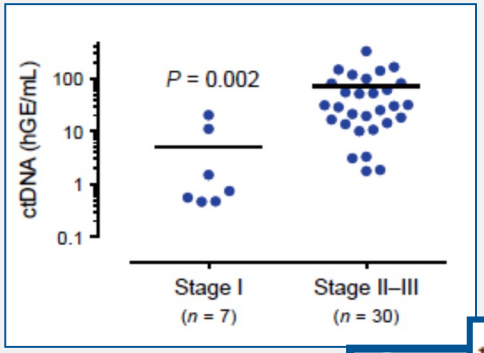
CAncer Personalized Profiling by deep sequencing (CAPP-seq)
a NGS-based method that tracks multiple mutations per patient, achieving lower **limits of detection ~0.002%**

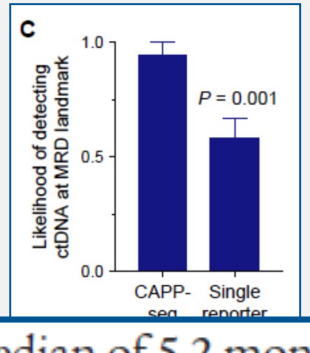
We retrospectively profiled 255 blood and tissue samples from **40 patients** with localized lung cancers being treated with curative-intent first-line therapies and **54 healthy adults**

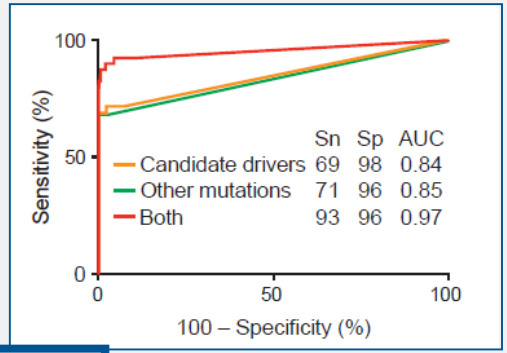


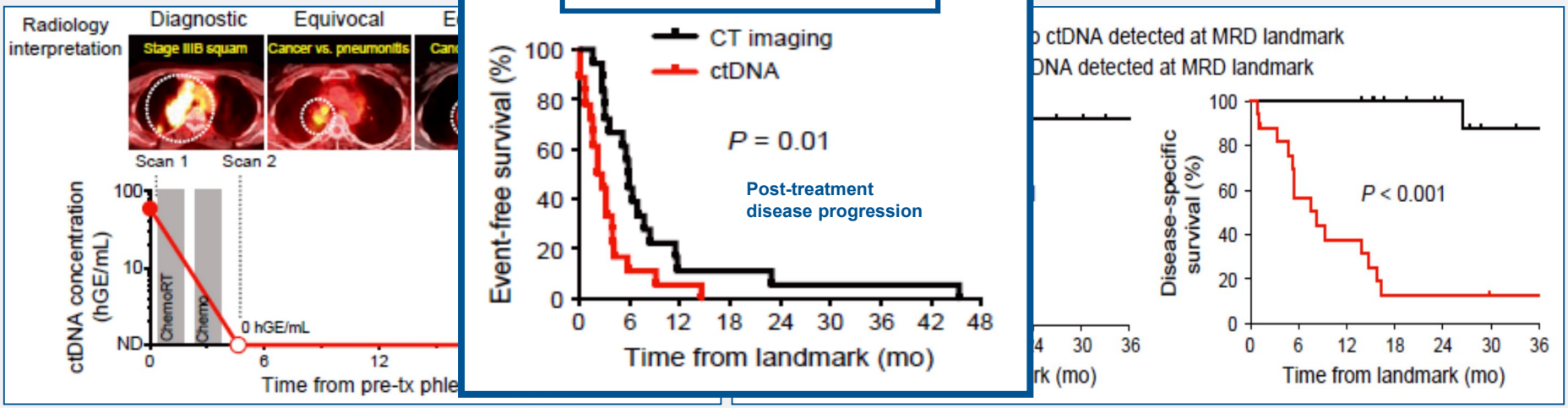


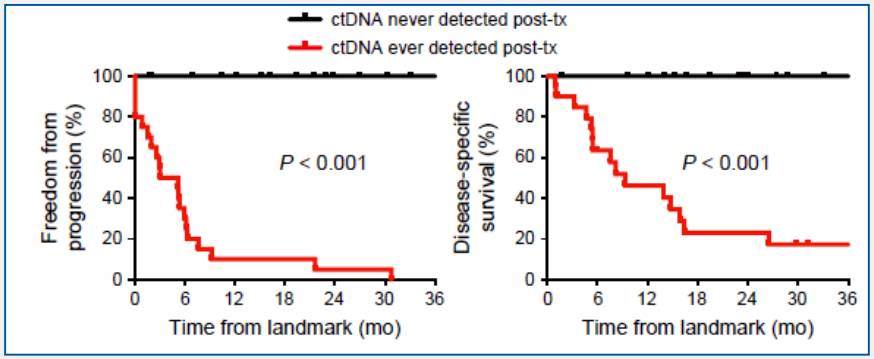
CANCER DISCOVERY DECEMBER 2017


ctDNA (hGE/mL)
100
10
1
0.1
P = 0.002
Stage I
(n = 7)
Stage II–III
(n = 30)


c
Likelihood of detecting ctDNA at MRD landmark
1.0
0.5
0.0
P = 0.001
CAPP-seq
Single reporter


Sensitivity (%)
100
50
0
Sn Sp AUC
Candidate drivers 69 98 0.84
Other mutations 71 96 0.85
Both 93 96 0.97
0
50
100
100 – Specificity (%)


Radiology interpretation
Diagnostic
Equivocal
Stage IIIB squam
Cancer vs. pneumonitis
Scan 1
Scan 2
ctDNA concentration (hGE/mL)
100
10
ND
ChemoRT
Chemo
0 hGE/mL
0
6
12
Time from pre-tx phle
median of 5.2 months
CT imaging
ctDNA
P = 0.01
Post-treatment disease progression
Event-free survival (%)
100
80
60
40
20
0
0 6 12 18 24 30 36 42 48
Time from landmark (mo)
o ctDNA detected at MRD landmark
DNA detected at MRD landmark
4 30 36
rk (mo)
Disease-specific survival (%)
P < 0.001
0 6 12 18 24 30 36
Time from landmark (mo)


ctDNA never detected post-tx
ctDNA ever detected post-tx
Freedom from progression (%)
100
80
60
40
20
0
P < 0.001
0 6 12 18 24 30 36
Time from landmark (mo)
Disease-specific survival (%)
100
80
60
40
20
0
P < 0.001
0 6 12 18 24 30 36
Time from landmark (mo)

Preclinical Comparison of Osimertinib with Other EGFR-TKIs in EGFR-Mutant NSCLC Brain Metastases Models, and Early Evidence of Clinical Brain Metastases Activity

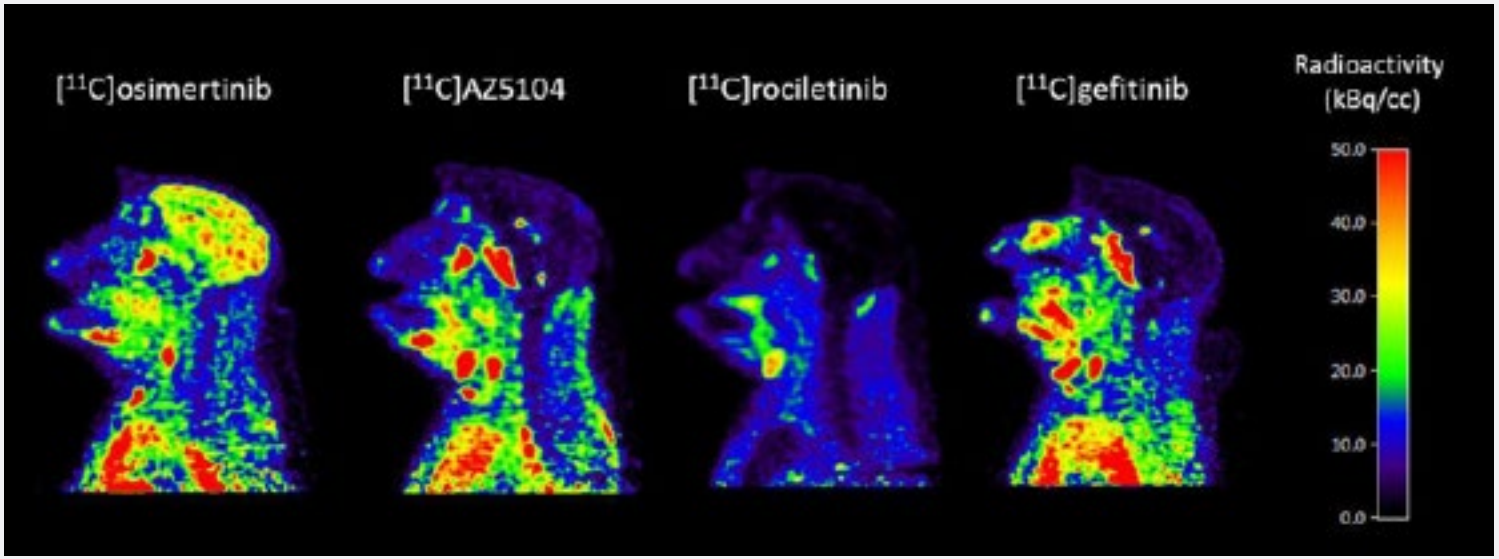
Peter Ballard[1], James W.T. Yates[2], Zhenfan Yang[3], Dong-Wan Kim[4], James Chih-Hsin Yang[5], Mireille Cantarini[6], Kathryn Pickup[1], Angela Jordan[1], Mike Hickey[7], Matthew Grist[1], Matthew Box[1], Peter Johnström[8,9], Katarina Varnäs[9], Jonas Malmquist[9], Kenneth S. Thress[10], Pasi A. Jänne[11], and Darren Cross[2]

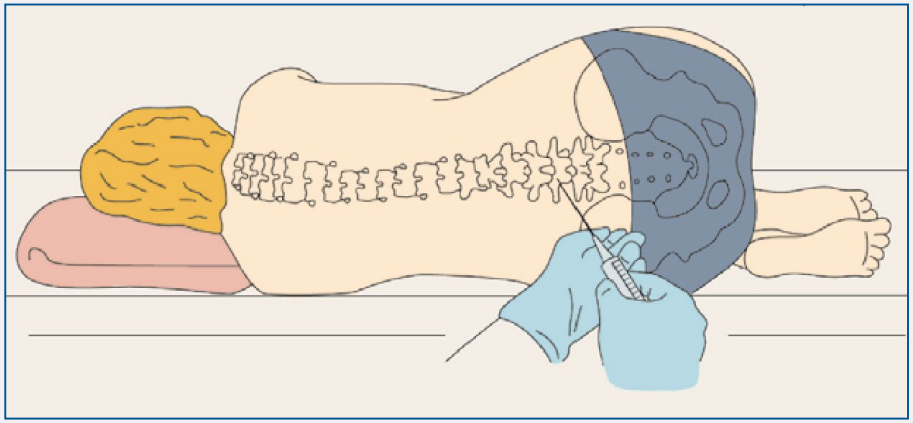
Clin Cancer Res; 22(20) October 15, 2016

Table 2. Distribution to mouse brain of osimertinib, gefitinib, rociletinib, and afatinib following oral administration

	Osimertinib	Gefitinib	Rociletinib	Afatinib
Dose (mg/kg)	25	6.25	100	7.5
Plasma C_{max} (μmol/L)	0.82	0.82	3.32	0.14
Brain C_{max} (μmol/L)	2.78	0.17	BLQ	BLQ
Brain/plasma C_{max} ratio	3.41	0.21	<0.08	<0.36

NOTE: Doses equivalent to clinical doses or reported previously.
Abbreviation: BLQ, below limit of quantification (rociletinib 0.25 μmol/L, afatinib 0.05 μmol/L); C_{max}, maximum plasma concentration.





T790M *EGFR* Mutation Detection in Cerebrospinal Fluid and Response to Osimertinib in a Lung Cancer Patient with Meningeal Carcinomatosis

Hugo Gortais, MD,
Catherine Daniel, MD,
François-Clément Bidard, MD
Department of Medical Oncology, Curie institute, Paris, France

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Journal of Thoracic Oncology
September 2017Volume 12, Issue 9, Pages e138–e139

Analysis of EGFR mutation status in blood and CSF in lung adenocarcinoma patients with EGFR mutation and CNS metastasis by ddPCR

Y. Sen, Q. Wang

ESMO Asia 2017

In which patients and clinical situations can plasma genotyping assays be utilized ?

- Current evidence from the various validation studies support the use of plasma genotyping in patients with newly diagnosed disease before they begin treatment and in patients with acquired resistance to treatment and clear disease progression.
- Patients with metastatic NSCLC and acquired resistance to targeted therapy represent an additional population in which validated plasma genotyping assays are useful in identifying resistance mechanisms and guiding subsequent therapy.
- The presence of increased disease burden (increasing number of metastatic sites, liver and bone metastases in particular) has been previously demonstrated to predict for increased sensitivity of plasma genotyping assays.
- Studies in primary central nervous system tumors suggest that the capability to detect tumor cfDNA in the plasma in this setting has limited sensitivity.
- Individual clinical situations in which initial biopsy yielded insufficient tissue for genotyping, repeat biopsy is anatomically difficult or an urgent clinical need exists to identify potentially targetable genomic alterations represent scenarios in which plasma genotyping may be potentially useful.

Existing challenges for liquid biopsy applications

- The clinical use of liquid biopsies will depend on the practical advantages for patients and clinicians, the infrastructure required and its cost-effectiveness.
- Tissue biopsies currently represent the standard of tumor diagnosis
- They only reflect a single point in time of a single site of the tumor
- Such a sampling method is thus inadequate for a comprehensive characterization of a tumor, as it has been demonstrated that various areas within the primary tumor or metastases can in fact harbor different genomic profiles
- Whether or not ctDNA does actually indeed offer a full representation of a patient's cancer (all existing metastases contribute to the ctDNA, CTCs, and exosomes found in the bloodstream, or if all tumor cells release an equal amount of ctDNA into the circulation)

Existing challenges for liquid biopsy applications

- The molecular genetic diversity within a tumor can also alter over time, rendering future treatment decisions based on historical biopsy information potentially inaccurate and suboptimal
- A more mature understanding of the biology behind ctDNA, CTCs, exosomes and platelets
- Variability in ctDNA levels in different stages of the disease
- Pre-analytical steps [collection of bioliquids (e.g., blood, serum, plasma), centrifugation settings, isolation reagents, and storage conditions] should be potentially standardized
- Analytical steps (mutational analysis procedures, NGS assays and sequencing platforms themselves, etc.) should be potentially validated for their use into clinical settings
- The need to evaluate the clinical relevance of ctDNA at various time points depending on the application (patient stratification, evaluation of treatment response, efficacy and resistance)

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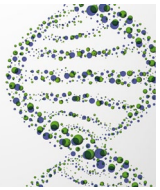

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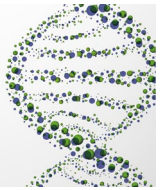

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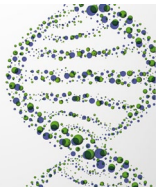

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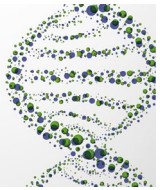

Molecular Adequacy of Image-Guided Rebiopsies for Molecular Retesting in Advanced Non-Small Cell Lung Cancer: A Single-Center Experience

Nadza Tokaca, MRCP, BM BCh,[a] Sarah Barth, MRCP,[a] Mary O'Brien, MRCP, MD,[a] Jaishree Bhosle, MRCP, PhD,[a] Nicos Fotiadis, FRCR, MD, PhD,[b] Andrew Wotherspoon, MRCPath, MB BCh,[c] Lisa Thompson, PhD,[d] Sanjay Popat, MRCP, PhD[e,*]

Journal of Thoracic Oncology Vol. 13 No. 1: 63-72

Table 2. Histological Discordance Rates

Original Histological Subtype	n	Rebiopsy Histologica
Adenocarcinoma	38	Adenocarcinoma
		NSCLC NOS
		Poorly differentiated
Squamous cell carcinoma	9	Squamous cell carcino
		Adenocarcinoma
		NSCLC NOS
		Pleomorphic carcinom
NSCLC NOS	4	NSCLC NOS
		Squamous cell carcino
Adenosquamous carcinoma	1	Adenocarcinoma
Total[a]	52	Concordant
		Discordant

[a]Total of 52 cases were evaluable for histological concordance. Of the 14 cases that were not (pathological fail), four were sent to a research laboratory, and two did not have the histologic
NOS, not otherwise specified; TTF-1, thyroid transcription factor 1.

63.6% of cases. The rates of complications were 15% for pneumothorax, 3% for pneumothorax requiring chest drain, and 8% for hemoptysis.