MOLECULAR TESTING IN LUNG CANCER- BASICS AND COMPARISONS OF METHODS USED AND GUIDELINES

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18/01/19

TOPICS TO BE DISCUSSED

1. Why and how to differentiate Squamous vs Adeno NSCLC ?
2. Target genetic alterations in NSCLC
3. Molecular testing methods
4. Need for Molecular testing –approved and non approved drugs
5. Samples to be tested
6. Most common genetic alterations and their testing methods
7. Role of liquid biopsy in lung cancer
8. Guidelines on molecular testing in lung cancer
9. Take home message

THERANOSTICS

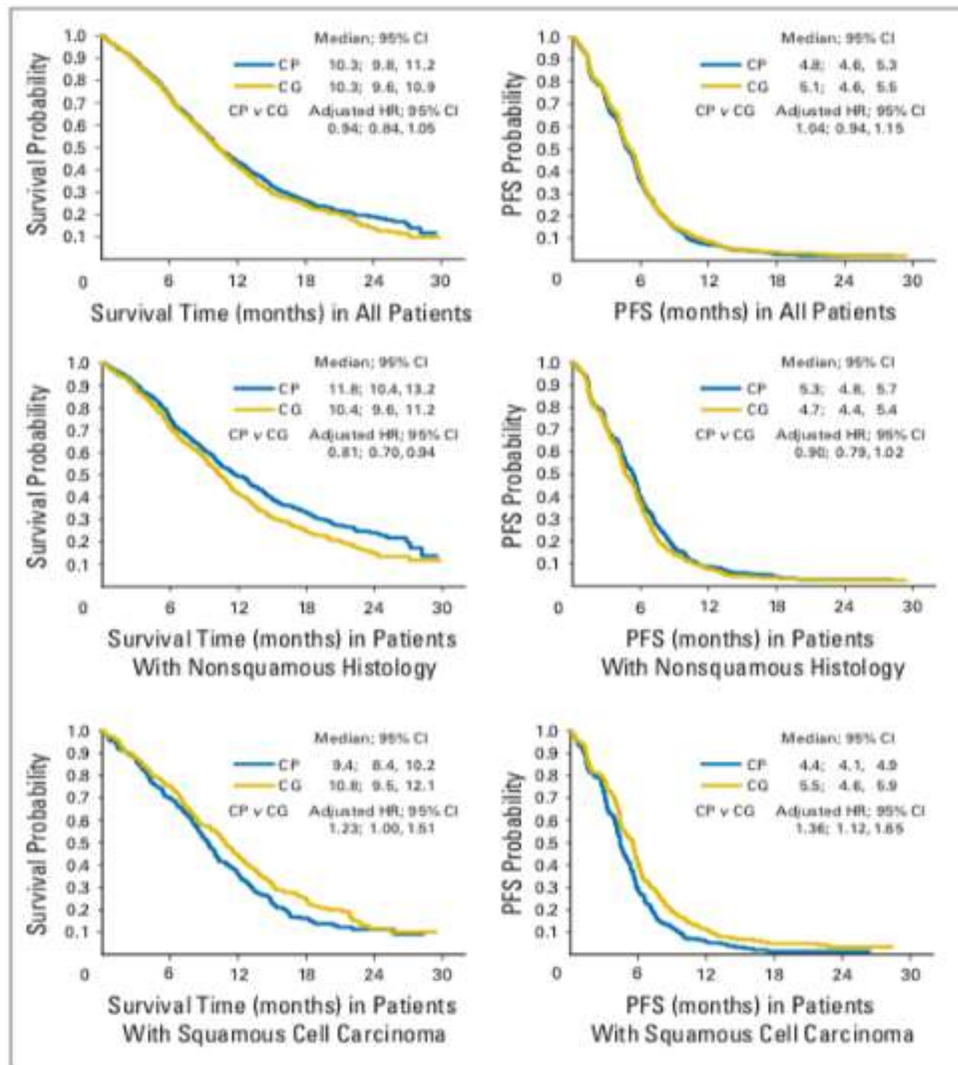
Therapeutics and diagnostics meaningfully combined to achieve personalised pharmacotherapy

NSCLC vs SCLC

- Molecular research advances have heralded major breakthroughs in the management of lung cancer, particularly for the more common (~80%) non-small cell lung cancer (NSCLC)
- Treatment for small cell lung cancer remains chemotherapy-based even now

Hughes et al. Transl Lung Cancer Res 2015;4(1):36-54

NSCLC - WHY SQUAMOUS VS NON SQUAMOUS IMPORTANT?



- Noninferiority, phase III, randomized study
- Stage IIIB or IV NSCLC
- N= 1725, ECOG 0-1
- Cisplatin 75 mg/m2 on day 1 + gemcitabine 1,250 mg/m2 on days 1 and 8 (n = 863) VS Cisplatin 75 mg/m2 + pemetrexed 500 mg/m2 on day 1 (n = 862) every 3 weeks (Total 6 cycles)

Toxicity	Cisplatin/ Pemetrexed (n = 839) No. of Patients	%	Cisplatin/ Gemcitabine (n = 830) No. of Patients	%	*P*
Hematologic					
Neutropenia	127	15.1	222	26.7	< .001
Anemia, hemoglobin	47	5.6	82	9.9	.001
Thrombocytopenia, platelets	34	4.1	105	12.7	< .001
Leukopenia	40	4.8	63	7.6	.019
Nonhematologic					
Febrile neutropenia	11	1.3	31	3.7	.002
Alopecia, any grade	100	11.9	178	21.4	< .001
Nausea	60	7.2	32	3.9	.004
Vomiting	51	6.1	51	6.1	1.000
Dehydration, any grade	30	3.6	17	2.0	.075
Fatigue	56	6.7	41	4.9	.143

Scagliotti et al. J Clin Oncol.2008 Jul 20;26(21):3543-51

HOW TO DIFFERENTIATE SQUAMOUS VS OTHER NSCLC?

Biopsy or cytology specimen intended for initial diagnosis

- Make an accurate diagnosis using the 2015 WHO classification
- Preserve the tissue for molecular studies, especially if the patient has advanced-stage disease
- "NSCC-NOS" should be reserved only for cases in which immunohistochemical testing is uninformative or ambiguous

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NSCLC CLASSIFICATION

- Adenocarcinoma – AIS vs MIA vs Invasive adeno carcinoma
  - AIS – lepidic growth, ≤ 3 cm
  - MIA- lepidic growth, ≤ 3 cm and ≤ 5 mm invasion
  - Invasive Adenocarcinoma - acinar, papillary, micropapillary, lepidic, or solid growth**,** > 5 mm invasion

- Squamous cell carcinoma - keratinization and/or intercellular bridges and express IHC for squamous differentiation

- Adenosquamous carcinoma (resection specimen preferred) - Each type constituting atleast 10 % of the tumour

- Large cell carcinoma - Undifferentiated NSCC , the diagnosis requires a thoroughly sampled resected tumor and cannot be made on non- resection or cytology specimens

- Sarcomatoid carcinoma-
  - Pleomorphic carcinoma - contains at least 10% spindle and/or giant cells
  - Carcinosarcoma- NSCC + sarcoma elements
  - Pulmonary blastoma – fetal adenocarcinoma + primitive mesenchymal stroma

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ROLE OF IHC IN INITIAL DIAGNOSIS

When adenocarcinoma or squamous cell carcinomas are poorly differentiated, and the defining morphologic criteria that would allow for specific diagnosis are inconspicuous or absent

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IHC MARKERS FOR NSCLC

1. TTF-1
2. NAPSIN-A
3. p63
4. p40

WHAT IS TTF-1?

- **TTF -1**
  - Nuclear transcription protein that is expressed in epithelial cells of the embryonal and mature lung and thyroid
  - Seen in 70-90 % primary lung adenocarcinoma cases
  - Metastatic adenocarcinoma to the lung - negative for TTF1
  - Thyroid metastases – positive for PAX 8 and thyroglobulin in addition to TTF-1
  - Rare cases of TTF-1 positivity from gynaecologic tract and pancreatobiliary system also possible (Radiology and clinical scenario important in these circumstances)

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WHAT IS NAPSIN A?

- **Napsin A –**
  - An aspartic proteinase expressed in normal type II pneumocytes and in proximal and distal renal tubules
  - Appears to be expressed in >80% of lung adenocarcinomas

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TTF -1 VS NAPSIN A

Neither TTF-1 nor Napsin A is specific for lung primary adenocarcinoma

	TTF-1 (+)	TTF-1 (−)
$N = 120$		
Lung primary adenocarcinoma		
Napsin A (+)	95 (79.2%)	10 (8.3%)
Napsin A (−)	4 (3.3%)	11 (9.2%)
$N = 37$		
Metastatic carcinomas		
Napsin A (+)	0	0
Napsin A (−)	8 (21.6%)	29 (78.4%)

Surgical archives of University of Rochester Medical Center, Rochester, NY, From 2004 to 2009

TTF -1 + Napsin A better than either alone

Ye et al. App Immunohistochem Mol Morphol. 2011 Jul;19(4):313-7

p63 AND p40

- p53 tumour suppressor gene – 2 isoforms producing p63 and p40
- p63 is responsible for proliferation and differentiation of epithelial progenitor cells
- Focal or weak p63 expression has been seen in upto 30 % adenocarcinoma cases
- p63 is more expressed in differentiated cells while p40 is seen in the stem-like cell populations

Affandi KA, et al. Journal of Pathology and Translational Medicine 2018; 52: 283-289

p40 VS p63 - WHICH IS BETTER ?

N= 70, Retrospective study
Previously diagnosed as primary lung squamous cell carcinoma (n=35) and lung adenocarcinoma (n=35)
January 2008 to December 2016

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	p-value
p40	77.1	100	100	81.4	<.001
p63	85.7	62.9	69.8	81.5	<.001

Affandi KA, et al. Journal of Pathology and Translational Medicine 2018; 52: 283-289

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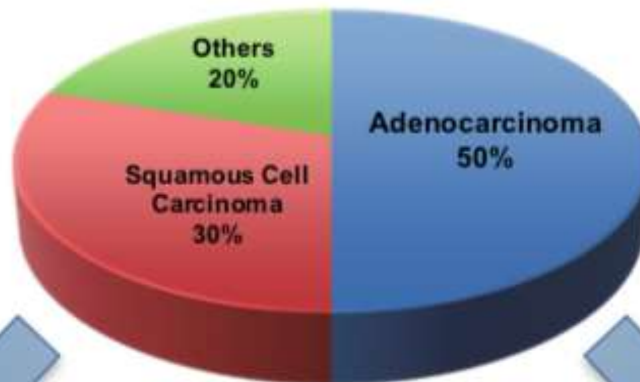
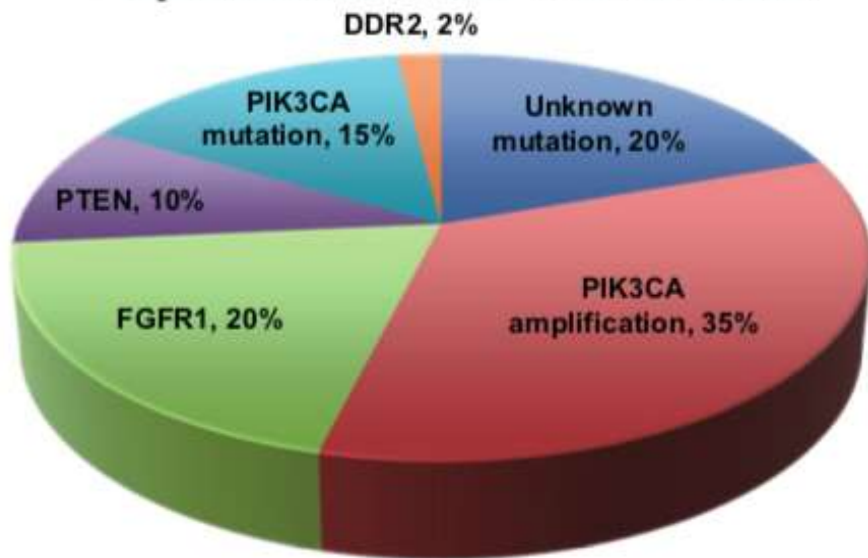
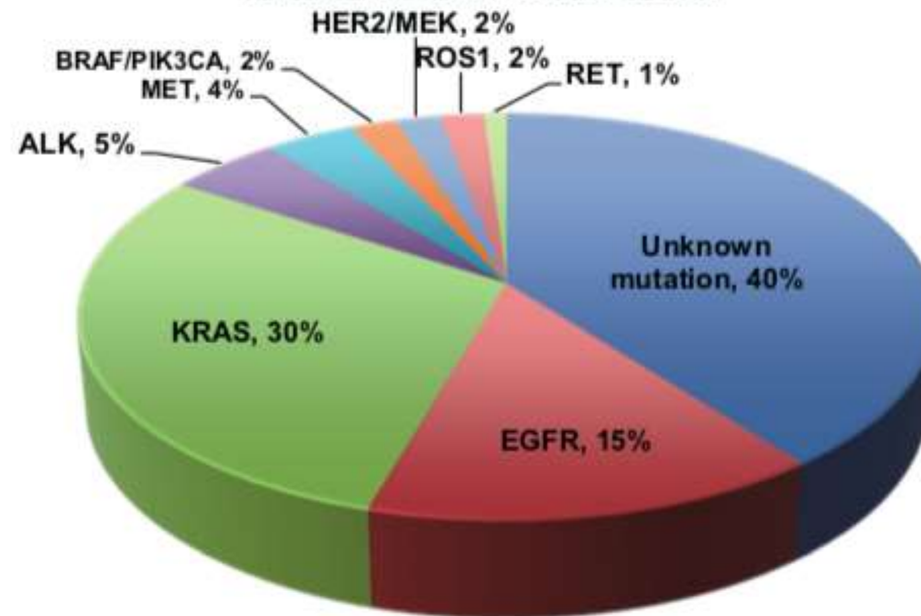
- A limited number of immunostains with one lung adenocarcinoma marker (TTF1, napsin A) and one squamous carcinoma marker (p40, p63) should suffice for most diagnostic problems
- Tumors that lack squamous cell morphology and show co-expression of p63 and TTF1 are preferably classified as adenocarcinoma
- A simple panel of TTF1 and p40 may be sufficient to classify most NSCC-NOS cases
- IHC should be used to differentiate primary lung adenocarcinoma from squamous cell carcinoma, large cell carcinoma, metastatic carcinoma, and primary pleural mesothelioma (particularly for pleural specimens)

GENETIC ALTERATIONS IN NSCLC







Chan et al. Transl Lung Cancer Res 2015;4(1):36-

TARGETABLE GENETIC ALTERATIONS IN MOLECULAR TESTING

(1) Mutations

EGFR
KRAS BRAF HER2
MET

ALK ROS1
RET NTRK1 FGFR1/3
NRG1

(2) Gene rearrangements

FGFR1 MET EGFR
HER2

(3) Amplifications

Shim et al. J Pathol Transl Med. 2017 May; 51(3): 242–254.

MOLECULAR TESTING METHODS

Category	Mutation	Gene rearrangement	Amplification
DNA	Direct sequencing	FISH	FISH
	PCR-based methods	NGS	qPCR
	NGS		NGS
RNA		RT-PCR (fusion transcript)	Real-time PCR (mRNA overexpression)
		NGS	
Protein	IHC (mutation-specific antibody)	IHC (protein expression)	IHC (protein overexpression)

Shim et al. J Pathol Transl Med. 2017 May; 51(3): 242–254.

NEED FOR MOLECULAR TESTING

GENER ATION	TKI	SELECTIVITY	REV/IRREVER	APPROVAL STATUS	FDA APPROVED DOSE/day	APPROVAL TIME
1ST	Geftinib	WT EGFR	Reversible	FDA, EMA	250 mg OD	As 1st line July 2015
	Erlotinib	WT EGFR	Reversible	FDA, EMA	150 mgOD	As 1st line May 2013
	Icotinib	WT EGFR	Reversible	CFDA	125 mg TDS	June 2011
2ND	Afatinib	WT EGFR + other HER	Irreversible	FDA, EMA, CFDA	40 mg OD	As 1st line July 2013
	Dacomitinib	WT EGFR + other HER	Irreversible	NO (awaiting)	-	-
3RD	Osimertinib	MUTANT EGFR	Irreversible	FDA, EMA	80 mg OD	As 2nd or 3rd line Nov 2015 AS 1ST LINE APRIL 2018
	Olmutinib	MUTANT EGFR	Irreversible	KFDA	800mg/day	May 2016

TARGETED THERAPIES APPROVED

ALK

1st Line therapy
Alectinib
Brigatinib
Ceritinib
Crizotinib

Subsequent therapy
Alectinib
Brigatinib
Ceritinib
Lorlatinib

ROS 1

Crizotinib
Ceritinib

BRAF V600E

1st line therapy
Dabrafenib/trametinib

Subsequent therapy
Dabrafenib/trametinib

PDL1

First-line therapy

- **Pembrolizumab (PDL 1 ≥ 50 %)**

NON SQUAMOUS

- **(Carboplatin or cisplatin)/ pemetrexed/pembrolizumab**
- **Carboplatin/paclitaxel/bevacizumab/ atezolizumab**

SQUAMOUS

- **(Carboplatin or cisplatin)/(paclitaxel or albumin-bound paclitaxel)/ pembrolizumab (squamous)**

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NON FDA APROVED TARGETED AGENTS

Gene	Frequency	Frequency
MUTATIONS		
KRAS	5%–10% in ADCs	MEK inhibitors
HER2	1%–2% in ADCs	Trastuzumab, afatinib
MET	3%–4% in ADCs	Crizotinib, cabozantinib
GENE FUSIONS		
RET	1% in ADCs	Cabozantinib, vandetanib, alectinib
NTRK1	< 1% in ADCs	Entrectinib
FGFR1/3	1% in NSCLCs	FGFR inhibitor
AMPLIFICATIONS		
FGFR1	13%–22% in SQCs	FGFR inhibitor
EGFR	8%–9% in SQCs,	EGFR inhibitor
MET	2%–4% in ADCs	Crizotinib
HER2	1%–2% in ADCs	Trastuzumab, afatinib

Shim et al. J Pathol Transl Med. 2017 May; 51(3): 242–254

SAMPLES TO TESTED

- Bronchial brushings
- Bronchial washings
- Sputum
- FNA biopsy
- Core needle biopsy
- Endobronchial biopsy and
- Transbronchial biopsy

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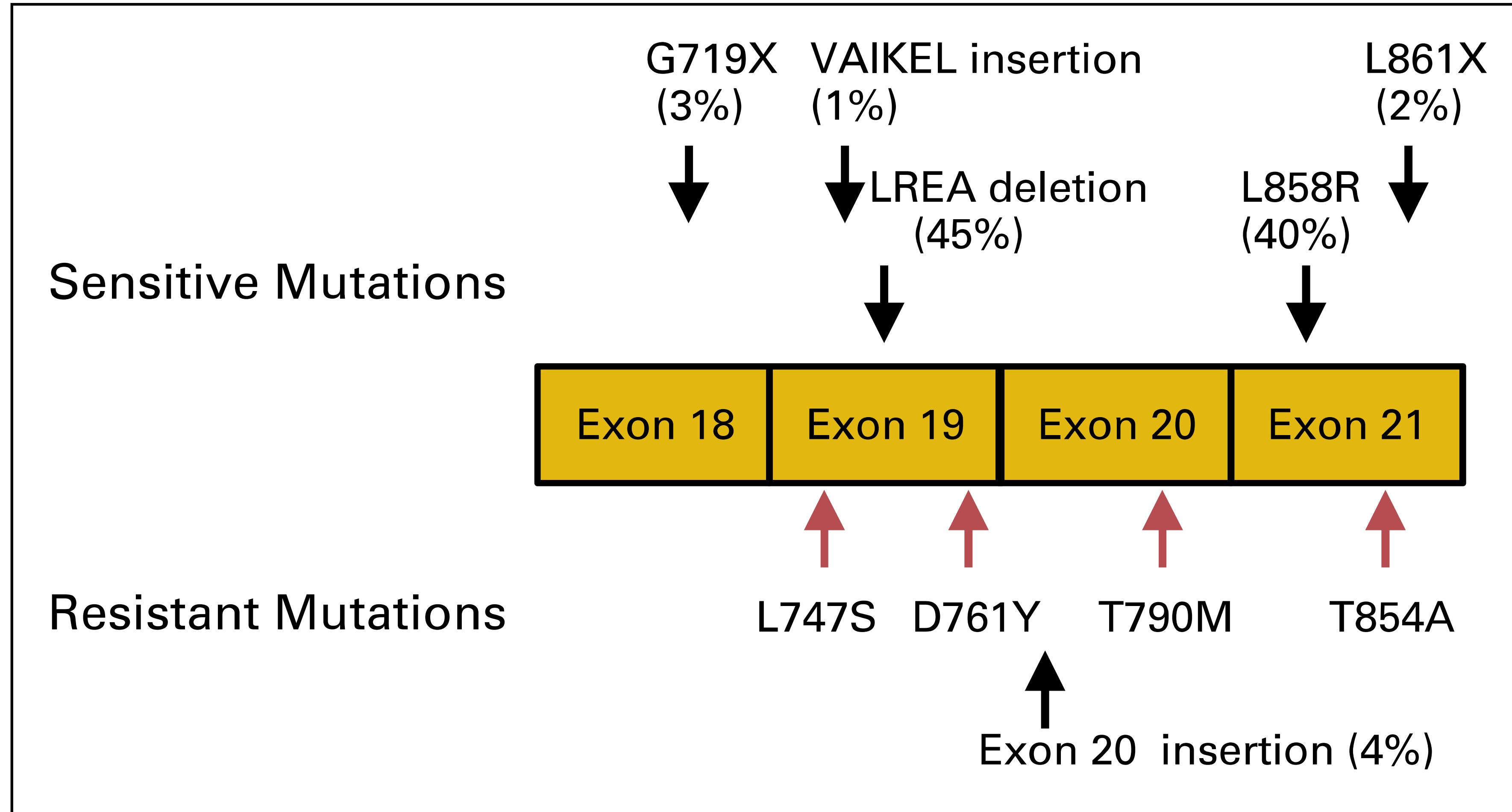
EGFR MUTATION

- EGFR gene is located on the short arm of chromosome 7 **(7p)**
- Transmembrane tyrosine kinase receptor

1. Extracellular, ligand-binding domain
2. Transmembrane domain
3. Cytoplasmic TK domain

Ohashi et al. Journal of Clinical Oncology. 2013;31(8):1070-1080

EGFR MUTATIONS



Ohashi et al. Journal of Clinical Oncology. 2013;31(8):1070-1080

EGFR TESTING METHODOLOGIES

SCREENING METHODS

1. Sanger sequencing,
2. Next Generation Sequencing (NGS),
3. High Resolution Melt Analysis (HRMA) and
4. Pyrosequencing

TARGETED METHODS

1. ddPCR
2. Real-time polymerase chain reaction (PCR)
3. Next-generation sequencing (NGS)

Transl Lung Cancer Res 2015;4(2):126-141

SANGERS SEQUENCING TECHNIQUE

- Also known as Dideoxy sequencing or chain termination
- Uses 2 nucleotides

1. Dideoxynucleotides (ddNTP’s) - contain a hydrogen group on the 3’ carbon instead of a hydroxyl group (OH)
2. Normal nucleotides (NTP’s)

- Principle

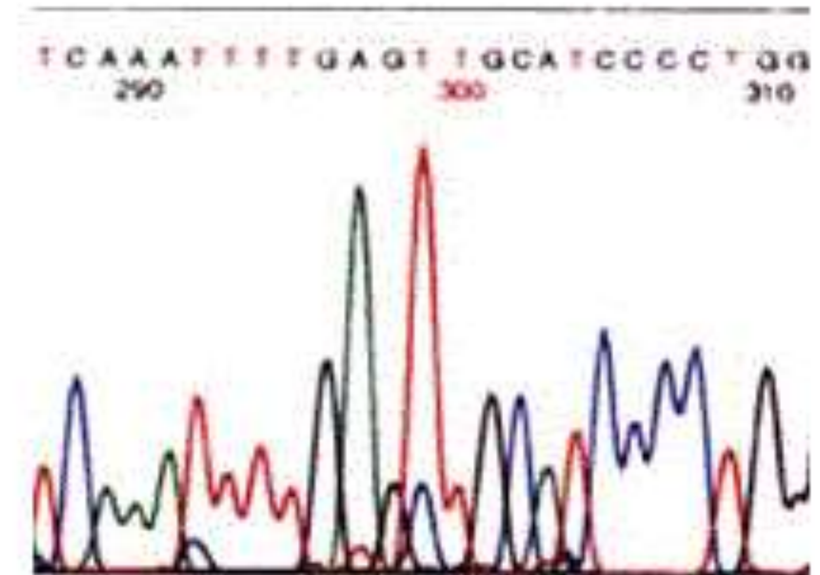
ddNTP’ s, when integrated into a sequence, prevent the addition of further nucleotides

Khoo et al. Transl Lung Cancer Res 2015;4(2):126-141

SANGERS SEQUENCING TECHNIQUE

Method

1. DNA heat denaturation
2. Fluorescent labelled primer addition to template strand
3. Addition of ddNTPs to the primer
4. Polyacrylmide gel electrophoresis
5. Laser reading of the bands
6. Results displayed as a chromatogram



Khoo et al. Transl Lung Cancer Res 2015;4(2):126-141

SANGERS SEQUENCING TECHNIQUE

- Advantages:

1. Considered the gold standard for characterizing all mutations
2. Wide variety of mutations can be detected (SNVs, small insertions/duplications/deletions)

- Disadvantages:

1. Limited in detecting gene copy number changes
2. Low sensitivity (mutant DNA should be present in atleast 20% of the total DNA)

Khoo et al. Transl Lung Cancer Res 2015;4(2):126-14

HIGH RESOLUTION MELT ANALYSIS (HRMA)

- Cheap, rapid and sensitive mutation screening (or scanning) method
- Principle:

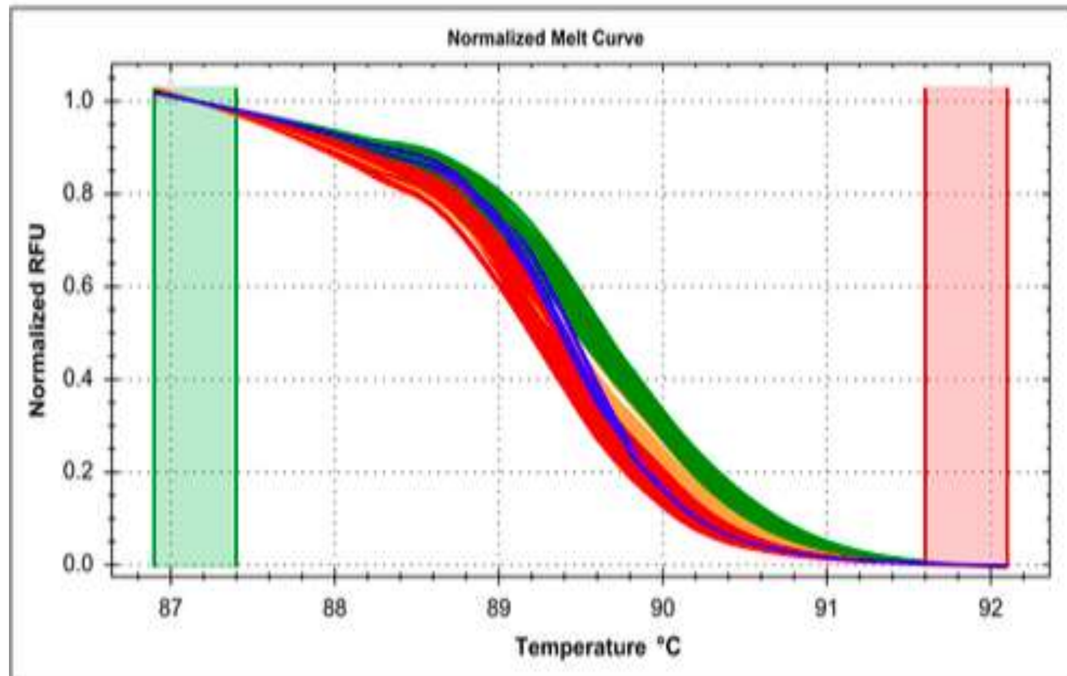
The DNA containing the mutation will “melt” at a different temperature compared to the wild type DNA

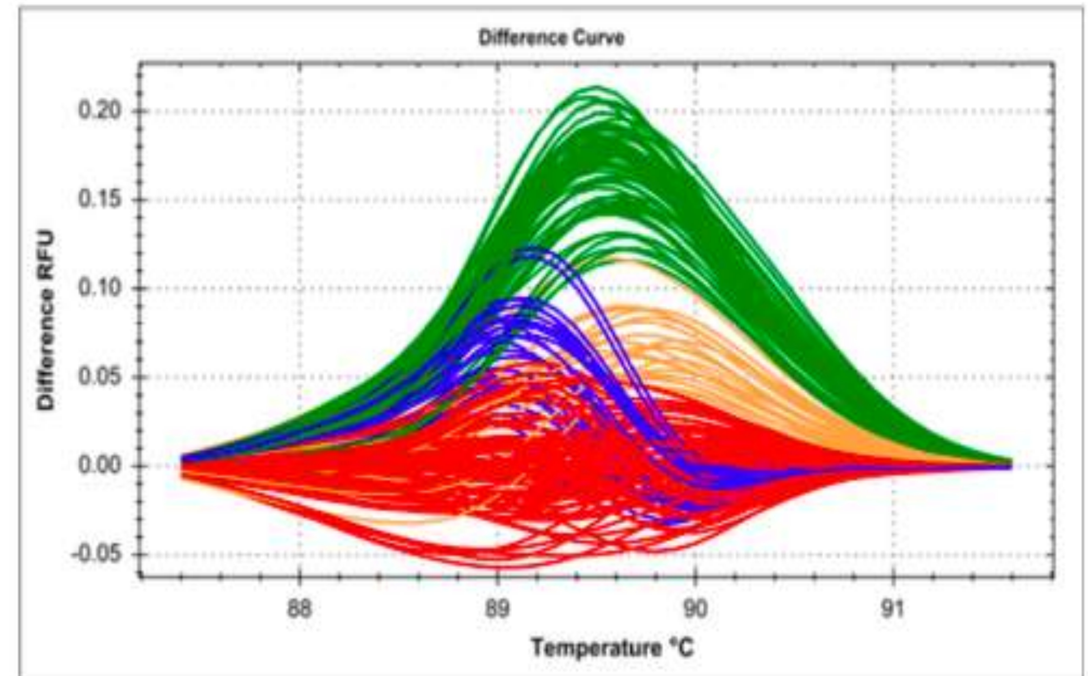
- Method:

1. DNA is amplified using RT-PCR with fluorescent dyes
2. Increments in temperature to a point where the double stranded DNA (with high fluorescence) will “melt apart” to become single stranded DNA fragments (low fluorescence)
3. This difference in melt curve signature is used to detect the presence or absence of a mutation

Khoo et al. *Transl Lung Cancer Res* 2015;4(2):126-14

HRMA





RFU- RELATIVE FLUORESCENCE UNITS

Slomka et al. Int. J. Mol. Sci. **2017**, 18, 231

PYROSEQUENCING

- Sequencing by synthesis
- Principle:

Chemiluminescent detection of inorganic pyrophosphate to detect specific base additions

- Method:
  - Utilises the template containing the region of interest, primers, DNA polymerase and a set of enzymes/substrates
  - Pyrophosphate is released each time a nucleotide is sequentially incorporated onto the 3'end of a DNA which through an enzymatic reaction results in light emission.

Slomka et al. Int. J. Mol. Sci. 2017, 18, 2316

PYROSEQUENCING

Advantage:

Allows detection of mutations in tumor samples as low as 5%

Best used to detect SNVs

Diasadvantage:

Limited in its ability to detect gene copy number changes

Slomka et al. Int. J. Mol. Sci. 2017, 18, 231

REAL TIME PCR FOR EGFR DETECTION

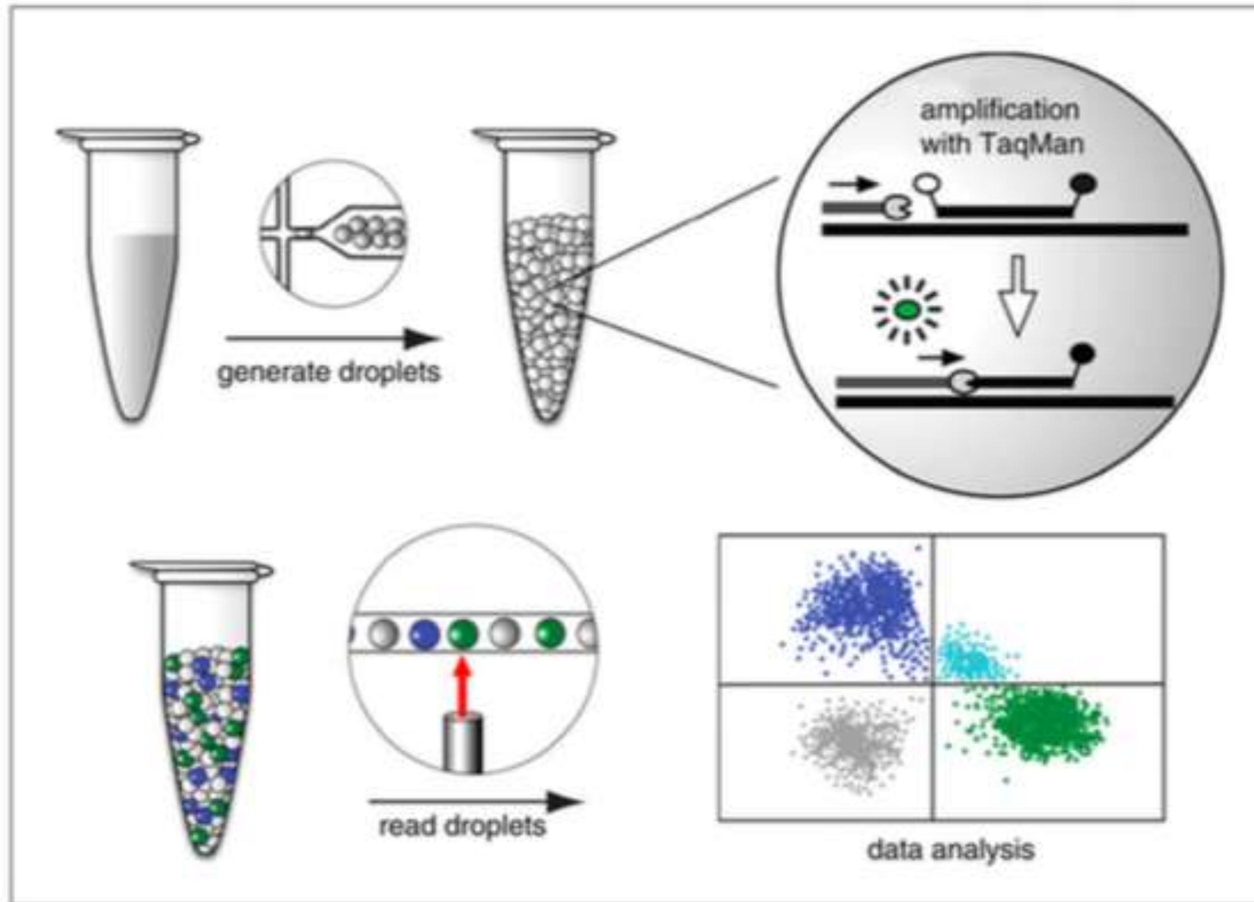
1. The **cobas** EGFR Mutation Test v2 (**cobas** EGFR test) is a real-time PCR test
2. Qualitative detection of 42 mutations in exons 18, 19, 20, and 21 of the (EGFR) gene
3. Tumor tissue and human plasma from non-small cell lung cancer (NSCLC) patients

n = 118 tissue samples
n = 74 plasma samples
RT PCR vs Next Generation Sequencing (NGS)

RESULTS
TISSUE
Limit of detection for detecting EGFR mutations of FFPET samples is ≤ 5% mutation level
Compared to NGS, had PPA of 87%, NPA of 100%, and OPA of 93%
PLASMA
LoD for detecting EGFR mutations in plasma samples is ≤ 100 cp/mL
compared to NGS had PPA of 80.0%, NPA of 94.9%, and OPA of 87.8%

O'Donnell et al. Journal of Clinical Oncology 2016 34:15

DIGITAL DROPLET PCR



- PCR mix is partitioned into thousands of nanoliter-sized droplets using specialized oil and microfluidics technology
- subjected to thermal cycling
- A PCR reaction only occurs in those droplets carrying the target DNA
- TaqMan probe hybridizes to an internal site of the PCR product
- The probe has a 5′ fluorophore whose fluorescence is quenched by a 3′ quencher
- Once the DNA polymerase reaches the probe during the extension step, the probe is cleaved by the polymerase's 5′ to 3′ exonuclease activity
- This action liberates the fluorophore from the quencher so that the fluorescence can be detected when excited by the appropriate wavelength of light

The counts of positive and negative droplets for each target are related to the target's concentration in the sample by the Poisson function

Mazaika and Homsy et al. Curr Protoc Hum Genet. ; 82: 7.24.1–7.24.13

DIGITAL DROPLET PCR

- Advantages:

1. Simpler, faster, and less error prone than real-time qPCR
2. CNV analysis, rare variant detection, SNP genotyping, and transcript quantification
3. Quantify nucleic acid concentration without the need for standard samples

Mazaika and Homsy et al. Curr Protoc Hum Genet. ; 82: 7.24.1–7.24.13

ddPCR vs RT PCR for EGFR MUTATION DETECTION

$$\text{iQC index} = \frac{iQC\ copies}{Input\ DNA\ copies}$$

Retrospective comparative clinical study n=228
ddEGFR and cobas EGFR (RT - PCR)tests for the detection of *EGFR* mutations in NSCLC

Retrospective comparison study, Applied iQC index ($n = 171$)		cobas EGFR Test		
		MD	MND	Total
ddEGFR Test	MD	111	10*	121
	MND	2	48	50
	Total	113	58	**171**
PPA (95% C.I.)	98.23% (93.75–99.78%)			
NPA (95% C.I.)	82.76% (70.57–91.41%)			
OPA (95% C.I.)	92.98% (88.06–96.32%)			
PPV (95% C.I.)	91.74% (85.33–95.97%)			
NPV (95% C.I.)	96.00% (86.29–99.51%)			

FFPE samples with iQC index $\geq$ 0.5 are preferred

Kim SS et al. Sci Rep. 2018 Jan 11;8(1):543

ddPCR vs RT PCR FOR EGFR MUTATION DETECTION

FFPE tumor samples obtained from patients between 2013 and 2014

Sample name	Gender	Age, years	NSCLC histology subtype	Stage	ARMS-qPCR	ddPCR (copy numbers)	Consistency
N001	F	58	Adenocarcinoma	IIB	WT	WT	Yes
N002	M	47	Adenocarcinoma	IIIA	L858R	L858R (164)	Yes
N003	F	57	Squamous cell	IIA	WT	WT	Yes
N004	M	68	Adenocarcinoma	IIA	Del	Del (84)	Yes
N005	M	72	Adenocarcinoma	IIB	WT	WT	Yes
N006	F	75	Adenocarcinoma	IIIB	WT	T790M (7)	No
N007	F	58	Adenocarcinoma	IIB	Del	Del (153)	Yes
N008	M	73	Adenocarcinoma	IIB	WT	WT	Yes
N009	M	62	Squamous cell	IIIA	WT	WT	Yes
N010	F	54	Squamous cell	IA	WT	WT	Yes

Mutation rates as low as 1% were stably detected by ARMS-qPCR,
While plasmid samples with mutation rates from 5 to 0.1% were reliably detected by ddPCR

Zhang *et al*. Exp Ther Med. 2015 Apr;9(4):1383-1388

RT PCR VS IHC VS DIRECT SEQUENCING

% mutant DNA relative to wild-type DNA	Therascreen EGFR Mutation Test kit (n = 18)[§], *	% mutant DNA relative to wild-type DNA	Direct sequencing (n = 19)[§], *
1%	9/18 (50%)	10%	1/19 (5%)
		20%	6/19 (31%)
3%	15/18 (83%)	25%	6/19 (31%)
		30%	12/19 (63%)
5%	18/18 (100%)	N.D.	7/19 (37%)

	Direct sequencing					
EGFR mutations	Sensitivity (%)	95% CI	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Exon 19 deletions	100	100–100	100	100	100	100
L858R	100	100–100	100	100	100	100
Exon 20 insertions	67.7	58.5–74.9	100	100	99.2	99.2
All mutations	94.7	90.9–98.6	100	100	99.1	99.2

DIRECT SEQUENCING VS RT PCR

Angulo et al. .PLoS One. 2012;7(8

RT PCR VS IHC VS DIRECT SEQUENCING

IHC	*EGFR* mutations	Direct sequencing Sensitivity (%)	95% CI	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
E746-A750	E746-A750 deletion	88.9	82.3–95.4	100	100	98.8	98.9
E746-A750	All exon 19 deletion	72.7	63.5–82	100	100	96.3	96.6
L858R	L858R	40	29.5–50.5	100	100	96.3	96.4
E746-A750 + L858R	E746-A750 deletion + L858R	71.4	61.6–81.2	100	100	94.4	95.1
E746-A750 + L858R	All mutations	46.7	35.9–57.5	100	100	89.3	90.2

IHC	*EGFR* mutations	Therascreen EGFR Mutation Test kit Sensitivity (%)	95% CI	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
E746-A750	All exon 19 deletion	66.7	56.6–76.7	100	100	96.2	96.4
L858R	L858R	40	29.3–50.7	100	100	96.2	96.2
E746-A750 + L858R	All exon 19 deletion + L858R	53.8	42.9–64.8	100	100	91.8	92.5
E746-A750 + L858R	All mutations	50	39–64.8	100	100	90.4	91.2

IHC – NOT GOOD METHOD TO SCREEN FOR EGFR MUTATIONS DUE TO LOW SENSITIVITY

Angulo et al. .PLoS One. 2012;7(8

NEXT GENERATION SEQUENCING

NEED:

- Previously discussed methods of sequencing allow for a few genes per run, resulting in a time consuming and expensive diagnostic
- Consider intratumor heterogeneity
- Higher sensitivity of NGS to detect low frequency mutations

Coco et al. Current Drug Targets, 2015, 16, 47-59

NEXT GENERATION SEQUENCING

- Also known as Massively parallel sequencing
- Simultaneous read of DNA sequences in a parallel way and is characterized by a high speed and relatively low cost
- Several NGS platforms

Coco et al. Current Drug Targets, 2015, 16, 47-59

NGS - STEPS

1. Sample preparation -DNA isolation, fragmentation/target region capture, and library construction
2. Sequencing - each fragment of the library is read multiple times
3. Analysis of bioinformatic data
    1. Alignment of sequencing reads to a reference genome using specific algorithms
    2. Filtering phase that removes all off-target reads and low quality data
    3. Coverage (read depth) that reports the number of reads for each region that has been sequenced and the value ranges from approximately 30X to 500X depending on NGS analysis
    4. Variant calling that reports all genetic variants (single nucleotide variation, insertion/deletion (indel), and copy number variations) using specific software
    5. Annotation step that defines all relevant variants linked to the pathogenesis

Coco et al. Current Drug Targets, 2015, 16, 47-59

NGS APPROACHES

- DISCOVERY NGS - investigate the whole mutational spectra of type/subtypes of cancer to discover any recurrent somatic mutations in a specific tumor
  - WGS - Whole genome sequencing
  - WES - Whole Exome sequencing
  - WTS - Whole Transcriptome sequencing
- TARGET NGS – cancer panels focus on a small number of genes frequently mutated in a specific cancer type or disease

Coco et al. Current Drug Targets, 2015, 16, 47-59

NGS APPROACHES

- Whole genome sequencing- Passenger mutations also identified (? Significance)
- Whole exome sequencing and Whole transcriptome sequencing-

1. Faster
2. Passenger mutations avoided

Coco et al. Current Drug Targets, 2015, 16, 47-59

TARGETED NGS IN LUNG CANCER

Variable	Total (%)
Tumors tested	22
Age, year (range)	64 (32-84)
Sex: Male	16 (72.7)
Lung adenocarcinoma	18 (81.8)
ROSE	12 (54.5)
Confirmed smokers	17 (77.3)
Stage IV	20 (91)

ROSE: Rapid on-site evaluation from pathology at the time of biopsy

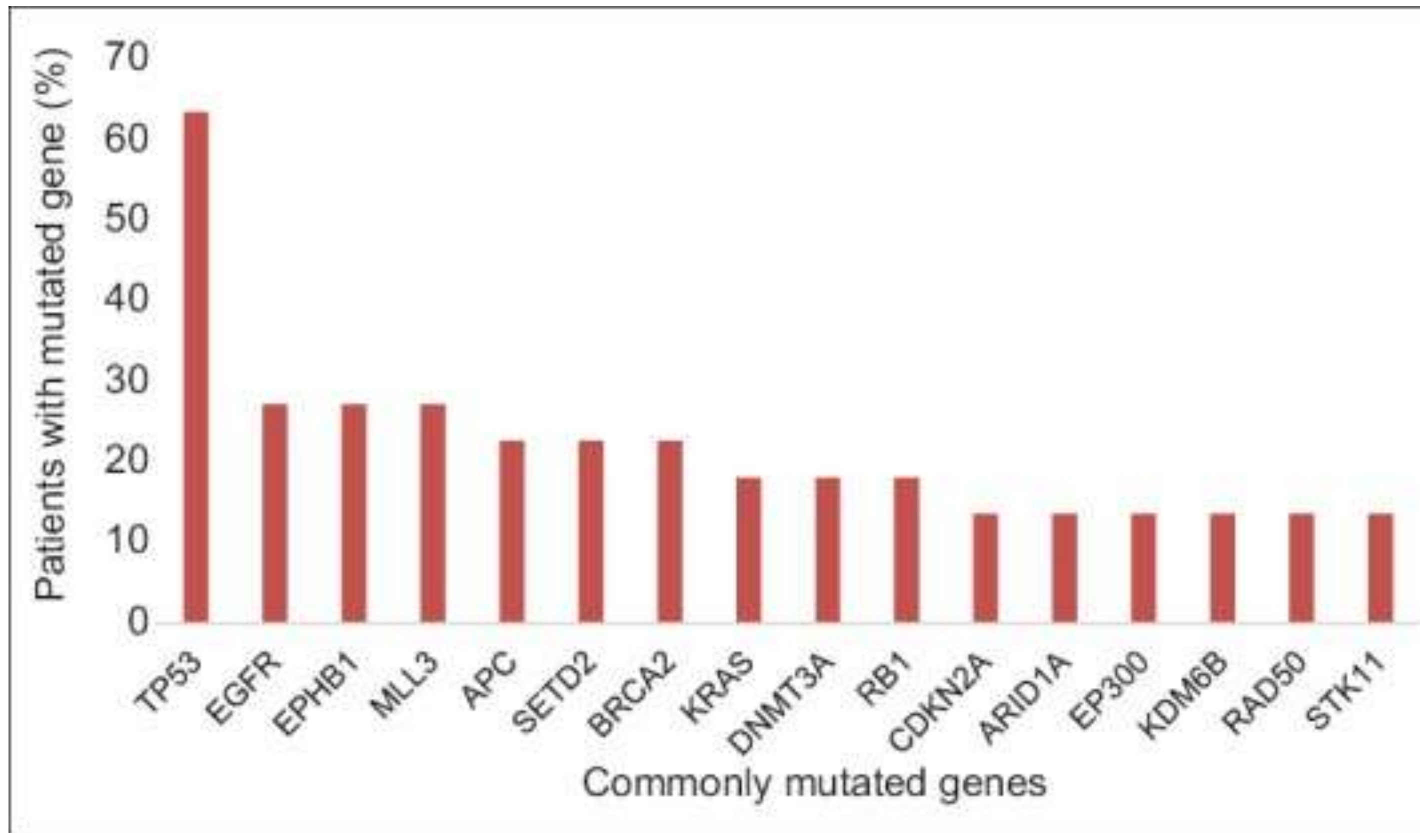
Biopsy Type	Full analysis	Tissue insufficient for analysis	Testing failure	Total
Surgical biopsy	5	0	0	5
Trans-thoracic CT-guided CNB	7	0	0	7
CNB-other	2	0	0	2
EBUS FNA	4	1	0	5
Endoscopic forceps biopsy	2	0	0	2
Effusion	1	0	0	1
Total				22

CT: Computed tomography, EBUS: Endobronchial ultrasound, FNA: Fine-needle aspiration, CNB: Core needle biopsy

N=22 NSCLC
RETROSPECTIVE STUDY January 2014 to July 2015
NGS Panel target- 467 cancer-associated genes

DiBardino et al. Cytojournal. 2017; 14: 7

FINAL VERDICT ON EGFR TESTING



NGS RESULTS ON NSCLC

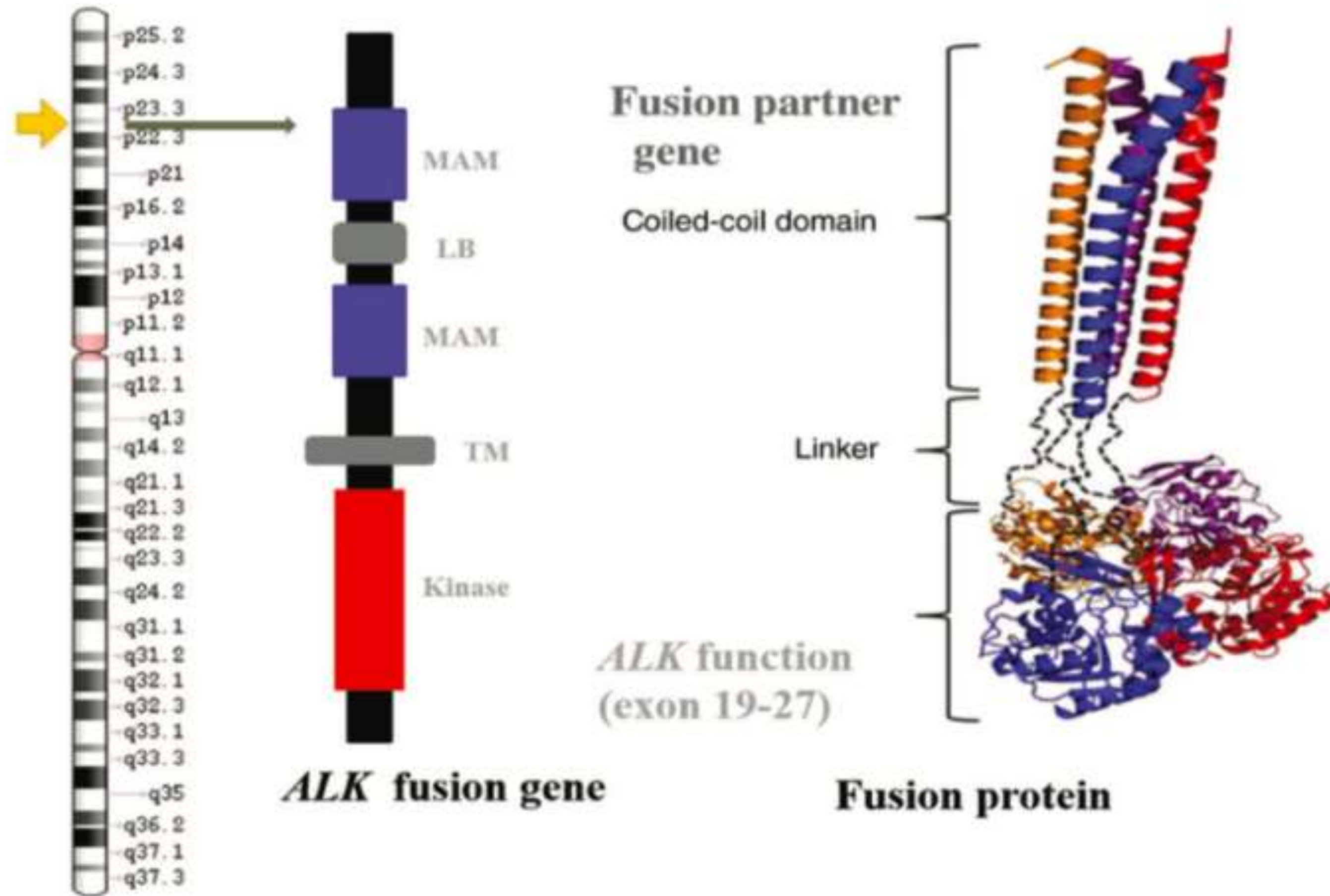
NGS using a large targeted gene panel was performed successfully in 94% of small biopsy and cytology samples compared to 100% of resections

VERDICT on EGFR TESTING– NGS > ddPCR> RT PCR > direct sequencing

DiBardino et al. Cytojournal. 2017; 14: 7

ALK REARRANGEMENT

5’ end from EML4 gene replaces native ALK 5’ end



- Located on the short arm of chromosome 2
- Most mutations of the ALK gene are translocations
- Most common ALK-EML4 fusion -inversion rearrangement at p21-p23
- Constitutive ALK kinase activity
- Rearrangements of the ALK gene with other partner genes have also been described

3’ end from native ALK gene

X. Du et al. Thoracic Cancer 9 (2018) 423–430

ALK TESTING METHODS

- Fluorescence in situ hybridization (FISH),
- Immunohistochemistry (IHC),
- Real Time-PCR (RT-PCR) and
- Next generation sequencing (NGS).

X. Du et al. Thoracic Cancer 9 (2018) 423–430

ALK TESTING BY FISH- PRINCIPLES

- Considered the Gold standard
- The Vysis LSI *ALK* break apart FISH probe kit (Abbott Molecular) - US Food and Drug Administration (FDA) approved
- Initially 50 tumor cells are counted
- A sample is considered positive if > 25 cells out of 50 (> 25/50 or > 50%) test positive
- A sample is considered equivocal if 5 to 25 cells (10 to 50%) test positive
- If the sample is equivocal, a second reader should evaluate the slide

Dacic et al. Oncotarget. 2016 Dec 13; 7(50): 82943–82952

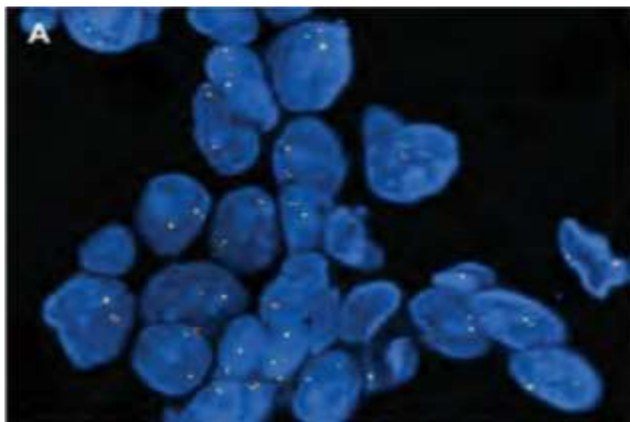
ALK TESTING BY FISH- PRINCIPLES

- Equivocal sample is considered to be positive for *ALK* rearrangement if at least 15% of tumor cells show rearrangement (15/100)
- Here both 3’ and 5’ part are given different colour signals to interpret positivity

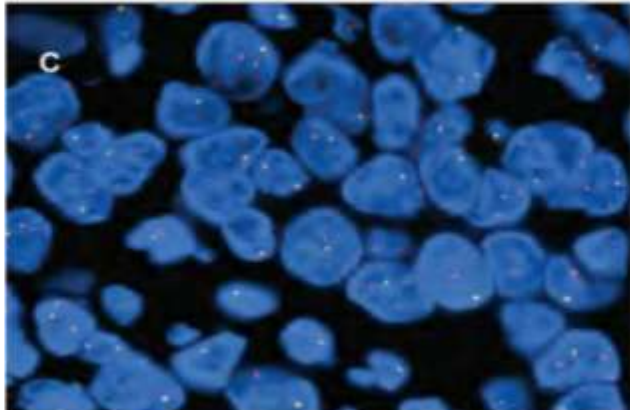
ALK TESTING

FISH

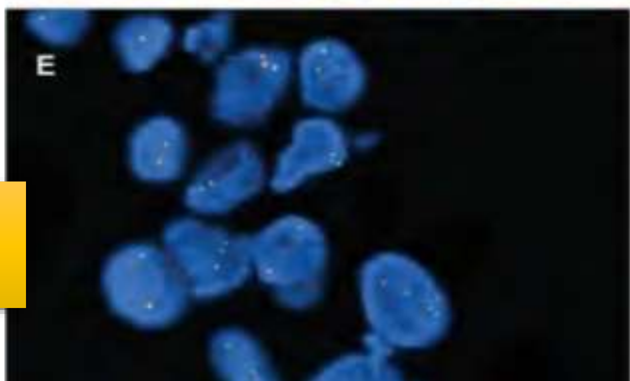
SPLIT SIGNAL



SINGLE RED SIGNAL FROM 3’ TELOMERIC PART

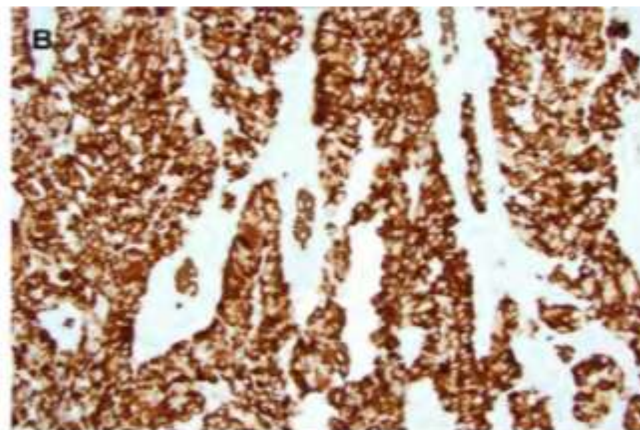


NEGATIVE
GREEN SIGNAL FROM 5’ CENTOMERIC PART OR FUSED YELLOW SIGNAL



IHC

IHC- STRONG +VE



IHC WEAK +VE

IHC -VE

Dacic et al. Oncotarget.2016 Dec 13; 7(50): 82943–82952

ALK TESTING BY FISH

- Mixed pattern in FISH (split signal + single orange signal) is considered negative
- Single orange signal pattern is prone to false positivity, hence should be correlated with IHC and/ NGS

Dacic et al. Oncotarget.2016 Dec 13; 7(50): 82943–82952

NEED FOR ALK TESTING BY IHC

- FISH +ve only indicates whether the *ALK* gene is broken at the DNA level and does not determine whether there has been a productive rejoining of the DNA producing a functional fusion protein
- Case reports indicate lack of therapeutic benefit with ALK inhibitors even in those with ALK +ve by FISH
- The “break-apart” signal pattern resulting from the intrachromosomal deletion and inversion event in the setting of polysomy typical of lung cancer is subtle and easily missed
- Not available in all labaratories

Dacic et al. Oncotarget.2016 Dec 13; 7(50): 82943–82952

ALK TESTING BY IHC

- N=153, Lung Adenocarcinomas, IHC by ALK1 vs D5F3 compared to FISH
- Mouse monoclonal antibody - ALK1
- Rabbit monoclonal antibody - D5F3

FISH was performed on FFPE tumor tissues using a break-apart probe specific to the *ALK* locus (Vysis LSI ALK Dual Color)

	LUNG ADENOCARCINOMA (n= 153)	
	D5F3 antibody	ALK1 antibody
Sensitivity(%)	100	67
Specificity (%)	99	97
Positive predictive value	96	78
Negative predictive value	100	95

Mino-Kenudson et al. *Clin Cancer Res*. 2010 March 1; 16(5): 1561–1571

COMPARISON OF ALK TESTING METHODS

METHOD	FISH	IHC	RT-PCR	NGS
FUSION TYPES	NO SPECIFICATION	NO SPECIFICATION	ONLY EML4-ALK FUSION	ALL KINDS
SENSITIVITY	10-15%	5-10%	1-5%	1-5%
TIME(DAYS)	2-3	0.5	1	5-7
COST	MEDIUM	LOW	MEDIUM	HIGH
FFPE MATERIAL	YES	YES	YES	YES
FRESH MATERIAL	NO	NO	YES	YES
AMOUNT OF TISSUE	ONE TISSUE SECTION (3 micrometre thick)	ONE TISSUE SECTION (3 micrometer thick)	0.1-0.5 microgram RNA	2-3 microgram DNA
OTHER MUTATIONS	NO	NO	NO	YES
TECHNICAL SKILL	MEDIUM	LOW	MEDIUM	HIGH
DIAGNOSTICIAN REQUIRED	MEDIUM	LOW	MEDIUM	HIGH
APPLICABILITY TO AVERAGE LAB	MOST	ALL	SOME	SOME

X. Du et al. Thoracic Cancer 9 (2018) 423–430

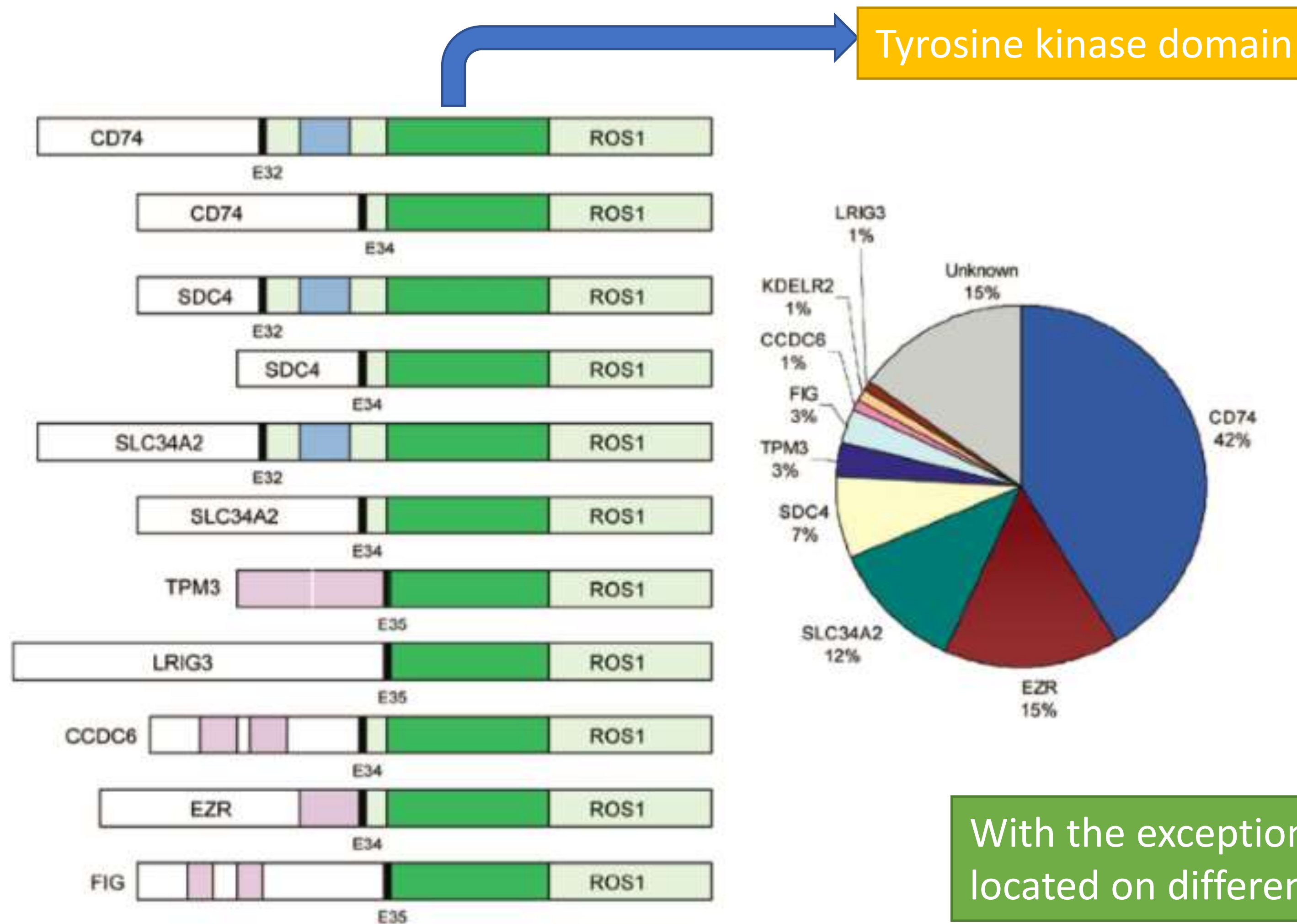
ROS -1

- Tyrosine kinase receptor
- Located in Chromosome 6
- 3 domains
  - (a) a glycoprotein-rich extracellular domain,
  - (b) a transmembrane domain, and
  - (c) an intracellular tyrosine kinase
- 9 different *ROS1* fusion partners have been identified in NSCLC
- *ROS1 - CD74 fusion*- the most common

Shaw et al. The Oncologist. 2013;18:865–875

ROS-1 REARRANGEMENTS

Tyrosine kinase domain



With the exception of *FIG* and *EZR*, *ROS1* fusion partners are located on different chromosomes than the native *ROS1* gene

Shaw et al. The Oncologist. 2013;18:865–875

COMPARISON OF FISH AND IHC FOR ALK AND ROS1

RETROSPECTIVE COHORT (already known genetic alterations)
Jan 2005-jan 2012, never smokers
IHC performed to detect ROS 1 and ALK
N= 219

FISH +ve if > 15 % tumour cells show split signal or single red/green signal
IHC performed using Ventana automated immunostainer
IHC clone for ROS1- D4D6
IHC clone for ALK - D5F3

PROSPECTIVE COHORT
Feb 2013- May 2013
IHC performed to detect ROS1 and ALK and confirmed by FISH
N= 111

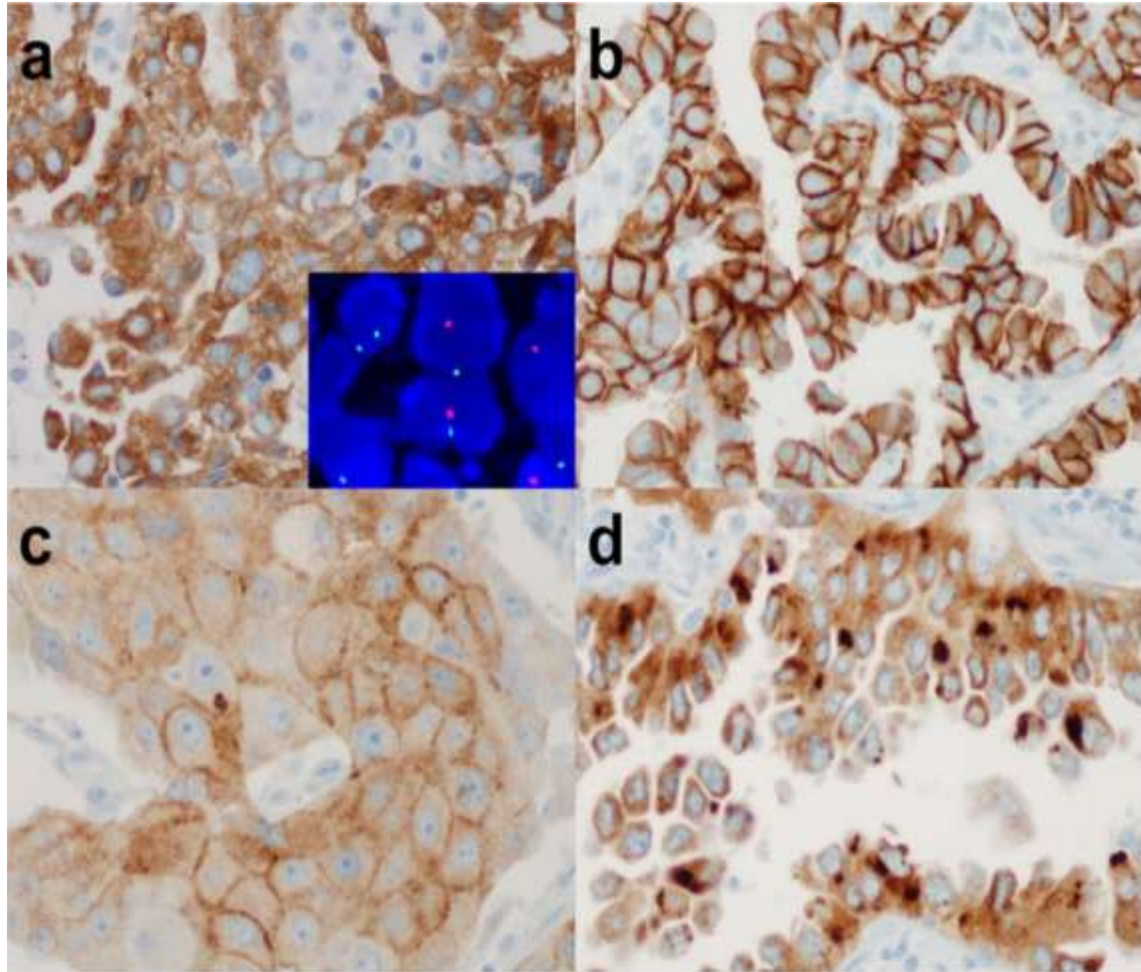
Cha et al. PLoS One. 2014 Jul 24;9(7)

COMPARISON OF FISH AND IHC FOR ALK AND ROS1

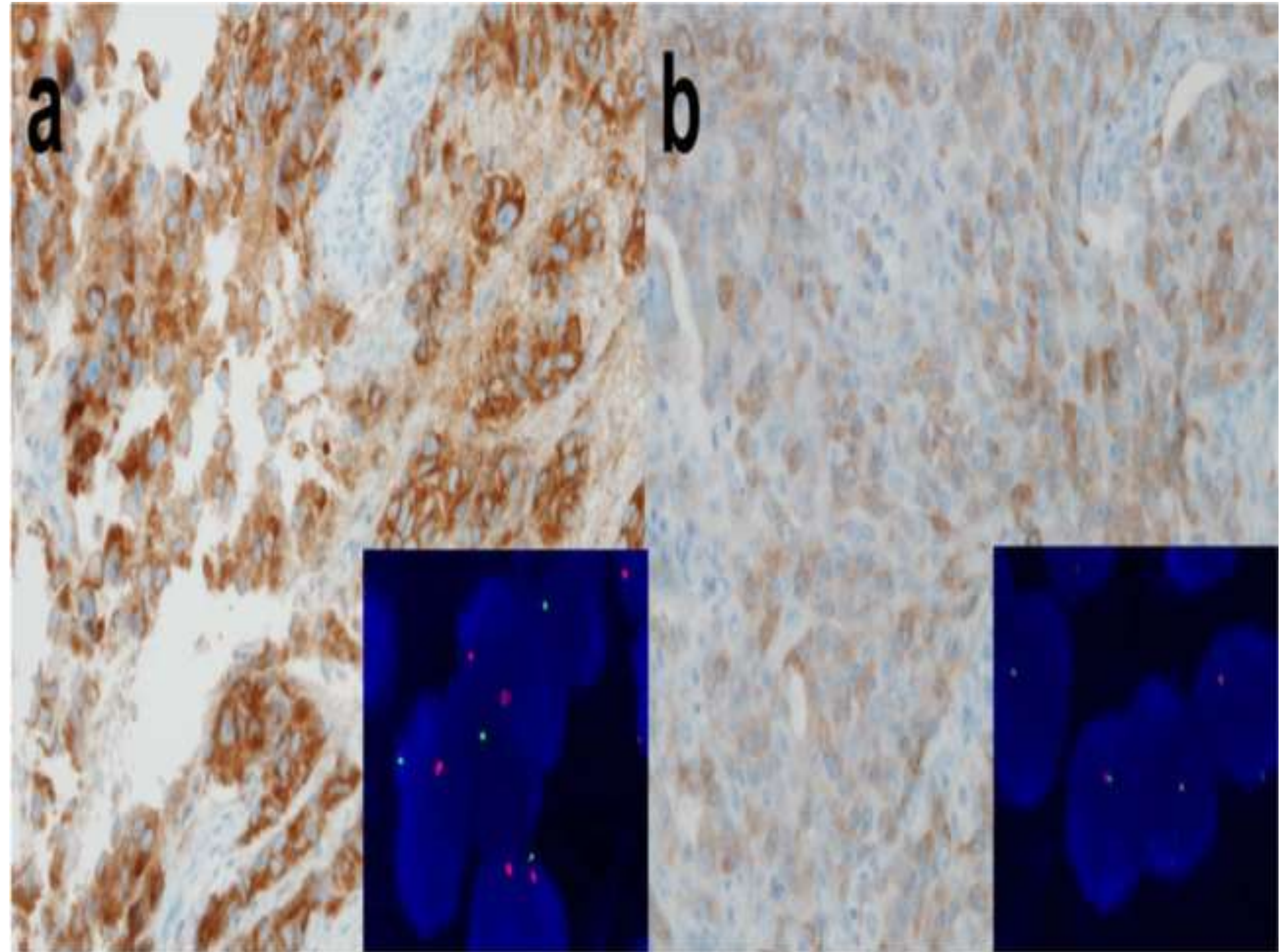
IHC results were scored by intensity (0/1/2/3) and extent (%) by 3 pathologists
Intensity= 0- no detectable staining,
1+ for weak reactivity mainly detectable at high magnification
2+ or 3+ for more intense (moderate or strong, respectively) reactivity easily detectable at low magnification
H – score (I*E)+(I*E)+(I*E) (score range 0-300)

		ROS1		ALK	
Cutoff		Sensitivity	Specificity	Sensitivity	Specificity
H-score	>0	100%	86.4%	100%	99.4%
	≥50	100%	94.3%	100%	99.7%
	≥100	**100%**	**97.8%**	**100%**	**100%**
	≥150	92.3%	98.4%	95.0%	100%
	≥200	84.6%	99.7%	75.0%	100%
	≥250	53.8%	100%	20.0%	100%
Extent	≥25%	100%	92.4%	100%	99.7%
	≥50%	100%	95.6%	100%	100%
	≥75%	**100%**	**96.8%**	**100%**	**100%**
Intensity	**≥2+**	**100%**	**95.0%**	**100%**	**99.4%**
	=3+	84.6%	98.4%	75%	100%

Cha et al. PLoS One. 2014 Jul 24;9(7)



FISH AND IHC FOR ROS1



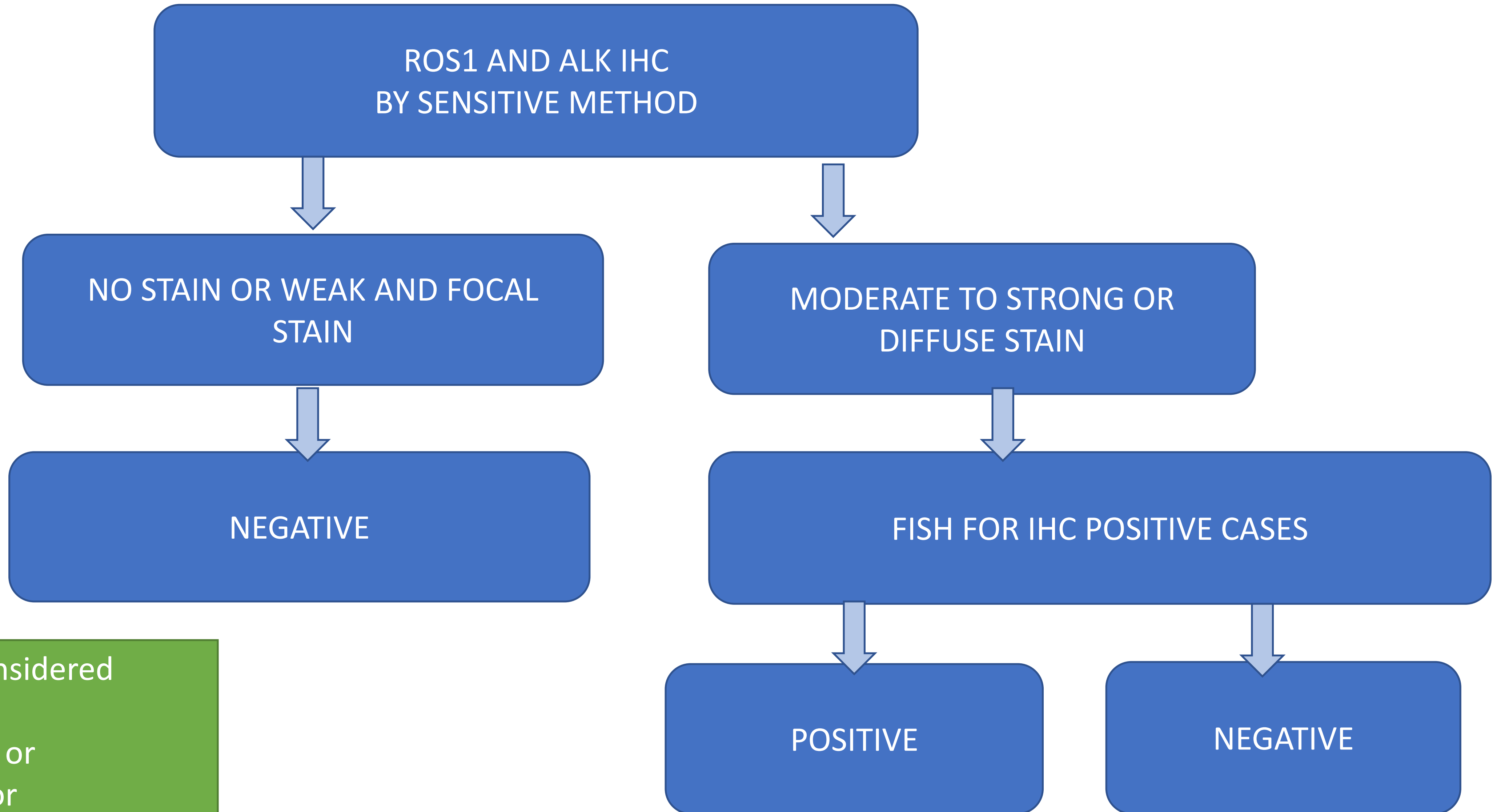
FISH AND IHC FOR ALK

Cha et al. PLoS One. 2014 Jul 24;9(7)

FISH VS IHC IN ROS -1 REARRANGEMENT

TECHNIQUE	FISH	IHC
SENSITIVITY	HIGHER	HIGHER
SPECIFICITY	HIGHER	LOWER
DETECT UNKNOWN/NOVEL FUSIONS	YES	YES
TECHNIQUE	CUMBERSOME	RELATIVELY EASY
FORMALIN FIXED TISSUE	YES	YES
SPECIFIC FUSION PARTNERS	NOT IDENTIFIED	NOT IDENTIFIED
INTRACHROMOSOMAL FUSIONS (eg FIG)	CAN BE MISSED	
TURNAROUND TIME	SLOW	RELATIVELY FAST
SMALL TISSUE	NO	YES

PROPOSED ALGORITHM



IHC – to be considered positive if
H-score ≥ 100, or
Extent ≥75%, or
Intensity - 2+ or 3+

Cha et al. PLoS One. 2014 Jul 24;9(7)

ROLE OF NGS IN ALK AND ROS-1

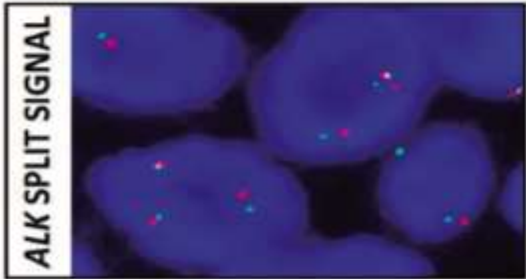
Retrospective cohort of NSCLC patients
N=42, ALK and ROS1 +ve by FISH

MEDIAN AGE – 60 YEARS
83% ADENO CARCINOMA

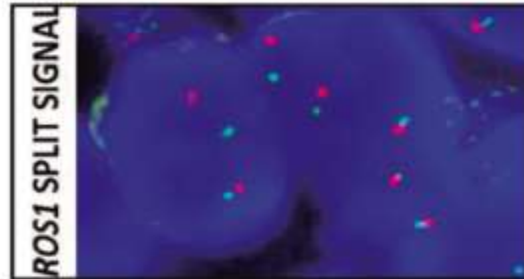
SUBJECTED TO IHC AND NGS

n= 34 *ALK*

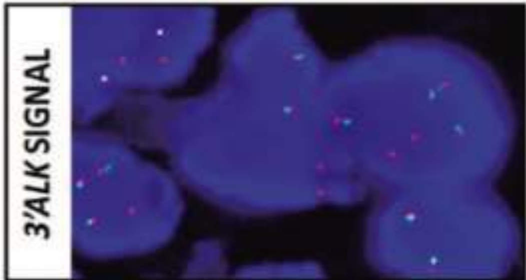
n= 8 *ROS1*



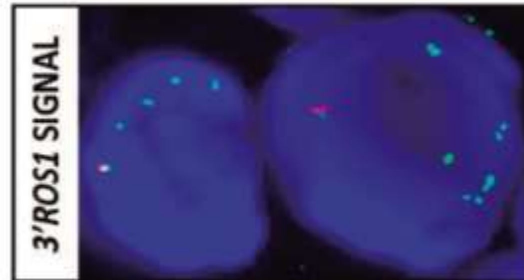
n= 15



n= 3

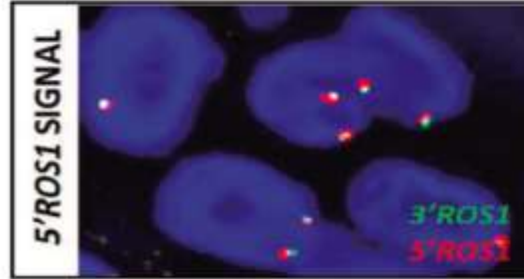


n= 17



n= 4

n= 2



n= 1

ISOLATED 5' - NEGATIVE

IHC \ FISH	SPLIT	3'	5'
POS	18	15	0
NEG	0	6	3

NGS \ FISH	SPLIT	3'	5'
POS	15	12	0
NEG	0	3	3
FAIL	3	6	0

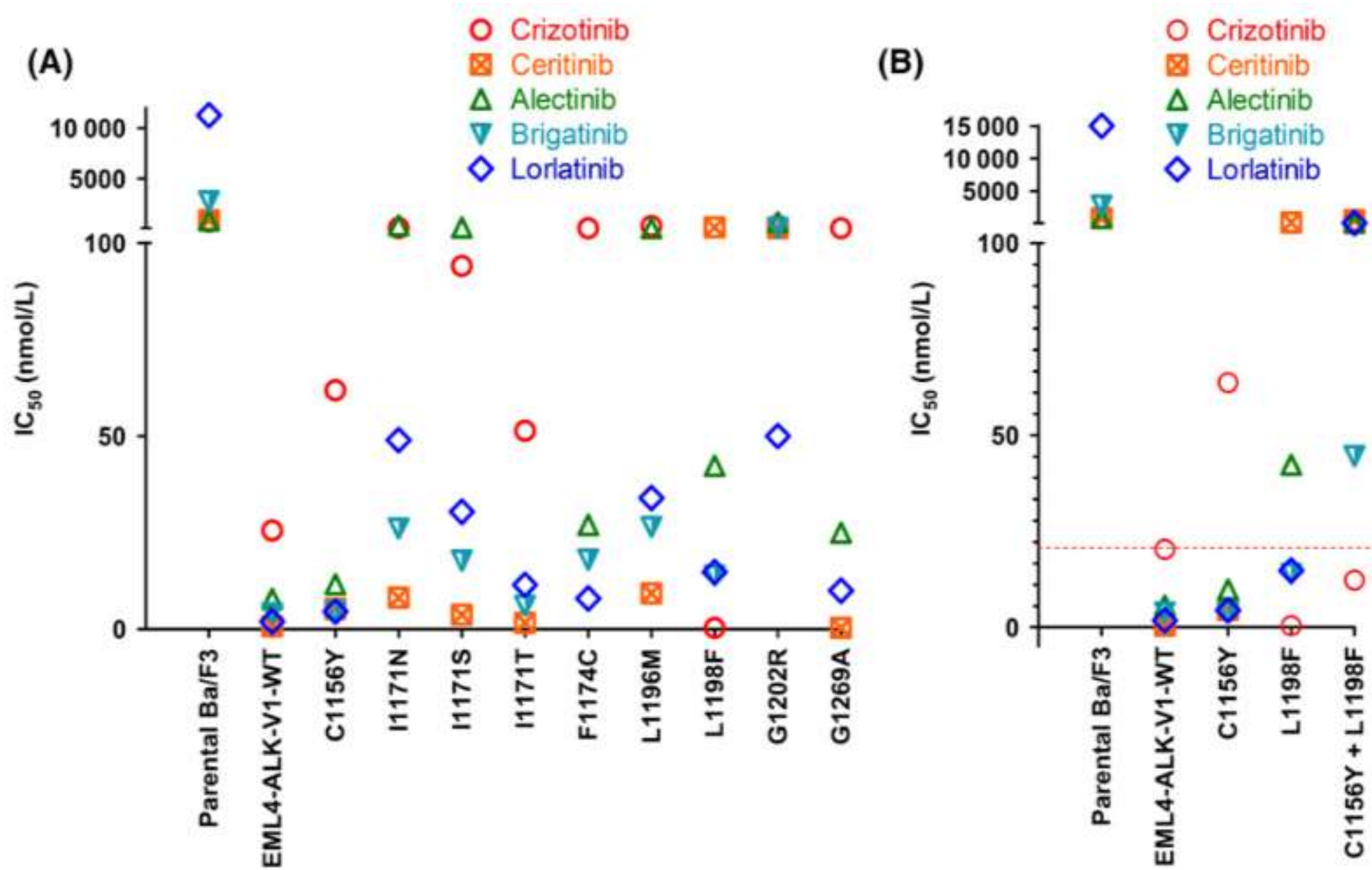
NGS \ IHC	POS	NEG
POS	27	0
NEG	0	6
FAIL	6	3

	LCC	1-2F1-2G *ROS1* FISH +	ROS1 IHC -	NGS -
CASE A		3'ROS1 5'ROS1		Analysis Results; Analysis Name; Total Mapped Fusion Panel Reads: 40502; ROS1 0.0 0

	ADC	1F1-2O *ALK* FISH +	ALK IHC -	NGS -
CASE B		3'ALK 5'ALK		Analysis Results; Analysis Name; Fusion Overall Call: NEGATIVE; Total Mapped Fusion Panel Reads: 43985

Torrus, Xavier. (2018). Detection of ALK and ROS1 Rearrangements Using Next Generation Sequencing in Lung Cancer: Comparison between FISH, IHC and NGS..

ALK RESISTANCE MUTATIONS



- SOME EVIDENCE OF BENEFIT IN TESTING ALK RESISTANCE PATTERN BEFORE CHANGING THERAPY
- NEED FURTHER STUDIES

Cancer Science. 2018;109:572–580

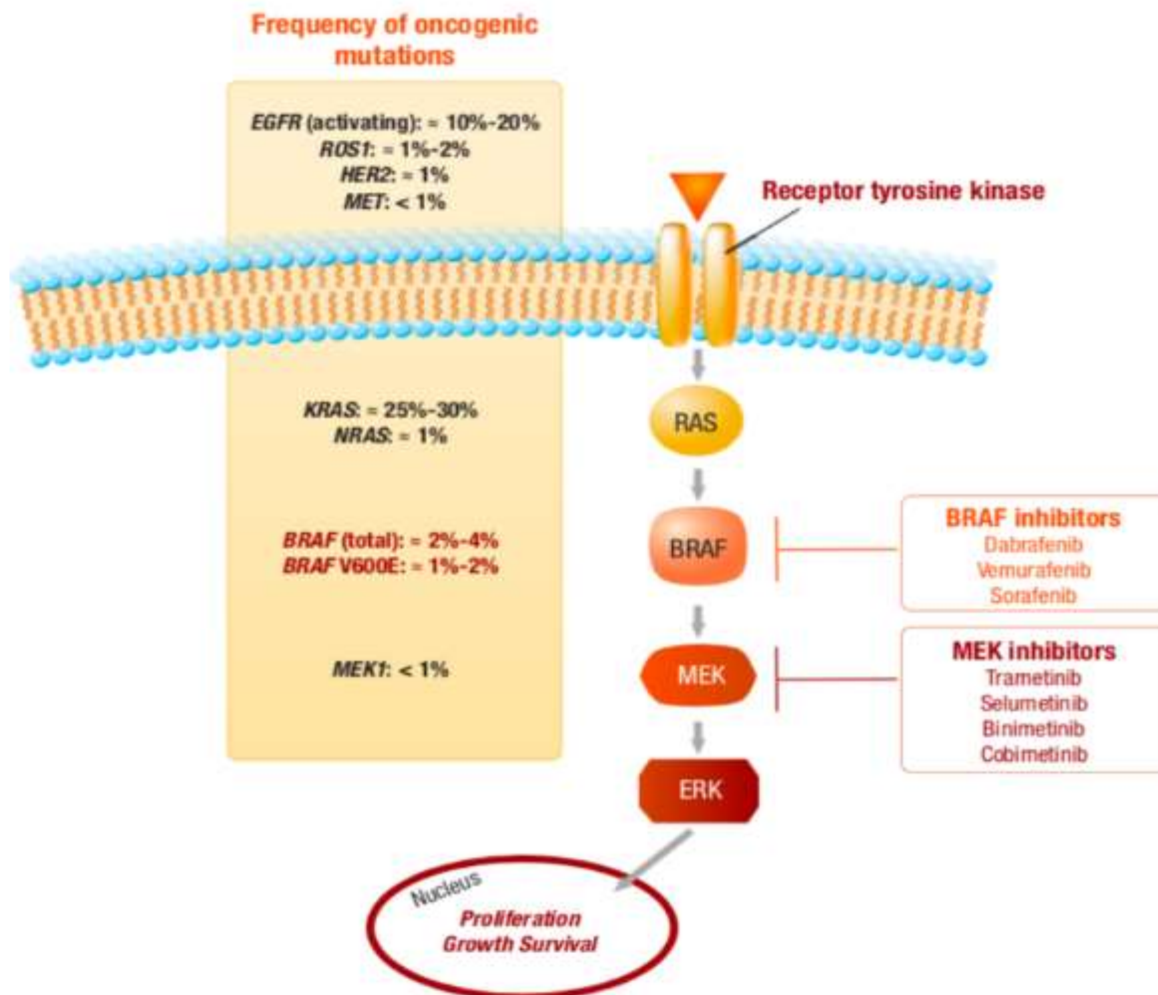
IHC VS FISH vs NGS IN ALK AND ROS-1 REARRANGEMENTS FINAL VERDICT

- FISH is considered the gold standard for both ALK and ROS -1
- NGS could be used as a screening test depending upon the initial tissue available
- NGS could be used to confirm discordant results between FISH and IHC

Torrus, Xavier. (2018). Detection of ALK and ROS1 Rearrangements Using Next Generation Sequencing in Lung Cancer: Comparison between FISH, IHC and NGS..

BRAF MUTATION

- *BRAF* is a proto-oncogene encoding a serine/threonine protein kinase which promotes cell proliferation and survival



- Most common - glutamate substitution for valine at codon 600 (V600E)
- Mutually exclusive from other known oncogenic driver mutations

TESTING METHODS

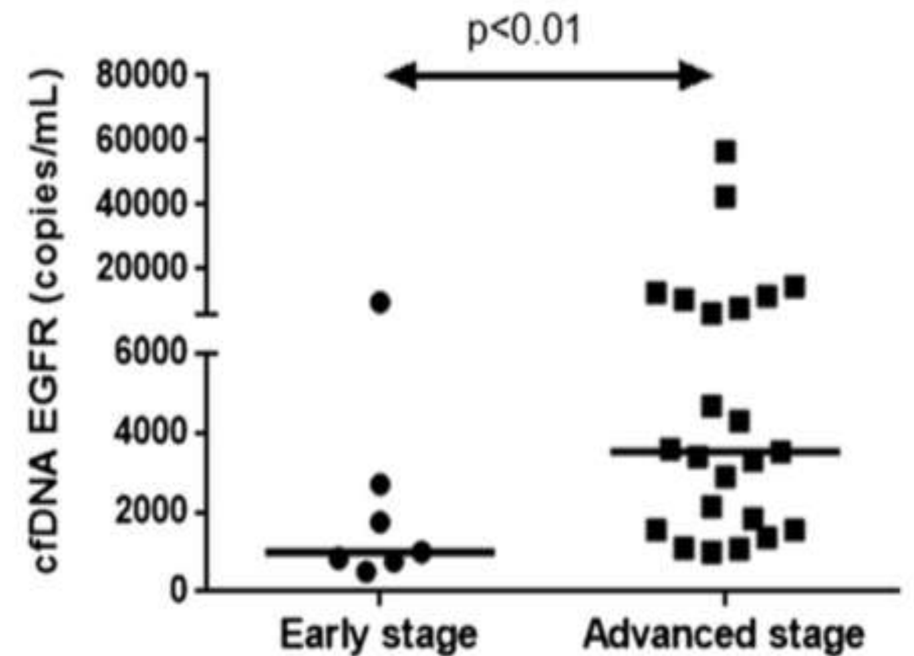
1. Direct sequencing
2. PCR
3. NGS

Baik et al. The Oncologist 2017;22:786–796

ROLE OF LIQUID BIOPSY IN EGFR MUTATION

n=450 , NSCLC

		Tumor samples				
		+	−	Total	Kappa index	Significance
L858R						
cfDNA	+	5 (22 %)	2 (9 %)	*7*	*0.679*	*P = 0.001*
	−	1 (4 %)	15 (6 %)	*16*		
	Total	*6*	*17*	*23*		
delE746-A750						
cfDNA	+	5 (22 %)	4 (17 %)	*9*	*0.310*	*P = 0.154*
	−	3 (17 %)	9 (43 %)	*12*		
	Total	*8*	*13*	*21*		

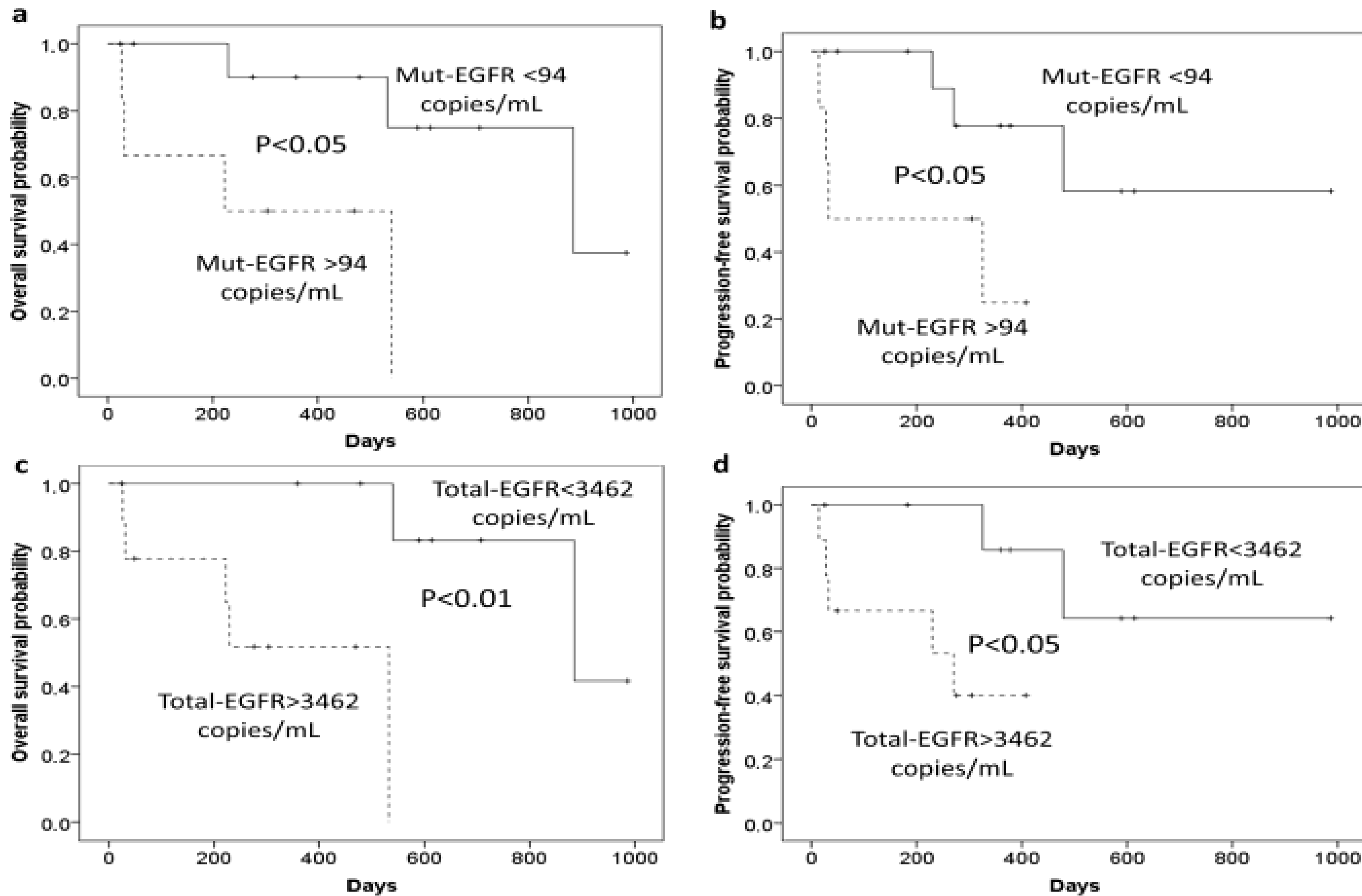


Concordance between L858R and delE746-A750 mutations analyzed in cfDNA by ddPCR and tumor samples from patients with advanced disease

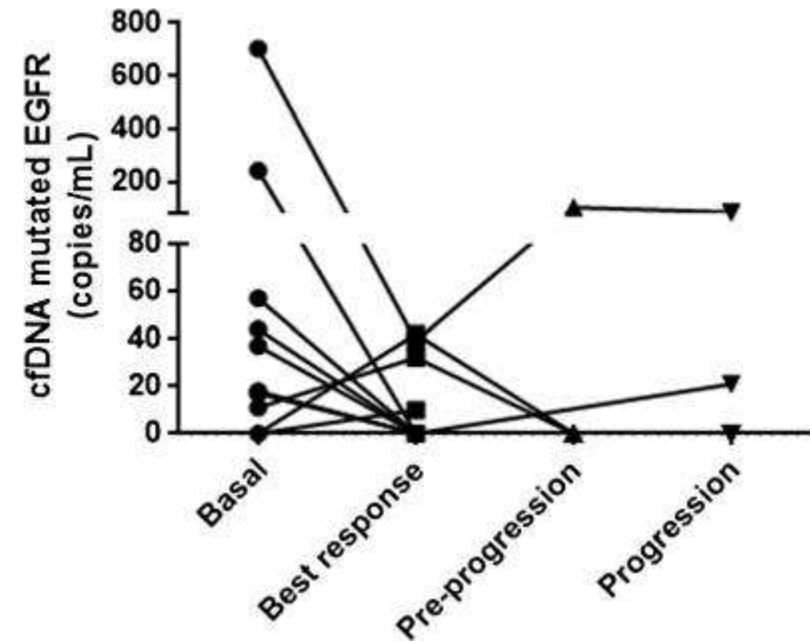
Circulating EGFR expression levels in patients with early and advanced NSCLCs

Alegre et al. Tumour Biol. 2016 Oct;37(10):13687-13694

ROLE OF LIQUID BIOPSY IN EGFR MUTATION



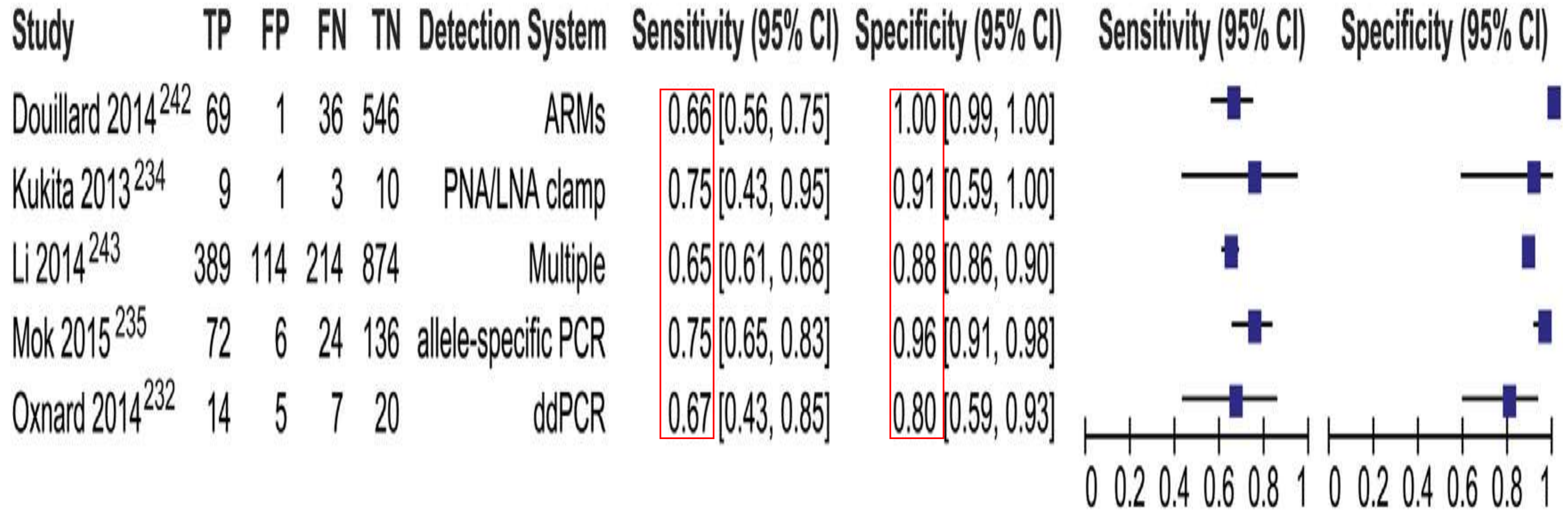
EGFR copy levels in cfDNA and survival



Longitudinal study of EGFR-mutated copy levels duri
treatment with EGFR-TKI

Alegre et al. Tumour Biol. 2016 Oct;37(10):13687-13694

T790M TESTING IN LIQUID BIOPSY



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 et al. Journal of Thoracic Oncology. 2018 (13): 323-358

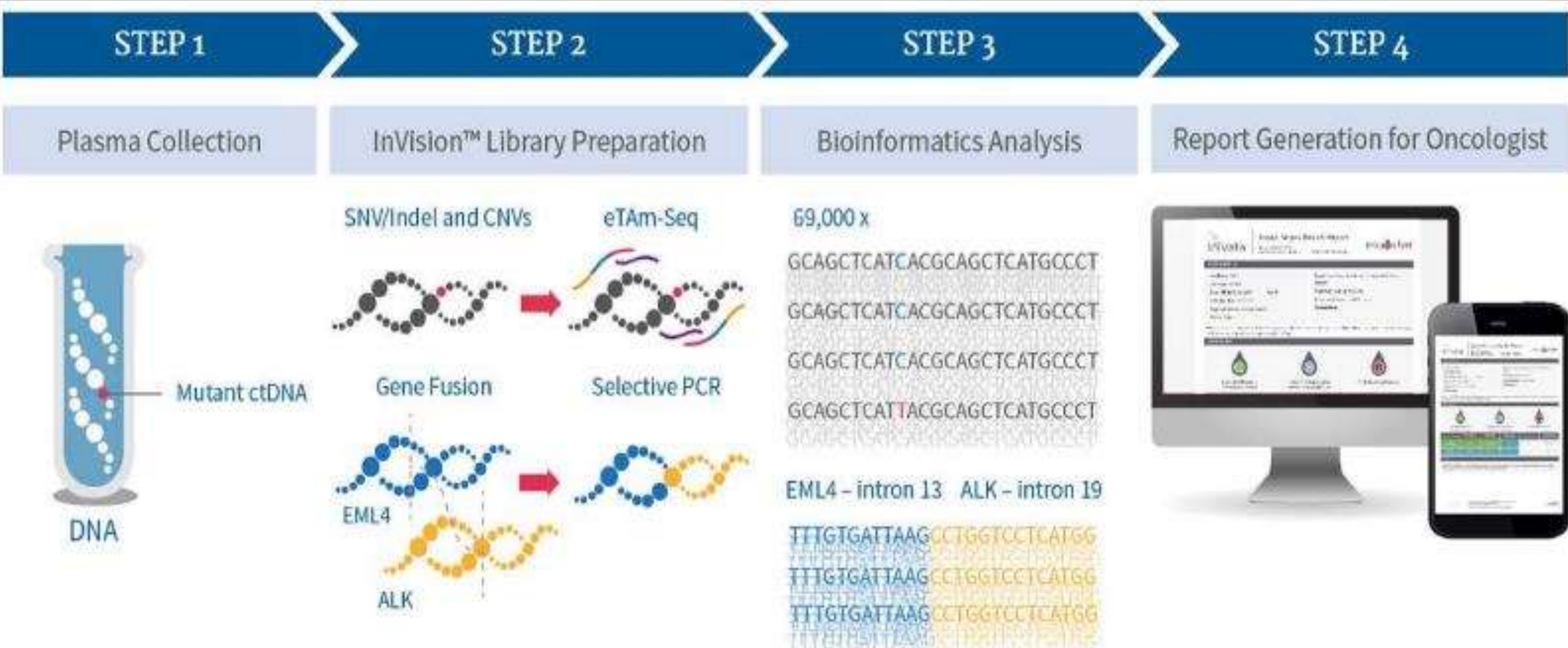
VERDICT ON LIQUID BIOPSY IN EGFR TESTING

- cfDNA can detect mutations not previously detected in tumor samples due to tumour heterogeneity
- T70M mutation status at baseline and follow up can also be detected by cfDNA which might be missed by tumour tissue analysis
- Levels of cfDNA at baseline and follow up have prognostic value
- EGFR mutation analysis in blood can be considered as a complementary test to tumor analysis, but not as subrogate

Alegre et al. Tumour Biol. 2016 Oct;37(10):13687-13694

ROLE OF LIQUID BIOPSY IN ALK AND ROS-1 REARRANGEMENTS

- Across 6 international centers
- Jan. 2015 to Aug. 2017
- ALK and ROS1 positive status was determined by standard of care practice at each institution (FISH/IHC or NGS)
- Analysis of EML4-ALK fusions and ROS1 fusions was performed using the InVision® platform



	Characteristics	Overall population (n=66, %)
Age	▪ Median (range)	57 (22-93)
Sex	▪ Male ▪ Female	29 (44%) 37 (56%)
Stage at diagnosis	▪ I-II / III ▪ IV	2 (3%) / 7 (11%) 56 (85%)
Histology	▪ Adenocarcinoma ▪ Squamous ▪ Other	63 (95%) 1 (2%) 1 (2%)
Smoking	▪ Non smoker ▪ Former smoker ▪ Current smoker	40 (61%) 18 (27%) 7 (11%)
Molecular diagnosis	▪ Immunohistochemistry ▪ FISH ▪ Other (NGS)	33 (50%) 45 (68%) 2 (3%)
Type of rearrangement	▪ *ALK* fusion ▪ *ROS1* fusion	59 (89%) 7 (11%)
Therapy at collection	▪ Treatment naïve ▪ 1st generation TKI ▪ 2nd generation TKI ▪ Next generation TKI ▪ Others	20 (30%) 11 (17%) 11 (17%) 18 (27%) 6 (9%)

Epstein M, et al. PLoS ONE (2018) 13(3): e0193802

ROLE OF LIQUID BIOPSY IN ALK AND ROS-1 REARRANGEMENTS

	ctDNA Positive *ALK* fusion N (%)
At diagnosis or treatment-*naïve* (n=21)	15 (71%)
During ongoing *response* (n=57)	7 (12%)
At *PD* (n=15)	8 (53%)

	ctDNA Positive *ROS1* fusion (N, %)
At diagnosis or treatment-*naïve* (n=6)	6 (100%)
During ongoing *response* (n=0)	0 (0%)
At *PD* (n=1)	1 (100%)

The detection of *ALK and ROS1* fusion in plasma is feasible in routine clinical practice, with good sensitivity for clinically actionable *ALK* and *ROS1* structural rearrangements in untreated advanced NSCLC patients

Sensitivity for ALK was 71% and 100% for ROS1 in treatment-naïve patients

Epstein M, et al. PLoS ONE (2018) 13(3): e0193802

ROLE OF LIQUID BIOPSY IN ALK AND ROS-1 REARRANGEMENTS

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NGS ON LIQUID BIOPSY

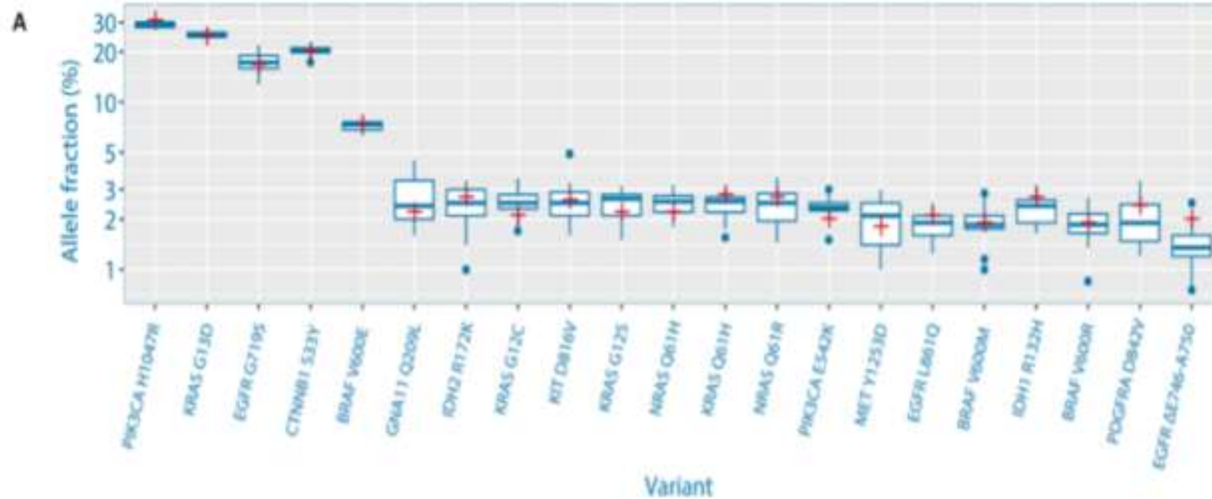
	Laboratory 1			Laboratory 2		
AF (%)*	Sensitivity (%)	90% CI (Lower)	90% CI (Upper)	Sensitivity (%)	90% CI (Lower)	90% CI (Upper)
1%-1.3%	99.17	97.40	99.85	100.00	98.96	100.00
0.5%-0.65%	99.63	98.26	99.98	97.66	95.43	98.97
0.25%-0.33%	89.17	85.29	92.30	90.28	86.91	93.00
0.13%-0.16%	69.26	64.31	73.89	67.71	62.88	72.26
0.06%-0.08%	37.41	32.50	42.52	30.86	26.10	35.95

In Vision NGS panel for liquid biopsy

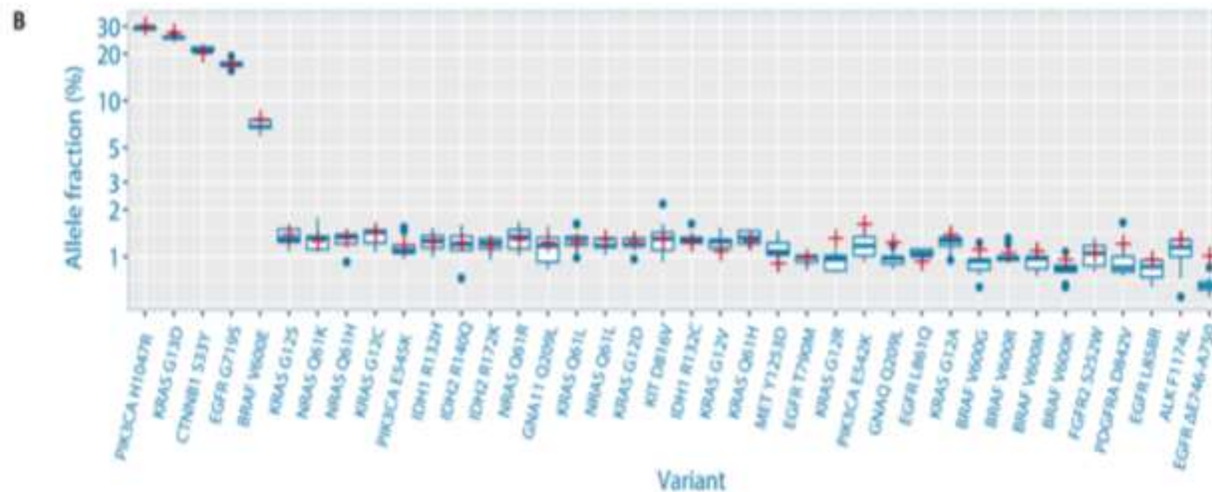
Enhanced version of TAm-Seq technology using multiplex PCR

Gale D et al. PLoS One. 2018 Mar 16;13(3):e0194630

NGS ON LIQUID BIOPSY



NGS VS ddPCR
Good correlation



It is possible to monitor tumor dynamics and assess evolution in plasma by analysis of multiple mutations in parallel across serially-collected samples rather than focusing on single hotspot mutations.

Gale D et al. PLoS One. 2018 Mar 16;13(3):e0194630

ctDNA EXTRACTION AND ANALYSIS REQUIREMENTS

- Ethylenediaminetetra-acetic acid (EDTA) tubes for plasma extraction or the use of preservative tubes designed specifically for cell-free DNA isolation
- The suggested maximum time from blood withdrawal to plasma extraction is 2 hours for EDTA tubes and 3 days for preservative tubes.
- 20 ml blood (no standard volume)
- Methods : qPCR (Cobas – FDA Approved), ddPCR, BEAMing (beads, emulsions, amplification and magnetics), NGS (Illumina /Ion- Torrent)

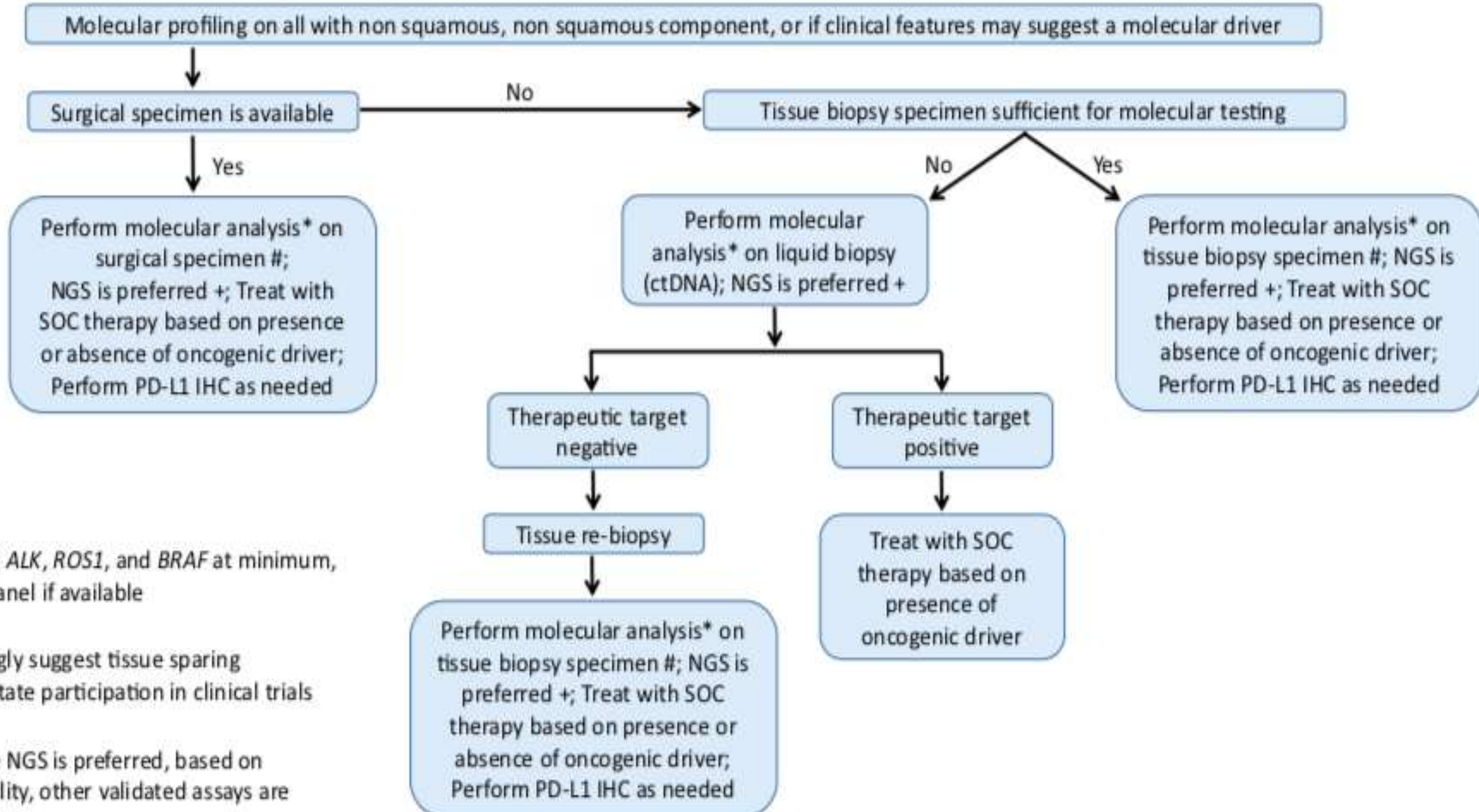
Rolfo, Christian et al. Journal of Thoracic Oncology. 2018; 13(9) ; 1248 -

IASLC RECOMMENDATIONS ON LIQUID BIOPSY IN MOLECULAR TESTING

- ddPCR could be considered for the detection of sensitizing EGFR mutations and a positive result should be sufficient for initiating therapy targeting these alter- ations; however, a negative result should prompt further evaluation with either a NGS-based test using ctDNA or using DNA from a tumor biopsy
- PCR-based methods should not be routinely used for ALK or ROS1 rearrangement detection from ctDNA.
- NGS platforms are reliable and preferred over PCR
- A negative result from NGS for oncogenic driver alterations is not sufficient to exclude therapy and requires a confirmation from tumor biopsy

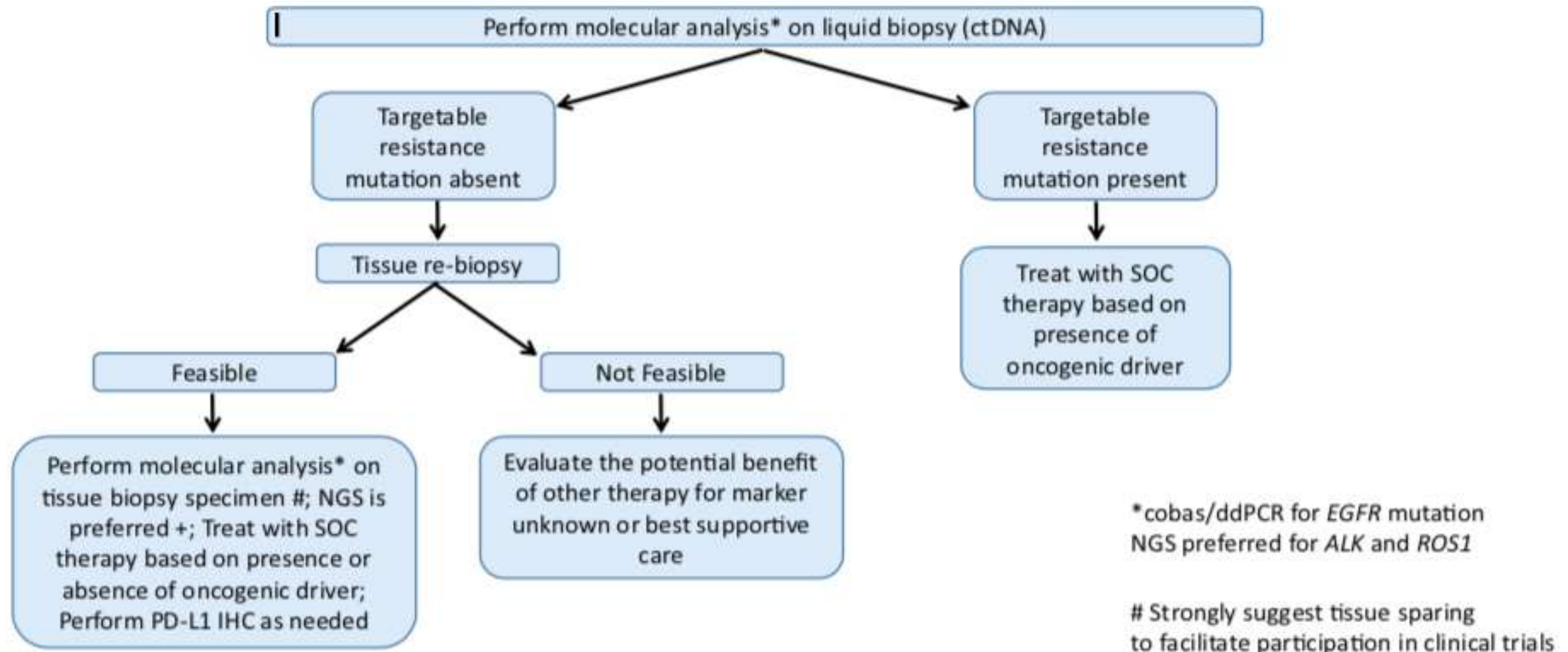
Rolfo, Christian et al. Journal of Thoracic Oncology. 2018; 13(9) ; 1248 -

IASLC RECOMMENDATIONS ON LIQUID BIOPSY IN MOLECULAR TESTING



Rolfo, Christian et al. Journal of Thoracic Oncology. 2018; 13(9) ; 1248 -

IASLC RECOMMENDATIONS ON LIQUID BIOPSY IN MOLECULAR TESTING



Rolfo, Christian et al. Journal of Thoracic Oncology. 2018; 13(9) ; 1248 - 68

PCR VS NGS IN LIQUID BIOPSY

LIQUID BIOPSY METHOD	PCR	NGS
TURNAROUND TIME	2-3 days	13 days
MULTIPLE MUTATIONS SIMULTANEOUS DETECTION	NO	YES
TUMOUR SUPPRESSOR GENES	NO	YES
MUTANT DNA QUANTIFICATION	NO	YES
COST	LOWER	HIGHER

Rolfo, Christian et al. Journal of Thoracic Oncology. 2018; 13(9) ; 1248 -

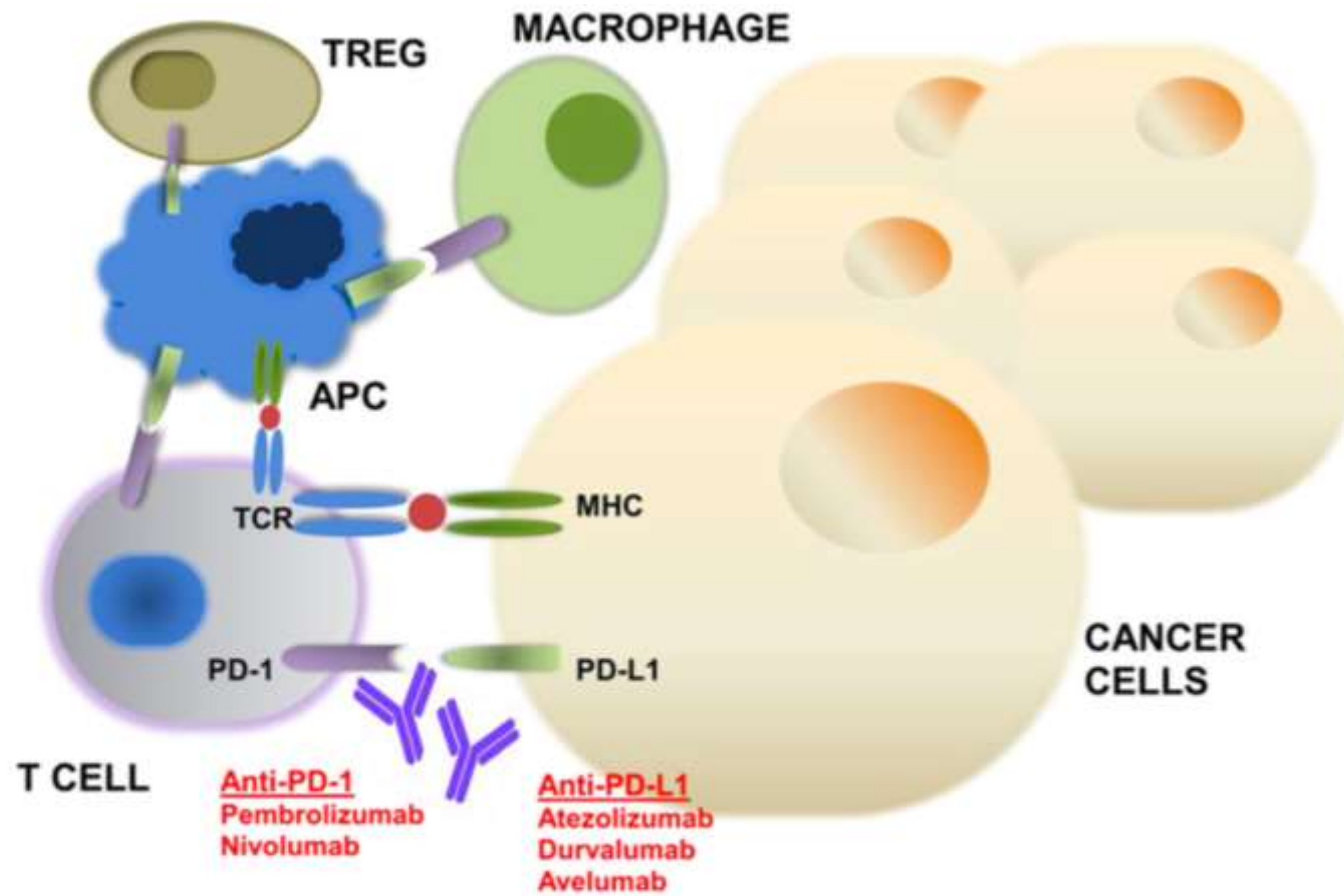
	ASCO (2018)	ESMO (2018)	NCCN (2019)
TISSUE	Cell blocks or smear	Cytology or smear	Biopsy or cytology
IHC for Adeno vs Squamous	No recommendation	Limited IHC panel	Limited IHC panel can be used if Morphology inconclusive
TYPE OF CANCER TO BE TESTED	1. An adenocarcinoma component; 2. Nonsquamous, non–small-cell histology 3. Any non–small-cell histology when clinical features indicate a higher probability of an oncogenic driver (eg, young age [< 50 years]; light or absent tobacco exposure)	Same	Same
MOLECULAR ASSAY USED	Should be able to detect in tissue with less than 20 % cancer cells	No recommendation	No recommendation
SITE OF TISSUE	Primary or Metastatic	Same	No recommendation

	ASCO (2018)	ESMO(2018)	NCCN(2019)
EGFR	All advanced adenocarcinoma to be tested	Same	1. RT PCR 2. Sangers technique with tumour enrichment 3. NGS
	IHC and FISH not recommended		
	Post progression, T790M to be tested using assays able to detect mutation in < 5% viable cells		Post progression,T790M to be tested using technique with analytic sensitivity of a minimum of 5% allelic fraction If T790M –ve, Other methods of resistance to be tested (MET amplification/HER2 amplification)

	ASCO (2018)	ESMO	NCCN
ROS1	All advanced adeno carcinoma to tested	Same	Same
	IHC - screening test in patients with advanced lung adenocarcinoma Positive ROS1 IHC results should be confirmed by a molecular or cytogenetic method	Same	Same RT PCR and NGS not advised as they might miss novel fusions
BRAF	All advanced lung adenocarcinoma to be tested	Same	Same Techniques: 1. RT PCR 2. Sangers technique 3. NGS
ALK	Routine testing advised in all adenocarcinoma	Same	Same
	IHC is an equivalent alternative to FISH	Same	• D5F3 IHC can be used to replace FISH, still FISH confirmation encouraged • RT PCR and NGS not advised as they might miss novel fusions
	Post progression on ALK TKI- Routine testing of ALK mutational status not recommended	May soon be recommended	Unclear

	ASCO (2018)	ESMO(2018)	NCCN (2019)
PDL1 testing	No recommendation	All advanced NSCLC	Metastatic NSCLC with negative EGFR, ALK, ROS and BRAF genetic variations
	No recommendation	• IHC 22C3 assay • 28-8 and SP263 IHC clones may be alternative tests	IHC advised
NGS	No statement	• Preferred over other methods if available	NGS may not detect all genetic alterations

PD1 AND PDL1 INHIBITORS



Gong et al. Journal for ImmunoTherapy of Cancer (2018) 6:8

TRIAL	DRUG	INDICATION	IHC CLONE USED	STAINING PLATFORM USED
KEYNOTE-001	pembrolizumab	PD-L1 positive (≥1%) progressing after platinum-based therapy (n=550)	22C3	DAKO
KEYNOTE-024	Pembrolizumab	Metastatic NSCLC with ≥50% PD-L1 expression (n = 305)	22C3	DAKO
KEYNOTE-021	Pembrolizumab	First line (in combination with platinum-doublet chemotherapy) n=123	22C3	DAKO
CheckMate 017	Nivolumab	Previously treated metastatic squamous NSCLC with platinum-based chemo n=272	28-8	DAKO
CheckMate 057	Nivolumab	Previously treated metastatic Non squamous NSCLC with platinum-based chemo n=582	28-8	DAKO
POPLAR	Atezolizumab	Second-line	SP142	VENTANA
PACIFIC	Durvalumab	Maintenance post concurrent chemo radiotherapy in locally advanced NSCLC	SP263	VENTANA

Sholl et al. Arch Pathol Lab Med—Vol 140, April 2016

Gong et al. Journal for ImmunoTherapy of Cancer (2018) 6:8

PDL1 TESTING BY IHC

STAINING PLATFORMS

IHC CLONES	Dako			Ventana		Leica	
	Center 1	Center 2	Center 3	Center 4	Center 5	Center 6	Center 7
28-8	*Ref.*	*0.94*	*0.79*	0.8	0.73	0.6	0.58
22C3	*Ref.*	*0.91*	*0.82*	0.81	0.77	0.5	0.62
SP263	0.83	0.83	0.86	*0.81*	*Ref.*	0.83	0.86
SP142	0.68	0.38	0.61	0.43	0.45	0.78	0.81
E1L3N	0.63	0.65	0.77	0.6	0.81	0.75	0.78

COMPARE PDL1 TESTING BY VARIOUS IHC CLONES AND STAINING PLATFORMS
7 CENTRES
n=41

ORANGE BOXES- STANDARDIZED KITS
REST OF THE BOXES – LABARATORY DEVELOPED KITS
GREEN BOXES – CONCORDANT RESULTS (Kappa value≥ 0.75)
RED BOXES – INSUFFICIENT CONCORDANCE (Kappa value < 0.75)

Adam et al. Annals of Oncology 29: 953–958, 2018

	ASCO (2018)	ESMO	NCCN
KRAS, RET, MET, ERBB2(HER2)	No routine testing	Same	KRAS can be tested as a prognostic marker at baseline
cfDNA Primary diagnosis	No role in primary molecular testing, But can be used when tissue is limited or insufficient	No recommendation	If a patient is medically unfit for invasive tissue sampling or tissue inadequate
cfDNA Post Progression	May be used to detect T790M after EGFR TKI progression (but no recommendation)	Same but negative test to be confirmed by tissue testing	Liquid biopsy can be used but negative results to be confirmed by tissue testing
Multiplexed genetic sequencing panels	Preferred	NGS preferred	NGS preferred

TAKE HOME MESSAGE

ADVANCED NSCLC WITH NON SQUAMOUS COMPONENT

TISSUE AVAILABLE – TISSUE BIOPSY/CYTOLOGY
TISSUE UNAVAILABLE – LIQUID BIOPSY

NGS AVAILABLE

NO

TEST FOR EGFR MUTATIONS BY ddPCR
ALK FUSION BY IHC(D5F3 CLONE)
ROS-1 FUSION BY IHC
BRAF BY ddPCR
PDL1 TESTING BY IHC FROM TISSUE

EGFR SENSITIVE MUTATION / BRAF MUTATION DETECTED – START THERAPY
ALK FUSION DETECTED BY D5F3 NEED NOT BE CONFIRMED- START THERAPY
ROS-1 FUSION DETECTED BY IHC NEED TO BE CONFIRMED BY FISH AND START THERAPY

YES

TARGETED NGS PANEL

TARGETED THERAPY

ADVANCED NSCLC - SQUAMOUS

PDL1 TESTING BY VALIDATED IHC PLATFORM FROM TISSUE