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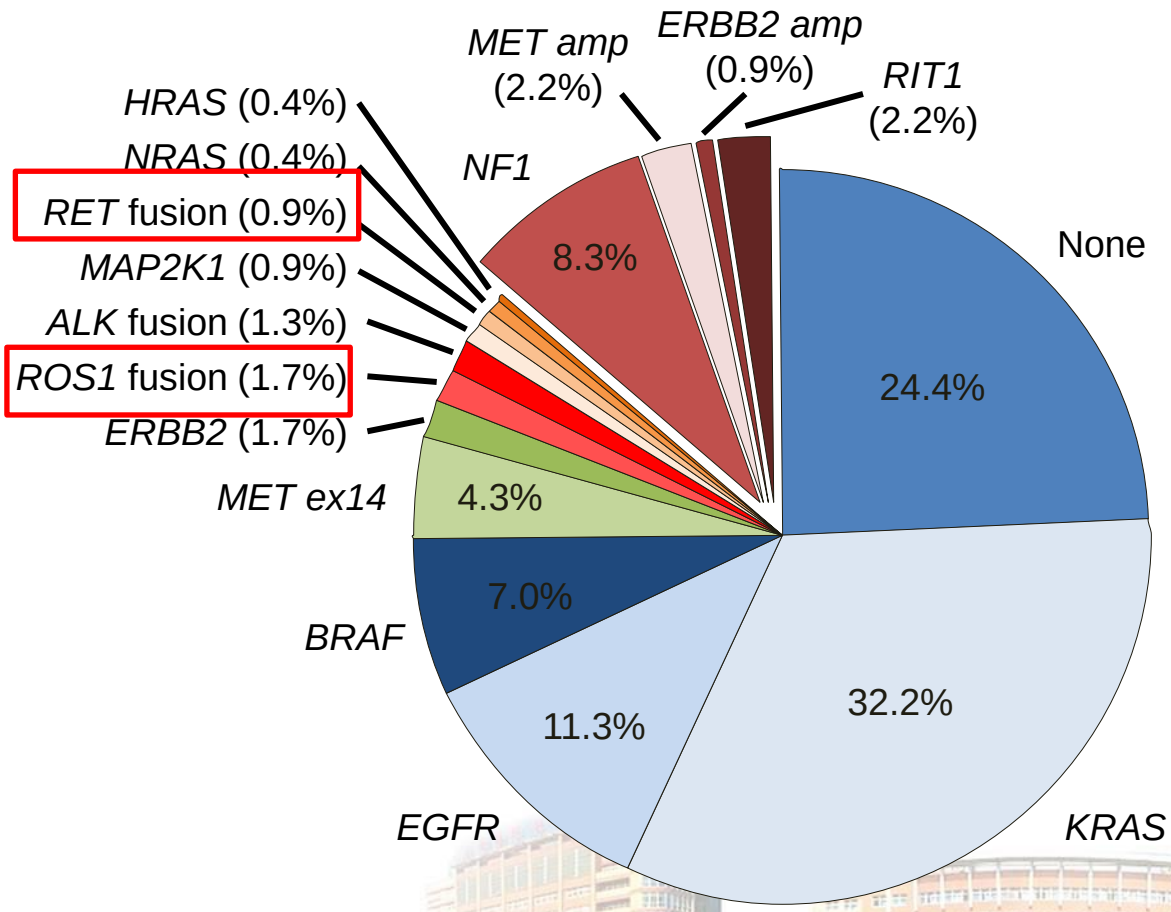
Biomarker testing beyond *EGFR* and *ALK*: Expanding the list of tests

Caicun Zhou
Shanghai Pulmonary Hospital,
Shanghai Tongji University, P.R.China

NSCLC Adenocarcinoma: Beyond *EGFR* Mutations and *ALK* Translocation



Govindan R. ISLAC 2013. Abstract PL05.1.

EMERGING TARGETED AGENTS FOR PATIENTS WITH GENETIC ALTERATIONS

Genetic Alteration (ie, Driver event)	Available Targeted Agents with Activity Against Driver Event in Lung Cancer
BRAF* V600E mutation	vemurafenib[1] dabrafenib[2]
***MET* amplification**	crizotinib[3,4]
***ROS1* rearrangements**	crizotinib[5]
***HER2* mutations**	trastuzumab[6] (category 2B) afatinib[7] (category 2B)
***RET* rearrangements**	cabozantinib[8] (category 2B)

*Non-V600E mutations have variable kinase activity and response to these agents.

[1]Gautschi O, Pauli C, Strobel K, et al. A patient with BRAF V600E lung adenocarcinoma responding to vemurafenib. J Thorac Oncol 2012;7:e23-24.
[2]Planchard D, Mazieres J, Riely GJ, et al. Interim results of phase II study BRF113928 of dabrafenib in BRAF V600E mutation-positive non-small cell lung cancer (NSCLC) patients [abstract]. J Clin Oncol 2013;31(Suppl 15): Abstract 8009.
[3]Ou SH, Kwak EL, Siwak-Tapp C, et al. Activity of crizotinib (PF02341066), a dual mesenchymal-epithelial transition (MET) and anaplastic lymphoma kinase (ALK) inhibitor, in a non-small cell lung cancer patient with de novo MET amplification. J Thorac Oncol 2011;6:942-946.
[4]Camidge RD, Ou S-HI, Shapiro G, et al. Efficacy and safety of crizotinib in patients with advanced c-MET-amplified non-small cell lung cancer. J Clin Oncol 2014;32(Suppl 5): Abstract 8001.
[5]Shaw AT, Ou S-HI, Bang Y-J, et al. Crizotinib in *ROS1*-rearranged non-small cell lung cancer. N Engl J Med 2014;371:1963-1971.
[6]Cappuzzo F, Bemis L, Varella-Garcia M. HER2 mutation and response to trastuzumab therapy in non-small-cell lung cancer. N Engl J Med 2006;354:2619-2621.
[7]Mazieres J, Peters S, Lepage B, et al. Lung cancer that harbors an HER2 mutation: epidemiologic characteristics and therapeutic perspectives. J Clin Oncol 2013;31:1997-2003.
[8]Drilon A, Wang L, Hasanovic A, et al. Response to cabozantinib in patients with RET fusion-positive lung adenocarcinomas. Cancer Discov 2013; 3:630-635.

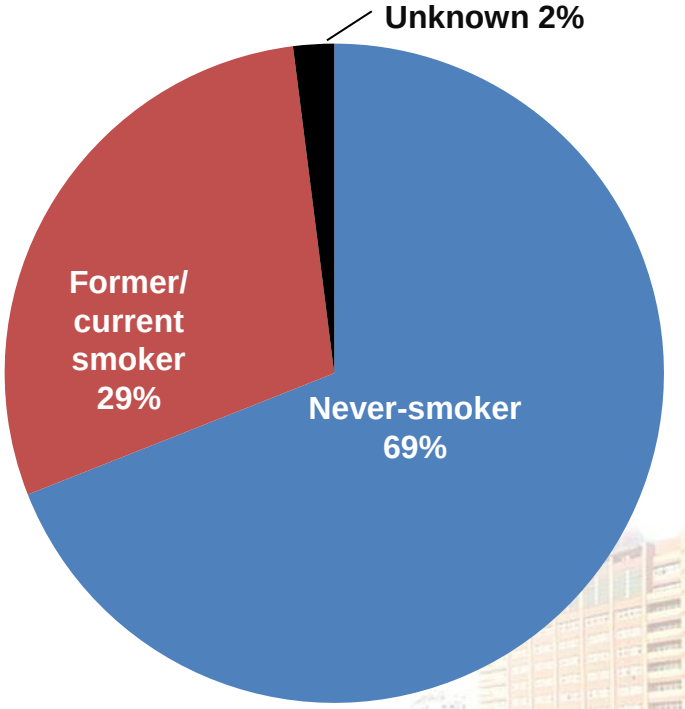
Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.

Test of biomarkers

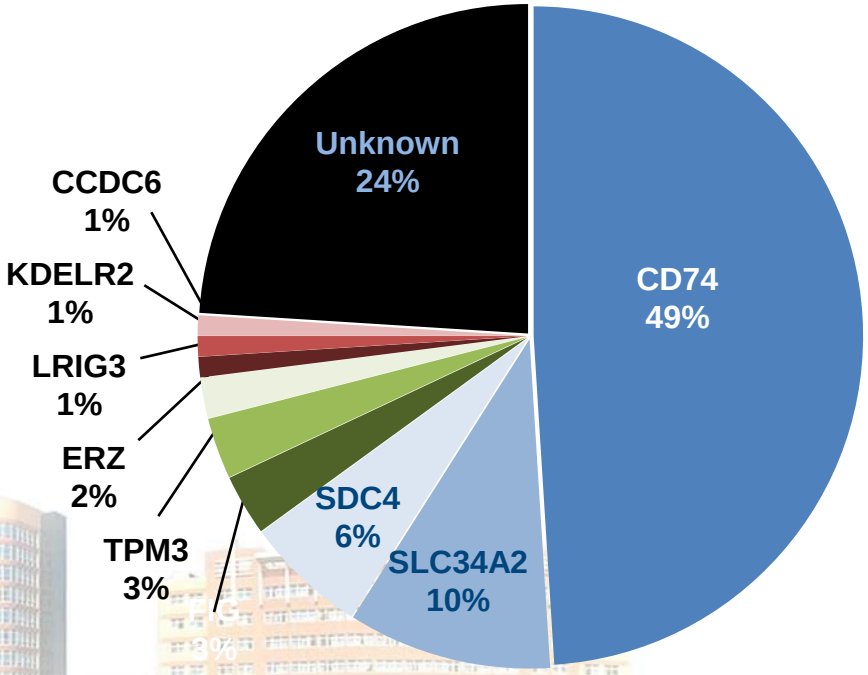
Methods	Advantage	Disadvantage
IHC	Rapid turnaround, effective, widespread availability, low cost	Low sensitivity, no provision of information of specific rearrangements
FISH	Formalin-fixed, paraffin-embedded tissue, detection of novel rearrangements, not uniformly available	No provision of information of specific rearrangements, expensive
(RT)PCR	Rapid turnaround time, limited tissue requirements, identification of specific fusion partners,	Specific primers required, no detection of novel fusion, high quality of RNA
NGS	Highly sensitive, not widespread availability	Not available in many centers, expensive

- *ROS1* rearrangement: 1.7%
- *ROS1* rearrangement mainly in young, non-smoking patients
- *ROS1 rearrangement* with adenocarcinoma of higher histological grade
- No OS difference between the *ROS1* positive and negative patients

Smoking status in NSCLC patients with *ROS1* rearrangements **(N=58)**



Fusion partners of *ROS1* rearrangement in NSCLC **(N=62)**



Ou S-HI. J Clin Pathol 2013; May 9 [Epub ahead of print]
Bergethon K, et al. J Clin Oncol. 2012 Mar 10;30(8):863-70.

Efficacy of Crizotinib in Patients with *ROS1*-rearrangement

Study	N	ORR	DCR
PROFILE 1001	42	51%	81%
EURROS1	28	77%	88%

ROS1 fusion testing

- Multiple studies investigated the incidence of the oncogenic fusion using a variety of techniques, including FISH, IHC, NGS of RNA and DNA, and polymerase chain reaction.

- No approved companion diagnostic yet available for this oncogene.

Morgensztern D, et al. J Thorac Oncol. 2015; 10(1 Suppl 1): S1-63.

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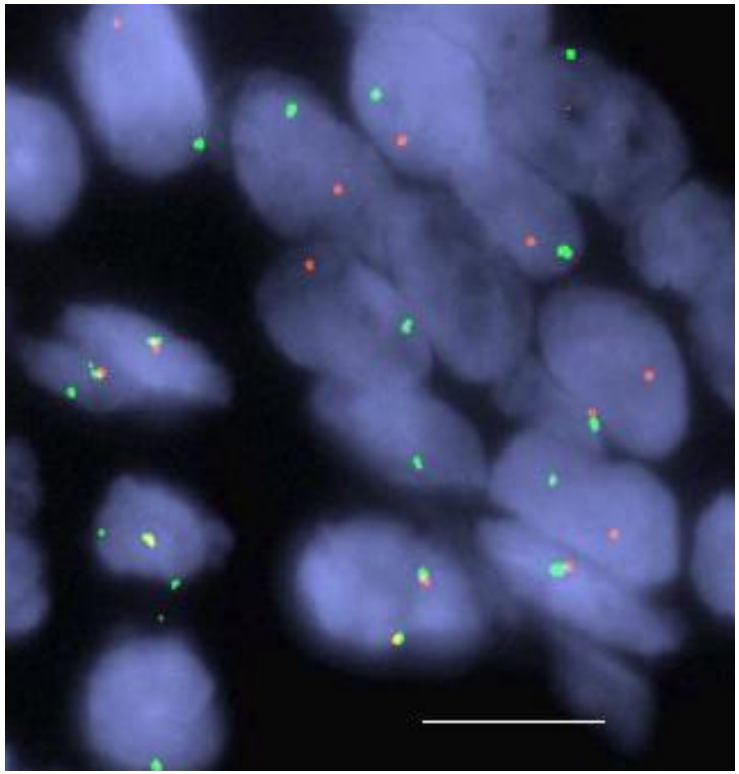
FISH Positive Definition in *ROS1*-rearranged

- A break-apart pattern with one fusion signal and two separated green and orange signals. Only signals that were more than one signal diameter apart from each other were counted as breaks. Another was an isolated 3’ green signal pattern.

- A case was considered positive for rearrangement if >15% of cells showed split signals or single green signals.



Lee SE et al. *Mod Pathol* 2015; 28: 468-79.

Comparison of methods in the detection of *ALK* and *ROS1* rearrangements in lung cancer

	ROS1 FISH+	*ROS1* FISH-	*ROS1* FISH Atypical	*ROS1* FISH No results
ROS1 dual-color CISH positive	3	2	0	0
ROS1 dual-color CISH negative	0	287	0	8
ROS1 dual-color CISH atypical	0	7	1	1
ROS1 dual-color CISH no result	0	17	0	36

The *ROS1* rearrangement status had a 97% (291 of 300) concordance between CISH and FISH.
1/3 samples with a *ROS1* rearrangement by FISH showed *ROS1* protein expression (33.3% sensitivity).

Rogers TM, Russel P, Wright G, et al. JTO 2015;10:611-8

ROS1 Immunohistochemistry

★ Intensity: 0 for absent expression
1^+ for weak staining
2^+ for moderate staining
3^+ for strong staining

★ H-score= (0× percentage of cells with absent cytoplasmic staining)+ (1x %1^+ cells) + (2 x % 2^+ cells) + (3 x % 3^+ cells). Scored only if ≥20 tumor cells present.

When the cutoff of *ROS1* H score is set at 100 to 130, a perfect correlation between IHC and RT-PCR results observed!

Sensitivity 100%

Specificity 100%

Boyle TA, Masgo K, Ellison KE, et al.
Clin Lung Cancer. 2015;16(2):106-11

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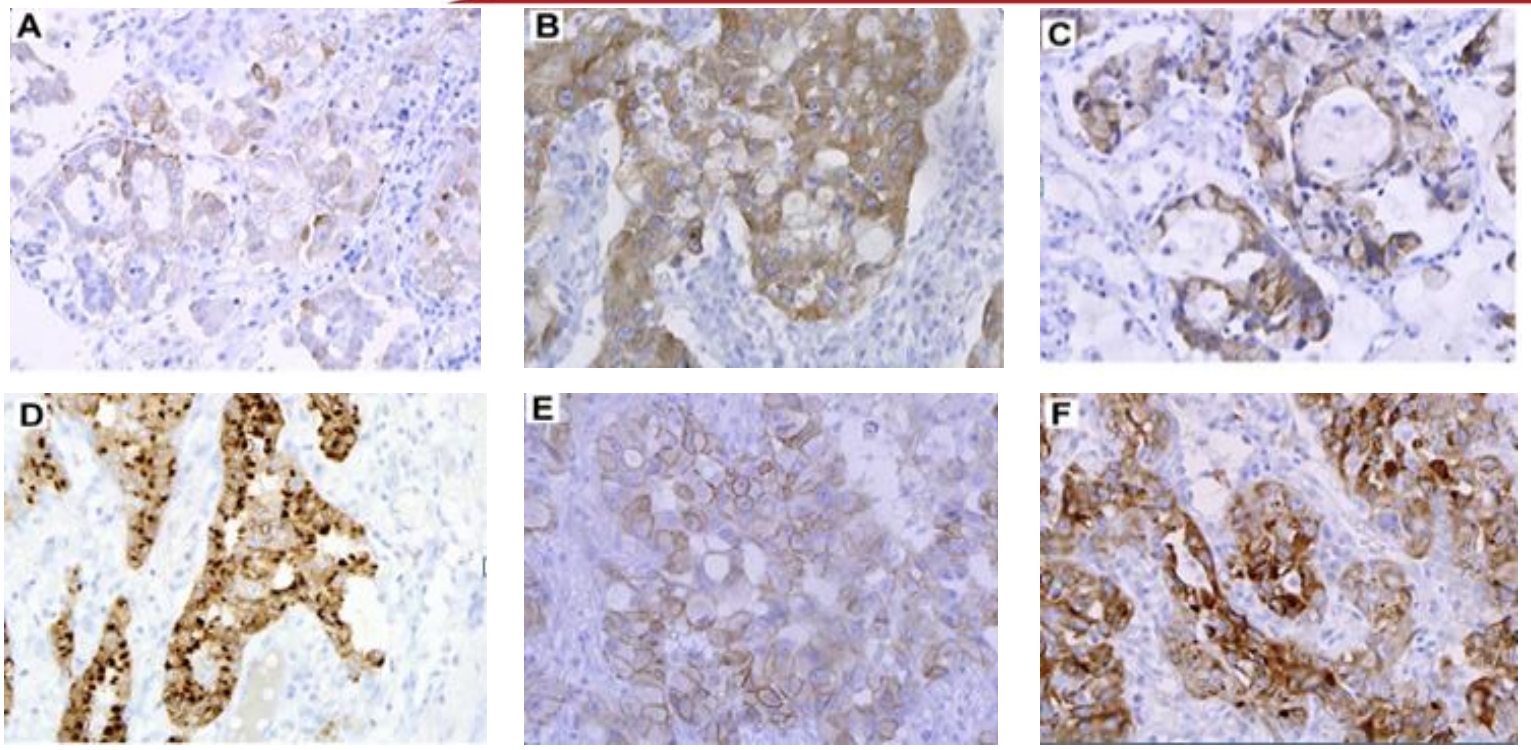
5/6 *ROS1* detected by FISH and 6/6 detected by IHC and RT-PCR.



Specimen	Fusion Partner	H-Score	Staining Pattern	Mucinous	Signet Ring	Cribriform	Predominant Pattern
1611	SCL34	170	Granular cytoplasmic	No	No	No	Solid
1958	CD74	130	Granular cytoplasmic	Yes	Yes(75%)	Yes(focal)	Solid/signet ring
2006	CD74	200	Cytoplasmic; focal globular	Yes	Yes(5%)	Yes	Acinar/solid
2087	CD74	170	Strongly globular(30%)	No	No	Yes	Lepidic
2604	EZR	200	Membranous; cytoplasmic	Yes	No	Yes	Solid/lepidic
2647	SDC4	270	Granular cytoplasmic	No	No	Yes	Solid

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BRAF-mutations in NSCLC: META-Analysis

Author	Year	Source of pts	Methods	No. of pts	Mut *BRAF* (%)	Female (%)	Smokers (%)	ADC (%)	Stage III/IV(%)
Pratilas	2008	4 countries	PCR+SEQ/MALDI -TOFMS	916	17(1.9)	577(63.0)	614(67.0)	623(68.0)	NA
Schmid	2009	Austria	PCR+SEQ	96	2(2.1)	38(39.6)	74(77.1)	NA	NA
Lee	2010	Korea	PCR+SEQ	173	2(1.2)	60(34.7)	117(67.6)	117(67.6)	NA
Kobayashi	2011	Japan	PCR+SEQ/SSCP	581	5(0.9)	204(35.1)	NA	382(65.7)	124(21.3)
Marchetti	2011	Italy	PCR+SEQ/HRMA	1046	37(3.5)	187(25.3)	542(73.3)	739(70.7)	218(29.5)
Paik	2011	USA	MALDI-TOF MS	697	18(2.6)	452(65.8)	386(56.2)	NA	NA
An	2012	China	HRMA	452	7(1.5)	NA	192(42.5)	307(67.9)	NA
Sasaki	2012	Japan	PCR+SEQ	305	6(2.0)	148(56.7)	NA	NA	NA
Cardarella	2013	USA	PCR+SEQ	883	36(4.1)	148(50.5)	229(78.4)	256(87.4)	237(80.9)
Ilie	2013	France	PCR+SEQ	450	40(8.9)	158(35.1)	403(89.6)	NA	352(78.2)

Total: 5599 cases

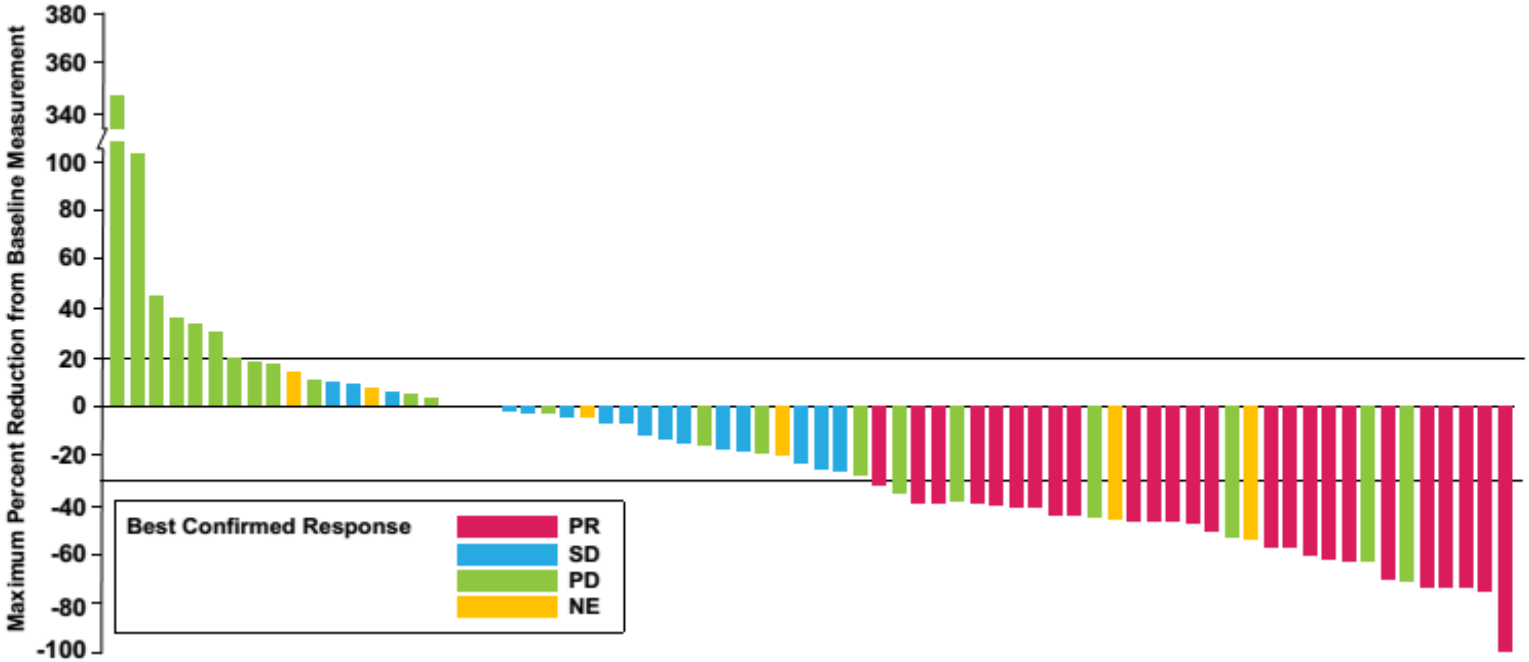
Mut *Braf*: 170 cases

3%= 170/5599

Chen, et al. PLOS one, 2014

Maximum reduction of sum of lesion diameters by best confirmed response in ≥ 2nd line(N=78).

Investigator assessed best confirmed response for > 2nd line: ORR 32%(21.9–43.6); DCR 56%(44.7–67.6).



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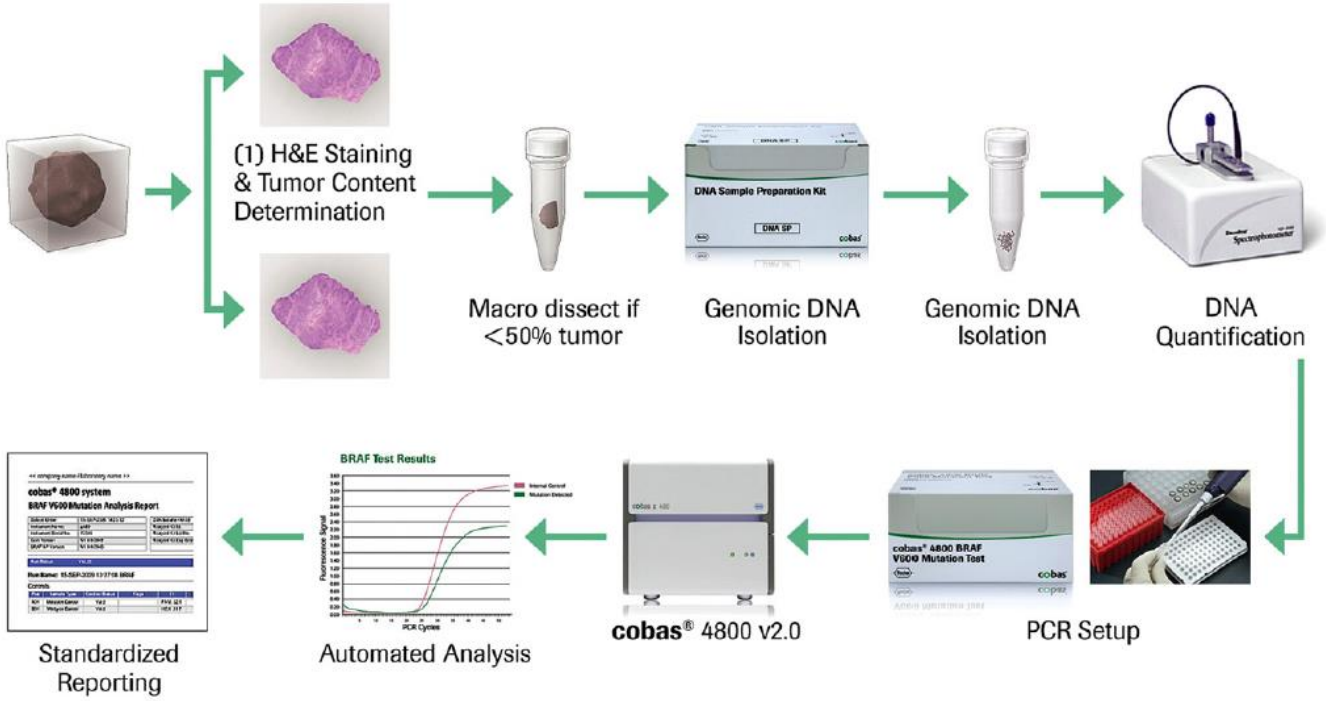
BRAF V600E mutation: real-time PCR

- **FDA approved companion biomarker real-time PCR(RT-PCR) assay on the Roche Cobas 4800**
- **This assay has been shown to be able to detect the mutation when the mutation constitutes only 10% of a mixture with wild-type *BRAF* gene(i.e., a ratio of 90:10 of wild-type: mutated *BRAF*)**



Ong FS, et al. Expert Rev Mol Diagn. 2012; 12(6): 593-602.
Cheng S, et al. N Biotechnol. 2012; 29(6): 682-8.

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MET Pathway Aberrations in NSCLC

MET Expression in Non-Small-Cell Lung Cancer: Testing, Treatment, and Future Directions (2013)

- ***MET* protein is overexpressed in 25-75% of NSCLC; overexpression is associated with poor prognosis**

- ***MET* oncogene amplification:**
  - **de novo in 3% to 7% of untreated NSCLC, therefore rarely the underlying primary resistance to EGFR TKIs**
  - **more frequently (21%) in tumors of patients previously treated with EGFR TKIs; an important underlying mechanism of acquired clinical resistance to EGFR TKIs in 5% to 20% of NSCLC**

1. Ichimura E, et al. Jpn J Cancer Res. 1996;87(10):1063-9.
2. Ma PC, et al. Cancer Res. 2005;65(4):1479-88.
3. Benedittini E, et al. Am J Pathol. 2010;177(1):415-23.
4. Cappuzzo F, et al. Ann Oncol. 2009;20:298-304.
5. Bean J, et al. Proc Natl Acad Sci U S A. 2007;104:20932–20937.
6. Engelman JA, et al. Science. 2007;316:1039–1043.
7. Sequist LV, et al. Sci Transl Med. 2011;3:75ra26-75ra26.

Randomized Phase II Trial of Onartuzumab in Combination With Erlotinib in Patients With Advanced Non–Small-Cell Lung Cancer

David R. Spigel, Thomas J. Ervin, Rodryg A. Ramlau, Davey B. Daniel, Jerome H. Goldschmidt Jr, George R. Blumenschein Jr, Maciej J. Krzakowski, Gilles Robinet, Benoit Godbert, Fabrice Barlesi, Ramaswamy Govindan, Taral Patel, Sergey V. Orlov, Michael S. Wertheim, Wei Yu, Jiping Zha, Robert L. Yauch, Premal H. Patel, See-Chun Phan, and Amy C. Peterson

MET IHC scoring system:

3+ ≥50% of tumor cells staining with strong intensity

2+ ≥50% of tumor cells with moderate or higher staining but <50% with strong intensity

1+ ≥50% of tumor cells with weak or higher staining but <50% with moderate or higher intensity

0+ no staining or <50% of tumor cells with any intensity

MET positivity was defined as a score of 2+ or 3+

JCO 2013; 31: 4105

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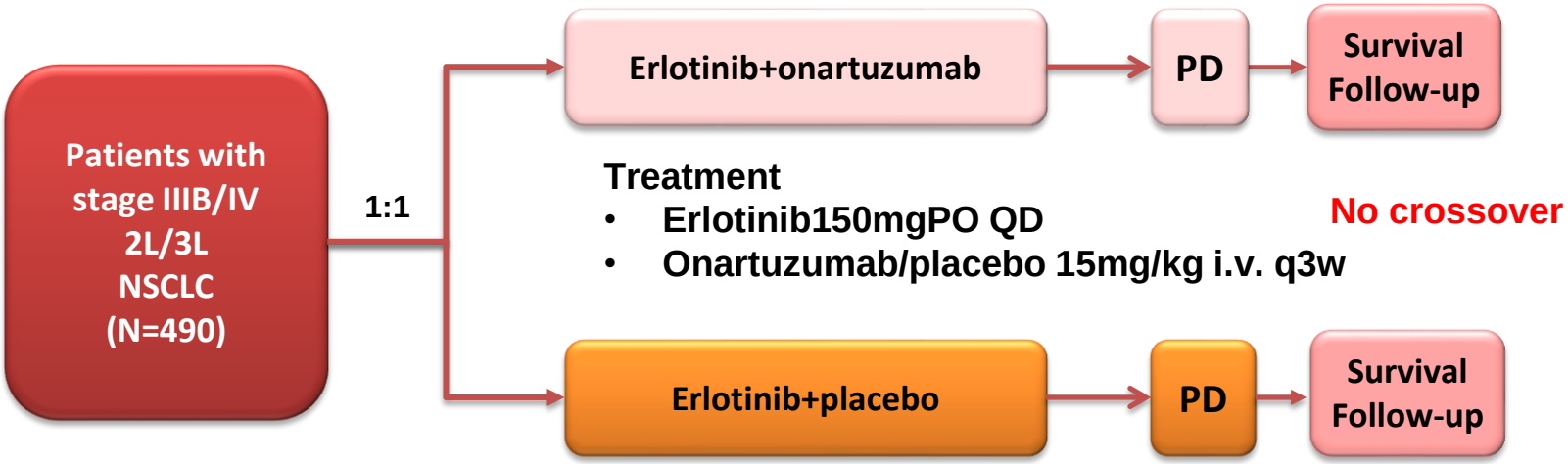
Global phase 3 trial (METLung) of Onartuzumab plus Erlotinib in NSCLC

Trial design:



Stratification criteria
- *EGFR* mut vs wt
- *MET* 2+ VS 3+
- Number of prior treatment
- Histology

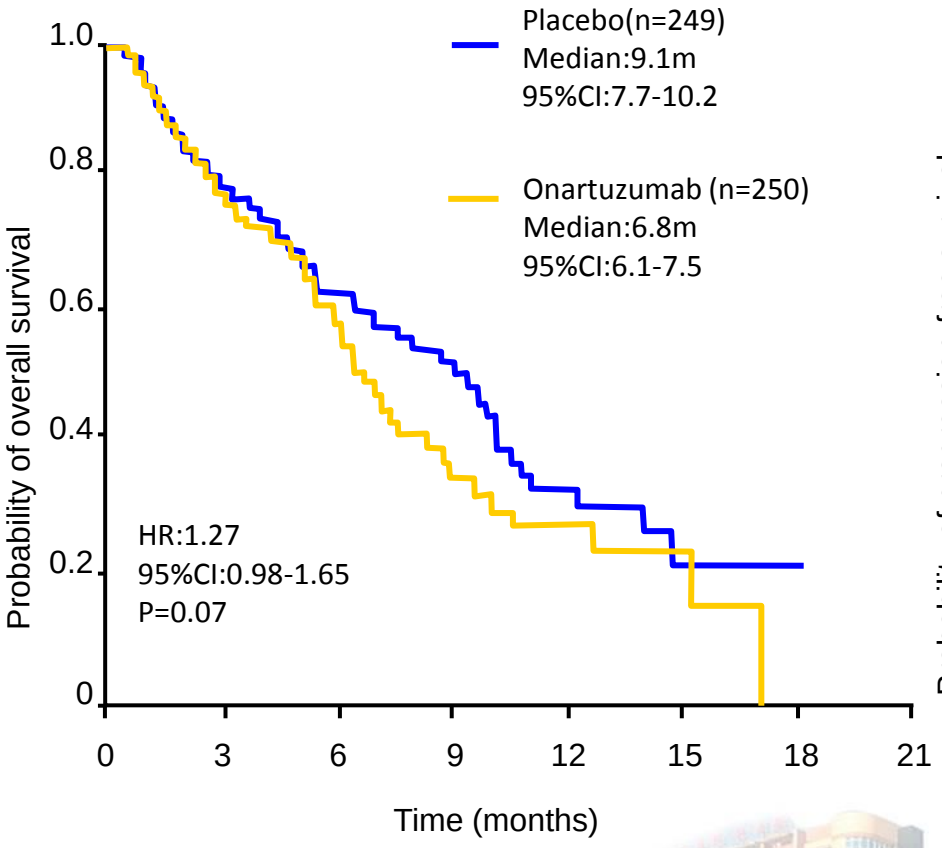
Key eligibility criteria
- *MET*-positive(2+ or 3+)
- 1 prior Pt-based treatment
- ECOG PS 0-1
- Central testing for
✓ *MET* IHC status
✓ *EGFR* mutation status

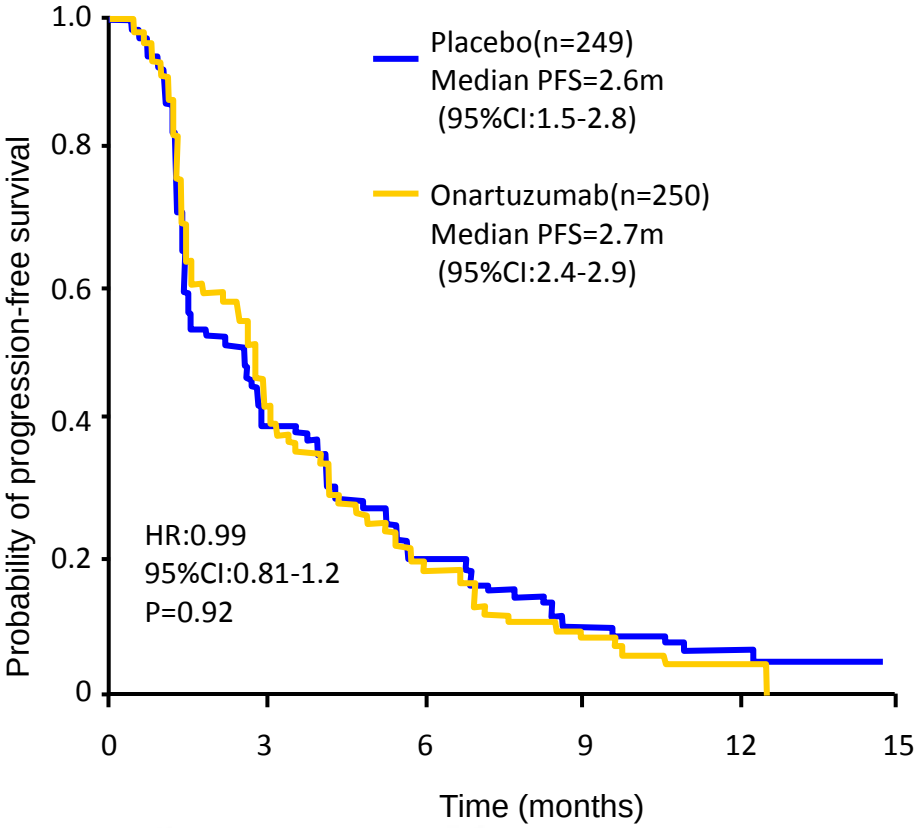
Primary endpoint
- OS

Secondary endpoints
- PFS
- ORR
- QoI
- Safety
- PK

Spigel DR, et al. 2014 ASCO Abstract 8000.

OS and PFS





Spigel DR, et al. 2014 ASCO Abstract 8000.

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Crizotinib in *MET* amp NSCLC: phase I trail

This study is part of phase I crizotinib study NCT00585195

patient(N=16)
- advanced NSCLC
- ≥ 18 year old
- Measurable disease
- Received no prior MET or HGF targeted therapy
- *MET* amp

→ Pts were classified to 3 category according to *MET* amp level
- If ≥2 objective responses occur in a category, 19 additional pts are to be enrolled

→ Crizotinib 250mg BID

→ Study Endpoint:
- ORR

In archival tumor tissue, *MET* amp was determined by FISH

MET(-)	Low *MET*	Inter *MET*	High *MET*
MET/CEP7 <1.8	MET/CEP7 1.8-2.2	MET/CEP7 2.2-5	MET/CEP7 >5

Camidge DG, et al. 2014 ASCO Abstract 8001.

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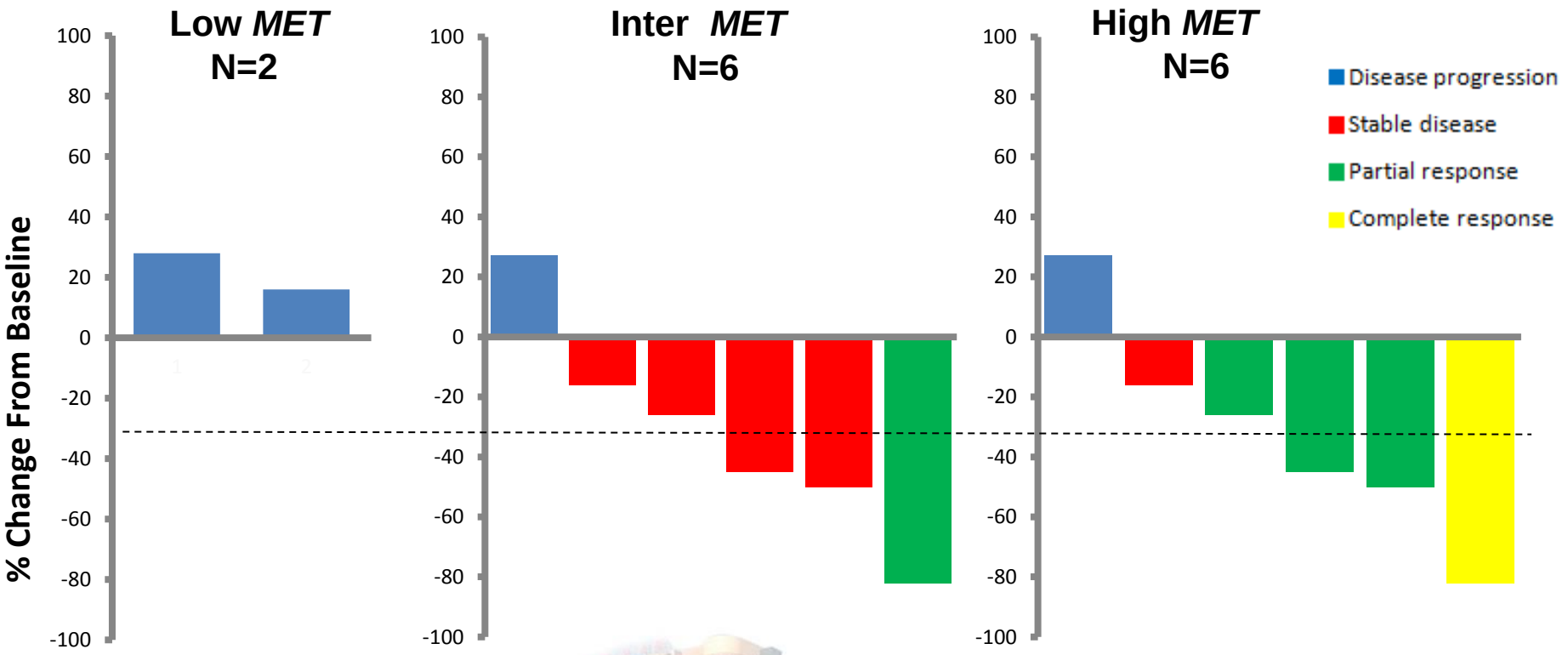
Tumor shrinking seen in intermediate and high *MET* amp cohorts



Camidge DG, et al. 2014 ASCO Abstract 8001.

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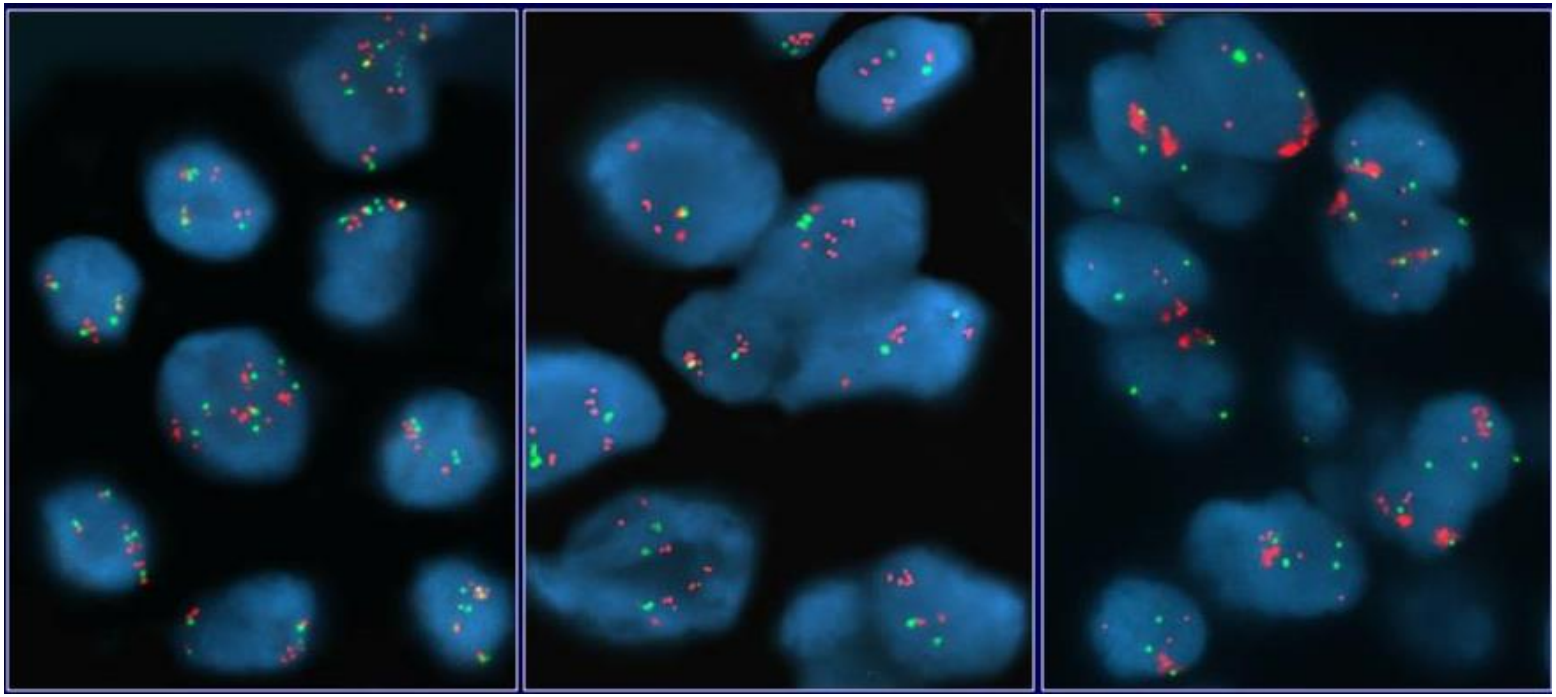
MET amplification cohorts determined by FISH



Low *MET* level	Intermediate *Met* level	High *Met* level
MET/CEP7 ratio≥1.8-≤2.2	*MET*/CEP7 ratio>2.2-<5.0	*MET*/CEP7 ratio≥5.0
Mean *MET* cell: 9.0	Mean *MET* cell: 7.0	Mean *MET* cell: 15.7
Mean CEP 7 cell: 4.7	Mean CEP 7 cell: 2.1	Mean CEP 7 cell: 2.8
Ratio: 1.9	Ratio: 3.3	Ratio: 5.6

Camidge DG, et al. 2014 ASCO Abstract 8001.

cMET

- ***MET*** **is a relevant target driving tumor growth in about 3% of NSCLC with *gene* amplification (ratio ≥2.2).**
- **Prospective studies need to define the best cut-off (ratio 2.2 versus 5).**
- ***MET*** **amplification is detectable in smokers irrespective of histology.**
- **IHC or *MET* copy numbers are not optimal for detecting patients potentially sensitive to anti-*MET* strategies.**
- **New studies with anti-*MET* agents should be conducted only in properly selected patients.**

HER2 testing

- **ASCO and CAP have recommended guidelines in *HER2* testing to ensure accuracy.**

- **The two methods currently approved for *HER2* testing are IHC and FISH.**

- **Mutations of *HER2* were also reported in lung adenocarcinoma. The mutations targeted never or light smokers, oriental ethnicity, and female gender,TTF-1 positive staining, high morphologic grade.**

FISH for *HER2* amplification

- **At least 20 cells per case were analyzed by two pathologists independently. An additional 20 cells were recounted to confirm their status as amplified or not amplified.**

- **Either high-level amplification (numerous loose or tight clusters of *HER2* signals, atypically large signals, or a *HER2*/centromere 17 ratio >5.0) or low-level amplification(*HER2*/centromere 17 ratio >2.0 and <5.0) were considered FISH-positive**

A. Yoshizawa et al. Lung Cancer, 2014; 85: 373-378.

HER2 IHC (+) in Advanced NSCLC

Study	Year	Population	N	Regimens	ORR (%)	PFS (m)	OS (m)
Clamon (CALGB)	2005	IHC 2+~3+	24	Trastuzumab	1/24	NR	NR
ECOG 2598	2004	IHC +~3+	56	PC+Trastuzumab	24.5	3.3	10.1
Gatzemeier (phase II)	2004	IHC +	51	GC+Trastuzumab	36	6.1	NR
			50	GC	41	7.0	NR

Clinical benefit was not observed in patients with *HER2*-positive NSCLC

Clamon G, et al. Cancer 103:1670-1675, 2005
Langer CJ, et al.J Clin Oncol 22:1180-1187, 2004
Gatzemeier U, et al. Ann Oncol 15:19-27, 2004

Incidence of *HER-2* mutation in adenocarcinomas

Study	N	*HER2* M(+)	incidence (%)
Tomizawa K et al. (Lung Cancer 2011)	504	13	2.58
Li C et al. (J Thor Oncol 2012)	224	8	3.57
Sun Y et al. (J Clin Oncol 2010)	52*	2	3.85
Arcila M et al. (Clin Cancer Res 2012)	560	25	4.46
Zhang Y et al. (Clin Cancer Res 2012)	349	16	4.58
Cardarella S et al. (J Thor Oncol 2012)	276	13	4.71
Li C et al. (Plos One 2011)	202**	12	5.94
Mazie`res et al (JCO 2013)	3800	65	1.8%
Barlesi,et al (ASCO ,2013)	10000	90	0.9%

Garrido-Castro AC, et al. Transl Lung Cancer Res 2013; 2(2):122-127.

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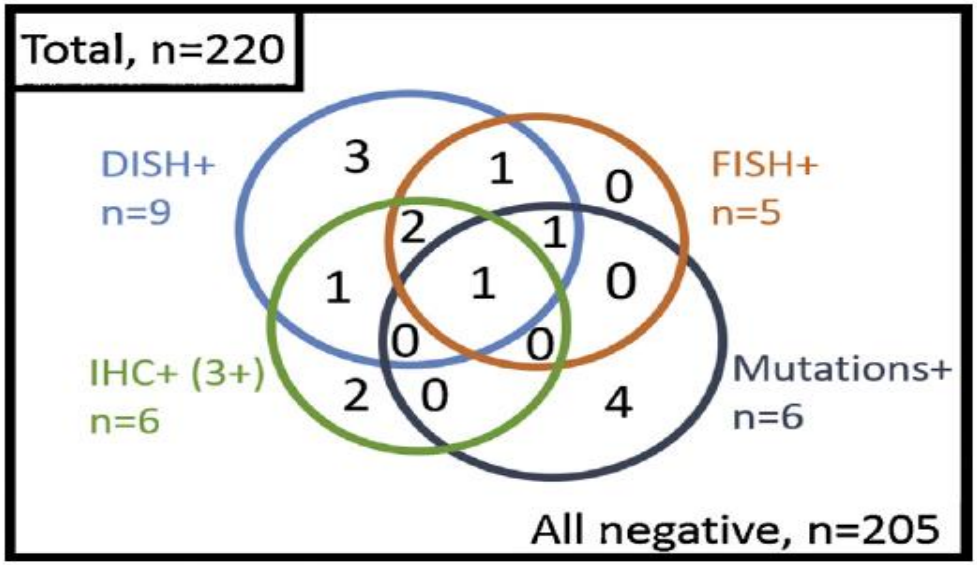
Correlations between *HER-2* mutations and IHC, FISH

		IHC			FISH		
		3+	0,1+,2+	P value	Amplified	Not amplified	P value
HER2 mut	Positive	1	5	0.329	2	4	<0.001
	Negative	5	209		3	211	



6 IHC(3+): 1 mutant, 2 amplification;

6 mutant: 2 amplification

5 FISH(+) : 2 mutant

No relationship between *HER2* IHC and mutations

A. Yoshizawa et al. Lung Cancer ,2014,373–378

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Lung Cancer That Harbors an *HER2* Mutation: Epidemiologic Characteristics and Therapeutic Perspectives

Patients	First-Line Treatment		Second-Line Treatment		Third-Line Treatment		Fourth-Line Treatment	
	Treatment	Best Disease Response	Treatment	Best Disease Response	Treatment	Best Disease Response	Treatment	Best Disease Response
11	VIN-HER	PR						
15	CAR-PAC-TRAS	SD						
19	TXT-MASA	PD						
24	VIN-TRAS	PR						
26	CAR-PAC-TRAS	PR						
27	VIN-TRAS	PR						
28	VIN-TRAS	SD						
30	LAP	PD						
31	NVB-HER	PR						
32	LAP	PD	TRAS-VIN	PR	AFA	SD	CAR-TRAS	SD
37	VIN-TRAS	PD						
41	DOC-TRAS	PR						
43	VIN-TRAS	PR	AFA	PR				
44	VIN-TRAS	PR	AFA	SD				
45	VIN-TRAS	SD	PAC-TRAS	SD				
47	TRAS	PR						

***HER2* mutations 1.7% in 3800 adenocarcinomas**

Trastuzumab-based therapy (n=15) 10PRs, DCR=93%

Afatinib (n=3) DCR =100%;Other *HER2* targeted drugs (n=3) DCR=0

Mazieres J, Peters S, Lepage et al. JCO 2013; 31: 1997

HER2 testing

- ***HER2*** **kinase domain mutations are most commonly in-frame insertions in exon 20 with duplication of amino acids YVMA at codon 775.**

- **Immunocytochemical staining for ERBB2 revealed no differences between tumors with or without *HER2* mutations, indicating that overexpression probably does not accompany the mutation.**

Morgensztern D, et al. J Thorac Oncol. 2015; 10(1 Suppl 1): S1-63.
Stephens P, et al. Nature. 2004; 431(7008): 525-6.

RET rearrangements in NSCLC screening studies

Study	Screening/validation techniques	Prevalence
Cai et al	RT-PCR, direct squencing	6/392 (1.5%)
Ju et al.	Whole-genome sequencing transcriptome sequencing, RT-PCR	2/21(14.3%)
Kohno ete al.	Whole-transcriptome sequencing, RT-PCR, FISH	7/433(1.6%)
Li et al.	Exon array analysis, RT-PCR	2/202(1%)
Lipson et al.	Next-generation sequencing, IHC, RT-PCR	12/667(1.8%)
Seo et al.	Whole-transcriptome sequencing, whole exon sequencing	4/200(2%)
Suehara et al.	Messenger RNA screen, RT-PCR,FISH	1/69(1.4%)
Takeuchi et al	FISH, RT-PCR	14/1529(0.9%)
Wang et al.	RT-PCR, IHC, FISH	13/936(1.4%)
Yokota et a l	RT-PCR, direct sequencing	3/371(0.8%)

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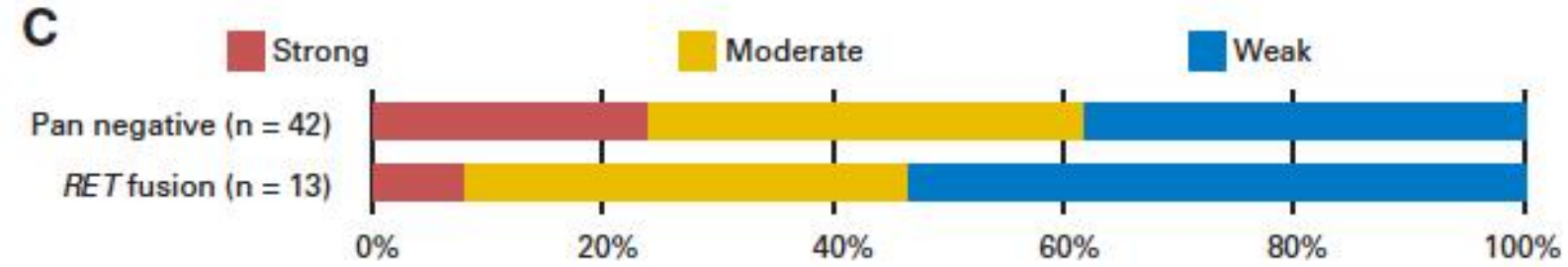
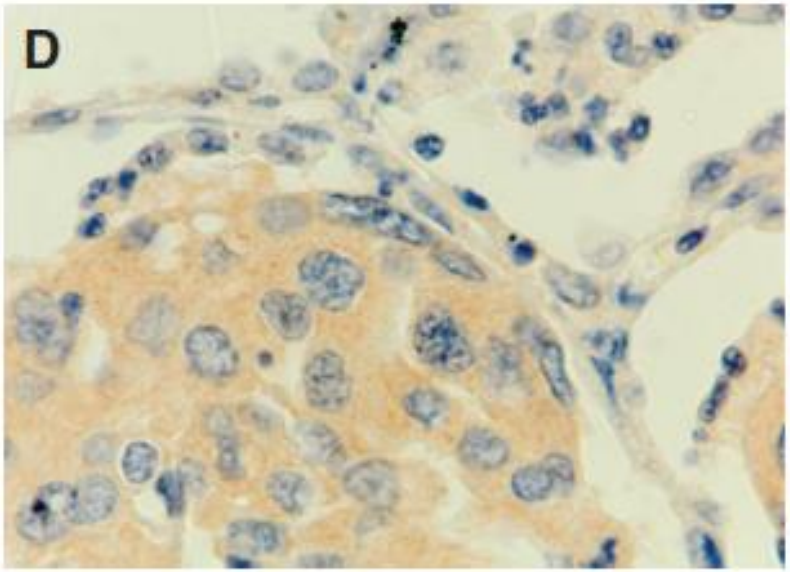
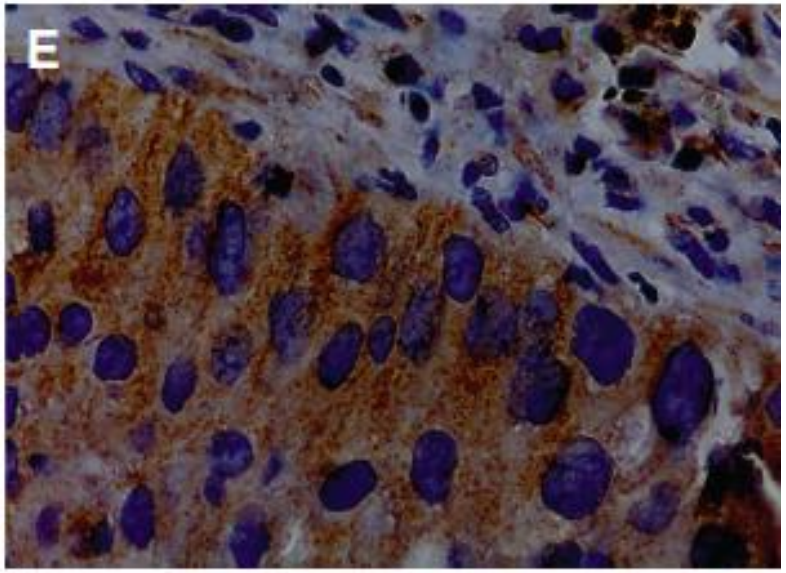
Response to Cabozantinib in Patients with *RET* Fusion-positive Lung Adenocarcinomas

Pati-ents	*RET* fusion	inhibitor	race	sex	Age (year)	Pathological diagnosis	Smoking	Response (reduse%)
1	*TRIM33-RET*	Cabozantinib	Whites	F	41	Papillary adenocarcinoma	Never	PR（66）
2	*KIF5B-RET*	Cabozantinib	African Ameri-cans	F	75	Poorly differentiated adenocarcinoma	Never	PR（32）
3	*KIF5B-RET*	Cabozantinib	whites	F	68	Mixed adenocarcinoma	Never	SD

RET fusions define a unique molecular and clinicopathologic subtype of NSCLC



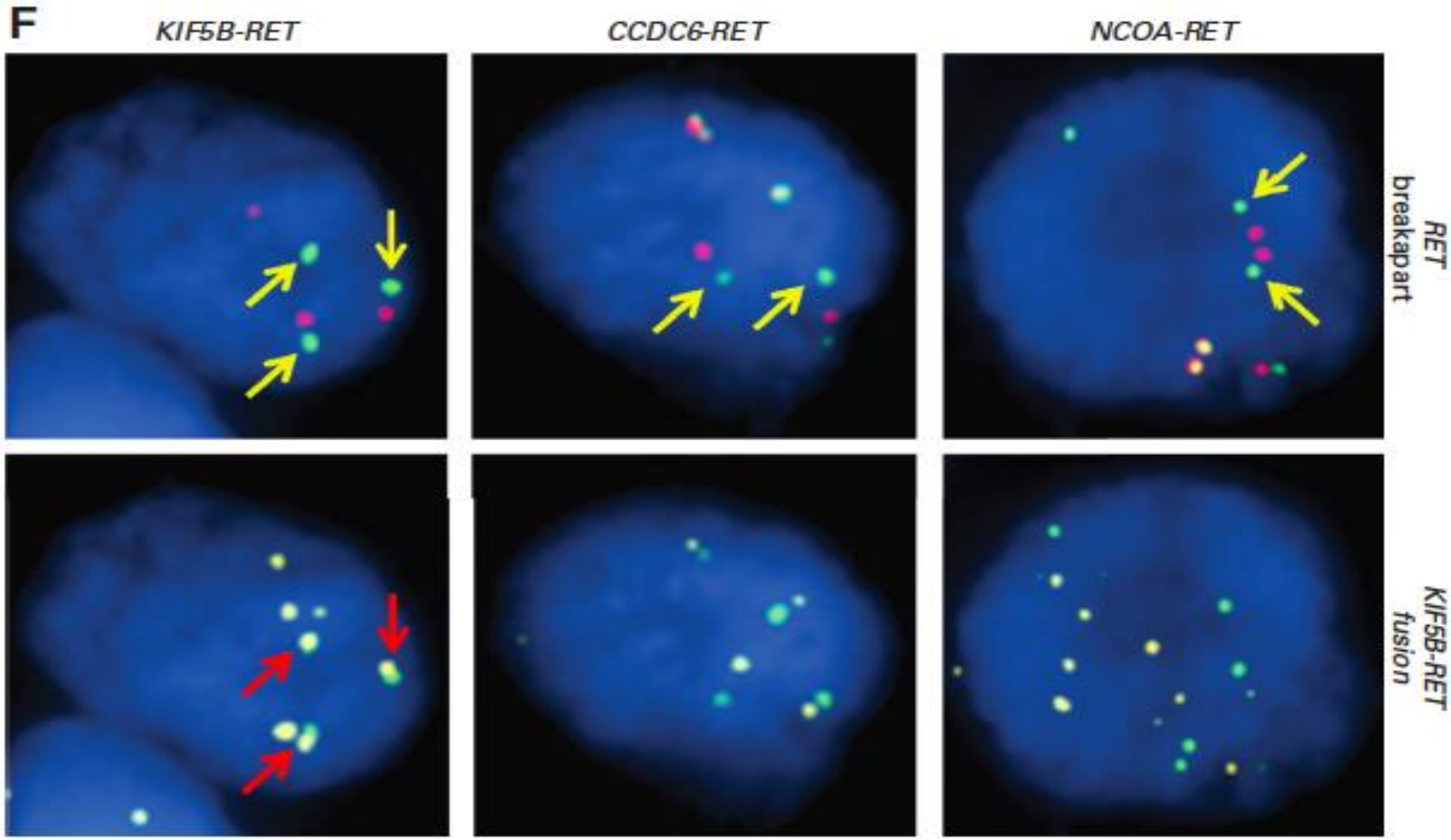




Wang: *RET* IHC staining with the antibody used has limited value in screening for *RET* fusions in NSCLC!

Drilon: *RET* IHC is not sufficiently reliable at present for diagnostic purposes.

Wang R, et al. J Clin Oncol. 2012; 30(35): 4352-9.
Drilon A, et al. Cancer Discovery 2013; 3: 630-635.



4/13 *RET* fusions were missed by FISH.
0/42 with negative *RET* fusions positive for FISH.
RT-PCR and FISH should be combined!!!

Wang R, et al. J Clin Oncol. 2012; 30(35): 4352-9

RET fusion testing

- **For the detection of *RET* fusions in lung cancer, RT-PCR alone is usually insufficient to detect new partners or isoforms.**

- **Although FISH is currently the most effective diagnostic technology to detect chromosomal rearrangements, the high cost and need for technical expertise limit its practical application.**

Wang R, et al. J Clin Oncol. 2012; 30(35): 4352-9.
Drilon A, et al. Cancer Discovery 2013; 3: 630-635.

Which population?

- Only adenocarcinoma?
- Never smokers or mild smokers?
- Adenosquamous carcinoma?
- Enriched population?

Screening for *RET* and *ROS1* fusions in an enriched cohort of pan-negative never-smokers with advanced lung adenocarcinomas to identify patients for treatment in targeted therapy trials

Subjects: 35 pan-negative, never-smoking patients with advanced adenocarcinoma (absence of mutations in *EGFR, KRAS, NRAS, BRAF, HER2, PIK3CA, MEK1,* and *AKT,* and *ALK* fusions)

Methods: Real-time via dual-probe FISH break apart assays, RT-PCR, and next-generation sequencing in selected cases

Results: Overall detection rate of *RET* or *ROS1* fusion: 31% (10/32)

RET **15%** (5/34) *ROS1* **15%** (5/33)

Alexander Edward Dela Cruz Drilon, et al.
JCO 31, 2013 (suppl; abstr 8067)

RET rearrangements detected by FISH in "pan-negative" lung adenocarcinoma

Subjects: 51 lung adenocarcinomas negative for *EGFR*, *KRAS*, *ALK* and *ROS1* (36 also negative for 7 other molecular markers)

RET fusion: by FISH

Incidence of *RET* fusion: **15%** (8 patients had rearrangements)

5 with *KIF5B-RET* fusions

2 with patterns consistent with the *CCDC6-RET* fusion

1 with extra copies of single 3'*RET* (loss of 5'*RET*)

Marileila Varella-Garcia,et al. JCO 2013; (suppl; abstr 8024)

Sequence of test

- One by one
  - Longer time
  - More tissue

- Panel of biomarkers
  - Fast
  - Less tissue
  - Exclusive with each other of biomarkers

同济大学附属
上海市肺科医院
SHANGHAI PULMONARY HOSPITAL

A single-tube multiplexed assay for detecting *ALK*, *ROS1*, and *RET* fusions in lung cancer

Table 2 RT-PCR/Sanger Sequencing Primers

Fusion variant	PCR forward primer	Sequence	RT-PCR reverse primer	Sequence
EML4-ALK; E2:A20	EML4 exon 2	5′-AAGATCATGTGGCCTCAGTG-3′	ALK exon 20R	5′-CTTGCTCAGCTTGTACTCAGG-3′
EML4-ALK; E6:A20	EML4 exon 6	5′-CTGCAGACAAGCATAAAGATG-3′	ALK exon 20R	5′-CTTGCTCAGCTTGTACTCAGG-3′
EML4-ALK; E13:A20	EML4 exon 13	5′-GACTCGGTGGAGTCATGC-3′	ALK exon 20R	5′-CTTGCTCAGCTTGTACTCAGG-3′
EML4-ALK; E18:A20	EML4 exon 18	5′-AGGTGGTTTGTTCTGGATGC-3′	ALK exon 20R	5′-CTTGCTCAGCTTGTACTCAGG-3′
EML4-ALK; E20:A20	EML4 exon 20	5′-CAGATATGGAAGGTGCACTG-3′	ALK exon 20R	5′-CTTGCTCAGCTTGTACTCAGG-3′
TFG-ALK; T5:A20	TFG exon 5F	5′-TCTACTCAGGTTATGGCAGCAA-3′	ALK exon 20R	5′-CTTGCTCAGCTTGTACTCAGG-3′
KIF5B-ALK; K17:A20	KIF5B exon 17F	5′-CCTTCAAAATGTGGAACAAAA-3′	ALK exon 20R	5′-CTTGCTCAGCTTGTACTCAGG-3′
KIF5B-ALK; K24:A20	KIF5B exon 24F	5′-TGAAAGCTTTGGAATCAGCA-3′	ALK exon 20R	5′-CTTGCTCAGCTTGTACTCAGG-3′
SLC34A2-ROS1; S4:R32	SLC34A2 exon 4F	5′-CTTCTCGGATTTCTCTACTTTTTC-3′	ROS1 exon 32R	5′-TCTTCAGCTTTCTCCCACTG-3′
SLC34A2-ROS1; S13del2046:R32	SLC34A2 S13del2046F	5′-GCAGGATGTCCCTGTCAAG-3′	ROS1 exon 32R	5′-TCTTCAGCTTTCTCCCACTG-3′
CD74-ROS1; C6:R32	CD74 exon 6F	5′-CATTGGCTCCTGTTTGAAATG-3′	ROS1 exon 32R	5′-TCTTCAGCTTTCTCCCACTG-3′
SDC4-ROS1; S2:R32	SDC4 exon 2F	5′-GAGCCCTACCAGACGATGAG-3′	ROS1 exon 32R	5′-TCTTCAGCTTTCTCCCACTG-3′
EZR-ROS1; E10:R34	EZR exon 10	5′-GGAGAGAGAGAAAGAGCAGATGA-3′	ROS1 exon 34R	5′-TGTAACAACCAGAAATATTCCAAC-3′
KIF5B-RET; K15:R12	KIF5B exon 15F	5′-AACGAGCAGCTGAGATGATG-3′	RET exon 12R-1	5′-TCCAAATTCGCCTTCTCCTA-3′
KIF5B-RET; K16:R12	KIF5B exon 16F	5′-AGAAAGCACACAAACTGAGAGC-3′	RET exon 12R-1	5′-TCCAAATTCGCCTTCTCCTA-3′
KIF5B-RET; K22:R12	KIF5B exon 22F	5′-TGGAAGAGACAGTGGCAAAA-3′	RET exon 12R-1	5′-TCCAAATTCGCCTTCTCCTA-3′
KIF5B-RET; K23:R12	KIF5B exon 23F	5′-CGCTGCTCAGAAGCAAAAA-3′	RET exon 12R-1	5′-TCCAAATTCGCCTTCTCCTA-3′
CCDC6-RET; C1:R12	CCDC6 exon 1F	5′-GCTGAAGATAGAGCTGGAGACC-3′	RET exon 12R-1	5′-TCCAAATTCGCCTTCTCCTA-3′
CUX1-RET; C10:R12	CUX1 exon 10F	5′-TCTCATCGGCCAATCACTCC-3′	RET exon 12R-2	5′-CCAAATTCGCCTTCTCCTAGAG-3′

Lira ME, Choi YL, Lim SM, et al. J Mol Diag

Concordance of *ALK* IHC and FISH analysis as well as *ROS1* and *RET* FISH with the NanoString Assay

	ALK IHC					*ALK* FISH			*ROS1* FISH			*RET* FISH		
Nano-String	0	1	2	3	Total	-	+	Total	-	+	Total	-	+	Total
+	1	3	18	70	92	0	46	46	0	4	4	0	11	11
-	84	2	0	1	87	6	0	6	42	0	42	4	0	4
Total	85	5	18	71	179	6	46	52	42	4	46	4	11	15

***ALK* IHC staining: 0,negative, 1, weak, 2, moderate, 3, strong; number of *ALK* positive and negative samples by NanoString assay.**
Accuracy of NanoString to IHC is 97.8%; sensitivity is 96.8%, specificity is 98.8%.
Accuracy of NanoString to *ALK* FISH is 100%. Accuracy of NanoString to *ROS1* FISH is 100% and to *RET* FISH 100%.

Lira ME, Choi YL, Lim SM, et al. J Mol Diag

Conclusion

- **Incidences of biomarkers beyond *EGFR* and *ALK* are 1-5%.**
- ***ROS1* rearrangements, *RET* rearrangements, *HER2* mutations and *BRAF* V600E are oncogenic drivers in lung cancer.**
- **FISH is used to test *ROS1*, *RET* and *cMET***
- ***HER2* mutations and *BRAF* mutation should be tested**
- ***ROS1* IHC can be used to screen *ROS1* fusions.**
- ***RET* IHC is not recommended.**
- **A panel of biomarkers will be tested in the future**

Thanks you for your attention!