MADRID 2014 ESMO congress

New insight of sequencing data in lung cancer

Kazuto Nishio, M.D. Ph.D.

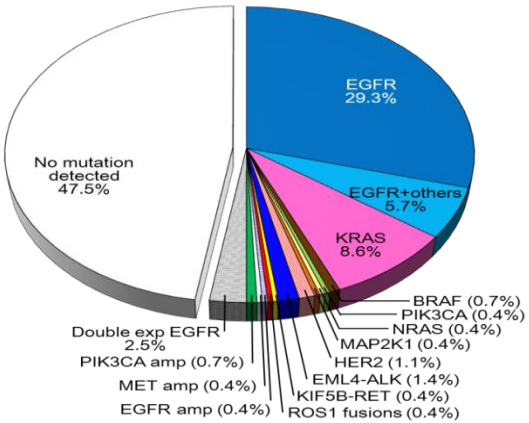
Department of Genome Biology, Kinki University
Faculty of Medicine

Disclosure slide

- I have the following financial relationships to disclose.
  - Honoraria（lecture fee) from: Daiichi Sankyo Co., Ltd　Chugai Pharmaceutical Co., Glaxosmithkline.co., Sumitomo Bakelite Co., Ltd. Eisai Co., Ltd.
  - Grant/Research funding from: Chugai Pharmaceutical Co., Pfizer Inc.

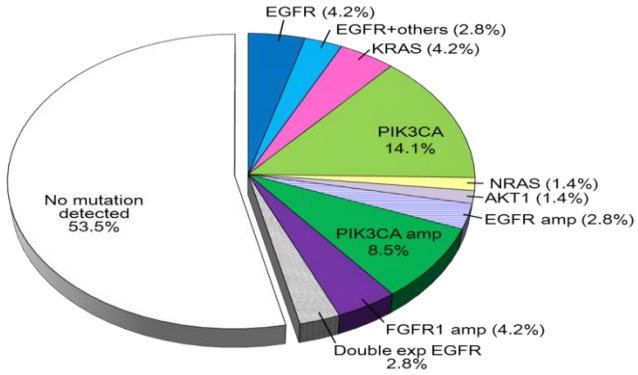
Mutations in patients with lung cancer

Adenocarcinoma



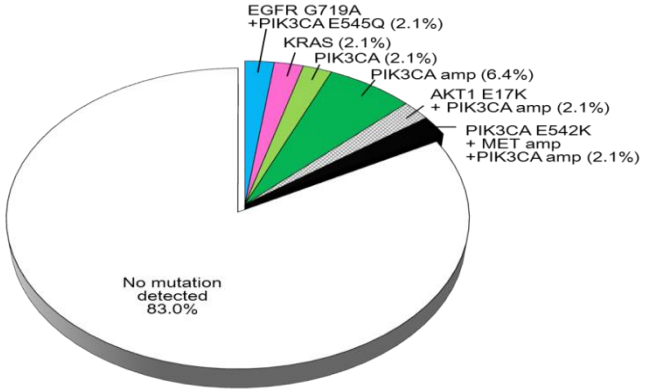
Mutations detected
in 52.5% (147/280)

Squamous cell carcinoma



Mutations detected
in 46.5% (33/71)

Small cell lung cancer



Mutations detected
in 17.0% (8/47)

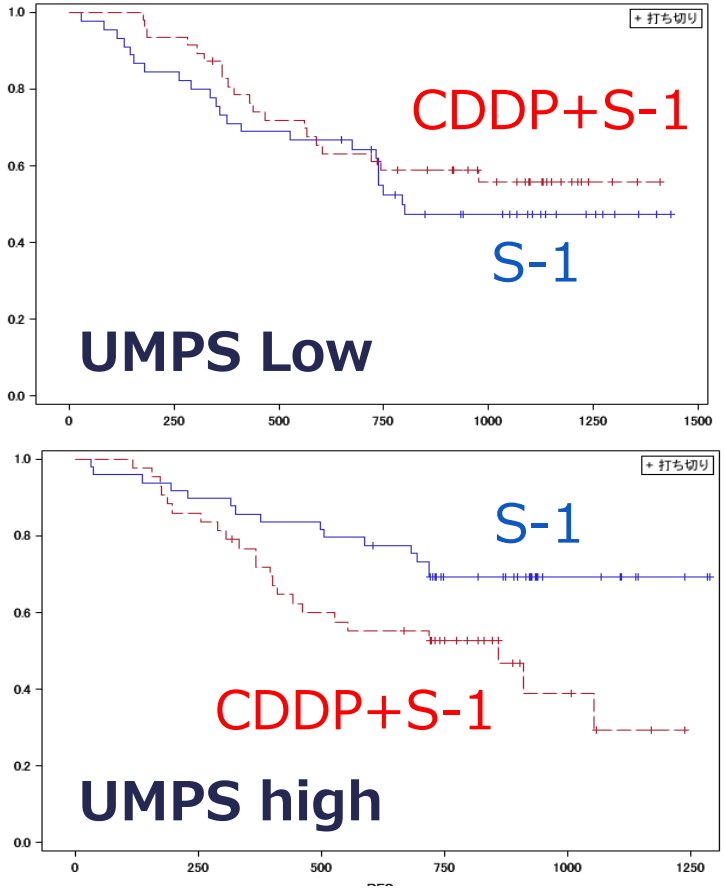
Exploratory analyses of mutations using samples from lung cancer patients enrolled in clinical trials of WJOG (2013)

For lung cancer patients;

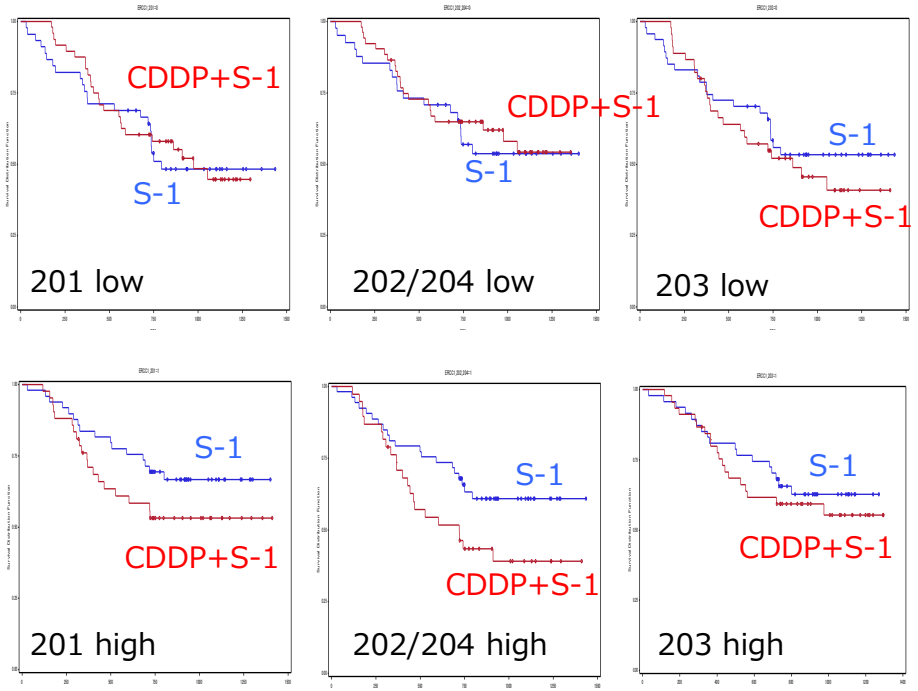
- LETS (CBDCA/TXL vs CBDCA/TS-1)
- WJOG4107 (adjuvant CDDP/TS-1 vs TS-1)
- JIPANG (adjuvant CDDP/VNR vs CDDP/PEM)
- IMPACT (adjuvant gefitinib vs CDDP/VNR)

Biomarker analysis of WJOG4107; A randomized phase II trial of adjuvant chemotherapy with S1 versus CDDP+S1 for resected stage II-IIIA non-small cell lung cancer (NSCLC)

Comparison of S-1 arm with CDDP+S-1 according to UMPS expression levels



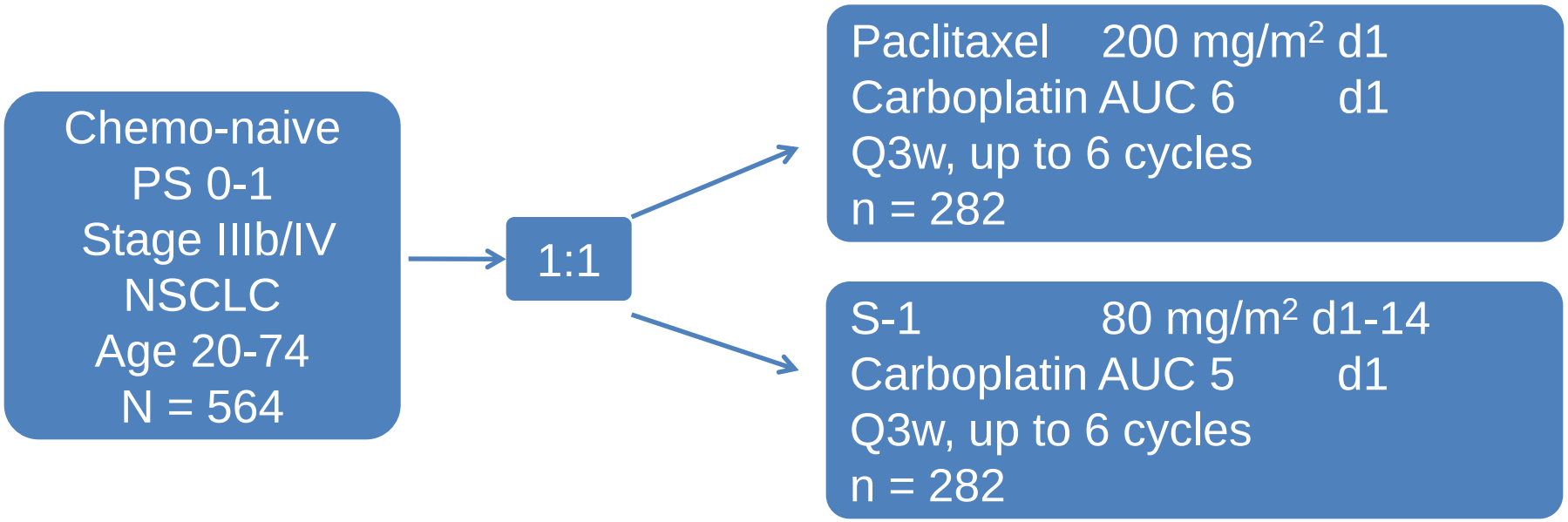
Comparison of S-1 arm with CDDP+S-1 according to ERCC1 isoform expression



Biomarker analyses in clinical trials for Non-Small Cell Lung Cancer ～WJOG6611LTR～

LETS study (Lung Cancer Evaluation of TS-1)

Phase III study of CBDCA/S-1 vs CBDCA/PTX



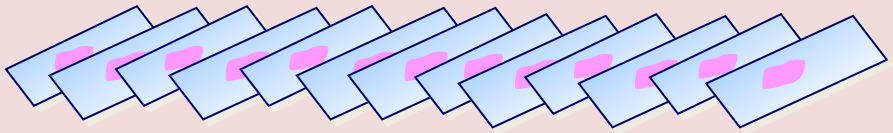
- ◆Objective ：Non-inferiority of CBDCA/S-1 compare to CBDCA/PTX
- ◆Planned Sample size：560
- ◆Primary Endpoint：Overall Survival

Okamoto et al. Journal of Clinical Oncology;28:5240-5246,2010

Biomarker analyses in clinical trials for Non-Small Cell Lung Cancer ～WJOG6611LTR～

Advanced NSCLC enrolled to LETS study (n=564)

If tumor FFPE* tissue specimens at diagnosis are available, the clinical centers send them to central lab**.



*Formalin-fixed paraffin-embedded

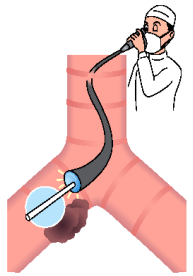
*~13 sections mounted on glass slides

**Kinki University Faculty of Medicine

P.I Kazuto Nishio

A total of 304 FFPE specimens were collected from 22 institutes

The majority (229 out of 304, 78%) of specimens were obtained by transbronchial biopsies.



WJOG6611LTR
Comprehensive genomic analyses in LETS study

WJOG

MassARRAY technology

Target DNA/RNA

1. PCR amplification

A
T

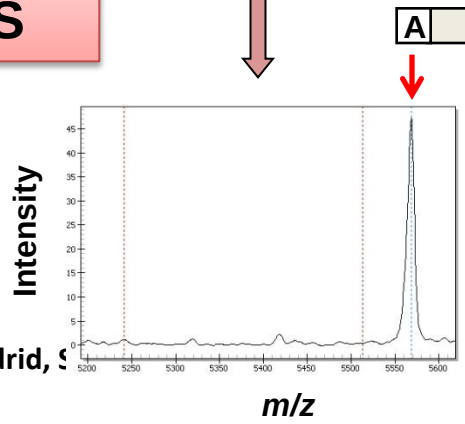
PCR product
(70-130bp)

2. Single-base extension

A
T

Single-base primer
extension product

3. Detection by MALDI-TOF MS



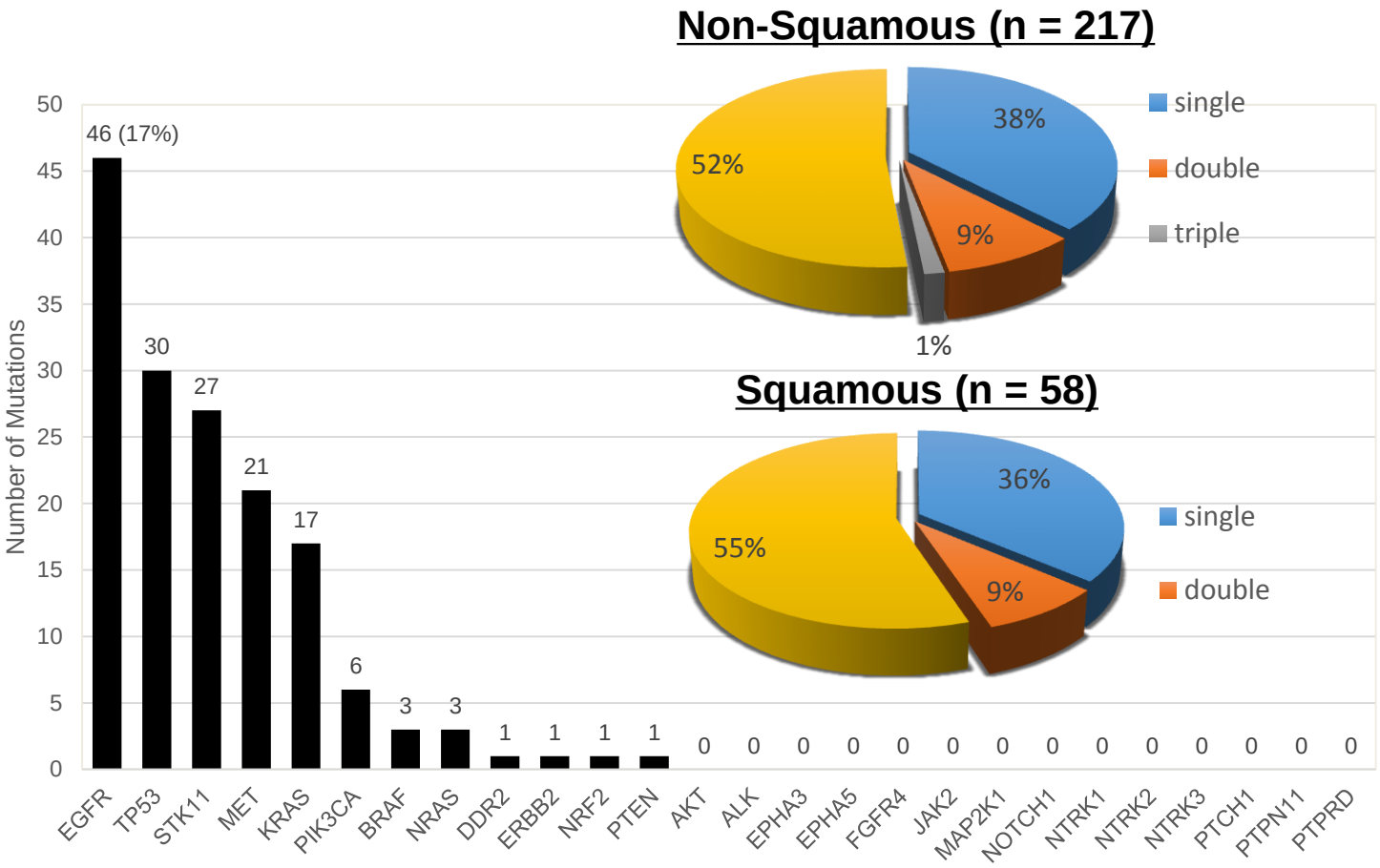
QGE
Quantitative
gene expression

Gene Mutations
LungCarta™ Panel

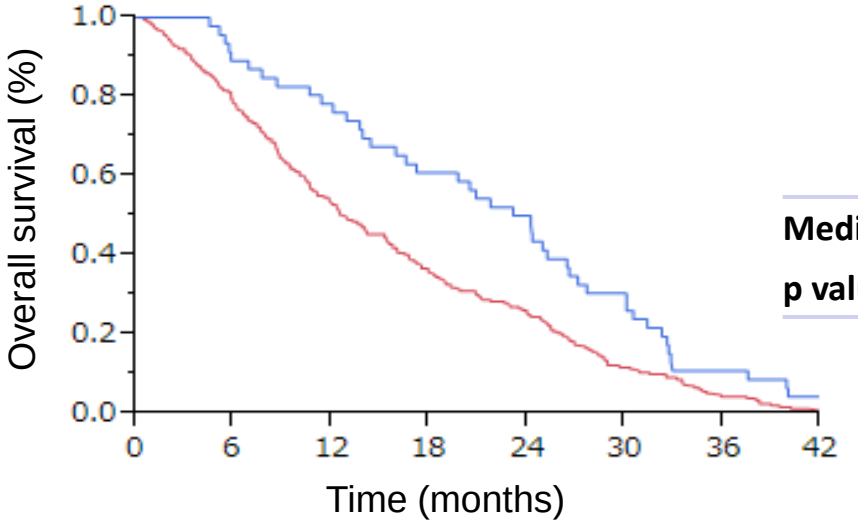
Fusion Gene
ALK, RET, ROS

LungCartaTM Panel: Analyses for somatic mutations

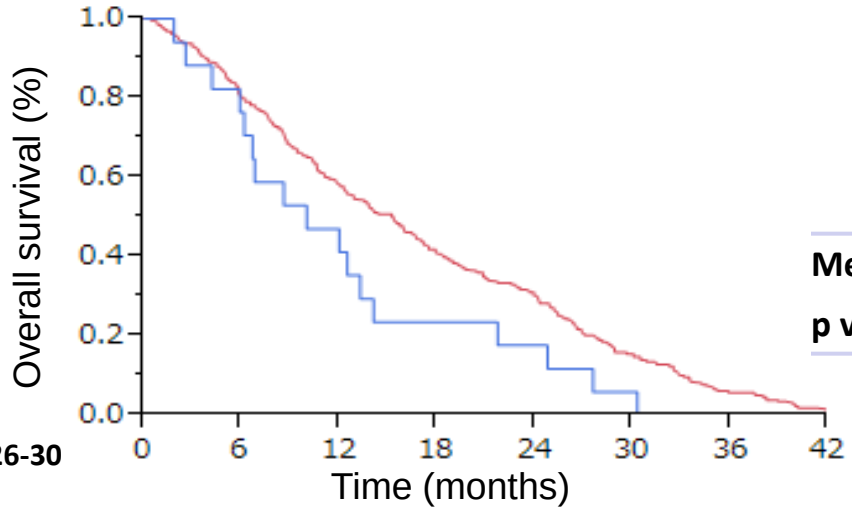
- **275 specimens (Non-Sq:217, Sq:58) provided available mutation profiles.**



LungCartaTM Panel: Analyses for somatic mutations



	EGFR mutation negative (n=229)	*EGFR* mutation positive (n=46)
Median, months	12.6	23.7
p value	0.004	



	KRAS mutation negative (n=258)	*KRAS* mutation positive (n=17)
Median, months	15.3	9.99
p value	0.04	

WJOG6611LTR
Comprehensive genomic analyses in LETS study

MassARRAY technology

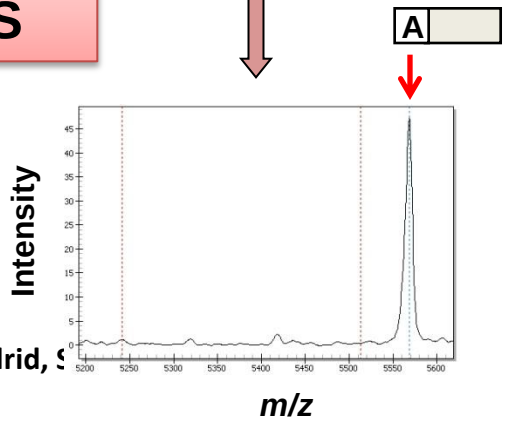
1. PCR amplification

PCR product (70-130bp)

2. Single-base extension

Single-base primer extension product

3. Detection by MALDI-TOF MS



QGE
Quantitative gene expression

Gene Mutations
LungCarta™ Panel

Fusion Gene
ALK, RET, ROS

LungFusion: Multiplex detection of ALK, RET, ROS1

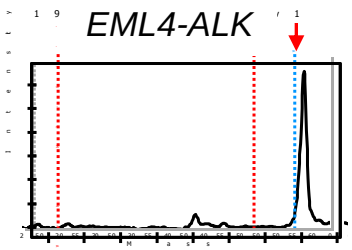
Multiplex 10 well reactions for 20 variants for fusion genes

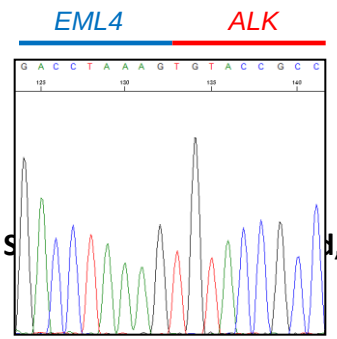
ALK fusion	*RET* fusion	*ROS1* fusion
EML4-ALK v1	*KIF5B-RETv2-K16-R12*	*CD74-ROS1-C6-R32*
EML4-ALK v2	*KIF5B-RETv4-K15-R12*	*CD74-ROS1-C6-R34*
EML4-ALK v3a	*KIF5B-RETv4-K22-R12*	*SLC34A2-ROS1*
KIF5B-ALK-K17-A20	*KIF5B-RETv4-K24-R11*	*SLC34A2-ROS1-long*
	CCDC6-RET-C1-R12	*SLC34A2-ROS1-short*
		LRIG3v1-ROS1
		SDC4-ROS1-S2-R32
		SDC4-ROS1-S4-R32
		SDC4-ROS1-S4-R34
		EZR-ROS1-E10-R34
		TPM3-ROS1

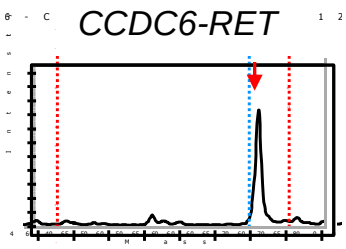
LungFusion: Multiplex detection of ALK, RET, ROS1

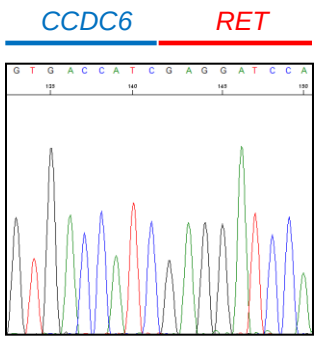
- **All 240 specimens referred for LungFusion were successfully tested.**

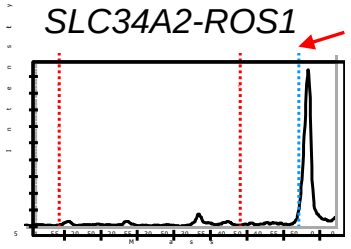
ALK fusion-positive (n=6)	*RET* fusion-positive (n=1)	*ROS1* fusion-positive (n=5)
6/240 =2.5%	**1/240 = 0.4%**	**5/240 = 2.1%**
EML4-ALK v1 n=3	*CCDC6-RET*-C1-R12 n=1	*SLC34A2-ROS1* n=3
EML4-ALK v2 n=2		*LRIG3v1-ROS1* n=1
EML4-ALK v3 n=1		*CD74-ROS1* n=1

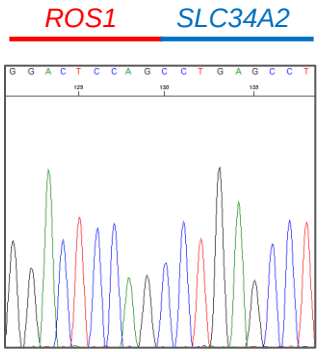








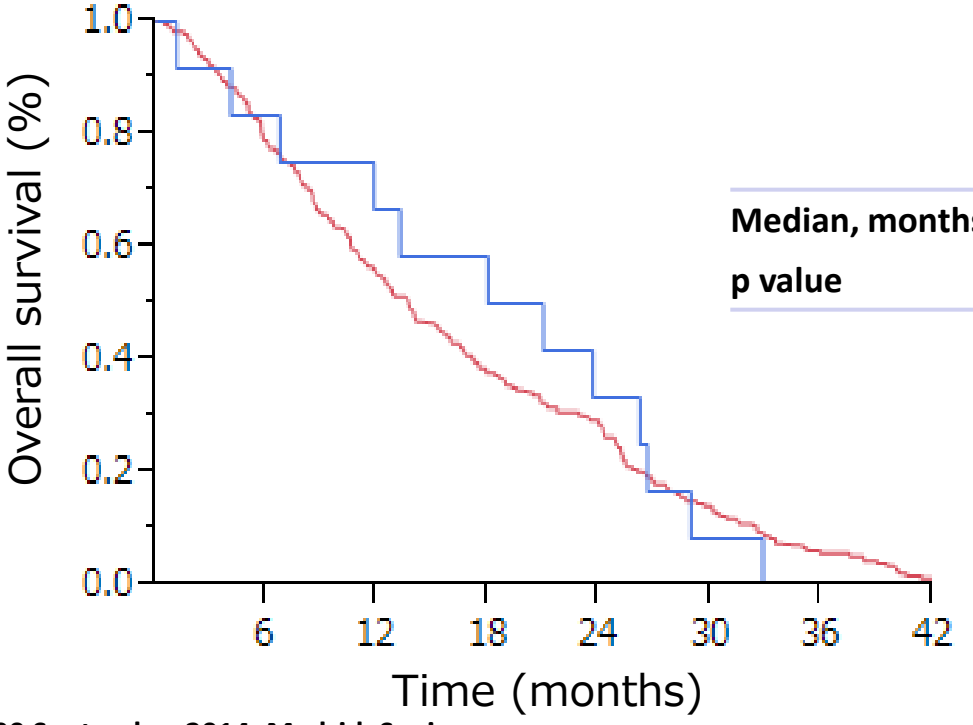




LungFusion: Multiplex detection of ALK, RET, ROS1

Clinicopathological characteristics of 12 patients with fusion(+) NSCLC.

- *ALK, RET, ROS1* rearrangements were mutually exclusive.
- Median age at diagnosis was 57 years old.

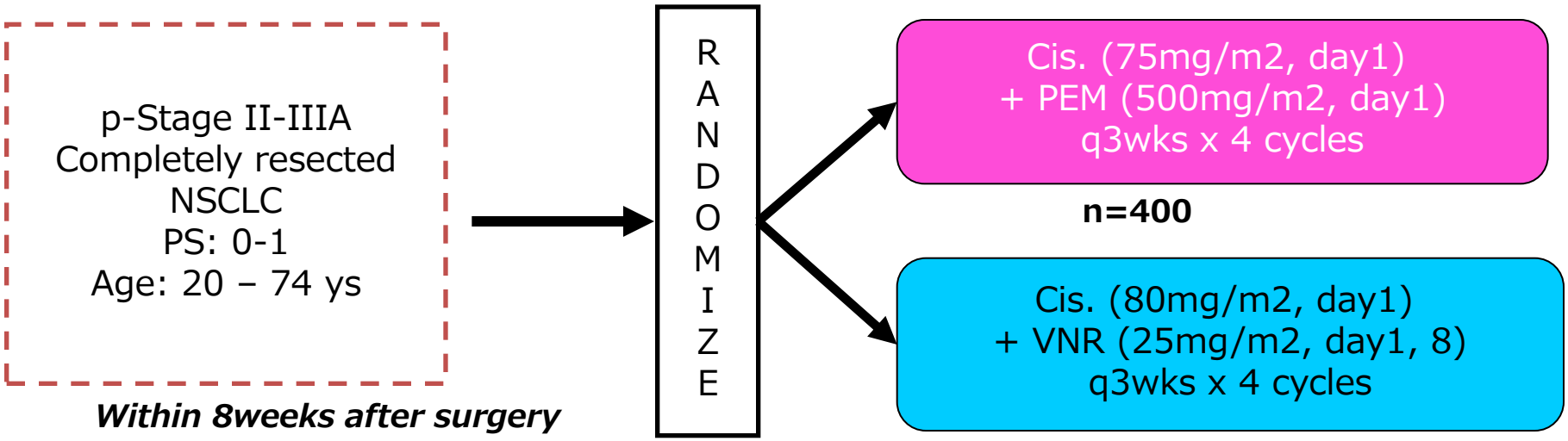


	Fusion gene negative (n=228)	Fusion gene positive (n=11)
Median, months	13.8	19.5
p value	0.89	

An intergroup study

JIPANG

Japan Intergroup Trial of Pemetrexed Adjuvant Chemotherapy for Non-squamous Non-small cell lung cancer



Stratified factors;
Institute, Gender, Stage, EGFR mut. status, Age (70)

P.I.; Tsuboi M.

Primary endpoint: Overall survival,
Secondary endpoints: Disease-free survival and toxicity

JIPANG Biomarker study

JIPANG

blood

FFPE

Lung Cancer Biomarker Consortium (LCBMC)
(Dr. Nishio, Kinki Univ Faculty of Med)

PGx
(blood)

Targeted
resequencing
(tissue DNA)

Sequenom
(DNA, RNA)

Transcriptome
(RNAseq)

Kindai Univ & Shizuoka Cancer Center

Somatic mutation assay for FFPE in WJOG

MiSeq	IonPGM/Proton	MassARRAY
TruSeq Amplicon Cancer Panel	Ion AmpliSeq Cancer Panel	LungCarta

Assay platform	DNA (ng)
LungCarta Panel (MassARRAY)	~240 ng
Ion AmpliSeq Cancer Panel (IonPGM)	**10 ng**
TruSeq Amplicon Cancer Panel (MiSeq)	~250 ng

Ion AmpliSeq™ Cancer Hotspot Panel v2

Cancer related 50 genes									
ABL1	*BRAF*	*EGFR*	*FGFR1*	*GNAQ*	*IDH2*	*KRAS*	*NPM1*	*PTPN11*	*SMO*
AKT1	*CDH1*	*ERBB2*	*FGFR2*	*GNAS*	*JAK2*	*MET*	*NRAS*	*RB1*	*SRC*
ALK	*CDKN2A*	*ERBB4*	*FGFR3*	*HNF1A*	*JAK3*	*MLH1*	*PDGFRA*	*RET*	*STK11*
APC	*CSF1R*	*EZH2*	*FLT3*	*HRAS*	*KDR*	*MPL*	*PIK3CA*	*SMAD4*	*TP53*
ATM	*CTNNB1*	*FBXW7*	*GNA11*	*IDH1*	*KIT*	*NOTCH1*	*PTEN*	*SMARCB1*	*VHL*

Ion AmpliSeq™ Colon and Lung Cancer Research Panel v2

Colon and Lung Cancer related 22 genes							
AKT1	*CTNNB1*	*ERBB2*	*FGFR1*	*KRAS*	*NOTCH1*	*PTEN*	*TP53*
ALK	*DDR2*	*ERBB4*	*FGFR2*	*MAP2K1*	*NRAS*	*SMAD4*	
BRAF	*EGFR*	*FBX7*	*FGFR3*	*MET*	*PIK3CA*	*STK11*	

RNA Fusion Lung Cancer Research Panel

Lung Fusion			
ALK	*RET*	*ROS1*	*NTRK1*

Custom Panel for prescreening of FGF/FGFR alteration

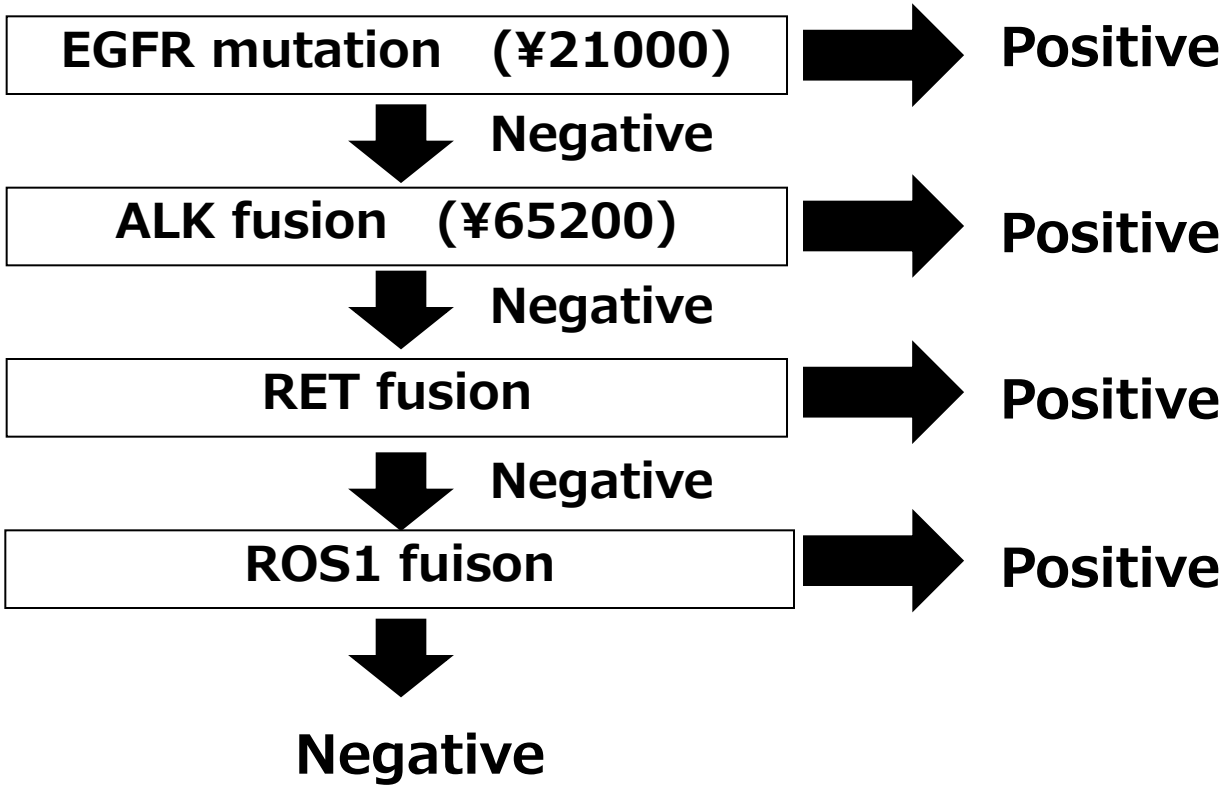
FGFR		
FGFR1	*FGFR2*	*FGFR3*

FGFR fusion		
FGFR1	*FGFR2*	*FGFR3*

Adaptor						
GRB2	*GAB1*	*SOS1*	*FRS2*	*PTPN11*	*SHC1*	*SHB*

Ext RAS/BRAF panel

The decision step on detecting driver mutation of NSCLC in clinical practice



- ✓ These genes are exclusive.
- ✓ More than 200,000 yen for a scan of their genetic profile.
- ✓ It takes approximately 1 month to evaluate gene mutations.

Lung cancer susp

Imaging CT/PET → Bronchoscopy → Pathological exam → EGFR mutation / ALK-IHC / ALK-FISH / ROS 1/RET

Multiplex assay is necessary.

Toward CoDx; from single to multi

Health and Labor Scientific Research Grants:Research on development of new drugs (H25-010)

2013 | 2014 | 2015 | 2016

MassArray

Device approval (FDA) → Device approval

LungCarta Panel	Feasibility Study	PMDA Preliminary consultation	*design* decision	Basic Performance	GMP production transfer	SOP	Clinical performance test	application for approval	
LungFusion kit	Feasibility Study	PMDA Preliminary consultation	*design* decision	Basic Performance	GMP production transfer	SOP	Clinical performance test	application for approval	

Ion Torrent PGM

Device approval (FDA) → Device approval

ColonLung Panel

LungFusion Panel

Feasibility Study	PMDA Preliminary consultation	*design* decision	Basic Performance	GMP production transfer	SOP	Clinical performance test	application for approval

OncoNetwork Consortium

1st panel

Colon and Lung cancer genes hot spot panel

KRAS, EGFR, BRAF, PIK3CA, AKT1 e.g.

2nd panel

ALK, RET, ROS1 Fusion panel

EML4-ALK, KIF5B-RET
FGFR-fusions e.g.
(on-going)

Ion AmpliSeq™ RNA Lung Fusion Panel

OncoNetwork Consortium

Prof. Harriet Feilotter
Dr Paul Park
Queen's University, Ontario
Canada

Prof. Ian Cree
Warwick Medical School
United Kingdom

Marjolijn J.L. Ligtenberg
Dr. Bastiaan Tops
Radboud University Nijmegen
Medical Centre, Netherlands

Cecily P. Vaughn
ARUP- Institute for Clinical and
Experimental Pathology
Utah, USA

Dr. Cristoph Noppen &
Dr. Henriette Kurth
VIOLLIER AG Basel ,
Switzerland

Prof. Orla Sheils
St James's Hospital Dublin,
Ireland

Prof. Kazuto Nishio
Faculty of Medicine, Kinki
University Osaka, Japan

Prof. Pierre Laurent Puig
Université Paris Descartes,
Paris, France

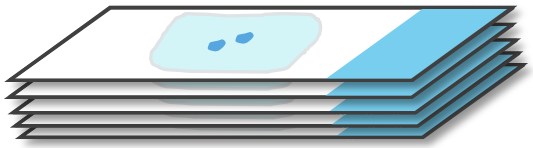
Prof. Aldo Scarpa
ARC-NET University of
Verona Italy

Dr. Ludovic Lacroix
Institut Gustave Roussy
Paris, France

Dr. Jose Costa
IPATIMUP Medical Faculty of Porto.
Portugal

Dr. Nicola Normanno
Centro Ricerche Oncologiche
Mercogliano, Italy

Screening of mutation and fusion in lung cancer



AllPrep DNA/RNA FFPE kit

10 ng DNA

Colon and Lung cancer genes hot spot panel

KRAS, EGFR, BRAF, PIK3CA, AKT1…
22 genes

Mutation：DNA

10 ng RNA

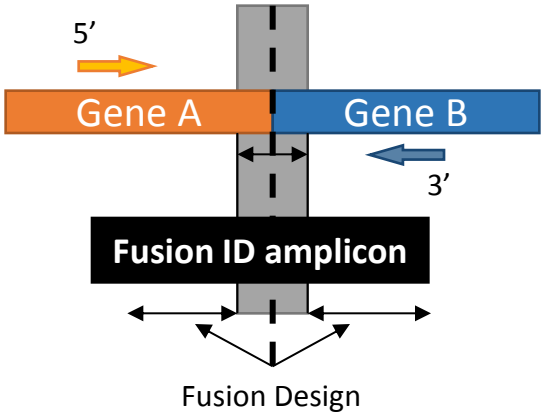
***ALK, RET, ROS1* Fusion panel**

EML4-ALK, KIF5B-RET, CD74-ROS1 …

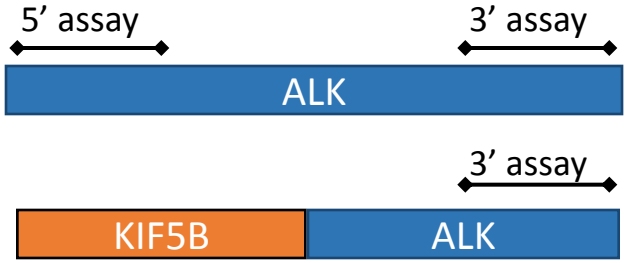
Fusion：RNA

Primer design of RNA Fusion Lung Cancer Research Panel

Fusion assays



Gene expression assays



Fusion specific amplicon provides nucleotide sequence through the junction of the two fusion genes

5’ amplicon and 3’ amplicon for ALK mRNA provides additional information on expression levels. Equivalent levels will be observed in normal samples, 3’ bias will be present in samples with ALK translocation

Susan M. Magdaleno et al, 2014 AACR annual meeting

RNA Fusion Lung Cancer Research Panel

***ALK* fusion**	*EML4-ALK.E6A19.COSF1296*
	EML4-ALK.E6A19.COSF1296.1
	EML4-ALK.E2A20.COSF479
	EML4-ALK.E2A20.COSF479.1
	EML4-ALK.E15A20.COSF413
	EML4-ALK.E17A20.COSF1367
	EML4-ALK.E17A20.COSF1367.1
	EML4-ALK.E17A20.COSF1366
	EML4-ALK.E17A20.COSF1366.1
	EML4-ALK.E14A20.COSF477
	EML4-ALK.E14A20.COSF477.1
	EML4-ALK.E13A20.COSF1062
	EML4-ALK.E13A20.COSF1062.1
	EML4-ALK.E13A20.COSF408
	EML4-ALK.E13A20.AB462411
	EML4-ALK.E18A20.COSF487
	EML4-ALK.E18A20.COSF487.1
	EML4-ALK.E2A20.COSF478
	EML4-ALK.E2A20.COSF478.1
	EML4-ALK.E20A20.COSF409
	EML4-ALK.E20A20.COSF409.1
	EML4-ALK.E20A20.COSF730
	EML4-ALK.E20A20.COSF730.1
	EML4-ALK.E6aA20.COSF411
	EML4-ALK.E6aA20.AB374361
	EML4-ALK.E6bA20.COSF412
	EML4-ALK.E6bA20.COSF412.1
	EML4-ALK.E6bA20.AB374362
	KIF5B-ALK.K15A20.COSF1381
	KIF5B-ALK.K15A20.COSF1060
	KIF5B-ALK.K17A20.COSF1257
	KIF5B-ALK.K24A20.COSF1058
	KLC1-ALK.K9A20.COSF1276
	EML4-ALK.E14A20.COSF1064
	HIP1-ALK.H28A20
	HIP1-ALK.H21A20
	TPR-ALK.T15A20

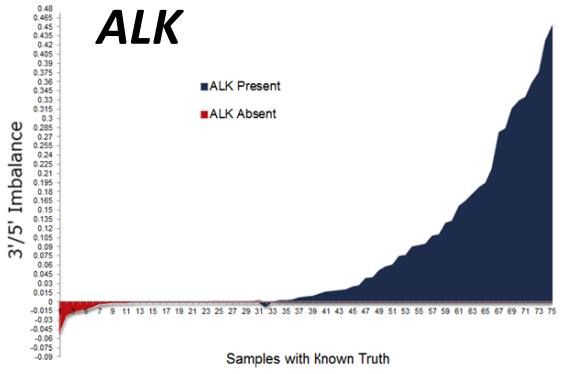
***RET* fusion**	*KIF5B-RET.K15R11.COSF1255*
	KIF5B-RET.K24R11.COSF1262
	KIF5B-RET.K24R8.COSF1236
	CCDC6-RET.C1R12.COSF1271
	KIF5B-RET.K15R12.COSF1232
	KIF5B-RET.K16R12.COSF1230
	KIF5B-RET.K22R12.COSF1253
	KIF5B-RET.K23R12.COSF1234
	CUX1-RET.C10R12
***ROS1* fusion**	*CD74-ROS1.C6R32.COSF1202*
	SDC4-ROS1.S2R32.COSF1265
	SDC4-ROS1.S4R32.COSF1278
	SLC34A2-ROS1.S13R32.COSF1259
	SLC34A2-ROS1.S4R32.COSF1197
	SDC4-ROS1.S2R34
	SDC4-ROS1.S4R34.COSF1280
	CD74-ROS1.C6R34.COSF1200
	EZR-ROS1.E10R34.COSF1267
	SLC34A2-ROS1.S4R34.COSF1198
	SLC34A2-ROS1.S13R34.COSF1261
	TPM3-ROS1.T7R35.COSF1273
	LRIG3-ROS1.L16R35.COSF1269
	GOPC-ROS1.G8R35.COSF1139
	GOPC-ROS1.G4R36.COSF1188
***NTRK1* fusion**	*CEL-NTRK1.C7N7*
	NFASC-NTRK1.N18N10
	IRF2BP2-NTRK1.I1N10
	TFG-NTRK1.T5N10
	SQSTM1-NTRK1.S5N10
	SSBP2-NTRK1.S12N12
	NTRK1-DYNC2H1.N17D86
	CD74-NTRK1.C3N13
	MPRIP-NTRK1.M14N13
	MPRIP-NTRK1.M18N13
	MPRIP-NTRK1.M21N13

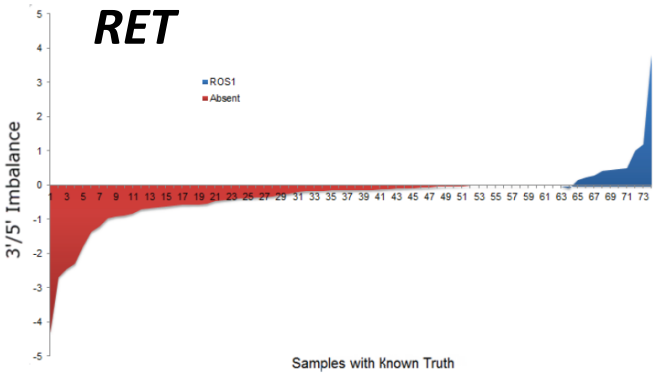
HK expression	*HMBS.ENCTRL.E8E9*
	TBP.ENCTRL.E3E4
	ITGB7.ENCTRL.E14E15
	MYC.ENCTRL.E2E3
	LMNA.ENCTRL.E3E4
expression	*ALK.5p_NM_004304.4.e5e6*
	ALK.3p_NM_004304.4.e23e24
	RET.5p_NM_020975.4.e6e7
	RET.3p_NM_020975.4.e18e19
	ROS1.5p_NM_002944.2.e11e12
	ROS1.3p_NM_002944.2.e38e39
	NTRK1.5p.eNST00000392302.e2e3
	NTRK1.3p.eNST00000392302.e17e18

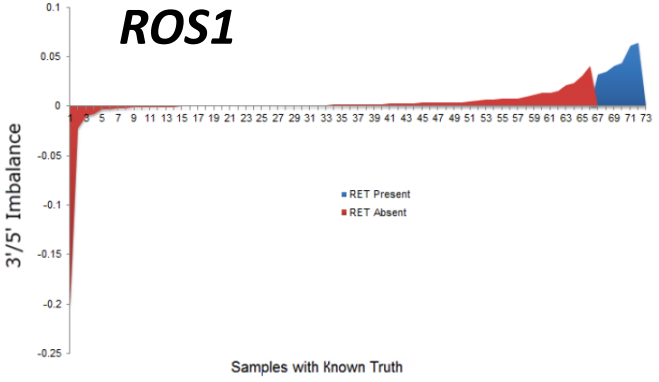
3’/5’ Imbalance in RNA Fusion Lung Cancer Research Panel

(3' reads - 5' reads) / (sum of all EXPR_CONTROL reads)

	No Evidence of a Fusion	Uncertain	Strong Evidence of a Fusion
ALK	≦0.001	0.001-0.025	≧0.025
RET	≦0.03	0.03-0.045	≧0.045
ROS1	≦0.2	0.2-0.5	≧0. 5
NTRK1	-	-	-



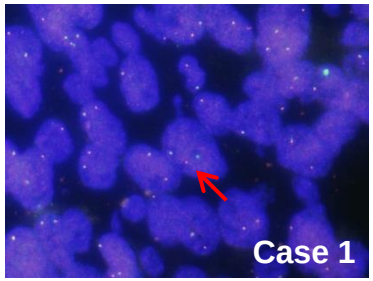




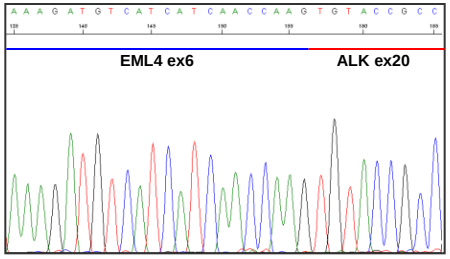
β-test in Kindai Univ

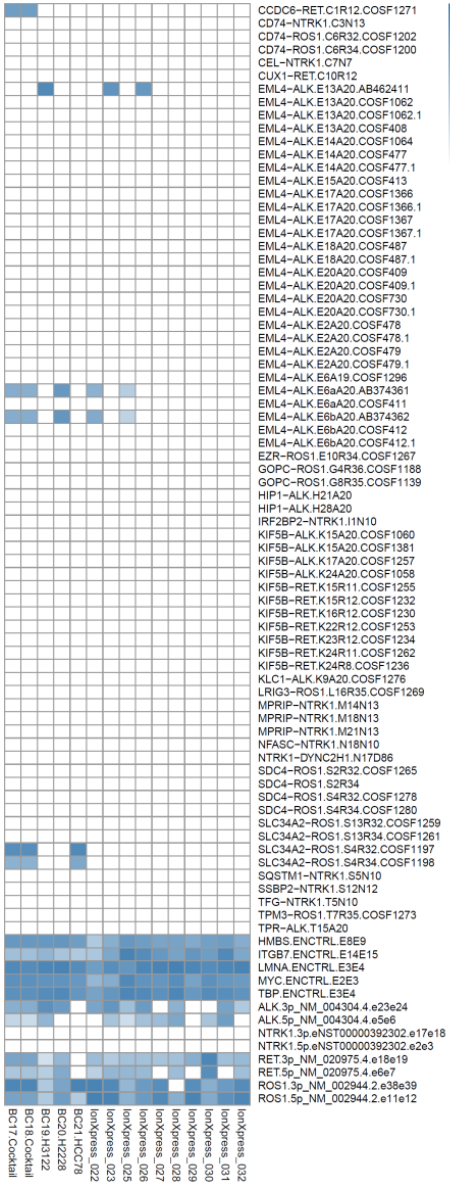
Fusion + cell lines; 3
EML4-ALK pos; 4
EML4-ALK negative: 6

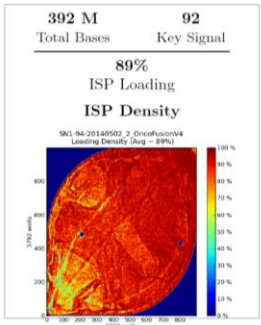
EML4-ALK positive

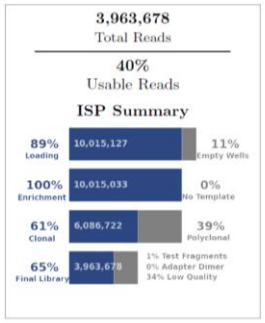


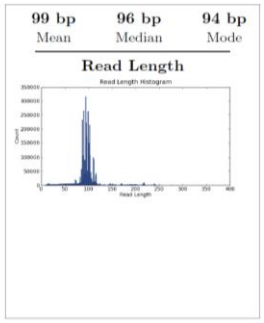
Case 1: variant 3a







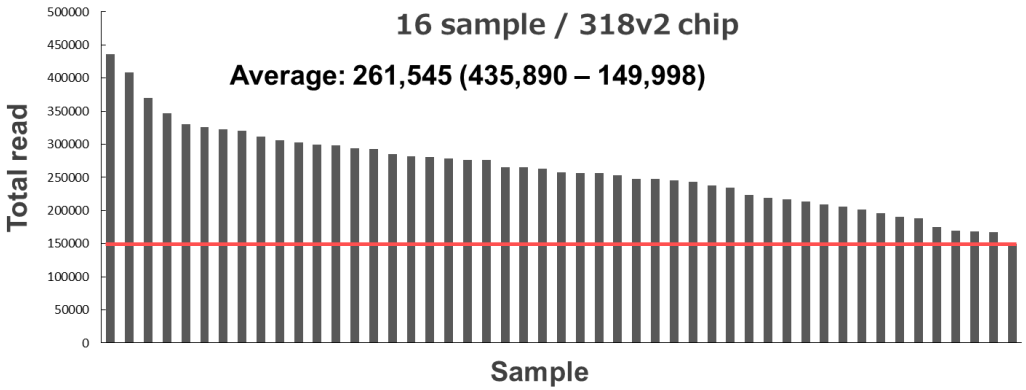




Assay QC

- Good run quality metrics overall
- Good distribution of reads to barcodes
- Good expression of all 5 housekeeping genes
- Detect *EML4-ALK* fusion transcripts down to 1% tumor RNA input

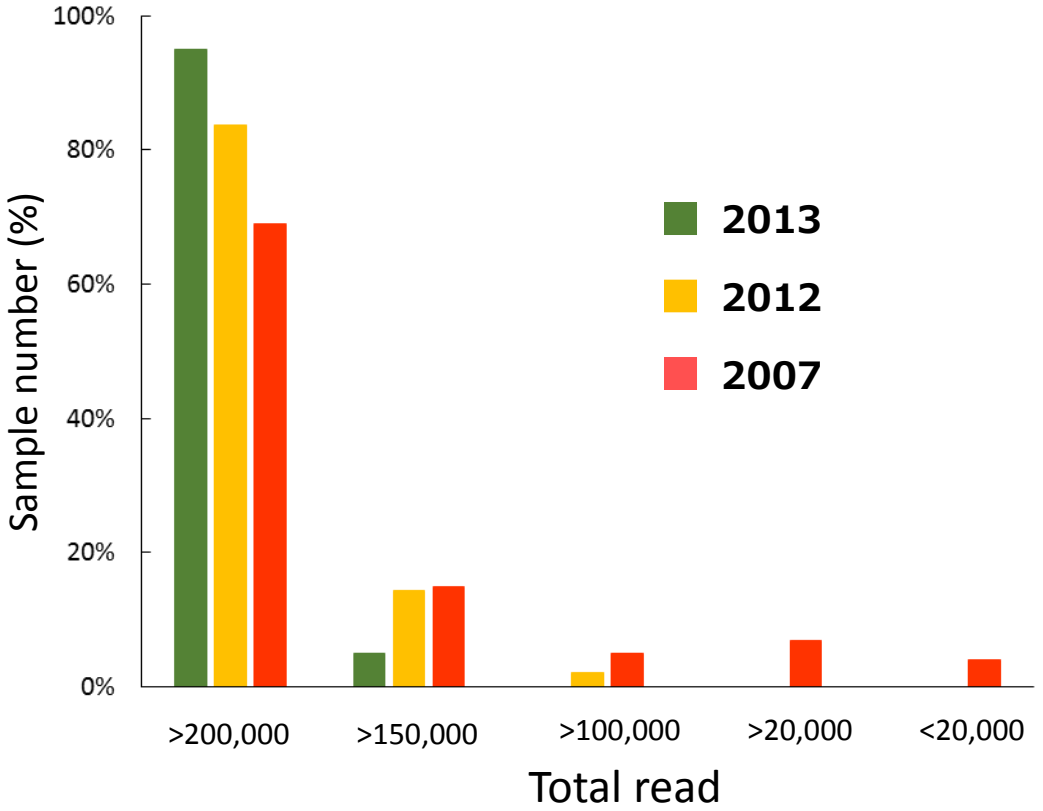
RNA Fusion Lung Cancer Research Panel for FFPE samples (in house)



samples

2007 : 102
2012 : 49
2013 : 20

>20,000 read: evaluable



Representative results
-RNA Fusion Lung Cancer Research Panel

Fusion negative

	7	4
EML4-ALK.E13A20.COSF408	0	0
EML4-ALK.E13A20.AB462411	0	0
EML4-ALK.E18A20.COSF487	0	0
EML4-ALK.E18A20.COSF487.1	0	0
EML4-ALK.E2A20.COSF478	0	0
EML4-ALK.E2A20.COSF478.1	0	0
EML4-ALK.E20A20.COSF409	0	0
EML4-ALK.E20A20.COSF409.1	0	0
EML4-ALK.E20A20.COSF730	0	0
EML4-ALK.E20A20.COSF730.1	0	0
EML4-ALK.E6aA20.COSF411	0	0
EML4-ALK.E6aA20.AB374361	0	0
EML4-ALK.E6bA20.COSF412	0	0
EML4-ALK.E6bA20.COSF412.1	0	0
EML4-ALK.E6bA20.AB374362	0	0
KIF5B-ALK.K15A20.COSF1381	0	0
KIF5B-ALK.K15A20.COSF1060	0	0
KIF5B-ALK.K17A20.COSF1257	0	0
KIF5B-ALK.K24A20.COSF1058	0	0
KLC1-ALK.K9A20.COSF1276	0	0
EML4-ALK.E14A20.COSF1064	0	0
HMBS.ENCTRL.E8E9	3312	1070
TBP.ENCTRL.E3E4	66250	26569
ITGB7.ENCTRL.E14E15	10089	4471
MYC.ENCTRL.E2E3	11069	4120
LMNA.ENCTRL.E3E4	154647	26940
ALK.5p_NM_004304.4.e5e6	98	67
ALK.3p_NM_004304.4.e23e24	136	23
RET.5p_NM_020975.4.e6e7	897	275
RET.3p_NM_020975.4.e18e19	887	475
ROS1.5p_NM_002944.2.e11e12	92613	154553
ROS1.3p_NM_002944.2.e38e39	77300	124608
NTRK1.5p.eNST00000392302.e2e3	0	3
NTRK1.3p.eNST00000392302.e17e18	141	62
ALK	0.0001	-0.0006
RET	0	0.0034
ROS1	-0.0611	-0.4864
NTRK1	0.0006	0.0009

ALK fusion positive

	48	90	31
EML4-ALK.E13A20.COSF408	0	0	0
EML4-ALK.E13A20.AB462411	1304	0	0
EML4-ALK.E18A20.COSF487	0	0	0
EML4-ALK.E18A20.COSF487.1	0	0	0
EML4-ALK.E2A20.COSF478	0	0	0
EML4-ALK.E2A20.COSF478.1	0	0	0
EML4-ALK.E20A20.COSF409	0	0	0
EML4-ALK.E20A20.COSF409.1	0	0	0
EML4-ALK.E20A20.COSF730	0	0	0
EML4-ALK.E20A20.COSF730.1	0	0	0
EML4-ALK.E6aA20.COSF411	0	0	0
EML4-ALK.E6aA20.AB374361	0	4130	1136
EML4-ALK.E6bA20.COSF412	0	0	0
EML4-ALK.E6bA20.COSF412.1	0	0	0
EML4-ALK.E6bA20.AB374362	0	0	0
KIF5B-ALK.K15A20.COSF1381	0	0	0
KIF5B-ALK.K15A20.COSF1060	0	0	0
KIF5B-ALK.K17A20.COSF1257	0	0	0
KIF5B-ALK.K24A20.COSF1058	0	0	0
KLC1-ALK.K9A20.COSF1276	0	0	0
EML4-ALK.E14A20.COSF1064	0	0	0
HMBS.ENCTRL.E8E9	909	1810	558
TBP.ENCTRL.E3E4	11888	27167	8988
ITGB7.ENCTRL.E14E15	1374	9153	3053
MYC.ENCTRL.E2E3	8475	5731	2358
LMNA.ENCTRL.E3E4	50817	136966	24333
ALK.5p_NM_004304.4.e5e6	59	0	0
ALK.3p_NM_004304.4.e23e24	5096	12253	2611
RET.5p_NM_020975.4.e6e7	65	0	0
RET.3p_NM_020975.4.e18e19	114	473	32
ROS1.5p_NM_002944.2.e11e12	77966	215607	179983
ROS1.3p_NM_002944.2.e38e39	66958	130706	85912
NTRK1.5p.eNST00000392302.e2e3	0	0	0
NTRK1.3p.eNST00000392302.e17e18	34	155	0
ALK	0.0678	0.0659	0.0645
RET	0.0005	0.0026	0.0008
ROS1	-0.1628	-0.4686	-2.3783
NTRK1	0.0005	0.0014	0

ROS1 fusion positive

	89
CCDC6-RET.C1R12.COSF1271	0
KIF5B-RET.K15R12.COSF1232	0
KIF5B-RET.K16R12.COSF1230	0
KIF5B-RET.K22R12.COSF1253	0
KIF5B-RET.K23R12.COSF1234	0
CD74-ROS1.C6R32.COSF1202	0
SDC4-ROS1.S2R32.COSF1265	0
SDC4-ROS1.S4R32.COSF1278	0
SLC34A2-ROS1.S13R32.COSF1259	0
SLC34A2-ROS1.S4R32.COSF1197	204841
SDC4-ROS1.S2R34	0
SDC4-ROS1.S4R34.COSF1280	0
CD74-ROS1.C6R34.COSF1200	0
EZR-ROS1.E10R34.COSF1267	0
SLC34A2-ROS1.S4R34.COSF1198	8730
SLC34A2-ROS1.S13R34.COSF1261	0
TPM3-ROS1.T7R35.COSF1273	0
LRIG3-ROS1.L16R35.COSF1269	0
GOPC-ROS1.G8R35.COSF1139	0
GOPC-ROS1.G4R36.COSF1188	0
CEL-NTRK1.C7N7	0
HMBS.ENCTRL.E8E9	155
TBP.ENCTRL.E3E4	936
ITGB7.ENCTRL.E14E15	191
MYC.ENCTRL.E2E3	689
LMNA.ENCTRL.E3E4	18799
ALK.5p_NM_004304.4.e5e6	0
ALK.3p_NM_004304.4.e23e24	0
RET.5p_NM_020975.4.e6e7	0
RET.3p_NM_020975.4.e18e19	211
ROS1.5p_NM_002944.2.e11e12	46594
ROS1.3p_NM_002944.2.e38e39	182881
NTRK1.5p.eNST00000392302.e2e3	0
NTRK1.3p.eNST00000392302.e17e18	0
ALK	0
RET	0.01
ROS1	6.552
NTRK1	0

-RNA Fusion Lung Cancer Research Panel

EML4-ALK.E6bA20.COSF412	0	0
EML4-ALK.E6bA20.COSF412.1	0	0
EML4-ALK.E6bA20.AB374362	0	0
KIF5B-ALK.K15A20.COSF1381	0	0
KIF5B-ALK.K15A20.COSF1060	0	0
KIF5B-ALK.K17A20.COSF1257	0	0
KIF5B-ALK.K24A20.COSF1058	0	0
KLC1-ALK.K9A20.COSF1276	0	0
EML4-ALK.E14A20.COSF1064	0	0
HIP1-ALK.H28A20	0	0
HIP1-ALK.H21A20	0	0
TPR-ALK.T15A20	0	0
KIF5B-RET.K15R11.COSF1255	0	0
KIF5B-RET.K24R11.COSF1262	0	0
KIF5B-RET.K24R8.COSF1236	0	0
CCDC6-RET.C1R12.COSF1271	0	0

HMBS.ENCTRL.E8E9	3877	499
TBP.ENCTRL.E3E4	48338	30509
ITGB7.ENCTRL.E14E15	4299	718
MYC.ENCTRL.E2E3	41144	13383
LMNA.ENCTRL.E3E4	75806	90876
ALK.5p_NM_004304.4.e5e6	41	0
ALK.3p_NM_004304.4.e23e24	71	3316
RET.5p_NM_020975.4.e6e7	32949	62
RET.3p_NM_020975.4.e18e19	60576	179
ROS1.5p_NM_002944.2.e11e12	2975	35232
ROS1.3p_NM_002944.2.e38e39	1807	17161
NTRK1.5p.eNST00000392302.e2e3	0	13
NTRK1.3p.eNST00000392302.e17e18	92	56

ALK	0.000	0.024
RET	0.170	0.001
ROS1	-0.007	-0.133
NTRK1	0.001	0.000

5’/3’Imbalance

Assay sensitivity; ~1%

Success rate; 97.6% (167/171) for FFPE samples (2007-2012)

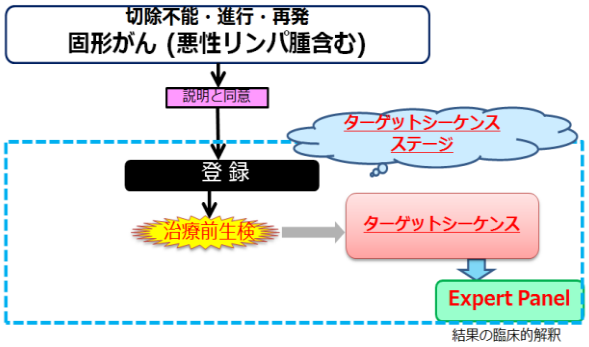
	No Evidence of a Fusion	Uncertain	Strong Evidence of a Fusion
ALK	≦0.001	0.001-0.025	≧0.025
RET	≦0.03	0.03-0.045	≧0.045
ROS1	≦0.2	0.2-0.5	≧0. 5

New fusions?

RACE on-going

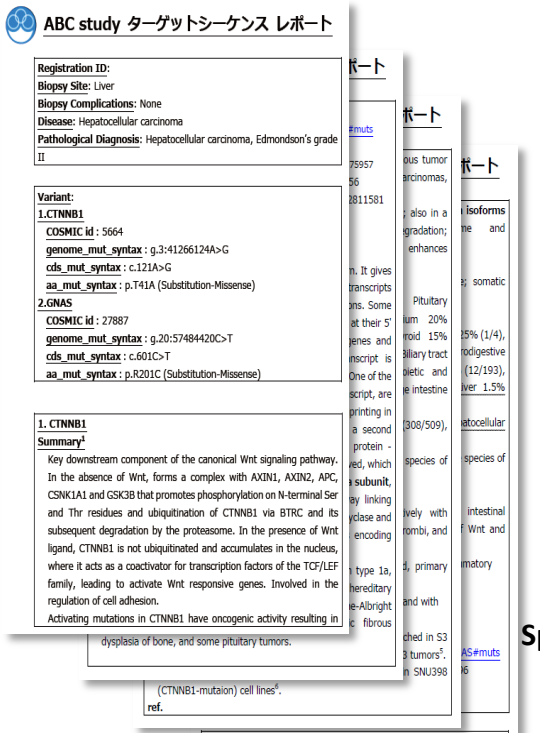
MADRID 2014 ESMO congress

Clinical Sequencing in Japan



***Pan-Cancer* Multi-Gene Cancer Panel**

Targetting report NCCE



ABC study ターゲットシーケンス レポート

Registration ID:
Biopsy Site: Liver
Biopsy Complications: None
Disease: Hepatocellular carcinoma
Pathological Diagnosis: Hepatocellular carcinoma, Edmondson's grade II

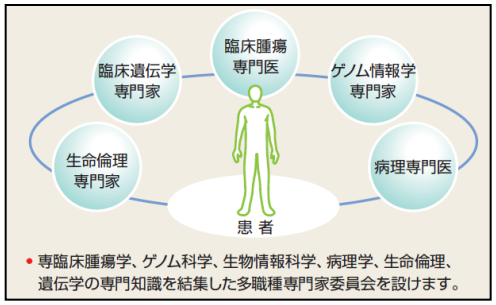
Variant:
1.CTNNB1
COSMIC id : 5664
genome_mut_syntax : g.3:41266124A>G
cds_mut_syntax : c.121A>G
aa_mut_syntax : p.T41A (Substitution-Missense)
2.GNAS
COSMIC id : 27887
genome_mut_syntax : g.20:57484420C>T
cds_mut_syntax : c.601C>T
aa_mut_syntax : p.R201C (Substitution-Missense)

1. CTNNB1
Summary[1]
Key downstream component of the canonical Wnt signaling pathway. In the absence of Wnt, forms a complex with AXIN1, AXIN2, APC, CSNK1A1 and GSK3B that promotes phosphorylation on N-terminal Ser and Thr residues and ubiquitination of CTNNB1 via BTRC and its subsequent degradation by the proteasome. In the presence of Wnt ligand, CTNNB1 is not ubiquitinated and accumulates in the nucleus, where it acts as a coactivator for transcription factors of the TCF/LEF family, leading to activate Wnt responsive genes. Involved in the regulation of cell adhesion.
Activating mutations in CTNNB1 have oncogenic activity resulting in

dysplasia of bone, and some pituitary tumors.

(CTNNB1-mutaion) cell lines[6].
ref.

Expert panel



Spain

KINDAI Clinical Sequencing Report

Registration ID:	*13-0002*
Block ID:	P12-3275-2, 9, 10, 15
Tumor Site:	Colon
Disease:	colorectal cancer
Pathological Diagnosis:	adenocarcinoma, sigmoid colon, resection

Assay Summary

DNA yield was enough. A260/A230 was >1.8. A260/A280 was >2.0. Twenty ng of DNA was applied to l
library preparation. DNA library yield was enough. Data volume was ~80Mb/sample. Run Summary was show
Appendix 1.
Sequencing was done for 5 specimens consisted with adenocarcinoma (block no 2 and 10) and polyps (block n
and 15). The duplicated samples of no.9 (9a and9b) were analyzed using Ion AmpliSeq Cancer Hotspot Panel
Gene list in this panel was as follows; *ABL1 EZH2 JAK3 PTEN AKT1 FBXW7 IDH2 PTPN11 ALK FGFR1 KI*
RB1 APC FGFR2 KIT RET ATM FGFR3 KRAS SMAD4 BRAF FLT3 MET SMARCB1 CDH1 GNA11 MLH1 S
CDKN2A GNAS MPL SRC CSF1R GNAQ NOTCH1 STK11 CTNNB1 HNF1A NPM1 TP53 EGFR HRAS NRAS
VHL ERBB2 IDH1 PDGFRA ERBB4 JAK2 PIK3CA.
The genes called as variants are listed as Table 1. The genes are selected by Valiant Caller Ver.3.4 (Life
Technologies). The genes with enough total and variant coverage are listed in Table 1. SNP was defined as the
variants observed in Japanese population frequently (>50%) (Table 2). The genes with Cosmic ID (designated
HotSpot) might be functional.

Ion AmpliSeq™ Cancer Hotspot Panel v2

Cancer related 50 genes									
ABL1	*BRAF*	*EGFR*	*FGFR1*	*GNAQ*	*IDH2*	*KRAS*	*NPM1*	*PTPN11*	*SMO*
AKT1	*CDH1*	*ERBB2*	*FGFR2*	*GNAS*	*JAK2*	*MET*	*NRAS*	*RB1*	*SRC*
ALK	*CDKN2A*	*ERBB4*	*FGFR3*	*HNF1A*	*JAK3*	*MLH1*	*PDGFRA*	*RET*	*STK11*
APC	*CSF1R*	*EZH2*	*FLT3*	*HRAS*	*KDR*	*MPL*	*PIK3CA*	*SMAD4*	*TP53*
ATM	*CTNNB1*	*FBXW7*	*GNA11*	*IDH1*	*KIT*	*NOTCH1*	*PTEN*	*SMARCB1*	*VHL*

Ion AmpliSeq™ Colon and Lung Cancer Research Panel v2

Colon and Lung Cancer related 22 genes							
AKT1	*CTNNB1*	*ERBB2*	*FGFR1*	*KRAS*	*NOTCH1*	*PTEN*	*TP53*
ALK	*DDR2*	*ERBB4*	*FGFR2*	*MAP2K1*	*NRAS*	*SMAD4*	
BRAF	*EGFR*	*FBX7*	*FGFR3*	*MET*	*PIK3CA*	*STK11*	

RNA Fusion Lung Cancer Research Panel

Lung Fusion			
ALK	*RET*	*ROS1*	*NTRK1*

Custom Panel for prescreening of FGF/FGFR alteration

FGFR		
FGFR1	*FGFR2*	*FGFR3*

FGFR fusion		
FGFR1	*FGFR2*	*FGFR3*

Adaptor						
GRB2	*GAB1*	*SOS1*	*FRS2*	*PTPN11*	*SHC1*	*SHB*

Ext RAS/BRAF panel

Custom FGFR fusion panel for prescreening of FGF/FGFR alteration

Fusion gene list in panel

FGFR fusion		
FGFR1	*FGFR2*	*FGFR3*

Genes	# of types	Fusion name
FGFR1	2 type	BAG4_FGFR1 and ERLIN2_FGFR1
FGFR2	7 type	FGFR2_AFF3, FGFR2_BICC1, FGFR2_CASP7, FGFR2_CCDC6, FGFR2_KIAA1967, FGFR2_OFD1 and SLC45A3_FGFR2
FGFR3	4 type	FGFR3_BAIAP2L1, FGFR3_TACC3_ex10, FGFR3_TACC3_ex11 and FGFR3_TACC3_ex4

Add to Gene Expression and endogenous control (FGFR1, FGFR2, FGFR3, HMBS, TBP, ITGB7, MYC, and LMNA)

Fusion junction detail

Fusion name	**5' Gene**	**Junction Sequence**	**3' Gene**
BAG4_FGFR1	*BAG4 exon 2*	AGACCAGAATTGCAAGGCCAG|GTCCGTTATGCCACCTGGAGC	*FGFR1* exon 9
ERLIN2_FGFR1	*ERLIN2* exon 10	CCGCAGAAACTACGAGTTGAT|GGTCAGTTTGAAAAGGAGGAT	*FGFR1* exon 4
FGFR2_AFF3	*FGFR2* exon 19	CTCACTCTCACAACCAATGAG|GAGAGTAGATCTGGAGAAACC	*AFF3* exon 8
FGFR2_BICC1	*FGFR2* exon 19	CTCACTCTCACAACCAATGAG|ATCATGGAGGAAACAAATACG	*BICC1* exon 3
FGFR2_CASP7	*FGFR2* exon 19	CTCACTCTCACAACCAATGAG|ATGGCAGATGATCAGGGCTGT	*CASP7* exon 4
FGFR2_CCDC6	*FGFR2* exon 19	CTCACTCTCACAACCAATGAG|CAAGCCAGGGCTGAGCAGGAA	*CCDC6* exon 2
FGFR2_KIAA1967	*FGFR2* exon 19	CTCACTCTCACAACCAATGAG|GGTGGGGAGAAACAGCGGGTC	*KIAA1967* exon 5
FGFR2_OFD1	*FGFR2* exon 19	CTCACTCTCACAACCAATGAG|ACACAACTTCGAAACCAGCTA	*OFD1* exon 3
SLC45A3_FGFR2	*SLC45A3* exon 1	ACAGCCGCGCGCCTCGGCCAG|TGACTGCAGCAGCAGCGGCAG	*FGFR2* exon 3b
FGFR3_BAIAP2L1	*FGFR3* exon 18	CTTACCGTGACGTCCACCGAC|AATGTTATGGAACAGTTCAAT	*BAIAP2L1* exon 2
FGFR3_TACC3_ex10	*FGFR3* exon 18	CTTACCGTGACGTCCACCGAC|GTGCCAGGCCCACCCCCAGGT	*TACC3* exon 10
FGFR3_TACC3_ex11	*FGFR3* exon 18	CTTACCGTGACGTCCACCGAC|GTAAAGGCGACACAGGAGGAG	*TACC3* exon 11
FGFR3_TACC3_ex4	*FGFR3* intron 18	GCTGAGGTGTGGGGCGGGCCT|TCTGGCCCAGGTGCCCTGGCT	*TACC3* exon 4

Clinical samples (ESCC, gastric, and lung)

FGFR3-TACC3 positive

Low quality RNA

	55	46	28	56	37	43	42	44	16	3_4	1	2	4	6	3_2	5	3_3	3_1	7	49	10	19	11	8	68	47	21
BAG4-FGFR1.B2F6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ERLIN2-FGFR1.E8F2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0	0	0
FGFR2-AFF3.F17A7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FGFR2-BICC1.F17B3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FGFR2-CASP7.F17C2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FGFR2-CCDC6.F17C2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1164	0	0	0	0	0
FGFR2-KIAA1967_CCAR2.F17K4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FGFR2-OFD1.F17O3	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SLC45A3-FGFR2.S1F2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FGFR3-BAIAP2L1.F17B2.COSF1347	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FGFR3-TACC3.F17T10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FGFR3-TACC3.F17T11.COSF1352	0	0	0	0	0	55406	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FGFR3-TACC3.F18T4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CD74-NTRK1.C6N10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MPRIP-NTRK1.M21N12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FGFR1.E7E8	4563	846	5125	721	7267	44	2953	3695	379	617	224	115	92	340	882	260	1328	499	193	0	0	0	4	0	1	0	0
FGFR2.E8E9	0	397	188	536	0	192	0	29	0	510	286	72	17	107	1646	373	2561	1818	0	0	0	0	0	0	0	0	0
FGFR3.E7E8	0	137	0	492	0	162	0	0	0	26	10	0	0	8	43	26	82	0	0	0	0	0	0	1	0	0	0
NTRK1.E2E3	0	10	0	0	0	19	0	0	0	0	0	10	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
HMBS.ENCTRL.E8E9	569	215	172	723	0	429	0	0	250	205	207	144	301	93	69	199	59	67	214	0	0	0	2	1	0	0	0
TBP.ENCTRL.E3E4	15209	6814	19202	15398	22213	6105	6038	26430	11762	3644	7207	6137	1412	2606	6252	3043	2437	2493	2536	4	686	6	46	69	13	0	8
ITGB7.ENCTRL.E14E15	11053	1648	3186	3525	1511	2997	0	758	1501	1153	304	490	511	156	2086	1299	1294	717	347	0	261	1	5	6	0	0	0
MYC.ENCTRL.E2E3	17434	37819	16927	12881	6811	6495	2956	1613	5331	####	13835	8545	11454	5313	7867	7631	3489	1801	2630	5	556	7	42	22	20	3	0
LMNA.ENCTRL.E3E4	1E+05	1E+05	1E+05	1E+05	1E+05	48672	75975	35076	41503	####	30619	27131	23333	27229	####	20161	####	####	12635	5898	972	118	729	123	48	61	5

HEPATOLOGY
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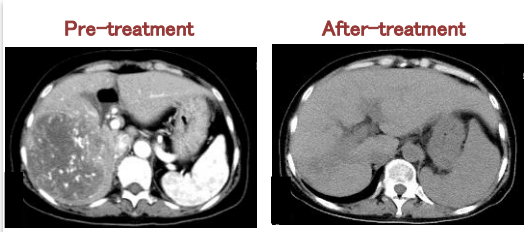
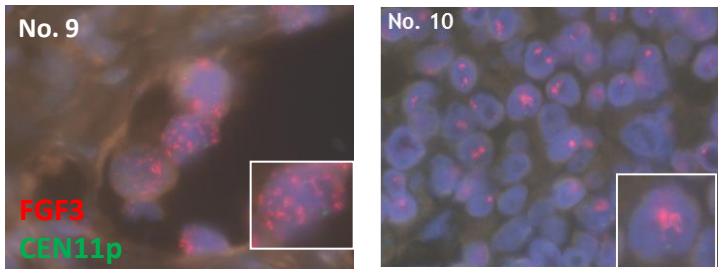
HEPATOBILIARY MALIGNANCIES

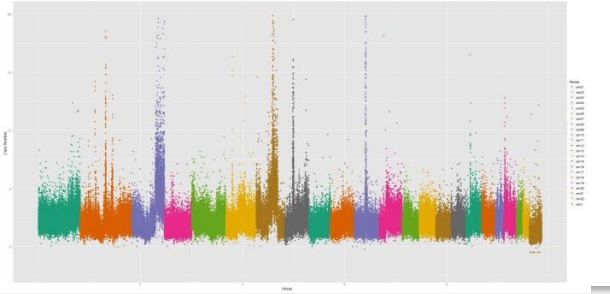
FGF3/FGF4 Amplification and Multiple Lung Metastases in Responders to Sorafenib in Hepatocellular Carcinoma

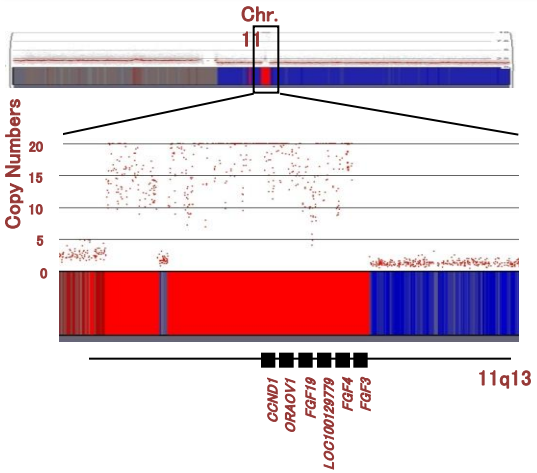
Tokuzo Arao,[1]* Kazuomi Ueshima,[2]* Kazuko Matsumoto,[1]* Tomoyuki Nagai,[1,2] Hideharu Kimura,[1]

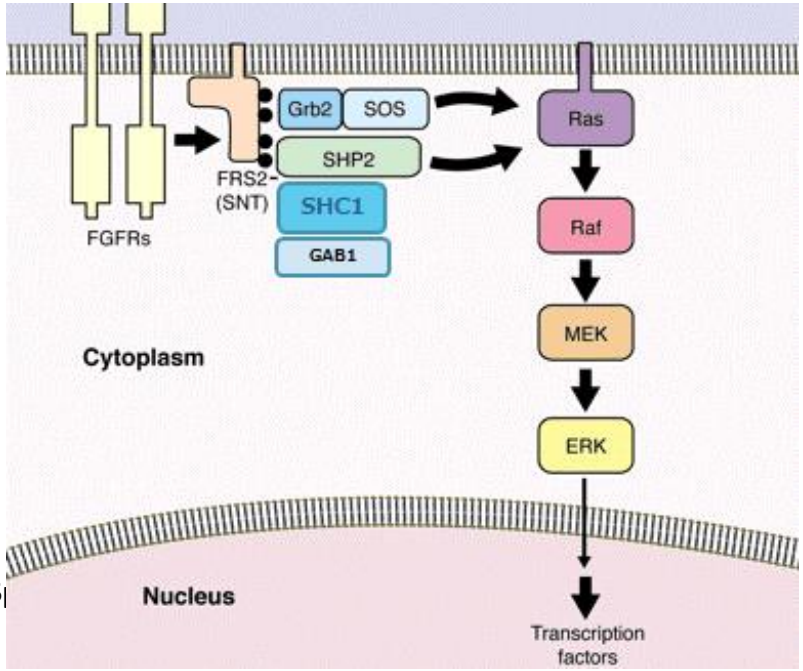
FGFR1 FGF3/4

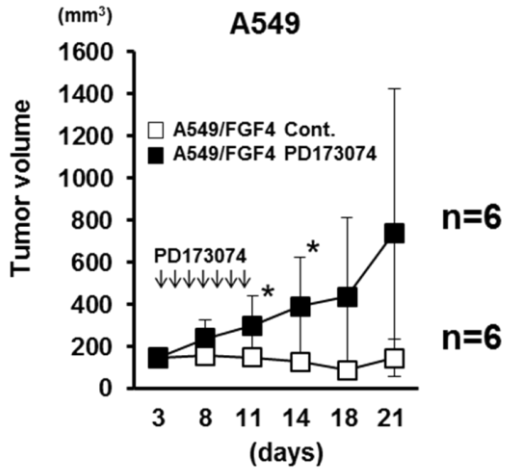




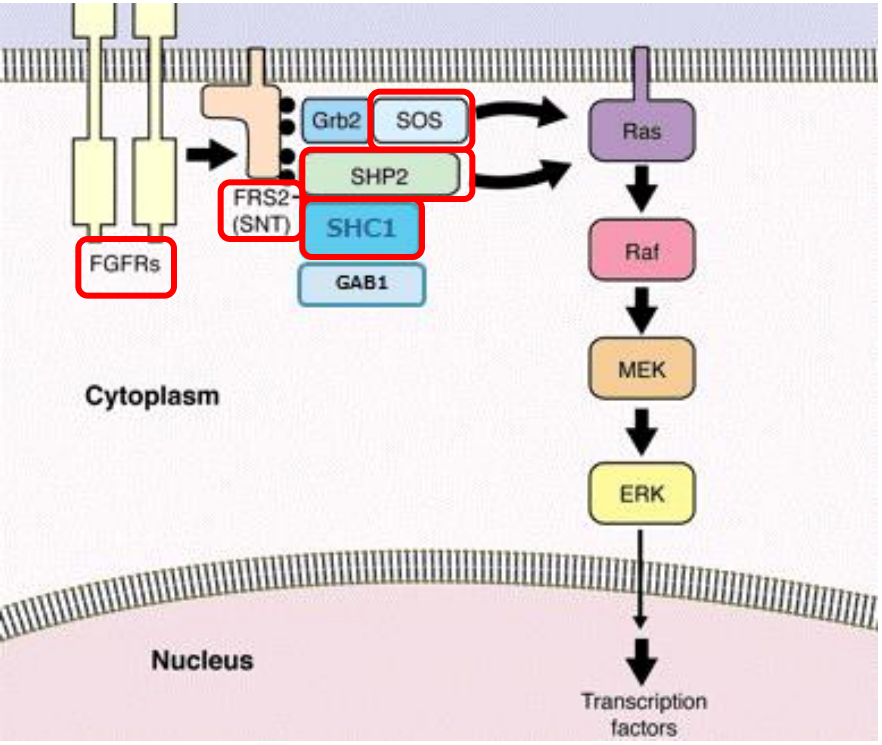








Detection of FGF-FGFRalt in responders by Kindai NGS adaptor panel



FGFR		
FGFR1	*FGFR2*	*FGFR3*

FGFR-fusion		
FGFR1	*FGFR2*	*FGFR3*

Adaptor						
GRB2	*GAB1*	*SOS1*	*FRS2*	*PTPN11*	*SHC1*	*SHB*

FGF CN		
FGF3	*FGF4*	*FGF19*

Chr	Gene			Location	AA change	Frequency
chr1	SHC1	Het	C/T	exonic	Non synominous	1/22
		Het	G/A	exonic	Non synominous	2/22
chr2	SOS1	Het	T/C	exonic	Non synominous	1/22
		Het	G/A	exonic	Non synominous	1/22
chr12	FRS2	Het	A/G	exonic	Non synominous	1/22
	PTPN11	Het	C/T	exonic	Non synominous	1/22

Chr	Gene			Location	AA change	Frequency
chr8	FGFR1	Het	G/A	exonic	Non synominous	4/14
		Het	G/A	exonic	Non synominous	1/14
		Het	G/A	exonic	Non synominous	1/14
		Het	G/A	exonic	Non synominous	1/14
chr10	FGFR2	Het	C/G	exonic	Non synominous	2/14
		Het	A/G	exonic	Non synominous	1/14
		Het	C/T	exonic	Non synominous	1/14
chr4	FGFR3	Het	G/A	exonic	Non synominous	1/14
		Het	G/A	exonic	Non synominous	5/14

Prescreening system of FGF/FGFRalt for FGFRi

Prescreeening in multi-center group (~300 inst)
At least one prior systemic or locoregional therapy, PS0-2

send FFPE or blood samples from primary or metastatic sites to Kinki Univ.

FGF/FGFR alt (NGS and ddPCR for FFPE and liquid biopsy)

(+): Registration

N=20-30
FGFR-I

Primary endpoint: RR
Secondary endpoints: DCR, PFS, OS, duration of DC
safety

(-)

Physician's choice

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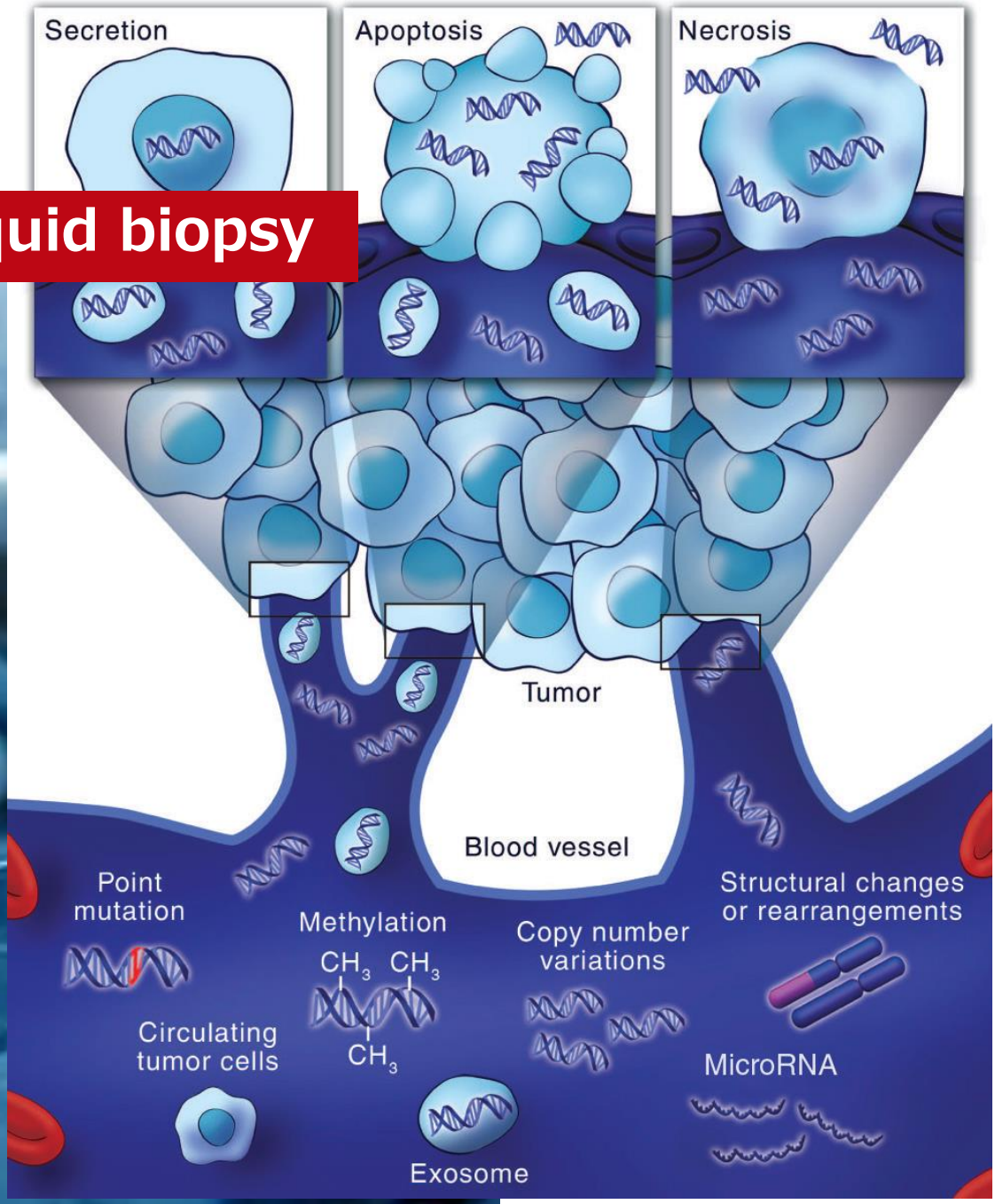
ddPCR and NGS for liquid biopsy



Luis A. Diaz Jr and Alberto Bardelli.
J Clin Oncol 32, 579-586, 2014

Ion AmpliSeq™ Cancer Hotspot Panel v2

Cancer related 50 genes									
ABL1	*BRAF*	*EGFR*	*FGFR1*	*GNAQ*	*IDH2*	*KRAS*	*NPM1*	*PTPN11*	*SMO*
AKT1	*CDH1*	*ERBB2*	*FGFR2*	*GNAS*	*JAK2*	*MET*	*NRAS*	*RB1*	*SRC*
ALK	*CDKN2A*	*ERBB4*	*FGFR3*	*HNF1A*	*JAK3*	*MLH1*	*PDGFRA*	*RET*	*STK11*
APC	*CSF1R*	*EZH2*	*FLT3*	*HRAS*	*KDR*	*MPL*	*PIK3CA*	*SMAD4*	*TP53*
ATM	*CTNNB1*	*FBXW7*	*GNA11*	*IDH1*	*KIT*	*NOTCH1*	*PTEN*	*SMARCB1*	*VHL*

Ion AmpliSeq™ Colon and Lung Cancer Research Panel v2

Colon and Lung Cancer related 22 genes							
AKT1	*CTNNB1*	*ERBB2*	*FGFR1*	*KRAS*	*NOTCH1*	*PTEN*	*TP53*
ALK	*DDR2*	*ERBB4*	*FGFR2*	*MAP2K1*	*NRAS*	*SMAD4*	
BRAF	*EGFR*	*FBX7*	*FGFR3*	*MET*	*PIK3CA*	*STK11*	

RNA Fusion Lung Cancer Research Panel

Lung Fusion			
ALK	*RET*	*ROS1*	*NTRK1*

Custom Panel for prescreening of FGF/FGFR alteration

FGFR		
FGFR1	*FGFR2*	*FGFR3*

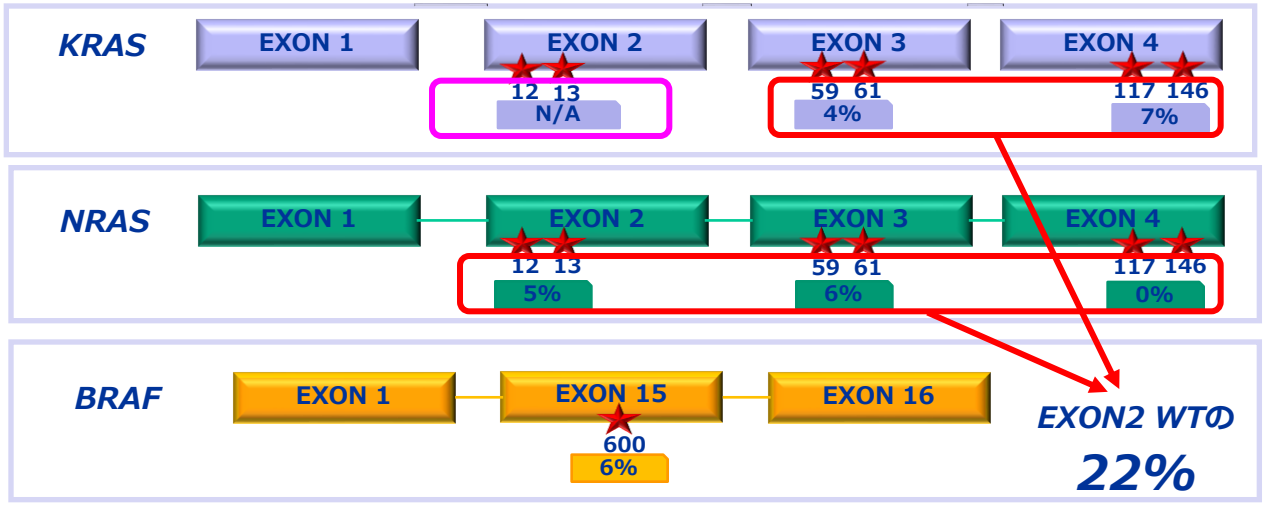
FGFR fusion		
FGFR1	*FGFR2*	*FGFR3*

Adaptor						
GRB2	*GAB1*	*SOS1*	*FRS2*	*PTPN11*	*SHC1*	*SHB*

Ext RAS/BRAF panel

KRAS/NRAS in PEAK Study

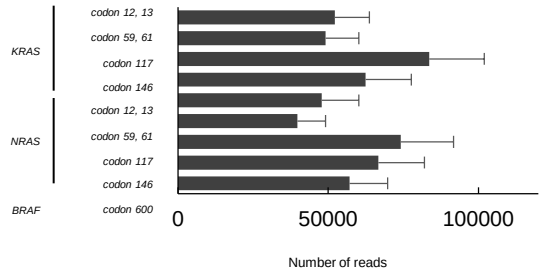
Phase II FOLFOX+BV vs.FOLFOX+Pmab



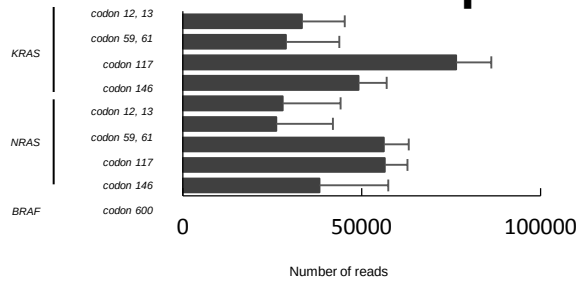
Extended RAS/BRAF assay by NGS (Ion PGM)

(RUO, Kindai Univ)

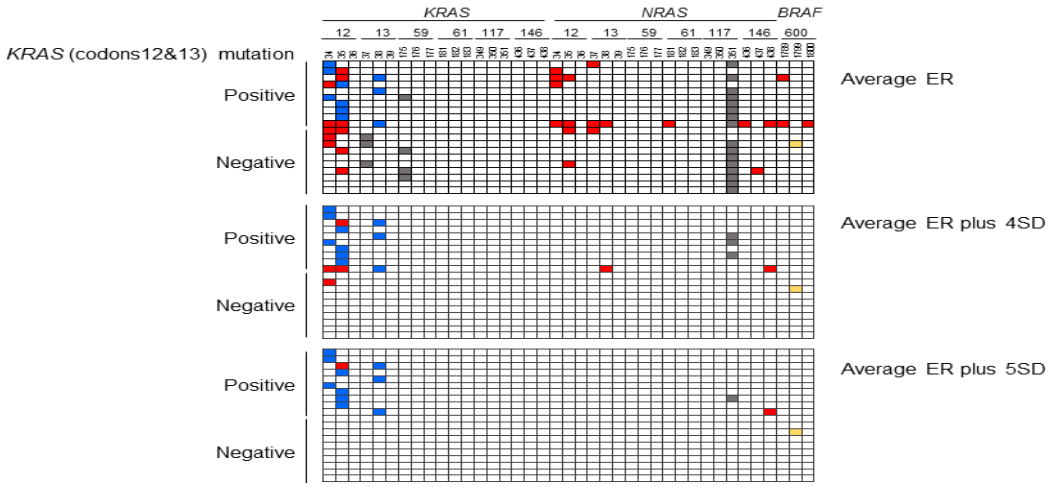
Read number
Normal DNA samples



CRC FFPE DNA samples



Adjustment of Poisson coefficient as cut-off value



20 FFPE samples

Ultra-deepsequencing allow us to detect the mutations of cfDNA.

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Extended RAS/BRAF kit by NGS (Ion PGM)

Minimum detection limit of the NGS mutation assay

	Position (Amino Acid)	Mutation rate (%)	NGS mutation assay
KRAS	c.34G>A (G12S)	12.31	Positive
		5.93	Positive
		2.40	Positive
		1.18	Negative
		0.66	Negative
		0.27	Negative
KRAS	c.38G>A (G13D)	11.66	Positive
		3.92	Positive
		2.30	Negative
		1.46	Negative
		0.50	Negative
		0.32	Negative
NRAS	c.181C>A (Q61K)	74.40	Positive
		5.19	Positive
		1.49	Positive
		0.58	Positive
		0.31	Negative
		0.07	Negative
BRAF	c.1798G>T (V600E)	8.19	Positive
		3.45	Positive
		1.85	Positive
		0.90	Negative
		0.52	Negative
		0.28	Negative

Concordance of plasma and tumor results for *RAS* mutations

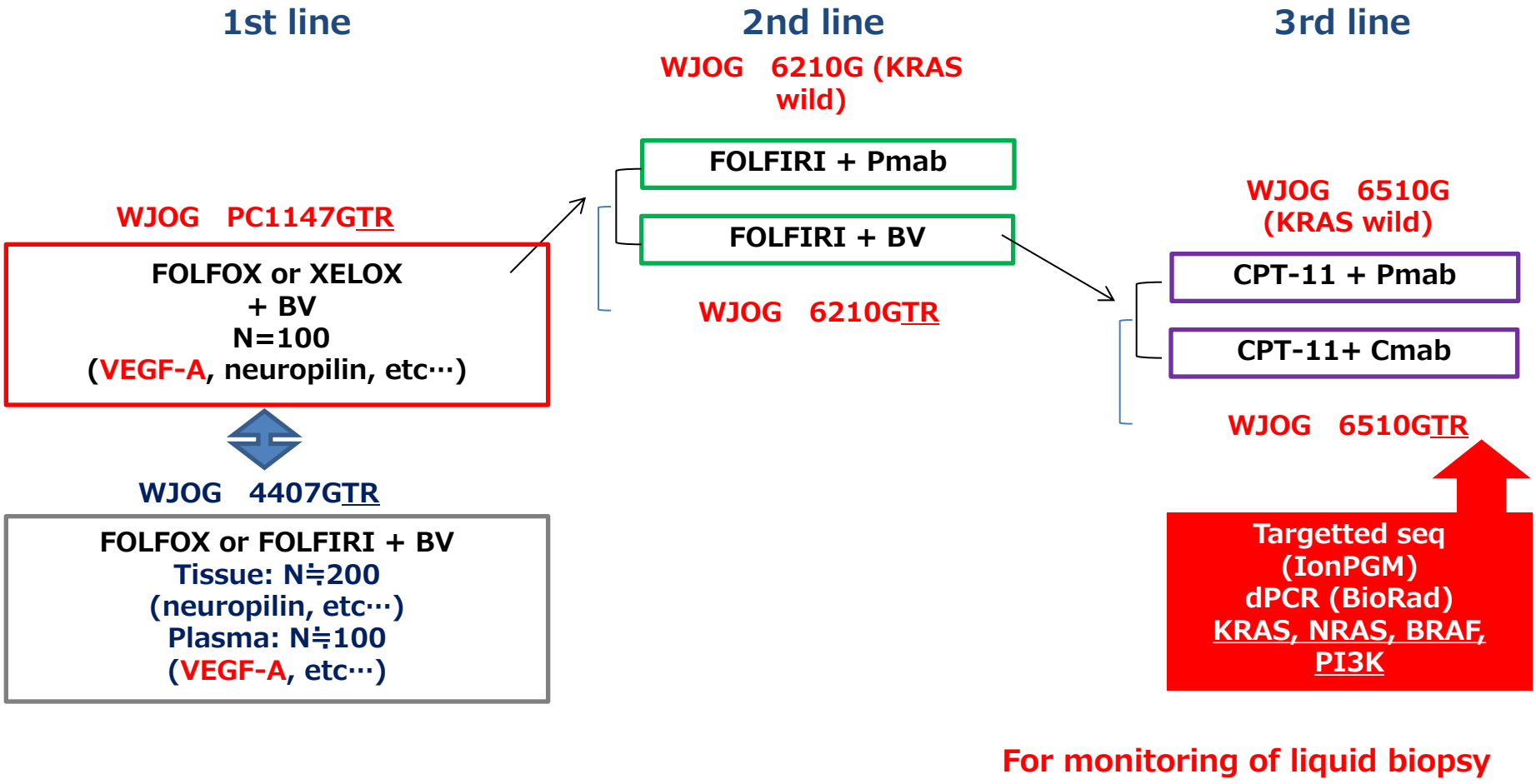
	KRAS		NRAS		BRAF	
	tumor	plasma	tumor	plasma	tumor	plasma
DS1						
DS3	G12S, G12D		G12S			
DS4						
DS6	G13D	G12S, G13D				
DS8	G12V	G12V				
DS9	G12S, A59E		G12S, Q61*			
DS22					V600E	V600E
DS23						
DS24						
DS25	G12S	G12S				
DS28			A146V, A146A			
DS29			G12S, G12D,Q61K			
DS30	G12S	G12S				
DS32	G12V	G12V				
DS33						

Comparison of Scorpion-ARMS and NGS mutation assay for *KRAS* mutations in archived FFPE tissues

		Scorpion-ARMS		total
		positive	negative	
NGS mutation assay	positive	35	8	43
	negative	0	57	57
total		35	65	100

Concordance of KRAS mutation between this assay and Scorpion-ARMS was 92% (92/100). DNA obtained from 15 plasma samples was also analyzed. KRAS and BRAF mutations were identified in both plasma and tissue samples of six patients.

WJOG TR study (Colorectal cancer)



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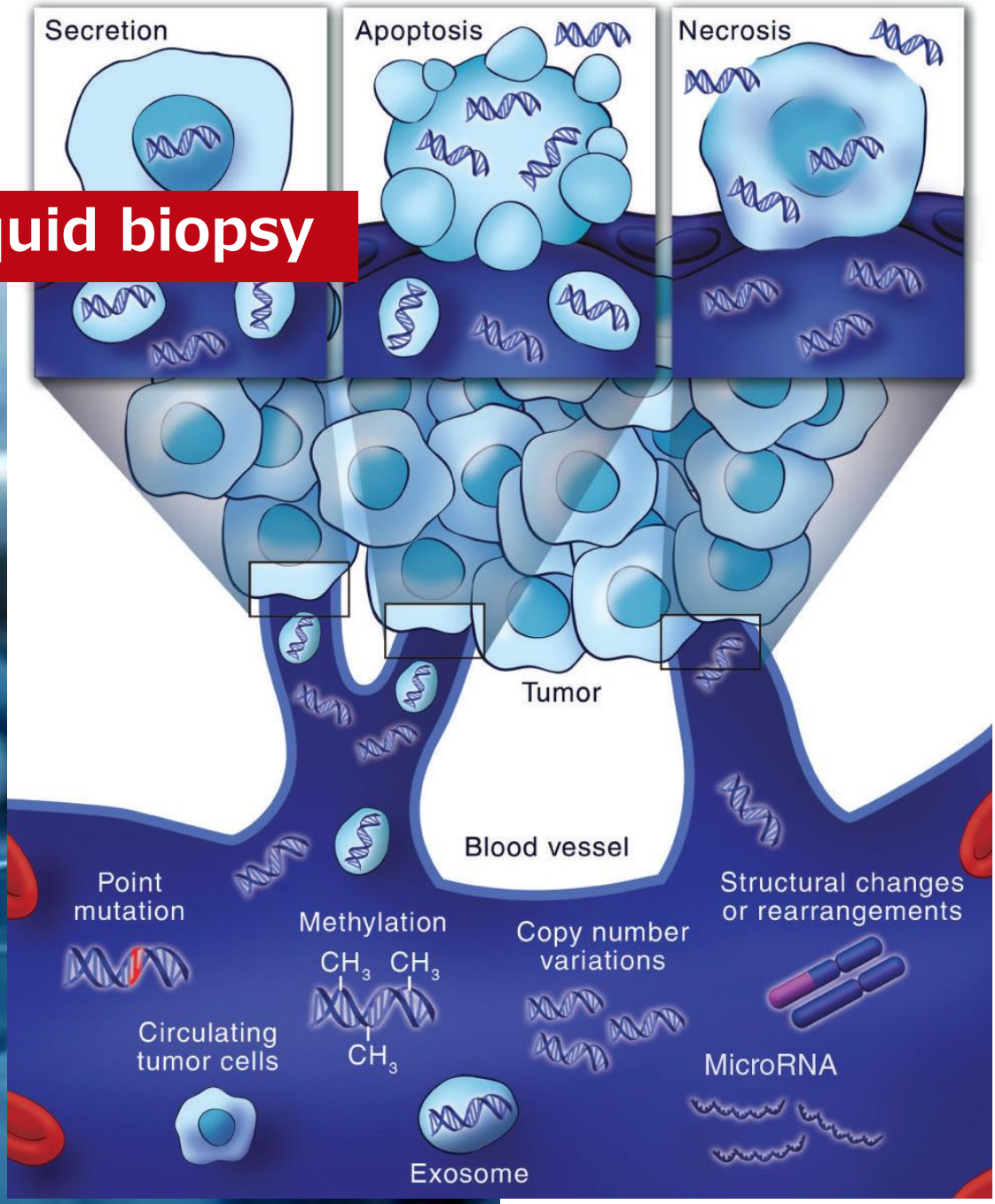
ddPCR and NGS for liquid biopsy



Luis A. Diaz Jr and Alberto Bardelli.
J Clin Oncol 32, 579-586, 2014

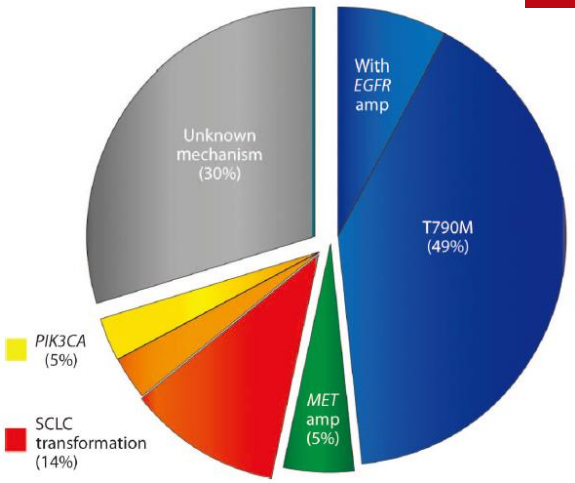
Detection of EGFR mutation in cfDNA

	Kimura H (Clin Cancer Res 2006)	Kimura H (Br J Cancer 2007)	Maheswaran S (NEJM 2008)	Bai H (JCO 2009)	Goto K (JTO 2012)	Sakai K (Cancer Sci)	Weber (BMC Cancer 2014)
Study design	Retrospective	Retrospective	Prospective	Retrospective	Prospective	Retrospective	Retrospective
Assay	SARMS	SARMS	SARMS	DHPLC	SARMS	SABER (MassArray)	Cobas
Samples	Serum	Serum	Plasma	Plasma	Serum	Serum	Plasma
Detection rate	48.10%	75%	35%	81.80%	43.10%	28% for T790M	12%
(blood/tissue DNA)	(8/11)	(6/8)	(7/20)	(63/77)	(22/51)		(179/196)

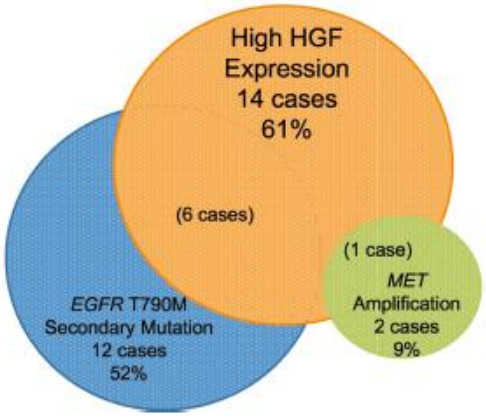
SARMS, Scorpion ARMS; DHPLC, Denaturing high-performance liquid chromatography

Epidermal growth factor receptor mutation status in circulating free DNA in serum: from IPASS, a phase III study of gefitinib or carboplatin/paclitaxel in non-small cell lung cancer. 2012; IPASS (IRESSA Pan-Asia Study), clinically selected patients with pulmonary adenocarcinoma received first-line gefitinib or carboplatin/paclitaxel.

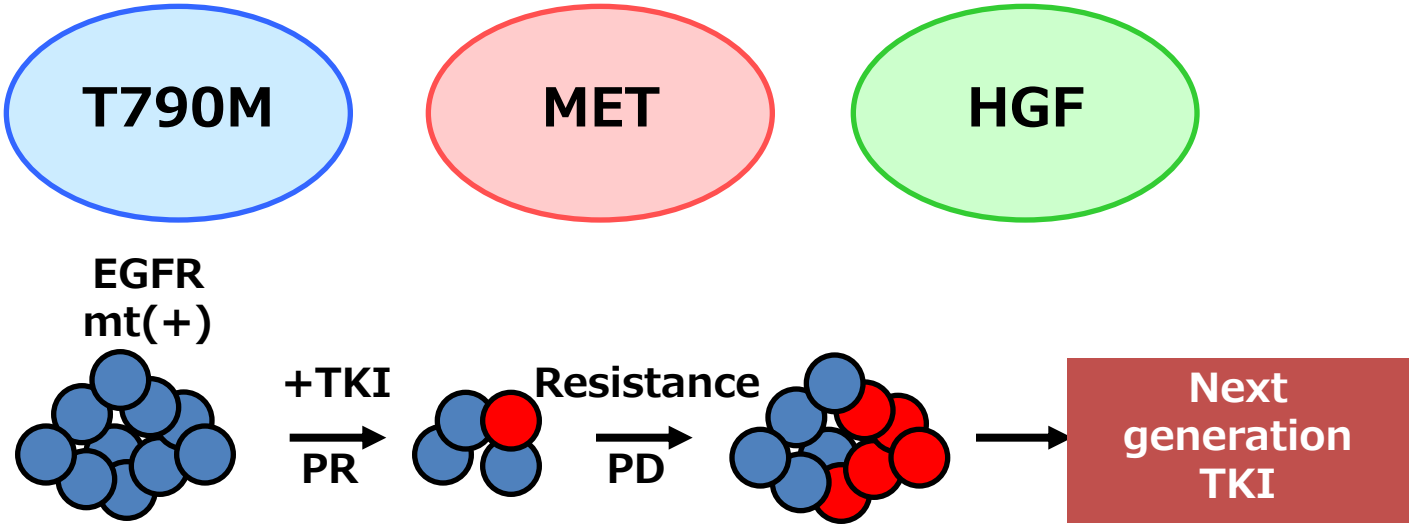
EGFR-TKI resistance



(Sequist LV., et al. Sci Transl Med, 2011)



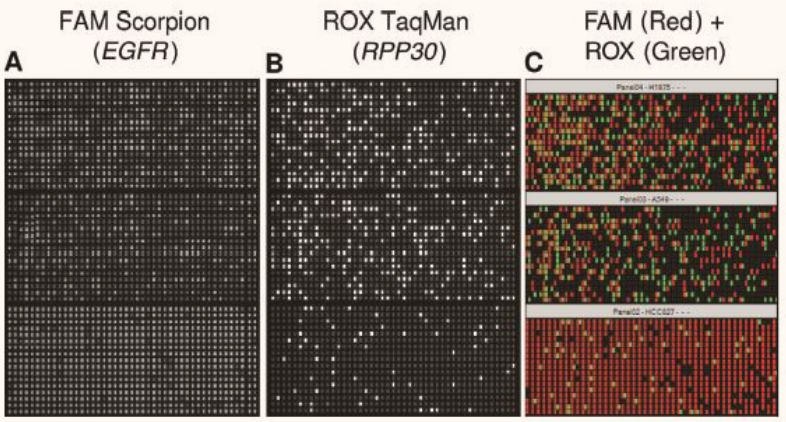
(Yano S., et al. J Thorac Oncol, 2011)



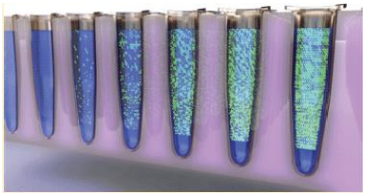
Rebiopsy is mandatory.

Digital PCR (3rd generation PCR)

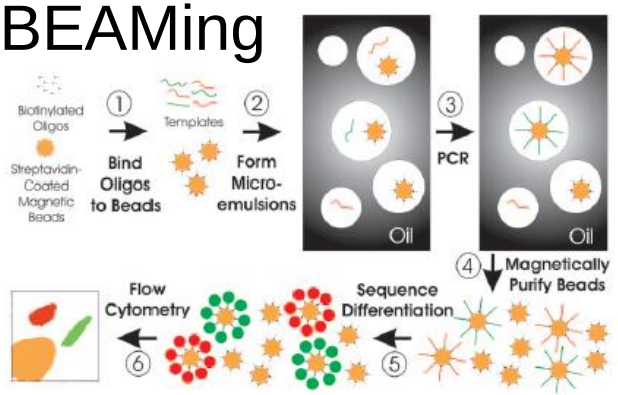
Nanofluidic Digital PCR Arrays



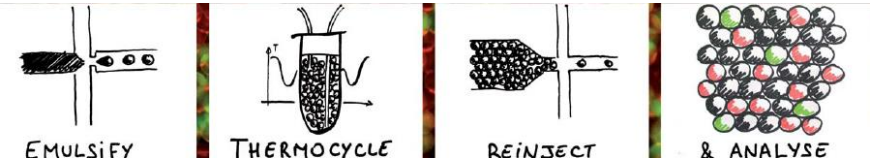
Emulsion PCR



BEAMing



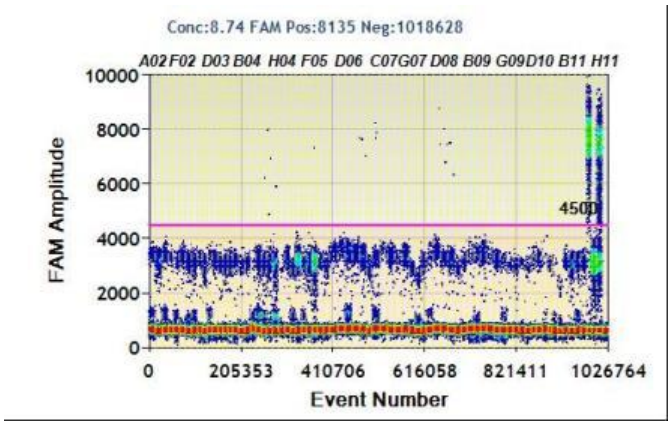
Droplet Digatal PCR

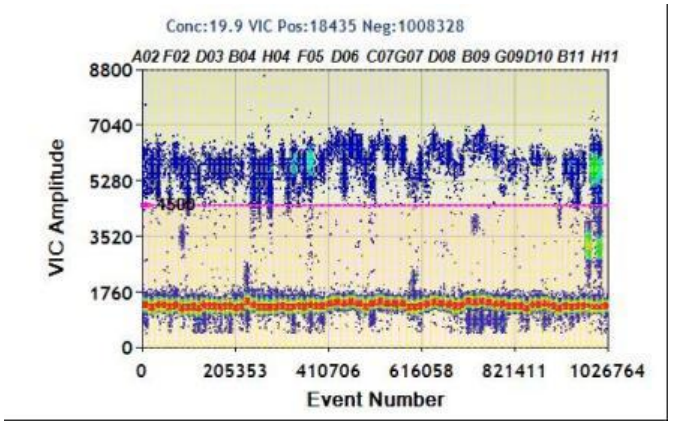


557 in AACR 2014

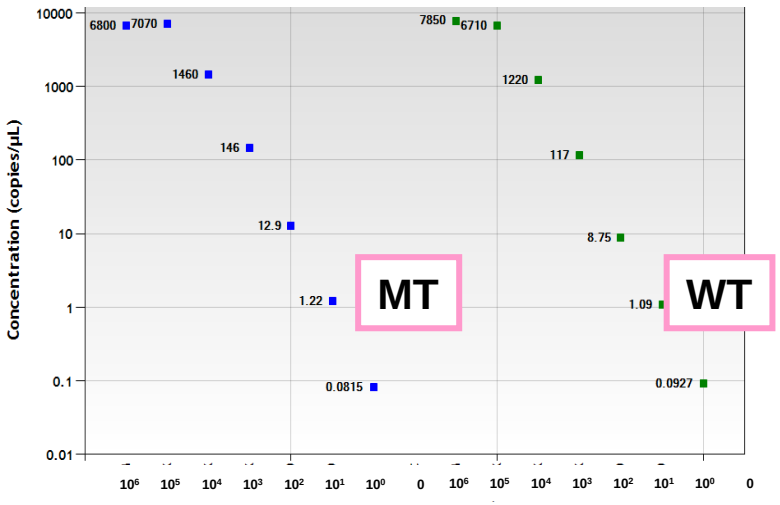
Noninvasive analysis of acquired resistance to EGFR-TKI

Hideharu Kimura[1], Makoto Nishio[2], Tsutomu Daito[3], Kazuto Nishio[4].
[1]Kanazawa University Hospital, Kanazawa, Japan; [2]Cancer Institute Hospital, Tokyo, Japan;
[3]Bio-Rad Laboratories K.K., Tokyo, Japan; [4]Kinki University School of Medicine, Osaka-Sayama, Japan





Assay sensitivity; 0.01~0.001%

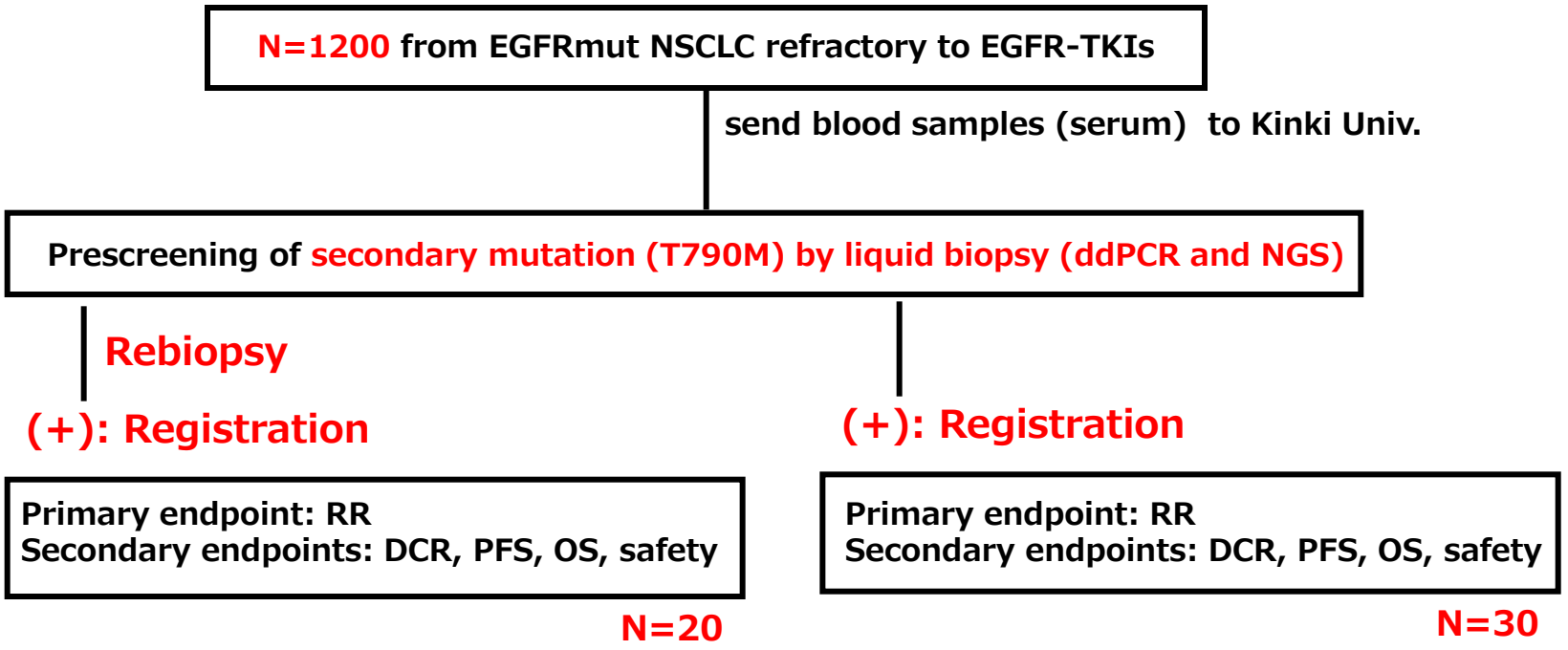


Positive in serum

	ddPCR	CH
positive	8	6
Post only	(6)	(5)
Pre and post	(1)	(0)
Pre only	(1)	(1)

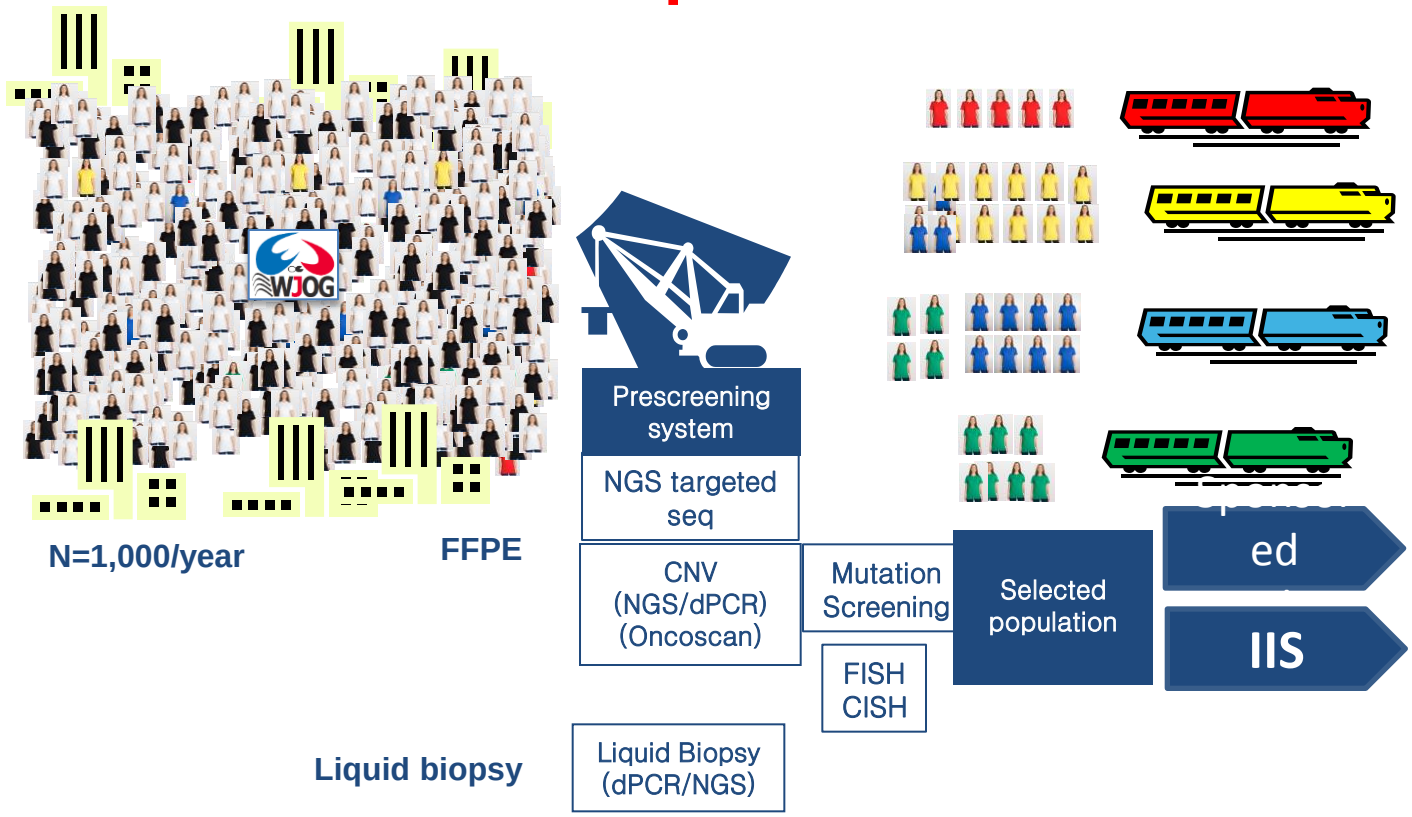
T790M positive in 8/ 38 cases
CH: colony hybridization assay

Prescreening system of T790M positive in cfDNA for 3rd generation EGFR-TKIs



Prescreening system for clinical studies of rare population

"Master protocol"



Conclusion

MassArray, NGS, and digital PCR
for
Prescreening system and Biobank
using
FFPE and Liquid Biopsy

Our biomarker studies will contribute to activation of drug development for rare population.

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- Department of Medical Oncology, Kindai Univ Faculty of Medicine; Nakagawa K, Takeda M, et al.
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