Circulating tumor DNA for prediction of antitumor effects

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COI Disclosure Information

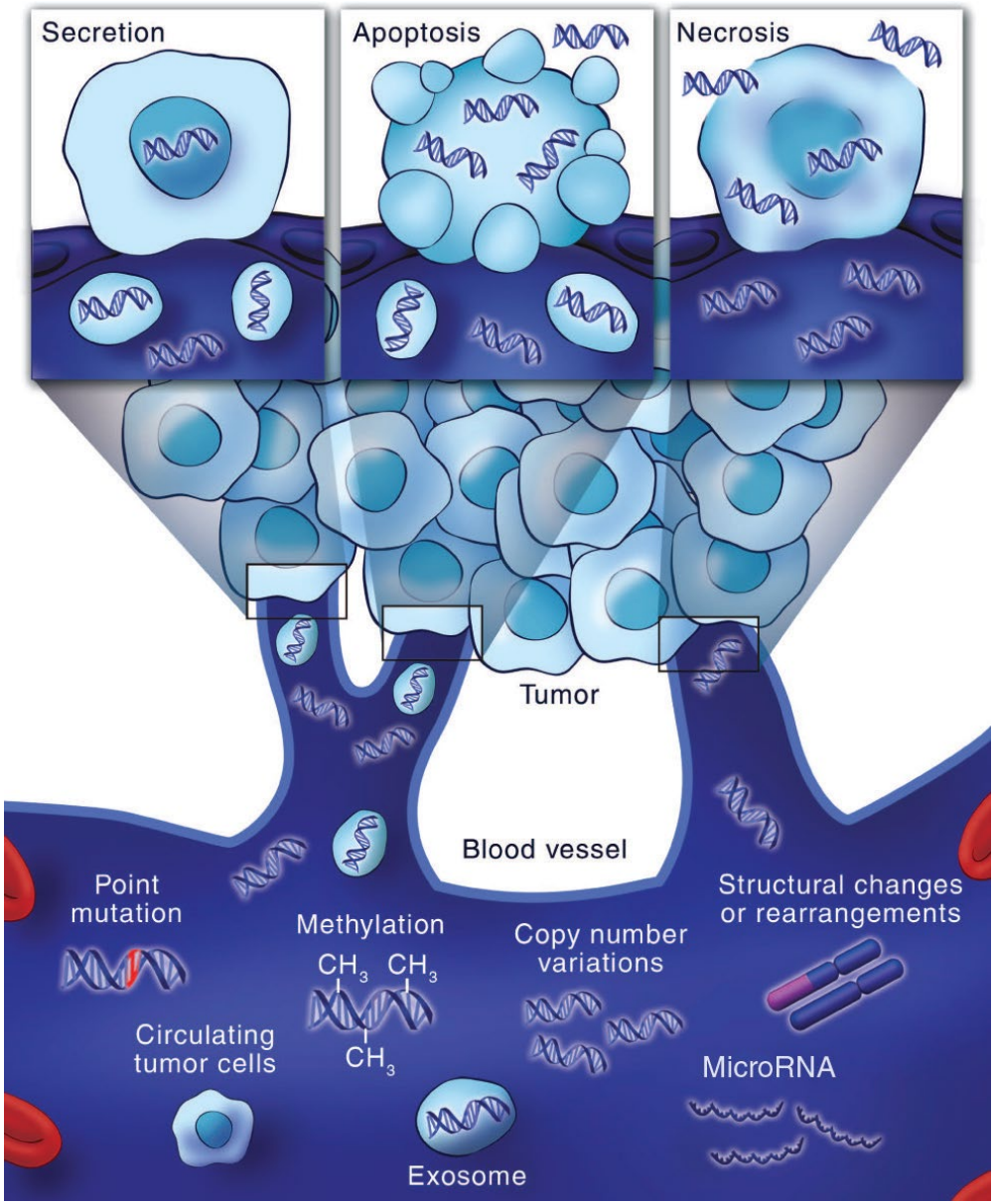
Presenter: Kazuto Nishio

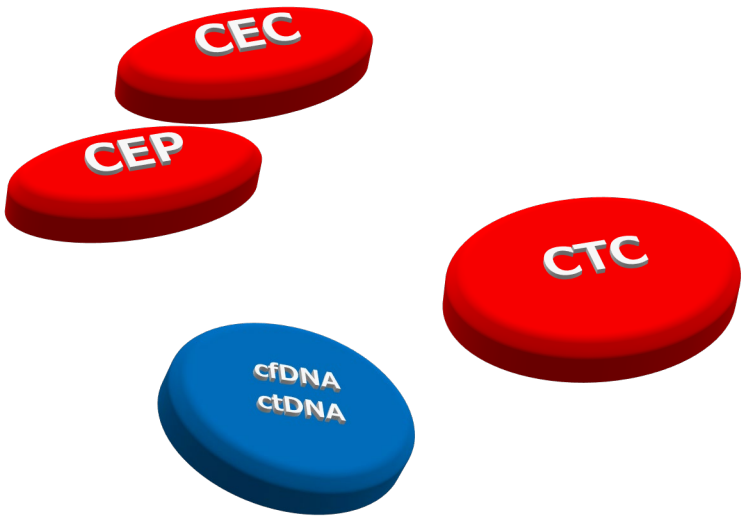
I have the following financial relationships to disclose.

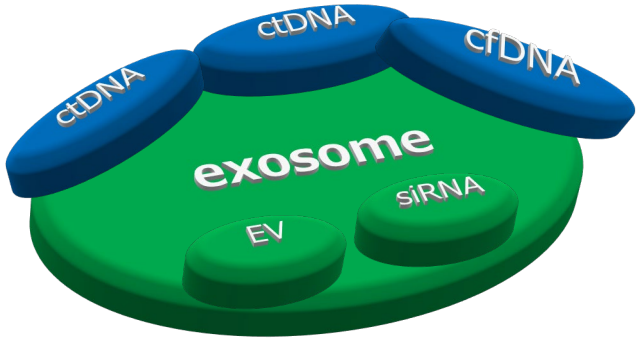
- ✓ **Honoraria（lecture fee) from:**
  AstraZeneca K.K., Nippon Boehringer Ingelheim Co., Ltd.
  Chugai Pharmaceutical Co.

- ✓ **Grant/Research funding from:**
  Korea Otsuka Pharmaceutical Co., Eli Lilly Japan K.K.
  Nippon Boehringer Ingelheim Co., Ltd.

Liquid biopsy







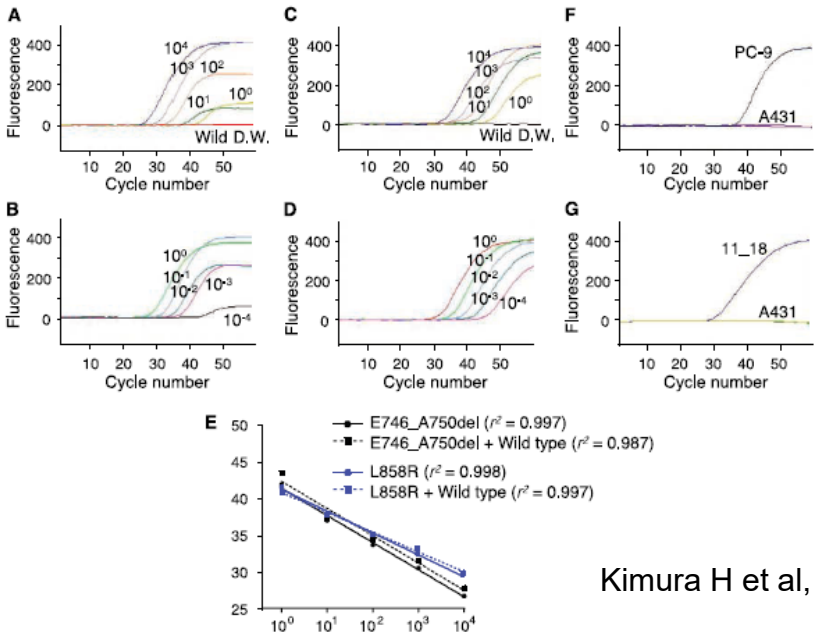
Diaz LA Jr et al, J Clin Oncol. 2014

Our history; Detection of EGFR gene mutation using blood cell free DNA

Detection of Epidermal Growth Factor Receptor Mutations in Serum as a Predictor of the Response to Gefitinib in Patients with Non –Small-Cell Lung Cancer 2006

Hideharu Kimura, Kazuo Kasahara, Makoto Kawaishi, et al.

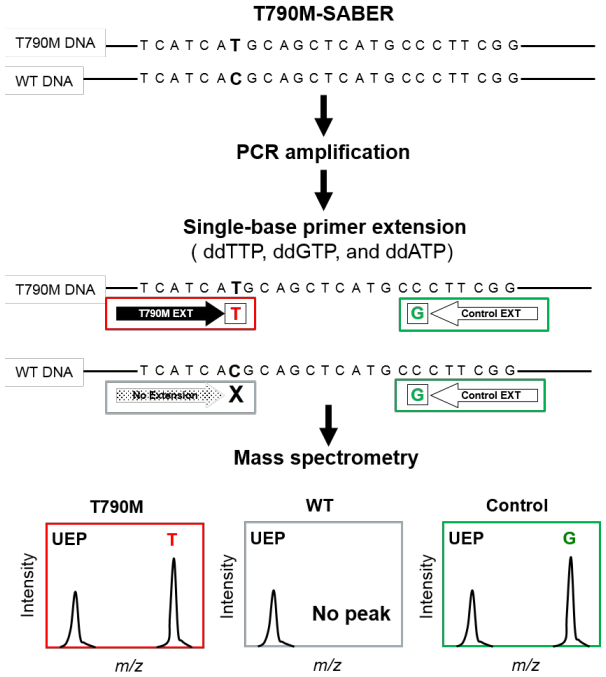
Clin Cancer Res 2006;12:3915-3921.



Kimura H et al, 2006

Detection of epidermal growth factor receptor T790M mutation in plasma DNA from patients refractory to epidermal growth factor receptor tyrosine kinase inhibitor 2013

Kazuko Sakai,[1] Atsushi Horiike,[2] Darryl L. Irwin,[3] Keita Kudo,[2] Yoshihiko Fujita,[1] Azusa Tanimoto,[2] Toshio Sakatani,[2] Ryota Saito,[2] Kyohei Kaburaki,[2] Noriko Yanagitani,[2] Fumiyoshi Ohyanagi,[2] Makoto Nishio[2] and Kazuto Nishio[1,4]



Sakai K et al, 2013

A more sensitive, multiplex assay is required.

Agenda

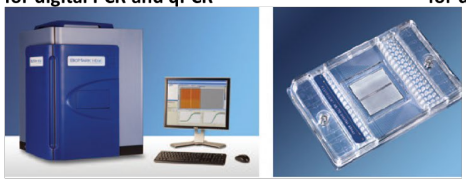
- ctDNA and mol targeted agents
- Ct DNA and ICIs

dPCR

NGS

Digital PCR on chips

✓Fluidigm Corporation's BioMark HD System for digital PCR and qPCR



✓Life Technologies' QuantStudio System for digital PCR and qPCR

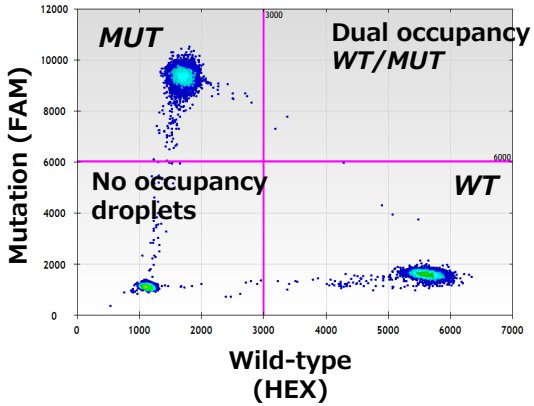
Digital PCR in droplets (ddPCR)

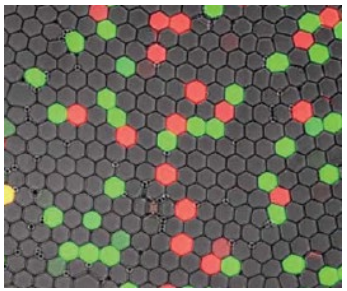
✓Bio-Rad's QX100 droplet digital PCR System

✓RainDrop Source and RainDrop Sense machines for droplet digital PCR

Monya BakerMonya Baker, Nature methods ,VOL.9 NO.6, 2012

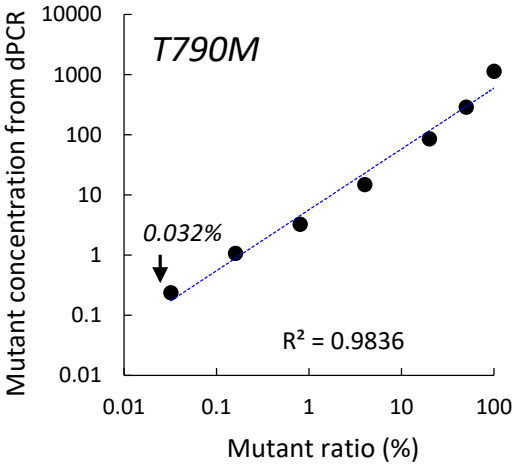
Next-Generation Sequencer

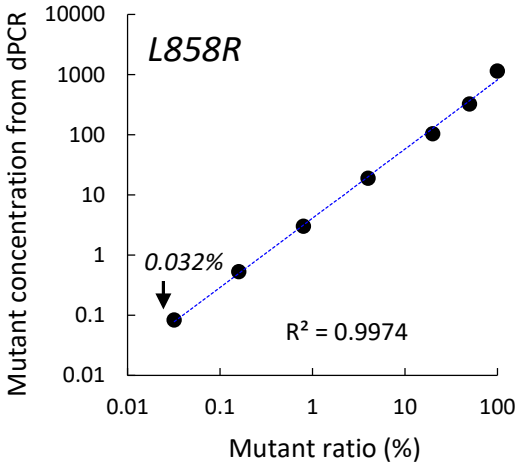


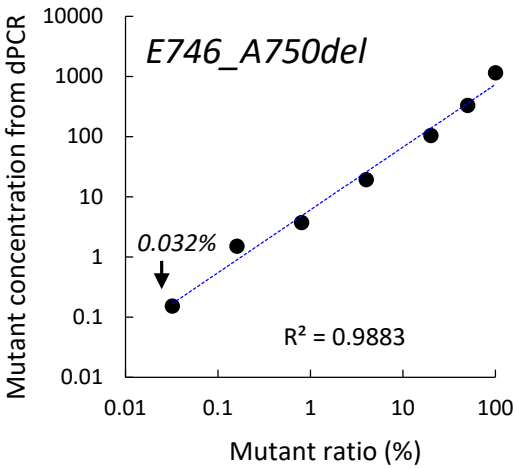


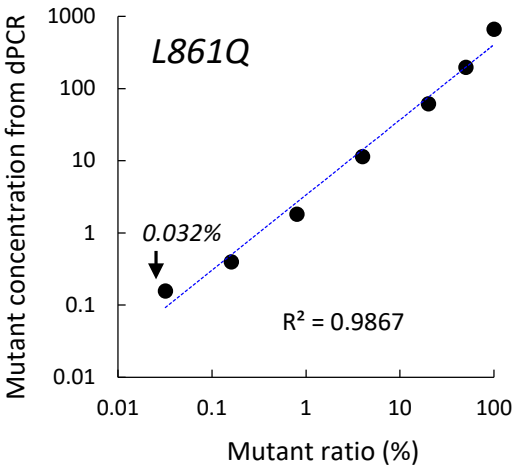
Minimum detection limit (sensitivity)

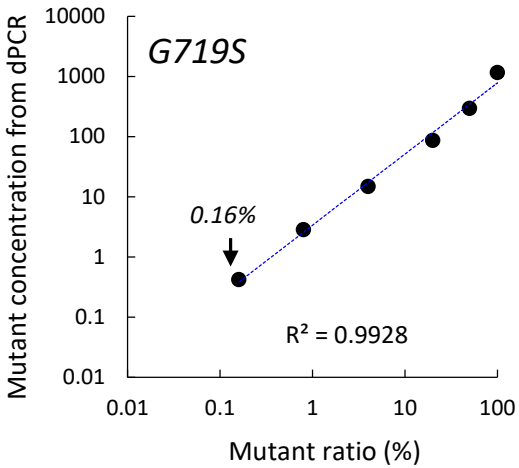
sensitivity
0.032～0.16%











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Could we detect cfDNA mutations in early stage cancer pts?

■ Questions

Collaboration with Dr Ohira and Dr Ikeda (Tokyo Med Univ)

1. Could we detect cfDNA mutations in early stage cancer pts?
2. ddPCR vs NGS (amplicon seq)

■ Samples

- Operatively resected tumor tissue and matched serum
  - Tokyo Medical University
  - Tumor 169, serum 168
  - Matched147

■ Assay platform

Tumor	Tumor DNA: CLv2 (IonProton) Tumor RNA: LungFusion (IonProton)
serum cfDNA :	NGS CLv2 (IonProton) and ddPCR

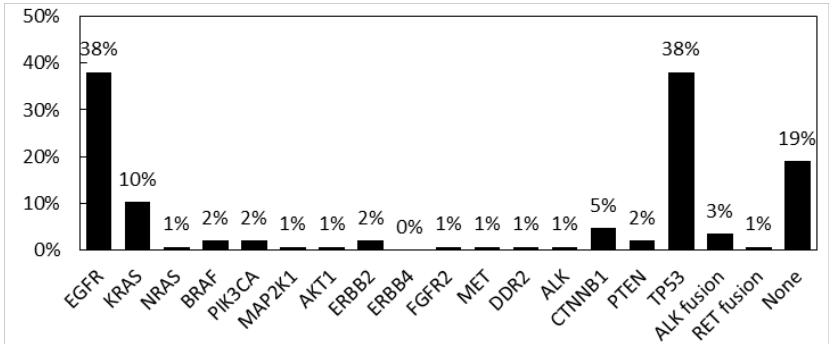
■ Status

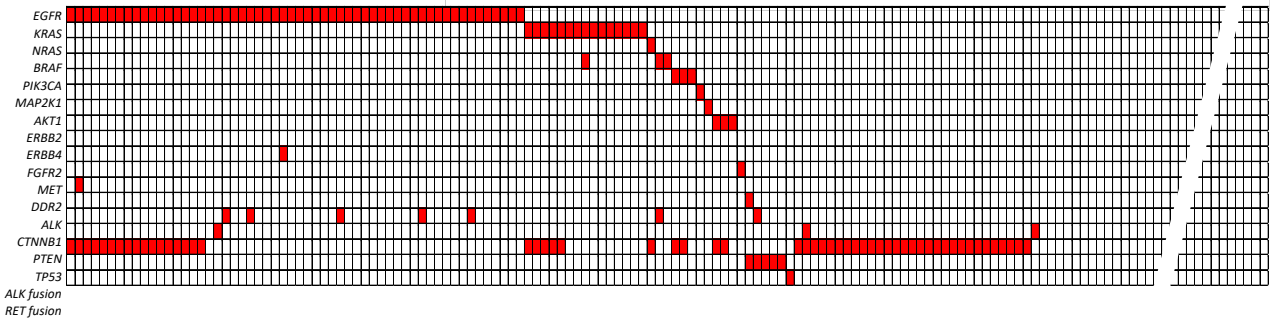
published

■ Tumor - NGS

Assay success rate : 147/147 (100%)

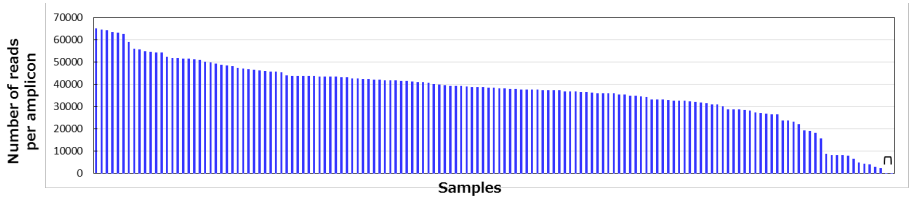
Total read : ave 1,756,501 (123,837 – 31,216,089)
Read/site : 19,092 (1,346 – 339,305)





■ Serum - NGS

Serum : 151 sample



cfDNA yield (copies) : average 16,524 (572 – 373,658)

Total read : average 3,407,623 (3,959 – 5,981,348)
Read/site : 37,039 (43 – 65,015)

Success rate : 145/147 (98.6%)

Detected **EGFR: 3, PIK3CA: 1, TP53: 5**

■ **low freq of cfDNA mutation** → **ddPCR vs NGS**

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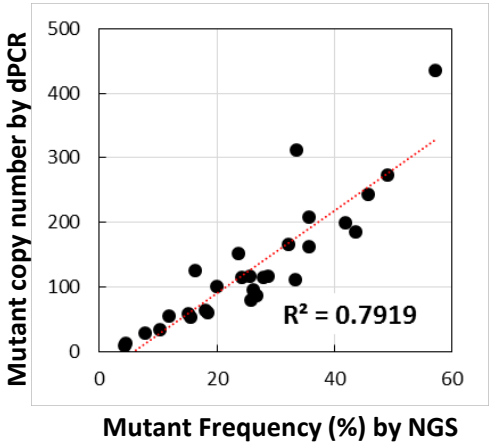
NGS vs ddPCR

Concordance between ddPCR and NGS

		EGFR L858R	KRAS G12C	PIK3CA E545K	PIK3CA H1047R
NGS	Tumor	30	9	2	1
	Serum	1	0	1	0
Digital PCR	Tumor	30	9	2	1
	Serum	1	1	1	0



■ concordance; 43/44 (except for *KRAS G12C*)

→ equivalent detection power for cfDNA mutation

***High correlation of EGFR L858R* detection**

Patient characteristics

Tumor/Serum paired sample; N=147

		No. (%)
Age, years	Median (range)	69 (23 - 85)
	> 65	97 (66.0)
	≦ 65	50 (34.0)
Sex	Male	78 (53.1)
	Female	69 (46.9)
Smoking status	Yes	95 (64.6)
	No	48 (32.7)
	Unknown	4 (2.7)
Stage	IA	76 (51.7)
	IB	35 (23.8)
	IIA	18 (12.2)
	IIB	7 (4.8)
	IIIA	11 (7.5)

Low detection in cfDNA was not due to assay sensitivity but sample characteristics?

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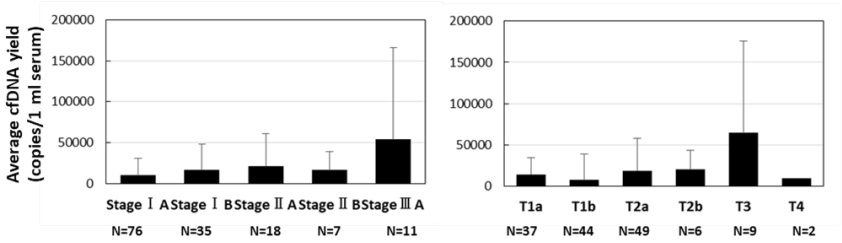
Can mutations in ctDNA of patients with early stage cancer ?

Stage and amount of serum cfDNA



		N	cfDNA yield (range)	*P* value (paired t-test)
Stage	Stage IA/IB	116	12,073 (572 – 146,390)	0.020
	Stage IIA/IIB/IIIA	36	30,848 (1,854 – 373,658)	
TNM Classification (1)	T1a/T1b	85	10,858 (915 – 146,390)	0.032
	T2a/T2b/T3/T4	97	24,921 (572 – 373,658)	
TNM Classification (2)	T1a/T1b/T2a	135	13,868 (572 – 163,125)	0.006
	T2b/T3/T4	17	42,080 (2,353 – 373,658)	

■ higher cfDNA amout in >=Stage II or >=T2

No.	EGFR	KRAS	NRAS	BRAF	PIK3CA	MAP2K1	AKT1	ERBB2	ERBB4	FGFR2	ALK	MET	DDR2	CTNNB1	PTEN	TP53	ALK fusion	RET fusion	Mas tumor size	New c-TNM(T)	New c-TNM(N)	New c-TNM(M)	New c-stage	recurrence
196																			4.6	T4	N1	M0	Stage Ⅲ A	No
215																			4.5	T3	N2	M0	Stage Ⅲ A	No
65																			8.1	T3	N0	M0	Stage Ⅱ B	Yes
188																			3.5	T3	N0	M0	Stage Ⅱ B	No
132																			1.4	T3	N0	M0	Stage Ⅱ B	No
79																			5.1	T2b	N1	M0	Stage Ⅱ B	Yes
64																			6.2	T2b	N0	M0	Stage Ⅱ A	No
59																			1.5	T4	N0	M0	Stage Ⅲ A	No
87																			5.5	T3	N1	M0	Stage Ⅲ A	No
106																			3.8	T3	N0	M0	Stage Ⅱ B	No
60																			2.8	T3	N0	M0	Stage Ⅱ B	No
115																			5.5	T2b	N1	M0	Stage Ⅱ B	No
168																			6.0	T2b	N0	M0	Stage Ⅱ A	No
172																			5.3	T2b	N0	M0	Stage Ⅱ A	No
82																			3.2	T2a	N2	M0	Stage Ⅲ A	No
75																			4.2	T2a	N2	M0	Stage Ⅲ A	No
56																			3.5	T2a	N2	M0	Stage Ⅲ A	Yes
95																			2.8	T2a	N1	M0	Stage Ⅱ A	Yes
164																			3.2	T2a	N1	M0	Stage Ⅱ A	No
126																			2.4	T2a	N1	M0	Stage Ⅱ A	No
66																			3.7	T2a	N1	M0	Stage Ⅱ A	No
152																			3.1	T2a	N1	M0	Stage Ⅱ A	No
221																			3.8	T2a	N1	M0	Stage Ⅱ A	No
219																			3.4	T2a	N1	M0	Stage Ⅱ A	No
94																			2.3	T2a	N1	M0	Stage Ⅱ A	No
81																			4.1	T2a	N1	M0	Stage Ⅱ A	No
189																			2.2	T1b	N2	M0	Stage Ⅲ A	No
169																			2.8	T1b	N1	M0	Stage Ⅱ A	No
149																			2.5	T1b	N1	M0	Stage Ⅱ A	No
138																			2.2	T1b	N1	M0	Stage Ⅱ A	No

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Tumor burden provides feasibility of cell-free DNA sequencing in NSCLC patients.

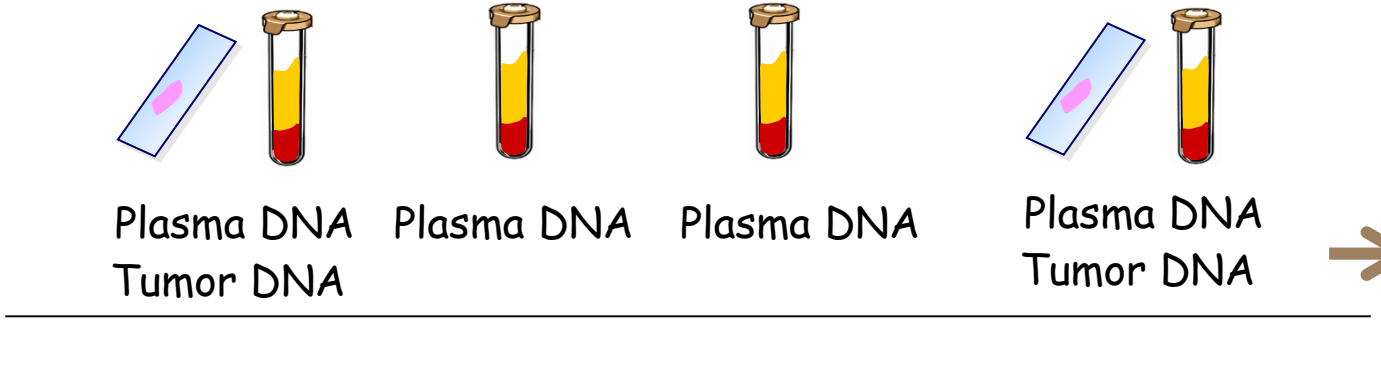
As a monitoring tool; detection of MRD

Monitoring of somatic mutations in circulating cell-free DNA by digital PCR and next-generation sequencing during afatinib treatment in patients with lung adenocarcinoma positive for EGFR activating mutations.

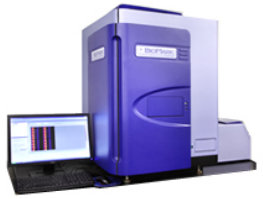
- adv NSCLC
- *EGFR* mut positive (Ex19 del, L858R)
- EGFR-TKIs naive
- PS0-1
- tissue sample avaliable

→ afatinib 40 mg/day →

Oligometastatic → PD → Systemic → PD



ddPCR

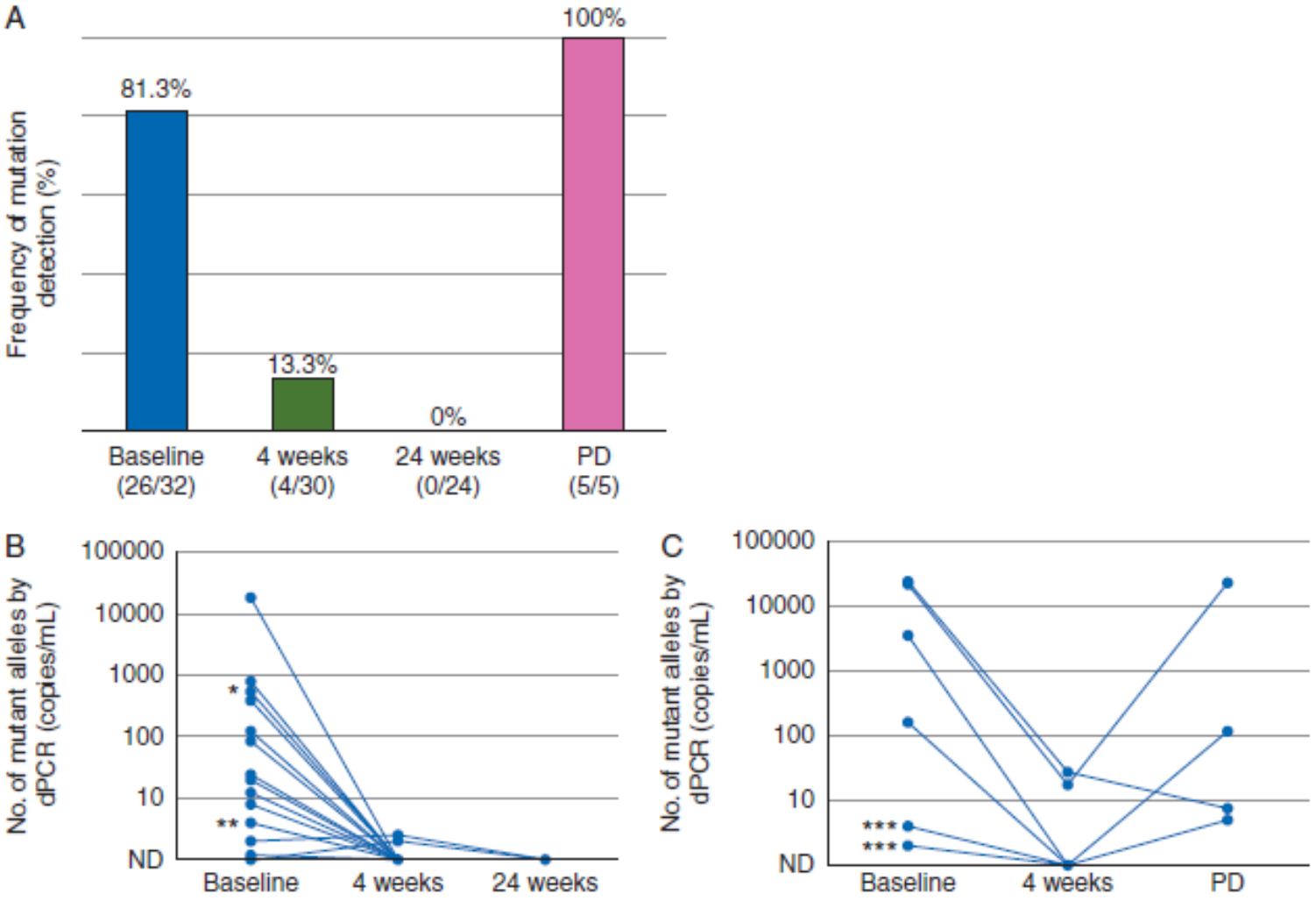


NGS

Iwama E et al. *Annals of Oncology* 28:136-141, 2017

As a monitoring tool; detection of MRD

Monitoring of EGFR activating mutations in cfDNA by dPCR during afatinib treatment.



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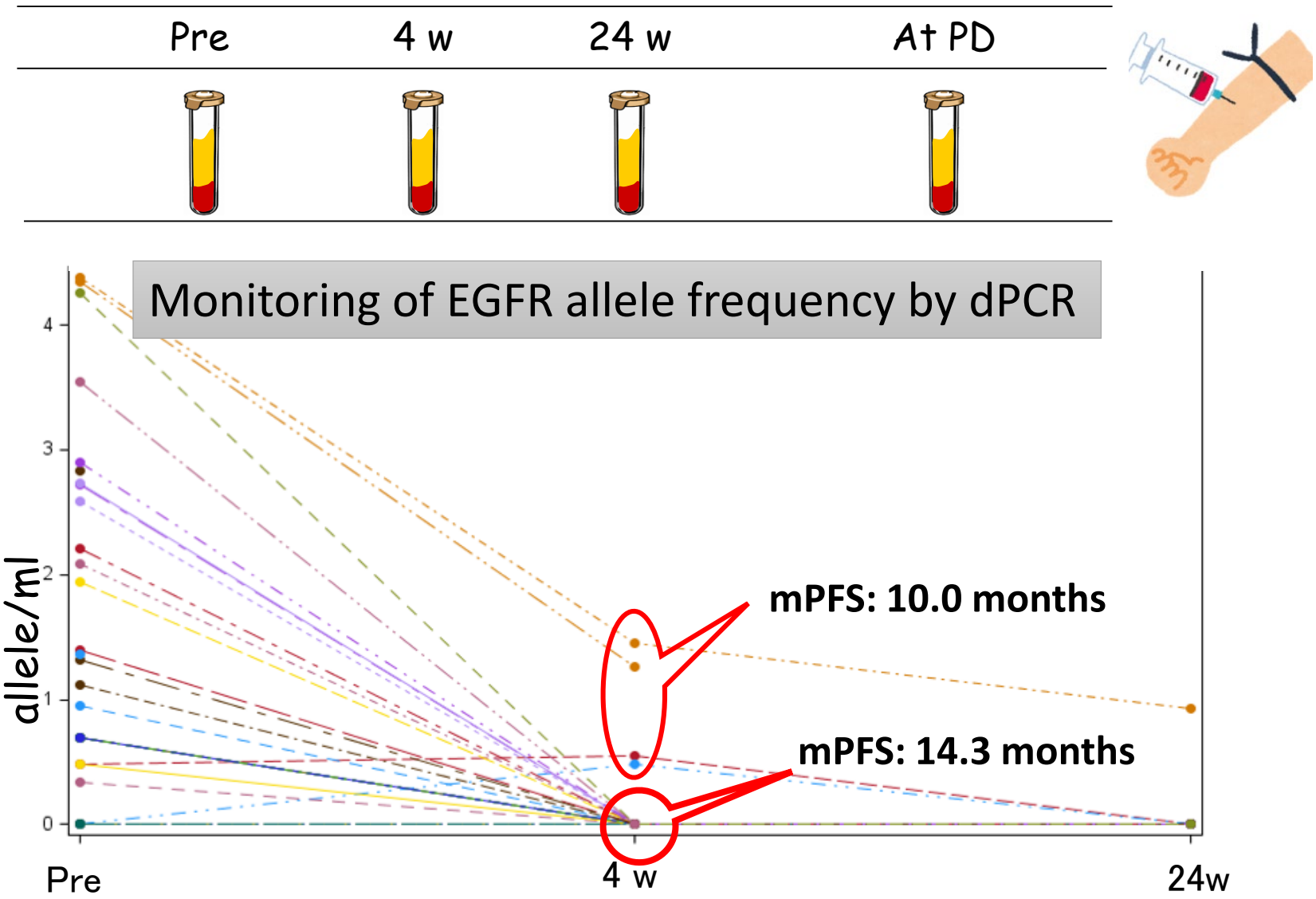

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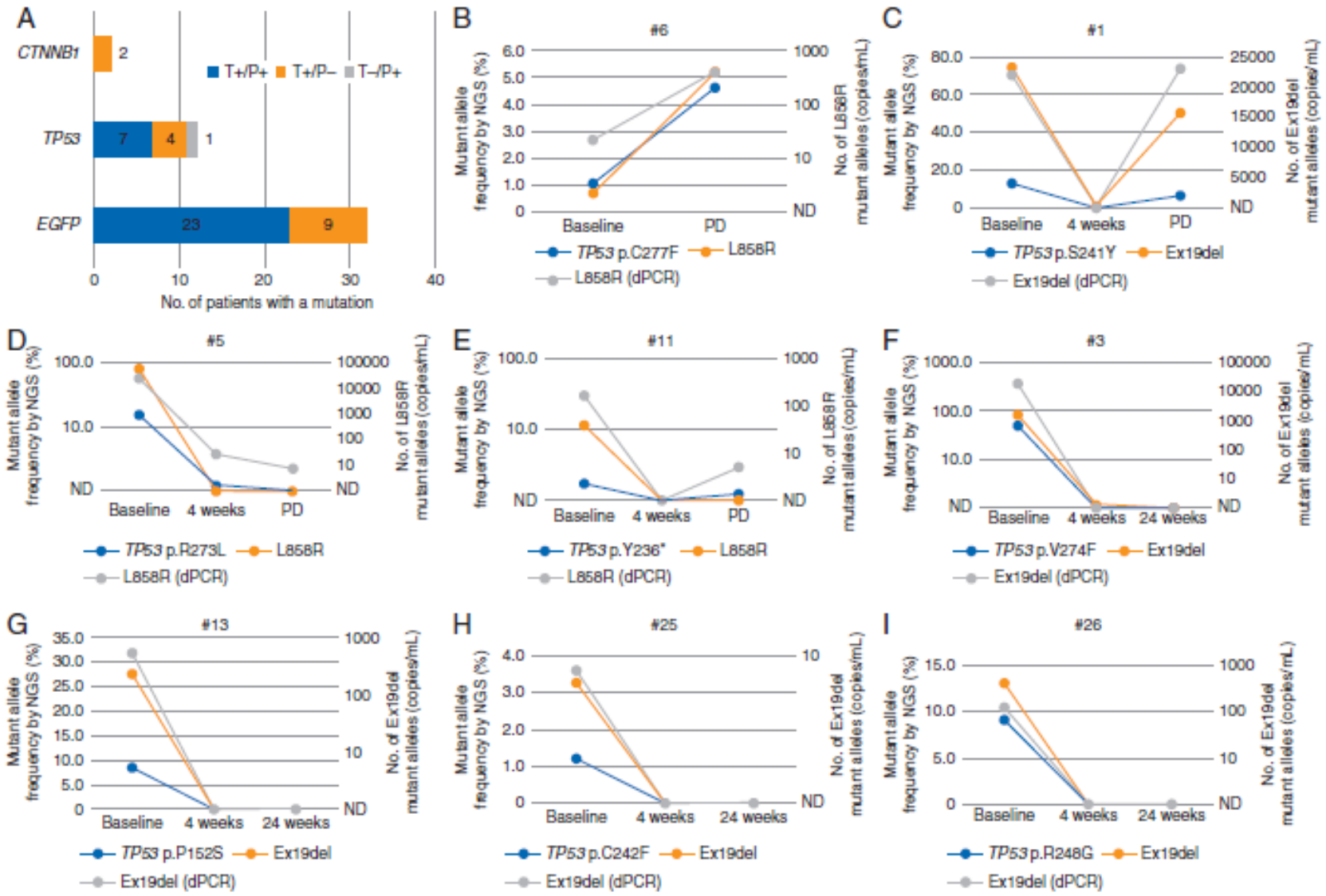
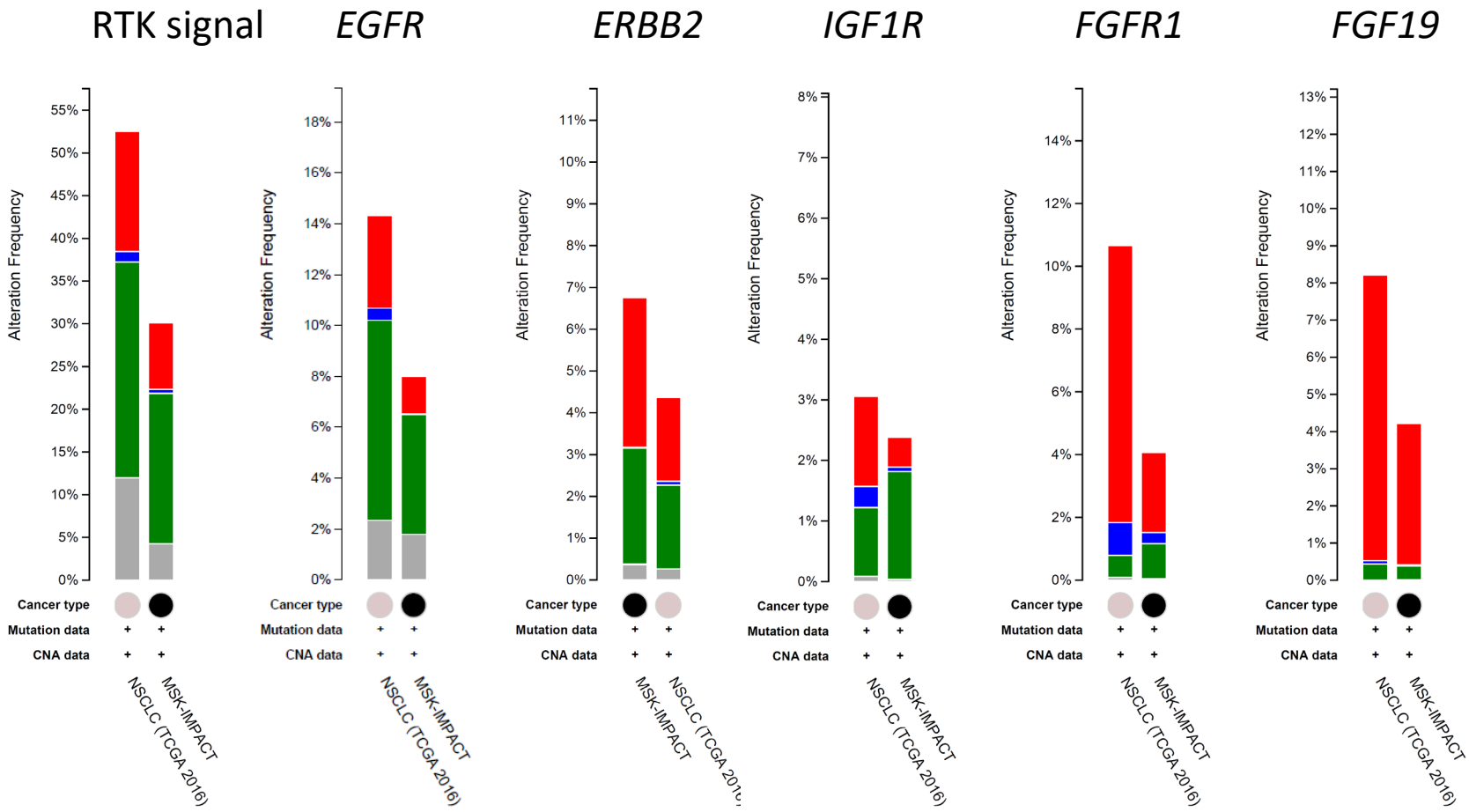
Monitoring of EGFR activating mutations in cfDNA by dPCR during afatinib treatment.



Iwama E et al. *Annals of Oncology* 28:136-141, 2017

Copy number alteration

Pan-Cancer

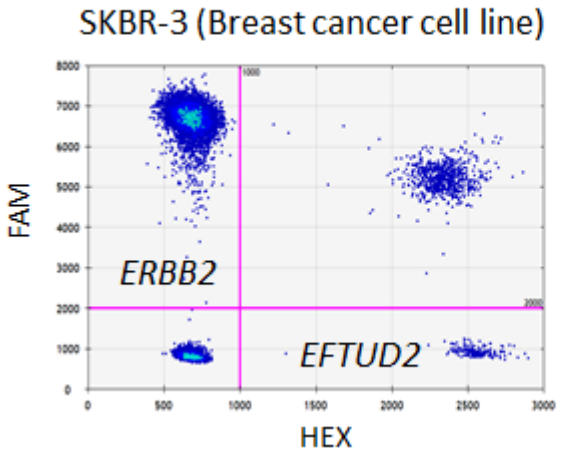


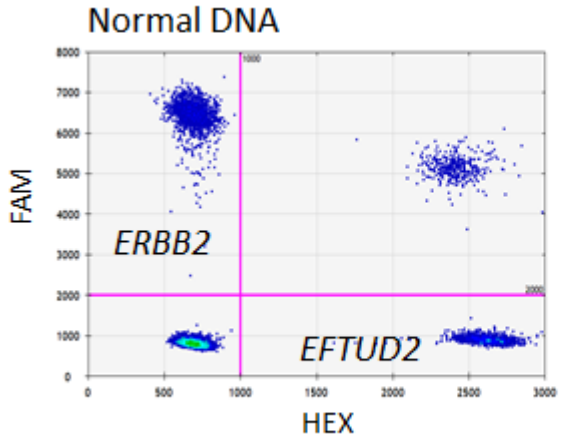
RTK signal: *EGFR ERBB2 ERBB3 ERBB4 PDGFA PDGFB PDGFRA PDGFRB KIT FGF1 FGFR1 IGF1 IGF1R VEGFA VEGFB KDR*

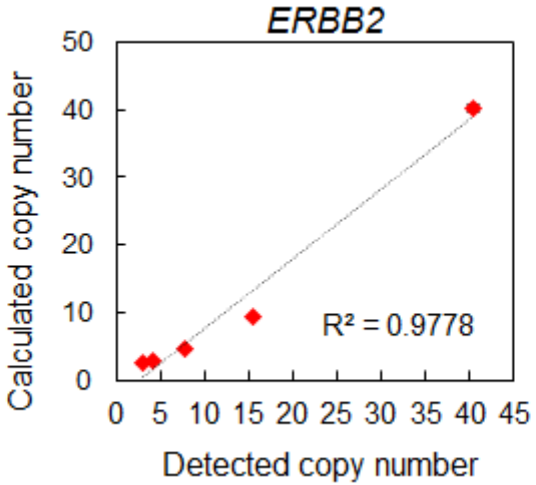
Mutation
Deletion
Amplification

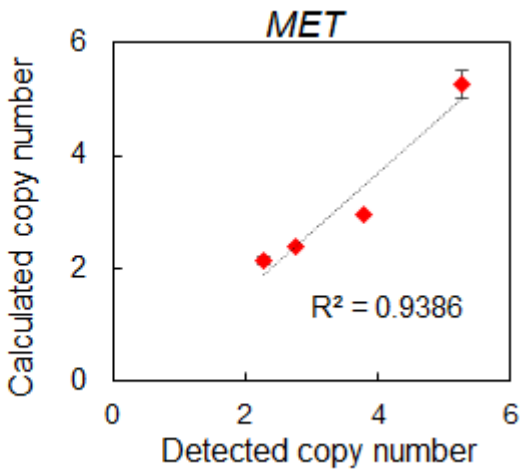
cBioPortal

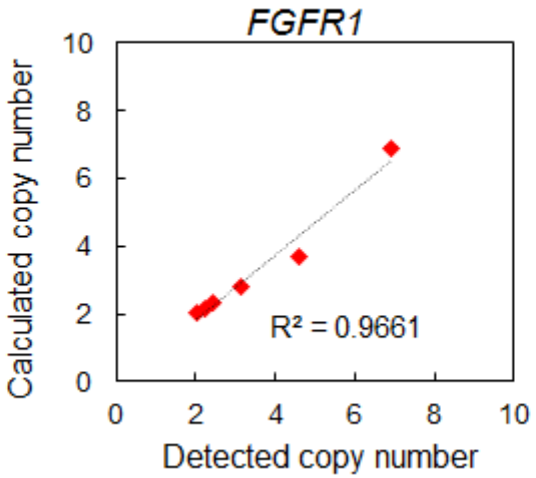
Detection of CCG by ddPCR

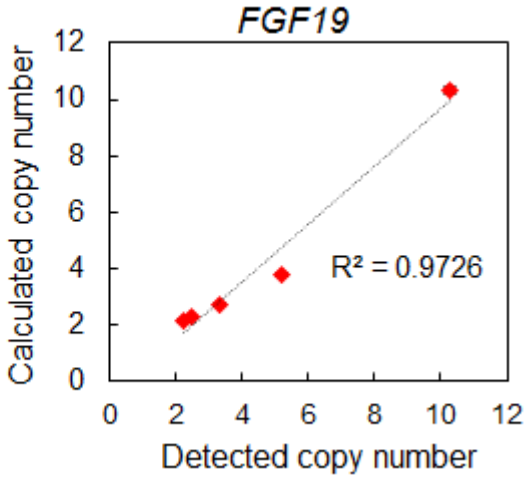






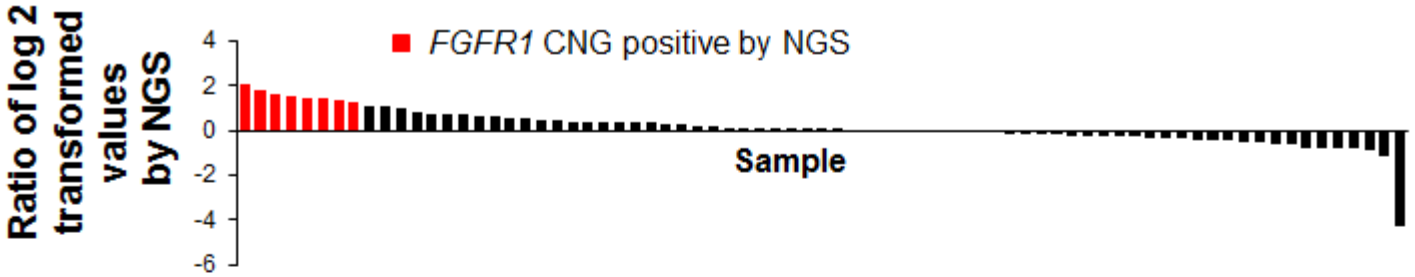




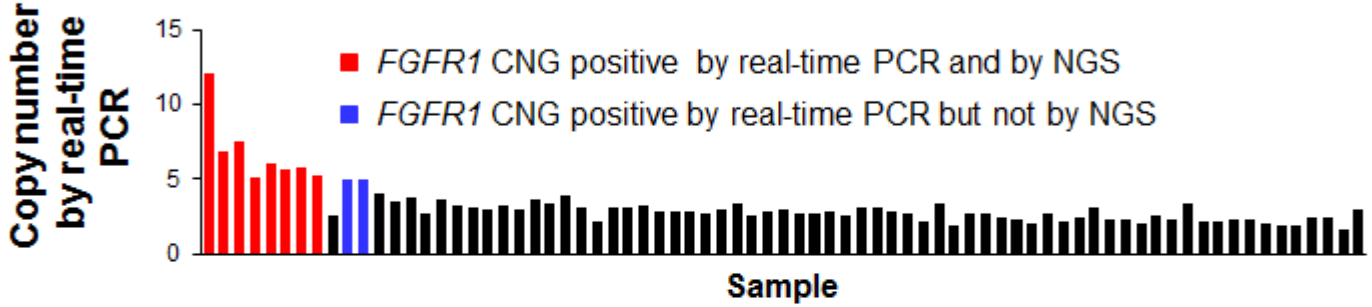


FGFR alteration in lung squamous ca

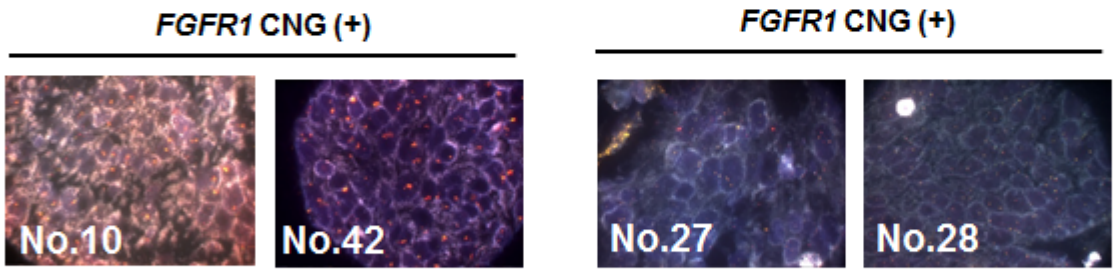
NGS (*FGFR1*)



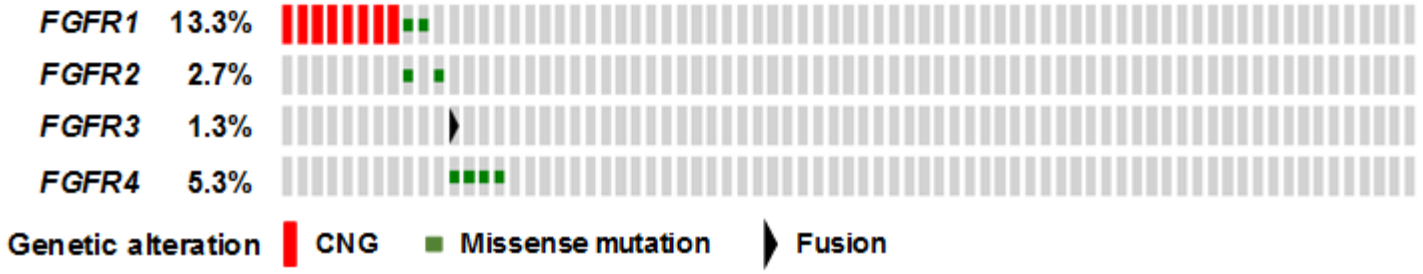
Taqman real-time PCR



FGFR1 FISH



NGS (*FGFRs* alteration)



Hibi M, Okamoto I, Nishio K, et al, 2016, Cancer Sci

Prediction of treatment effect by liquid biopsy - Breast cancer

WJOG6110B/ELTOP:

A randomized phase II trial of trastuzumab plus capecitabine versus lapatinib plus capecitabine in patients with HER2-positive metastatic breast cancer previously treated with trastuzumab and taxanes:

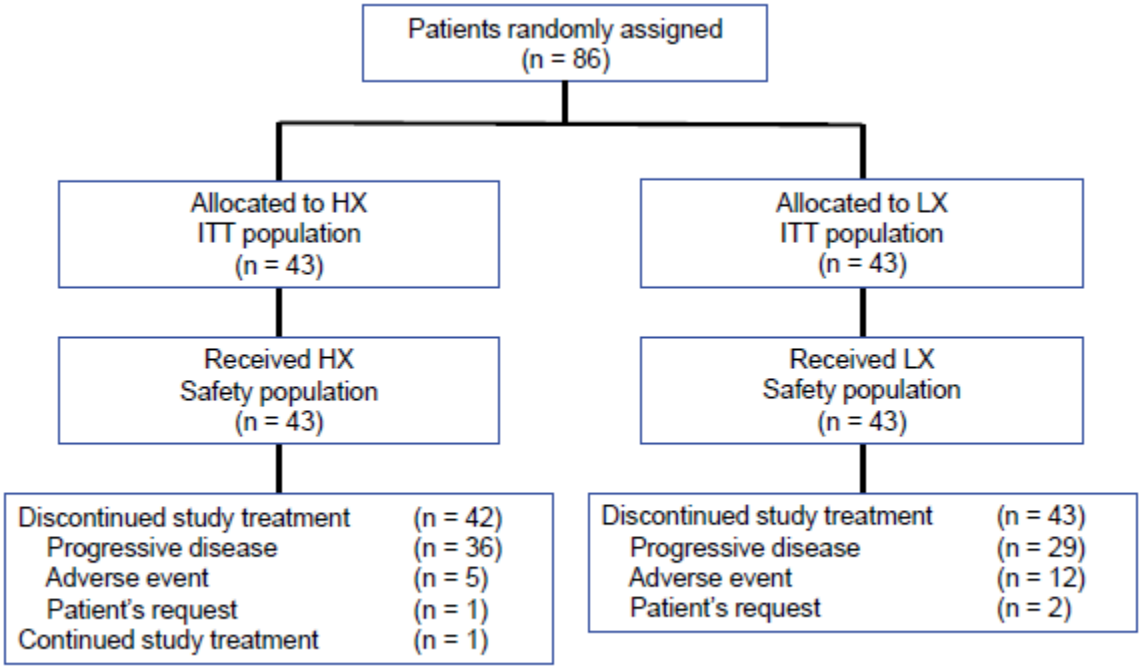
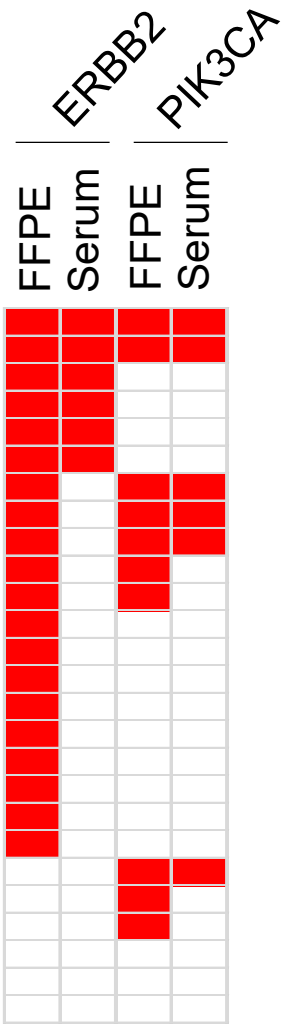


Fig. 1. CONSORT diagram. Abbreviation: HX, trastuzumab plus capecitabine; LX, lapatinib plus capecitabine; ITT, intent-to-treat.

The Breast 40 (2018) 67e75

Prediction of treatment effect by liquid biopsy - Breast cancer



ERBB2

Concordance: 46.26%

Sensitivity: 30.0%

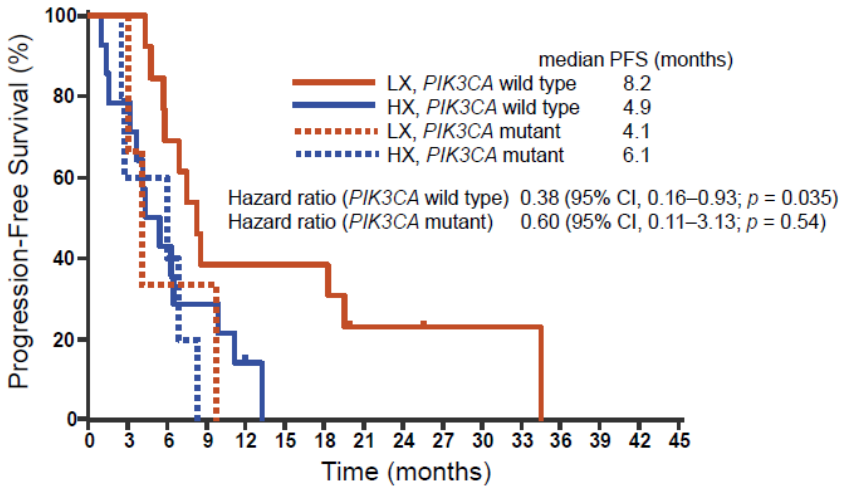
Specificity: 100%

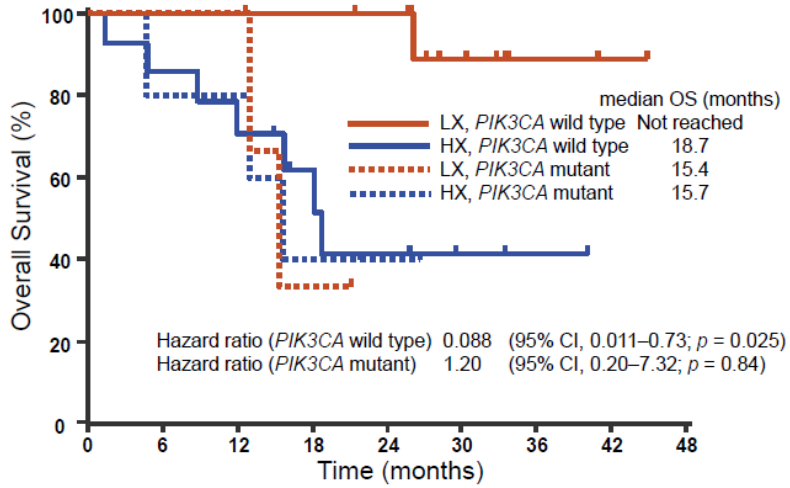
PIK3CA

Concordance: 84.6%

Sensitivity: 60.0%

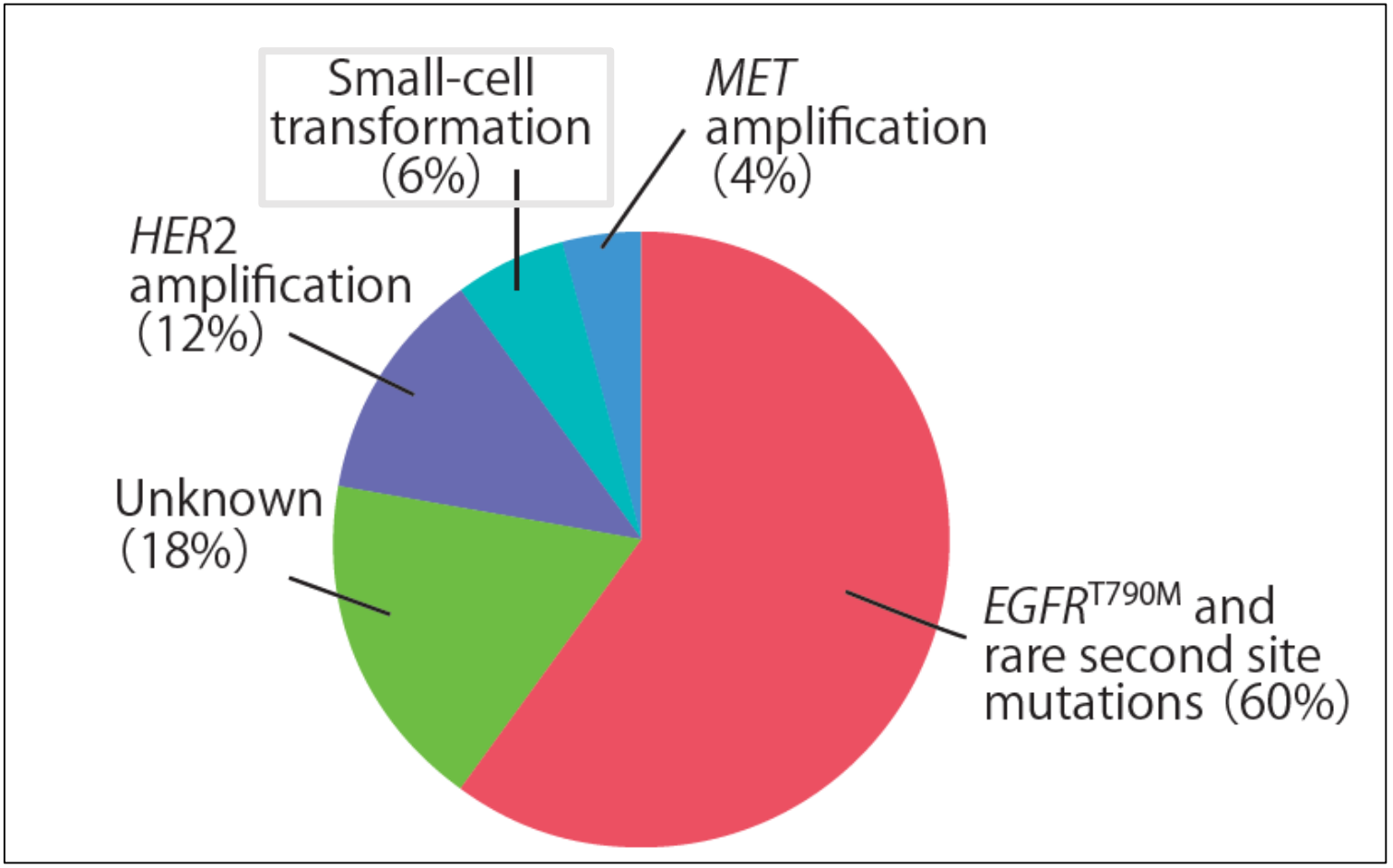
Specificity: 100%





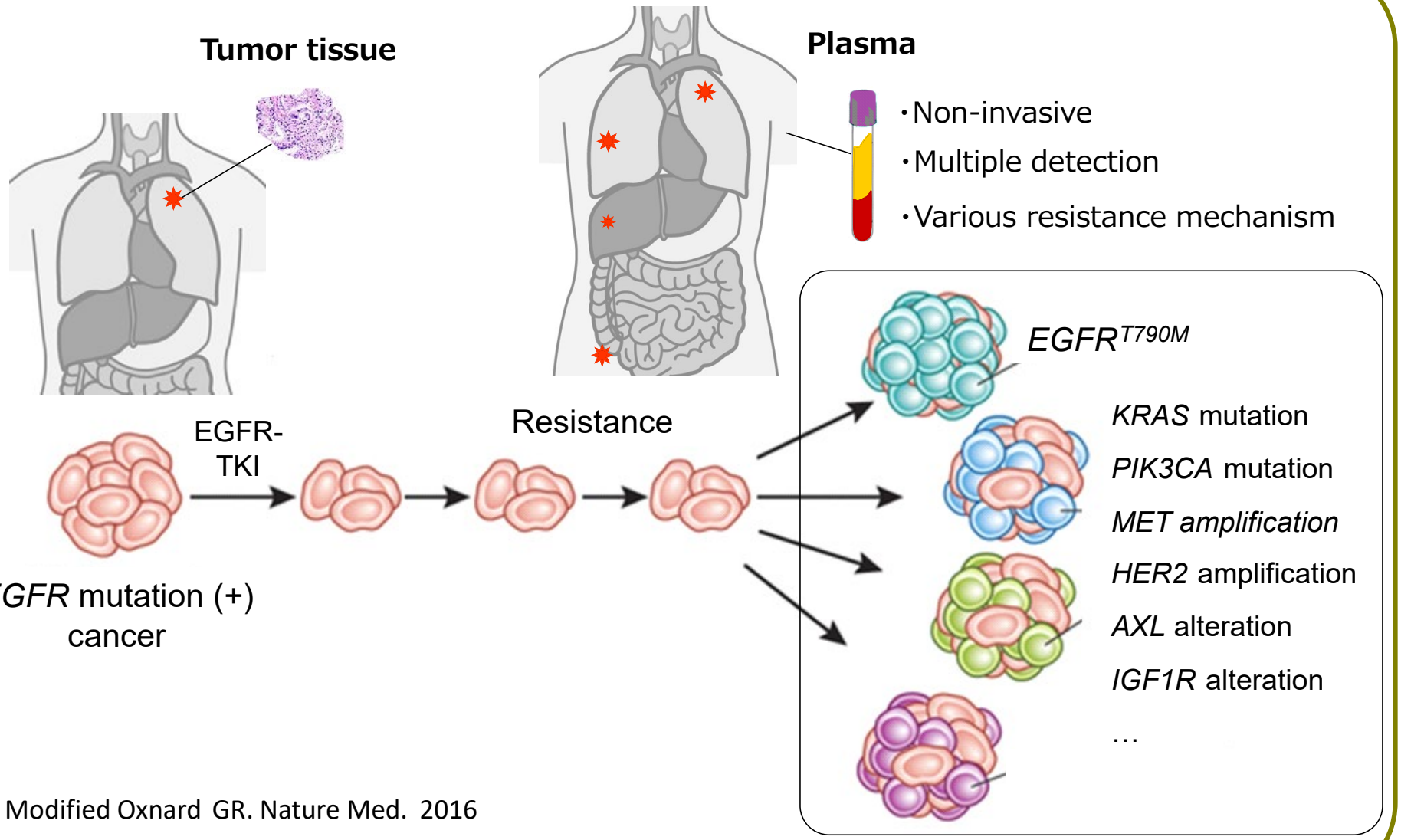
T Takano et al. Breast 2018

Mechanisms of resistance to EGFR-TKI (Japanese population)



日本内科学会雑誌105 巻9 号 (2016)

Detection of resistant mechanisms by LB



Modified Oxnard GR. Nature Med. 2016

RESEARCH ARTICLE | CANCER

Activation of ERBB2 Signaling Causes Resistance to the EGFR-Directed Therapeutic Antibody Cetuximab

Kimio Yonesaka[1,2,3,4], Kreshnik Zejnullahu[1,2], Isamu Okamoto[3], Taroh Satoh[3], Federico Cappuzzo[5], John Souglakos[6,7], Dalia E...
+ See all authors and affiliations

Science Translational Medicine 3:99, 2011

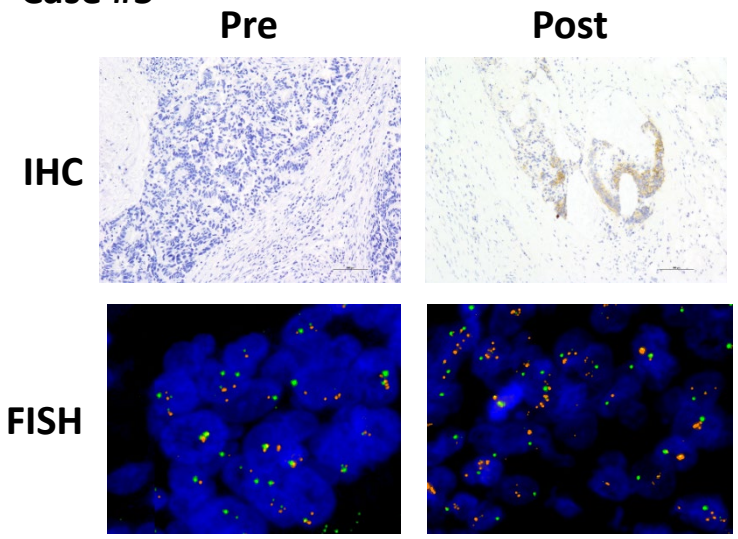
Activation of HER2/HER3 signal by heregulin (HER3 ligand)and HER2 copy number gain: Nobel resistant mechanism of anti-EGFR antibody.
↓
Can HER2 gene amplification be detected in blood samples?

HER2 genomic amplification in circulating tumor DNA from patients with cetuximab-resistant colorectal cancer

Naoki Takegawa[1], Kimio Yonesaka[1*], Kazuko Sakai[2], Hiroto Ueda[1], Satomi Watanabe[1], Yoshikane Nonagase[1], Tatsuya Okuno[1], Masayuki Takeda[1], Osamu Maenishi[3], Junji Tsurutani[1], Taroh Satoh[4], Isamu Okamoto[5], Kazuto Nishio[2], Takao Tamura[1], and Kazuhiko Nakagawa[1]

No.	Relative quantitation of HER2 gene in plasma	HER2 ECD protein , ng/mL
1	1.23	11.4
2	0.97	10.1
3	0.94	12.6
4	1.08	8.7
5	5.18*	13.5
6	1.07	15.9
7	1.20	9.0
8	1.09	5.2
9	1.09	15.8
10	1.33*	17.9
11	1.09	12.2
12	1.08	9.9
13	1.09	7.7
14	1.29*	13.1
15	1.11	-
16	1.01	15.3
17	1.40*	9
18	1.15	-

Case #5



Prediction of treatment effect by liquid biopsy - colorectal cancer

WJOG6210G: Randomized Phase II of 2nd-line FOLFIRI plus Panitumumab or Bevacizumab for *KRAS* Wild Colorectal Cancer

Eligibility
1. Progressive disease during or within 3 months after 1st-line with FU, oxaliplatin, and Bmab
2. *KRAS* exon 2 (codon 12 or 13) wild-type by a validated method
3. PS0-2; ≥20 years
4. Adequate organ function

UMIN ID：UMIN000005216

R

1:1

Stratification:
- Institution
- Köhne index

FOLFIRI+Pmab (n=60)

Pmab 6 mg/kg every 2 weeks
FOLFIRI every 2 weeks (Irinotecan:150 mg/m^2, l-LV:200 mg/m^2, 5-FU:400 mg/m^2/iv, 2400 mg/m^2/civ)

FOLFIRI+Bmab (n=60)

Bmab 5 mg/kg every 2 weeks
FOLFIRI every 2 weeks (Same as above)

Prediction of treatment effect by liquid biopsy - colorectal cancer

WJOG6210G

Serum of wt KRAS CRC refractory to 1st generation of chemotherapy

serum : 1.0 ml (range; 0.2 – 2.7)

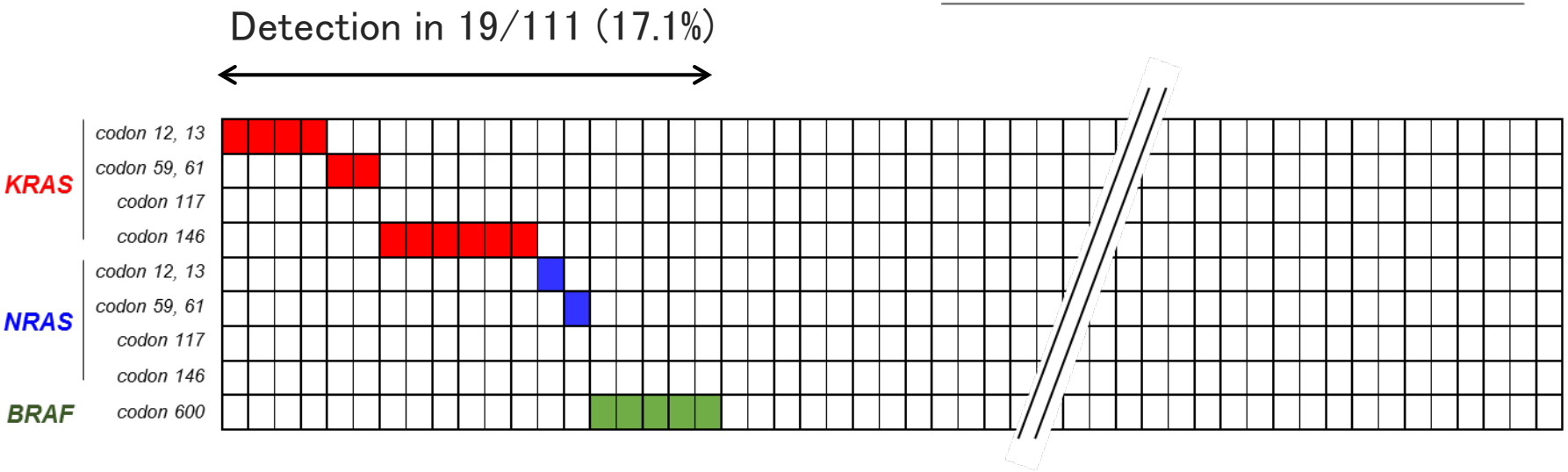
cfDNA yield: 15,376 copies (range; 1848 – 62936)

Success rate 100% (N=111)

The NGS panel for ext RAS

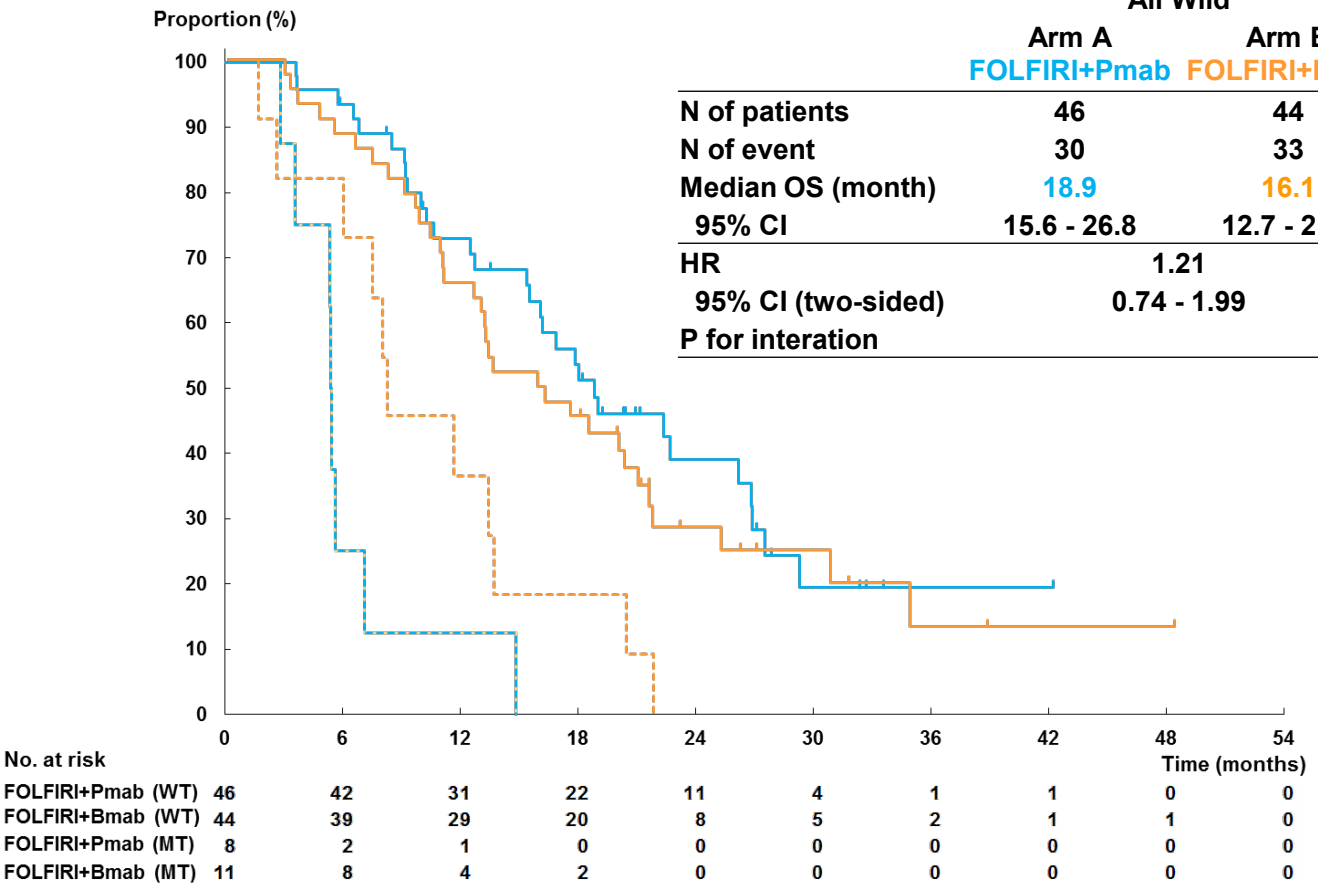
PLoS One. 2015 May 8;10(5):e0121891

Gene	Exon	Codon	Amplicon length
KRAS	2	12, 13	102
	3	59, 61	96
	4	117	100
	4	146	96
NRAS	2	12, 13	100
	3	59, 61	99
	4	117	102
	4	146	98
BRAF	15	600	105



Prediction of treatment effect by liquid biopsy - colorectal cancer

OS according to *RAS* and *BRAF* mutation status in ctDNA



	All Wild		RAS/BRAF mutant*	
	Arm A FOLFIRI+Pmab	Arm B FOLFIRI+Bmab	Arm A FOLFIRI+Pmab	Arm B FOLFIRI+Bmab
N of patients	46	44	8	11
N of event	30	33	8	11
Median OS (month)	18.9	16.1	5.4	8.2
95% CI	15.6 - 26.8	12.7 - 21.1	3.6 - 7.1	6.0 - 13.7
HR	1.21		0.42	
95% CI (two-sided)	0.74 - 1.99		0.15 - 1.12	
P for interation	p=0.026			

K Shitara et al. Cancer Sci. 2016

Concordance of Genomic Alterations by Next Generation Sequencing (NGS) in Tumor Tissue vs. Cell-Free DNA in Stage I-IV NSCLC

N=141

cfDNA: AVENIO ctDNA Surveillance Kit
Tumor DNA: AVENIO FFPE assay

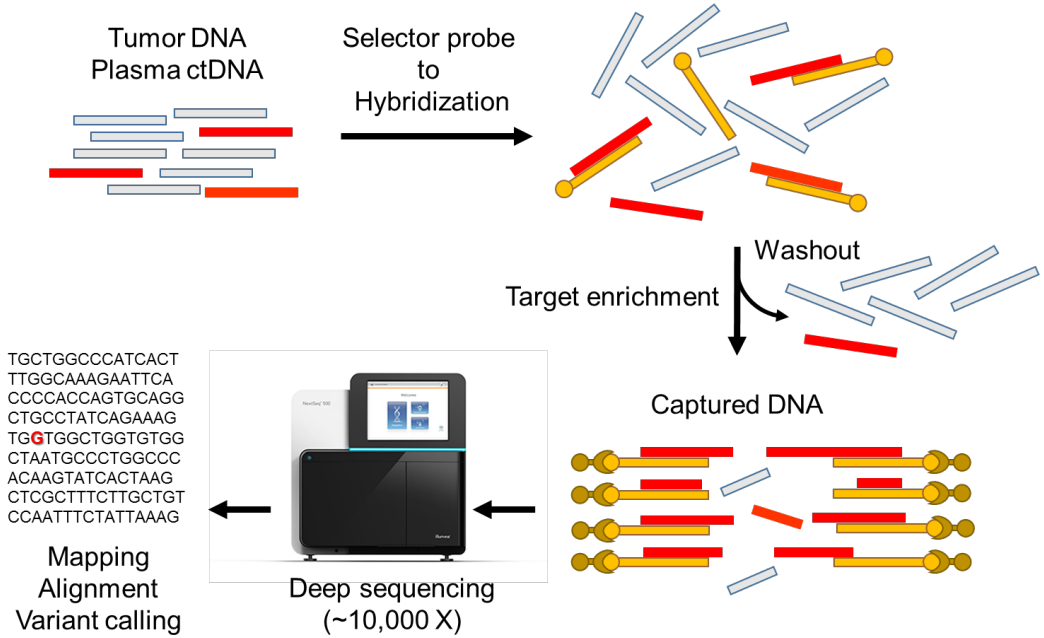
ステージ	患者数	患者あたりの平均SNV数 - 組織	患者あたりの平均SNV数 - 血漿	一致した患者数
I	48	6.9	4.8	13 (27.1%)
II	37	6.6	8.3	30 (81.1%)
III	33	7.4	7.6	25 (75.8%)
IV	23	7.8	10.7	20 (87.0%)

AMP 2017

John Jiang, Hans-Peter Adams, Lijing Yao, Preeti Lal, Aarthi Balasubramanyam, Frederike Fuhlbrueck, Nalin Tikoo, Stephanie Yaung, Sebastian Froehler, Li Tai Fang, Jost Achenbach, Oliver Oster, Olaf Schega, Rainer Krügel, John Palma, Andre Rosenthal

How to detect the low mut allele frequency?

CAncer Personalized Profiling by deep Sequencing (CAPP-Seq)



- NGS based high sensitivive gene mutation analysis
- Method based on molecular barcode method, capture sequence and error suppression algorithm
- Number of genes: 197
- Also detect fusion gene, CNV INDEL
- Required amount of sample: 4 mL plasma (10-50 ng DNA)

Reagent kit → **Illumina NextSeq** → **Analysis Package**

3 days → 31 hours → 16 hours

AVENIO ctDNA Surveillance Panel

Gene	Seq Target	SNV	Indel*	Fusion**	CNV**
ALK	Selected Regions	▪	▪	▪	▪
APC	All Coding Regions	▪	▪		
BRAF	Selected Regions				
BRCA1	All Coding Regions				
BRCA2	All Coding Regions				
DPYD	Selected Regions				
EGFR	All Coding Regions	▪	▪		▪
ERBB2	All Coding Regions	▪	▪		▪
KIT	Selected Regions	▪	▪		

Gene	Seq Target	SNV	Indel*	Fusion**	CNV**
KRAS	All Coding Regions	▪			
MET	All Coding Regions	▪	▪		▪
		▪			
		▪			
		▪		▪	▪
		▪		▪	▪
TP53	All Coding Regions	▪			
UGT1A1	Selected Regions	▪			

Actionable mutation/fusion/CNA

Frequently mutated select regions of these genes included to monitor tumor burden (n=180)

ABCC5	CHRM2	DOCK3	GBP7	IFI16	LRRTM1	NXPH4	RNASE3	TMEM200A
ABCG2	CNTN5	DSC3	GJA8	IL7R	LRRTM4	NYAP2	ROBO2	TNFRSF21
ACTN2	CNTNAP2	DSCAM	GPR139	INSL3	LTBP4	OPRD1	SEMA5B	TNN
ADAMTS12	CPXCR1	EGFLAM	GRIA2	ITGA10	MAP2	P2RY10	SLC18A3	TNR
ADAMTS16	CPZ	EPHA5	GRIK3	ITSN1	MAP7D3	PAX6	SLC39A12	TRHDE
ARFGEF1	CRMP1	EPHA6	GRIN2B	KCNA5	MKRN3	PCDH15	SLC6A5	TRIM58
ASTN1	CSMD1	EYS	GRIN3B	KCNB2	MMP16	PDYN	SLC8A1	TRPS1
ASTN2	CSMD3	FAM135B	GRM1	KCNC2	MTX1	PDZRN3	SLITRK1	UGT3A2
AVPR1A	CTNNB1	FA					SLITRK4	USH2A
BCHE	CTNND2	FA					SLITRK5	USP29
BPIFB4	CYBB	FA					SLPI	VPS13B
C6	DCAF12L1	FA					SMAD4	WBSCR17
C6orf118	DCAF12L2	FA					SOX9	WIPF1
CA10	DCAF4L2	FBN2	HEBP1	KIF19	NLGN4X	POLE	SPTA1	WSCD2
CACNA1E	DCLK1	FBXL7	HECW1	KLHL31	NLRP3	POM121L12	ST6GALNAC3	ZC3H12A
CDH12	DCSTAMP	FBXW7	HS3ST4	KPRP	NMUR1	PREX1	STK11	ZFPM2
CDH18	DD11	FCRL5	HS3ST5	LPPR4	NOL4	PTPLA	SV2A	ZIC1
CDH8	DLGAP2	FOXG1	HTR1A	LRFN5	NPAP1	RALYL	T	ZIC4
CDH9	DMD	FRYL	HTR1E	LRP1B	NROB1	RFX5	THSD7A	ZNF521
CDKN2A	DNTTIP1	GBA3	HTR2C	LRRC7	NRXN1	RIN3	TIAM1	ZSCAN1

Tumor mutation burden

Evaluation of clinical outcomes by analysis of mutations in tumor tissue and ctDNA using NGS from STEAM, a prospective, randomized, multi-center study in metastatic colorectal cancer (mCRC)

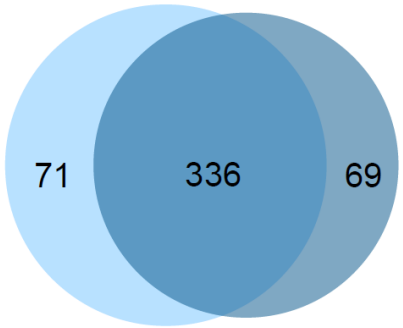
N=118 (metastatic CRC)

cfDNA: AVENIO ctDNA Expanded Kit

All 77 genes in the Expanded Panel

Tissue
(n=118)
407 variants

Pre-Induction Plasma
(n=118)
405 variants



Gene	Overall Agreement
KRAS	**91.5%**
NRAS	**99.2%**
BRAF	**100.0%**
PIK3CA	**94.9%**
APC	**94.1%**
SMAD4	**97.5%**
TP53	**93.2%**

Overall Concordance = 336/407 = **83%**

ASCO 2017

Lee JJ, Palma JF, Yao L, Lovejoy AF, Yaung S, Zhang D, Wingate-Pearse N, Yau M, Williams C, Pimentel M, Munoz A, Mayol K, Mancao C, Nicholas A, Sommer N, Hurwitz HI, Bendell J, Rohr UP

Agenda

- ctDNA and mol targeted agents
- Ct DNA and ICIs

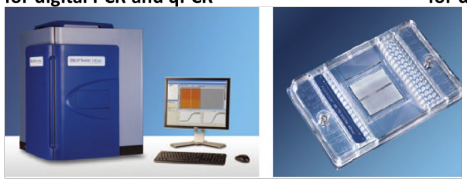
dPCR

NGS

Next-Generation Sequencer

Digital PCR on chips

✓Fluidigm Corporation's BioMark HD System for digital PCR and qPCR



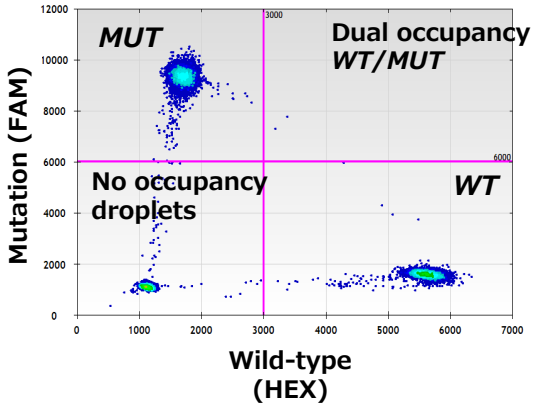
✓Life Technologies' QuantStudio System for digital PCR and qPCR

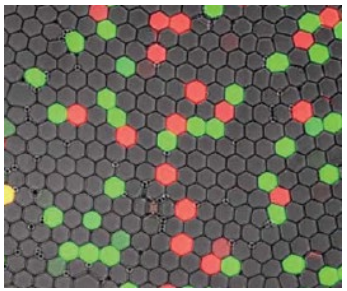
Digital PCR in droplets (ddPCR)

✓Bio-Rad's QX100 droplet digital PCR System

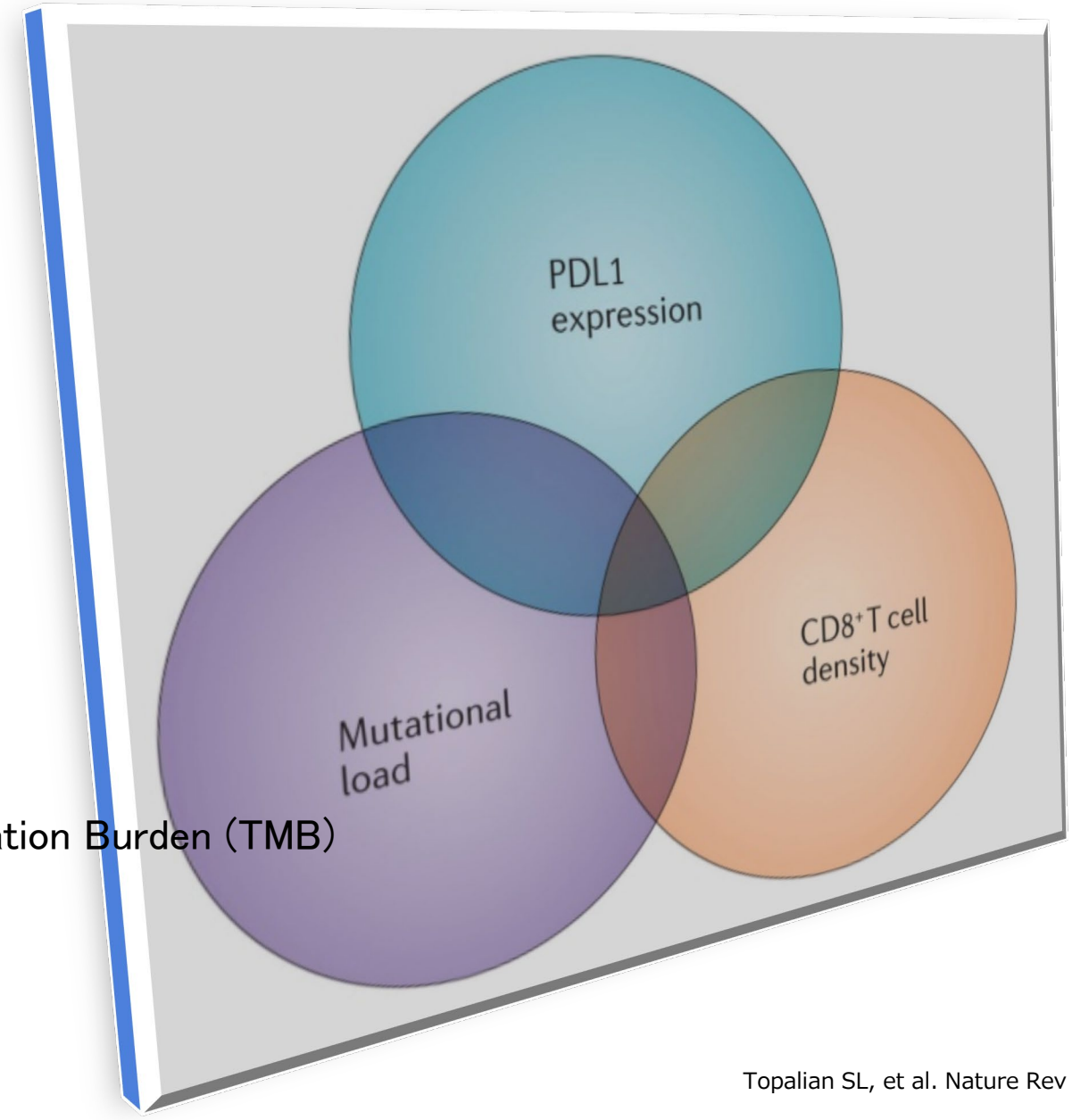
✓RainDrop Source and RainDrop Sense machines for droplet digital PCR

Monya BakerMonya Baker, Nature methods ,VOL.9 NO.6, 2012





Predictive biomarkers for ICI is necessary



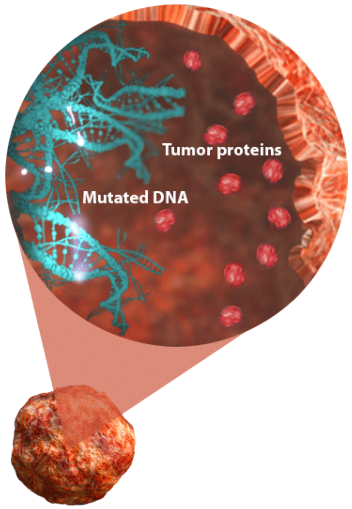
Tumor Mutation Burden (TMB)

Topalian SL, et al. Nature Rev Cancer 2016; 16: 275–287.

TMB high

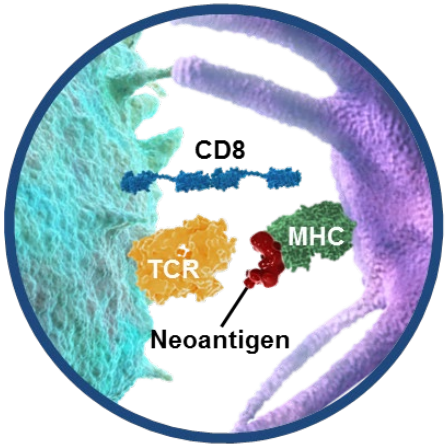
TMB: No of missense mutation/Mb
Tumor Mutational Burden and Neoantigens

TMB high…[1,2]

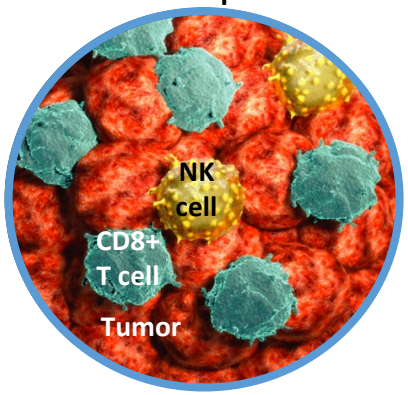


Tumor cell

…neoantigen load↑[1,2]



…… which can lead to high tumor immunogenicity and increased T-cell reactivity and anti-tumor response[2-5]

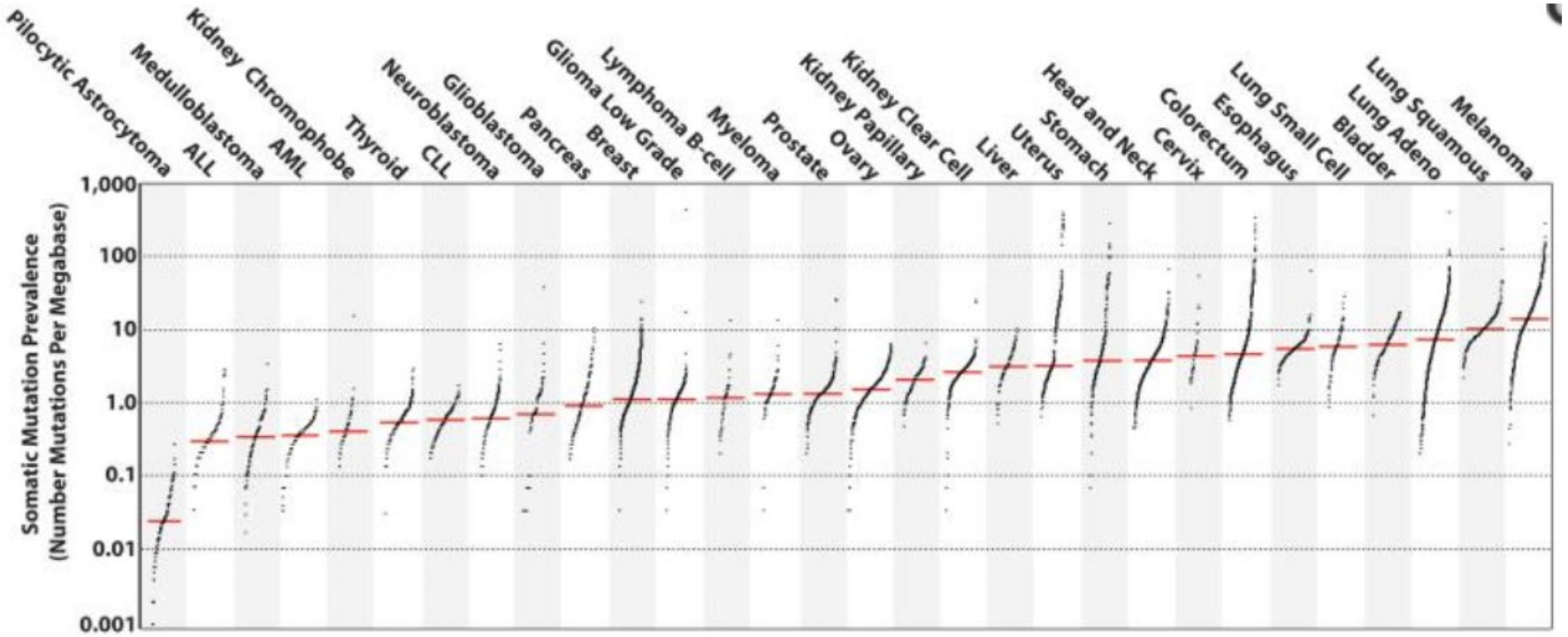


…which can lead to high tumor immunogenicity and increased T-cell reactivity and anti-tumor response[2-5]

1. Schumacher TN, Schreiber RD. *Science.* 2015;348(6230):69-74. 2. Kim JM, Chen DS. *Ann Oncol*. 2016;27(8):1492-1504.
3. Liontos M et al. *Ann Transl Med*. 2016; 4(14):264. 4. Sharma P, Allison JP. *Science*. 2015;348(6230):56-61. 5. Giannakis M et al. *Cell Rep*. 2016;15:857-865.

一部改変

Nonsynonymous Mutation Burden Associated with Clinical Benefit of Anti–PD-1 Therapy

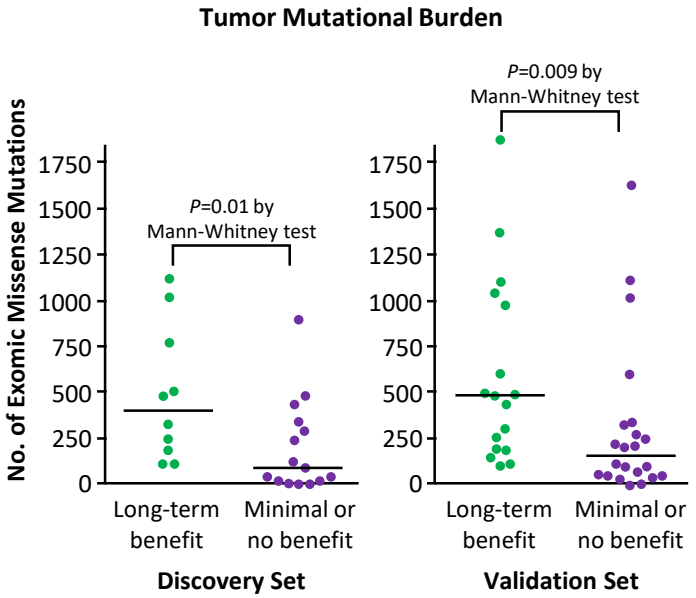


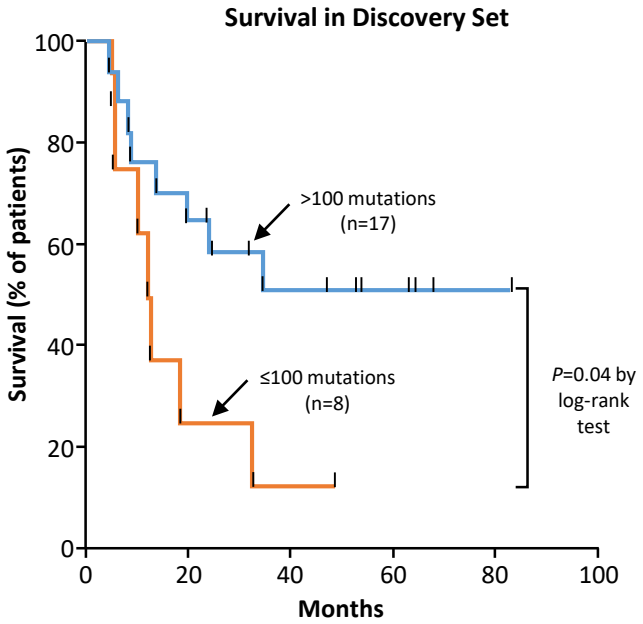
Alexandrov LB, et al. Nature. 2013; 500: 415–421.

First Demonstration of High TMB Correlated With Clinical Benefit With a CTLA-4 Inhibitor in Patients With Melanoma

Methods/Design

- Treated with commercial ipilimumab or treated as part of 1 of 11 clinical trials with ipilimumab (discovery set, n=25), or ipilimumab or tremelimumab (validation set, n=39)[1,2]
- **WES by Illumina HiSeq 2000[2]**
- **TMB high defined as >100 mut/tumor[2]**

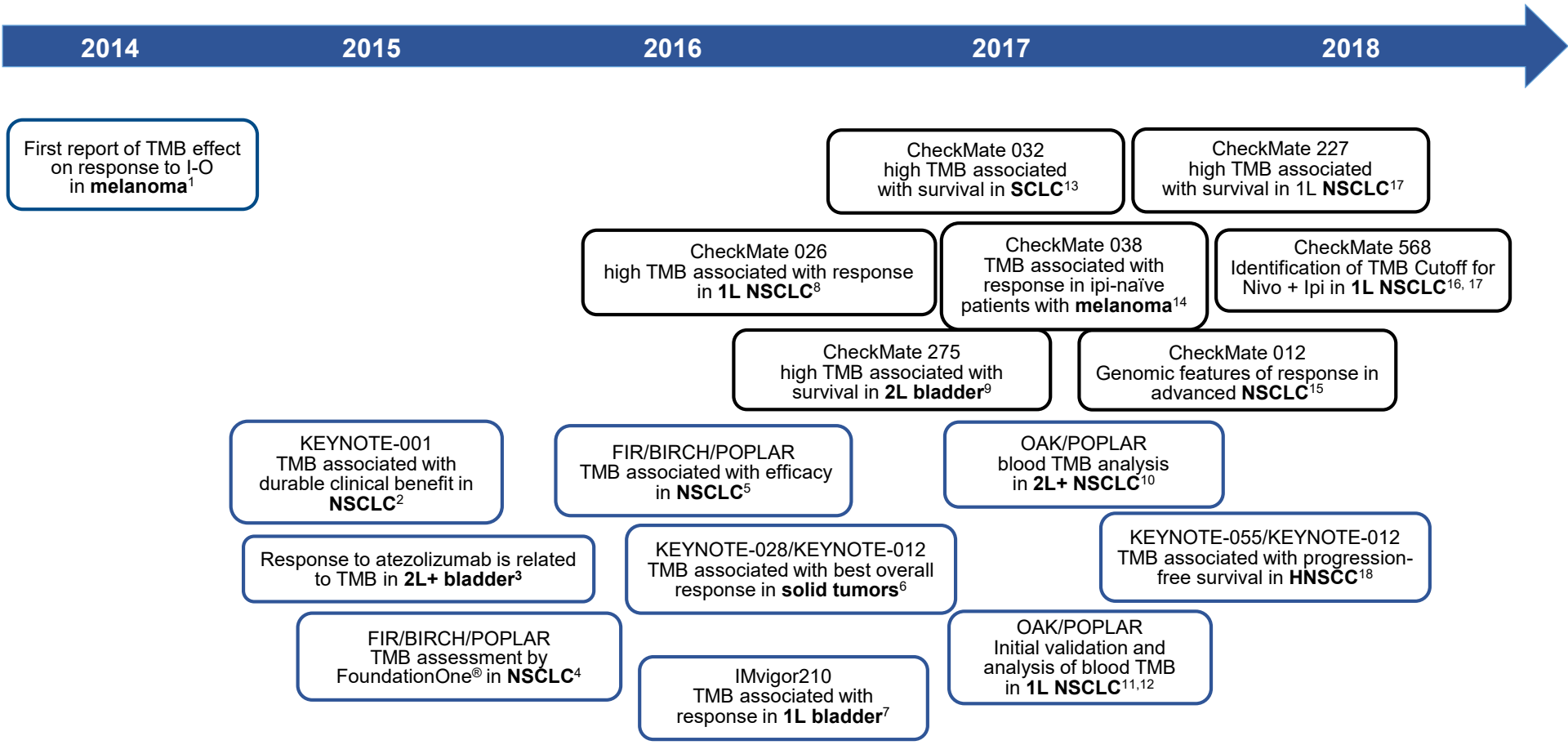




Patients with high TMB showed significant improvement in outcomes compared with those with low TMB[2]

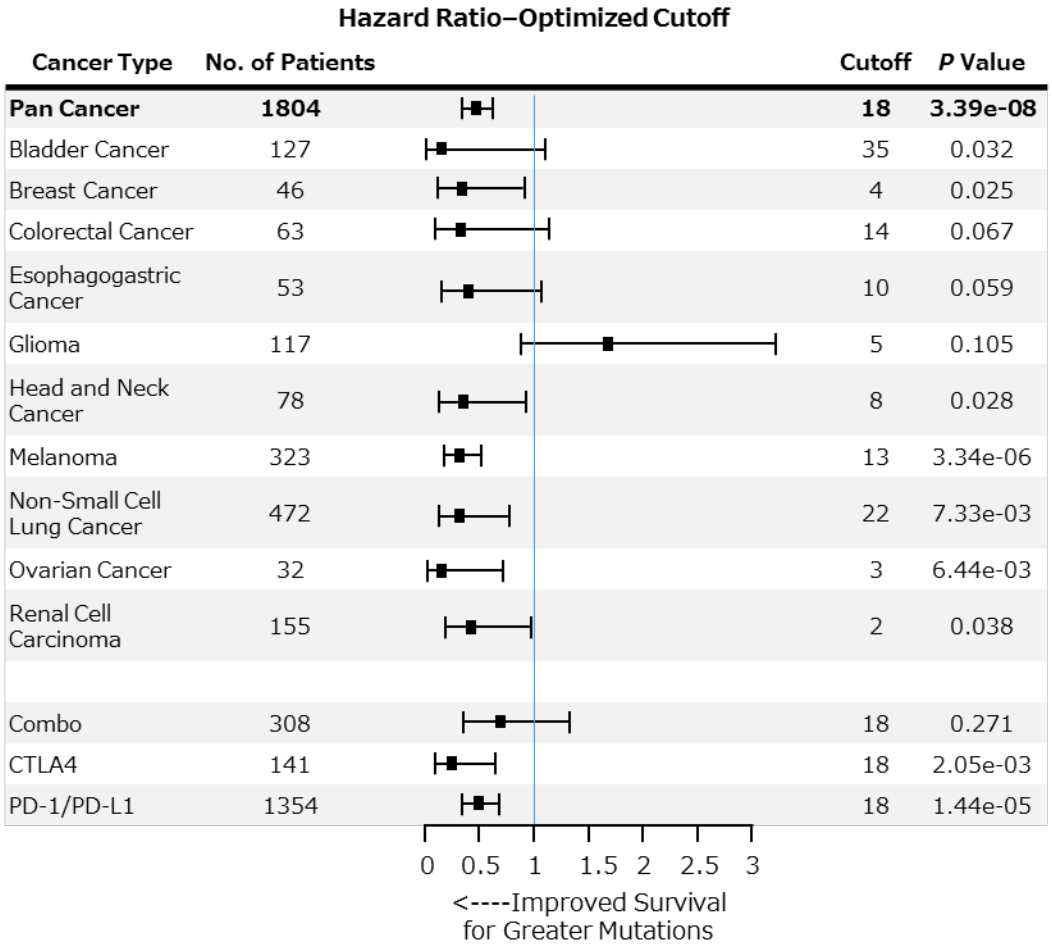
1. Snyder A et al. *N Engl J Med*. 2014;371(23):2189-2199 [supplementary appendix]. 2. Snyder A et al. *N Engl J Med*. 2014;371(23):2189-2199.

TMB Clinical Data Timeline: Increasing Knowledge Over Time



1. Snyder A et al. *N Engl J Med.* 2014;371(23):2189-2199. 2. Rizvi NA et al. *Science*. 2015;348(6230):124-128. 3. Rosenberg JE et al. *Lancet.* 2016;387(10031):1909-1920. 4. Kowanetz M et al. Poster presentation at ESMO 2016. 77P. 5. Kowanetz M et al. Oral presentation at WCLC 2016. 6149. 6. Cristescu R et al. Poster presentation at ASCO-SITC 2017. 7. Balar AV et al. *Lancet*. 2017;389:67-76. 8. Carbone DP et al. *N Engl J Med.* 2017;376(25):2415-2426. 9. Galsky MD et al. Poster presentation at ESMO 2017. 848PD. 10. Gandara DR et al. Oral presentation at ESMO 2017. 1295O. 11. Fabrizio DA et al. Poster presentation at ESMO 2017. 102P. 12. Mok T et al. Poster presentation at ESMO 2017. 1383TiP. 13. Antonia SJ et al. Oral presentation at WCLC 2017. 11063. 14. Riaz N et al. *Cell*. 2017;171(4):934-949. 15. Hellmann MD et al. *Cancer Cell*. 2017; https://doi: 10.1016/j.ccell.2018.03.018. 16. Ramalingam SS et al. Oral presentation at AACR 2018. CT078 17. Hellmann MD et al. *N Engl J Med.* 2018; doi: 10.1056/NEJMoa1801946. [Supplementary Appendix] 18. Seiwert TY et al. Oral presentation at AACR 2018. LB-339.

Pan-Tumor Assessment of TMB and Clinical Benefit with Checkpoint Inhibitors



Chan TA. Oral presentation at ASCO-SITC 2017. 2. Cheng DT et al. *J Mol Diagn*. 2015;17:251-264.

AVENIO ctDNA Surveillance Panel

Gene	Seq Target	SNV	Indel*	Fusion**	CNV**
ALK	Selected Regions	▪	▪	▪	▪
APC	All Coding Regions	▪	▪		
BRAF	Selected Regions				
BRCA1	All Coding Regions				
BRCA2	All Coding Regions				
DPYD	Selected Regions				
EGFR	All Coding Regions	▪	▪		▪
ERBB2	All Coding Regions	▪	▪		▪
KIT	Selected Regions	▪	▪		

Gene	Seq Target	SNV	Indel*	Fusion**	CNV**
KRAS	All Coding Regions	▪			
MET	All Coding Regions	▪	▪		▪
		▪			
		▪			
		▪		▪	▪
		▪		▪	▪
TP53	All Coding Regions	▪			
UGT1A1	Selected Regions	▪			

Actionable mutation

Frequently mutated select regions of these genes included to monitor tumor burden (n=180)

ABCC5	CHRM2	DOCK3	GBP7	IFI16	LRRTM1	NXPH4	RNASE3	TMEM200A
ABCG2	CNTN5	DSC3	GJA8	IL7R	LRRTM4	NYAP2	ROBO2	TNFRSF21
ACTN2	CNTNAP2	DSCAM	GPR139	INSL3	LTBP4	OPRD1	SEMA5B	TNN
ADAMTS12	CPXCR1	EGFLAM	GRIA2	ITGA10	MAP2	P2RY10	SLC18A3	TNR
ADAMTS16	CPZ	EPHA5	GRIK3	ITSN1	MAP7D3	PAX6	SLC39A12	TRHDE
ARFGEF1	CRMP1	EPHA6	GRIN2B	KCNA5	MKRN3	PCDH15	SLC6A5	TRIM58
ASTN1	CSMD1	EYS	GRIN3B	KCNB2	MMP16	PDYN	SLC8A1	TRPS1
ASTN2	CSMD3	FAM135B	GRM1	KCNC2	MTX1	PDZRN3	SLITRK1	UGT3A2
AVPR1A	CTNNB1	FA					SLITRK4	USH2A
BCHE	CTNND2	FA					SLITRK5	USP29
BPIFB4	CYBB	FA					SLPI	VPS13B
C6	DCAF12L1	FA					SMAD4	WBSCR17
C6orf118	DCAF12L2	FA					SOX9	WIPF1
CA10	DCAF4L2	FBN2	HEBP1	KIF19	NLGN4X	POLE	SPTA1	WSCD2
CACNA1E	DCLK1	FBXL7	HECW1	KLHL31	NLRP3	POM121L12	ST6GALNAC3	ZC3H12A
CDH12	DCSTAMP	FBXW7	HS3ST4	KPRP	NMUR1	PREX1	STK11	ZFPM2
CDH18	DD11	FCRL5	HS3ST5	LPPR4	NOL4	PTPLA	SV2A	ZIC1
CDH8	DLGAP2	FOXG1	HTR1A	LRFN5	NPAP1	RALYL	T	ZIC4
CDH9	DMD	FRYL	HTR1E	LRP1B	NROB1	RFX5	THSD7A	ZNF521
CDKN2A	DNTTIP1	GBA3	HTR2C	LRRC7	NRXN1	RIN3	TIAM1	ZSCAN1

Mutation burden

Ongoing; concordance of bTMB and tTMB (FMI)

Acknowledgement

・WJOG data center (chairman Dr. Nakamura S)
・WJOG lung cancer group (chairman Prof. Yamamoto)
・Life Technologies
Katayama J, Furukawa T, Murakami S
・Kindai University Faculty of Medicine
Dept Surgery
Mitsudomi T
Dept Medical Oncology
Kitano M, Tsurutani J, Takeda M, Takahama T, Watanabe S, Hayashi H, Nakagawa K
Dept Pathology
Shimizu S, Ito A
Dept Genome Biology
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