CIRCULATING TUMOUR DNA: A PARADIGM SHIFT IN PATIENT MANAGEMENT

RODNEY J. SCOTT

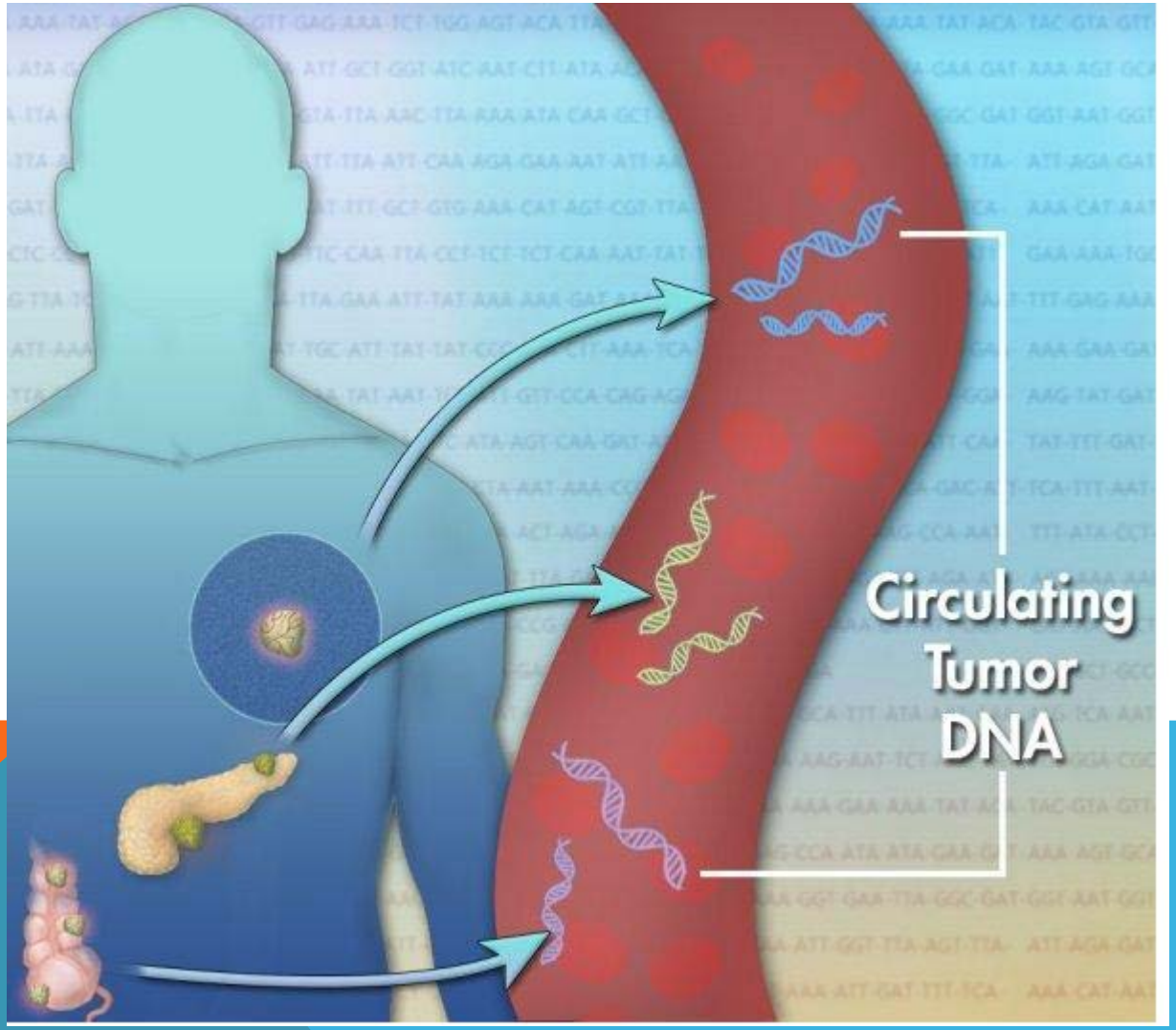
Kathmandu, Bir Hospital visit, August 2018

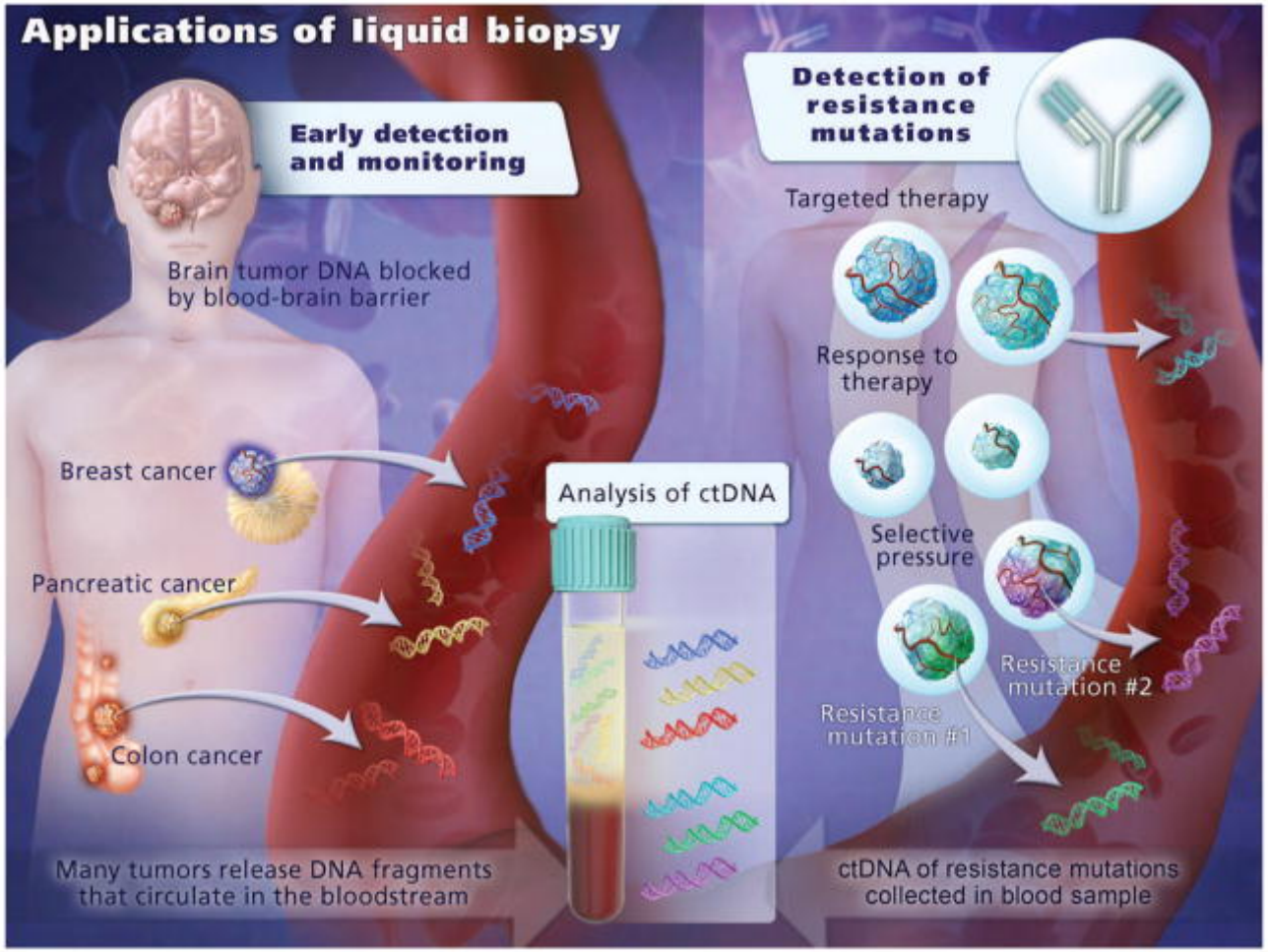
Laureate Professor Rodney Scott

Laureate Professor
School of Biomedical Sciences and Pharmacy
(Medical Genetics)

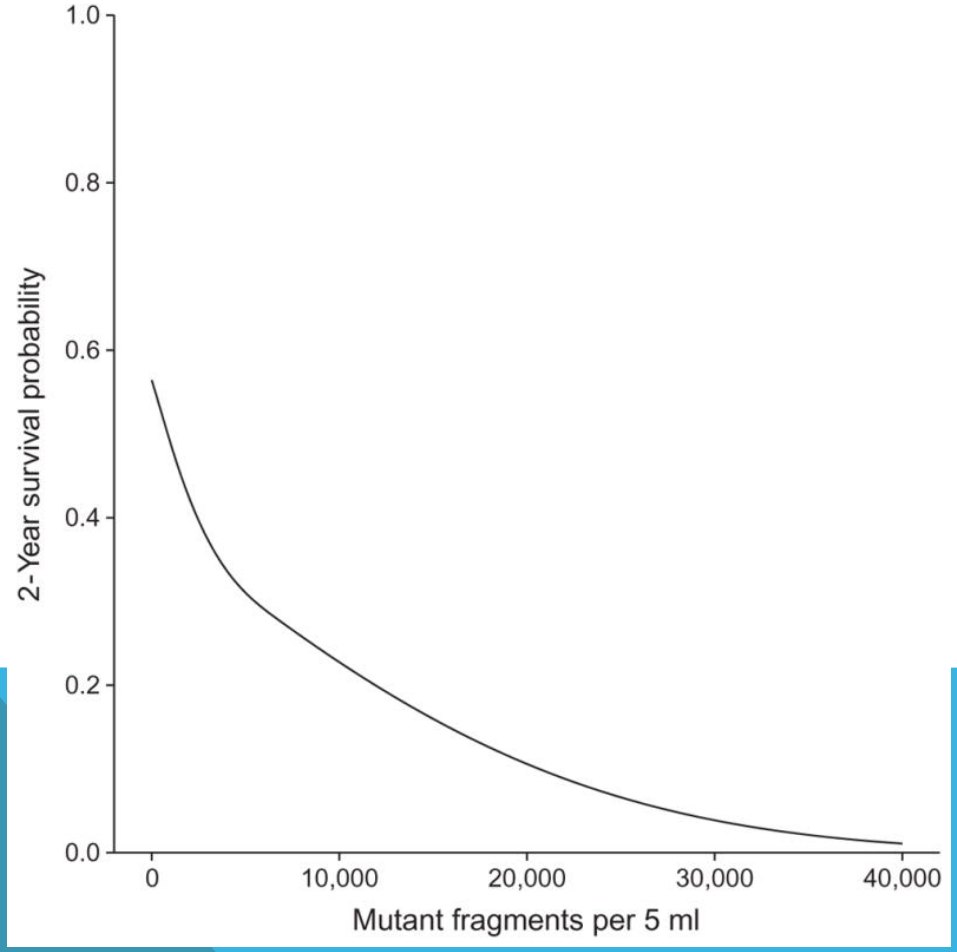
rodney.scott@newcastle.edu.au
(02) 4921 4974

CIRCULATING TUMOUR DNA

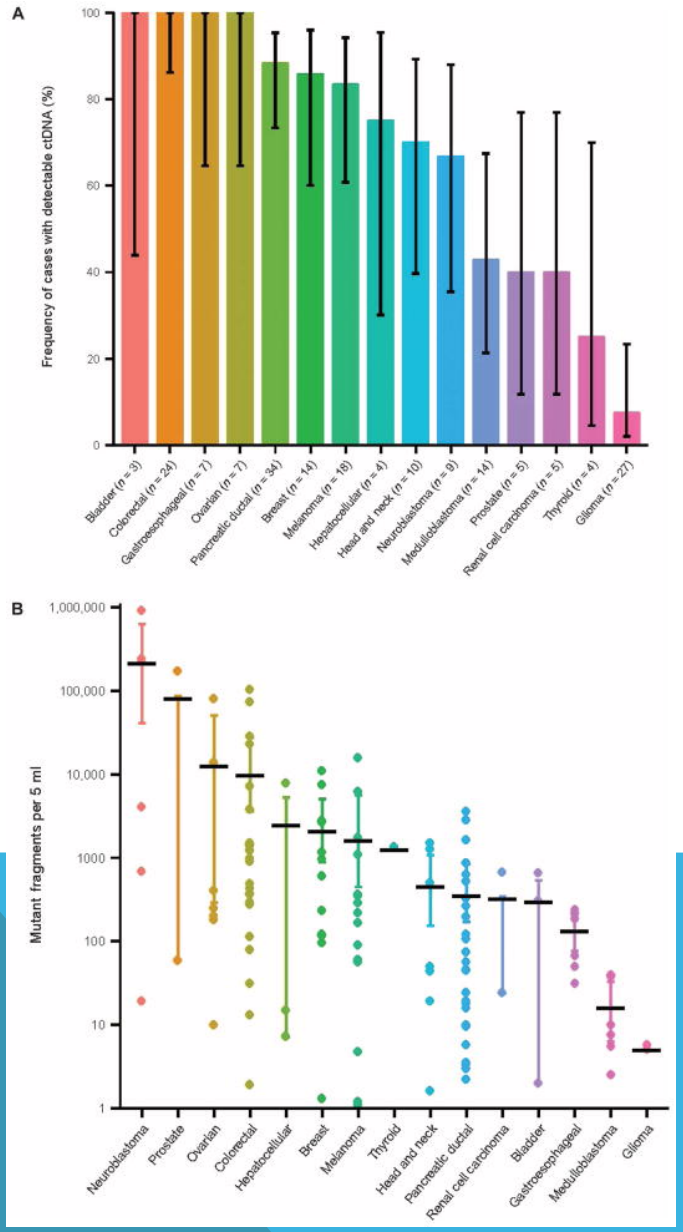



Applications of liquid biopsy
Early detection and monitoring
Brain tumor DNA blocked by blood-brain barrier
Breast cancer
Pancreatic cancer
Colon cancer
Many tumors release DNA fragments that circulate in the bloodstream
Analysis of ctDNA
Detection of resistance mutations
Targeted therapy
Response to therapy
Selective pressure
Resistance mutation #1
Resistance mutation #2
ctDNA of resistance mutations collected in blood sample

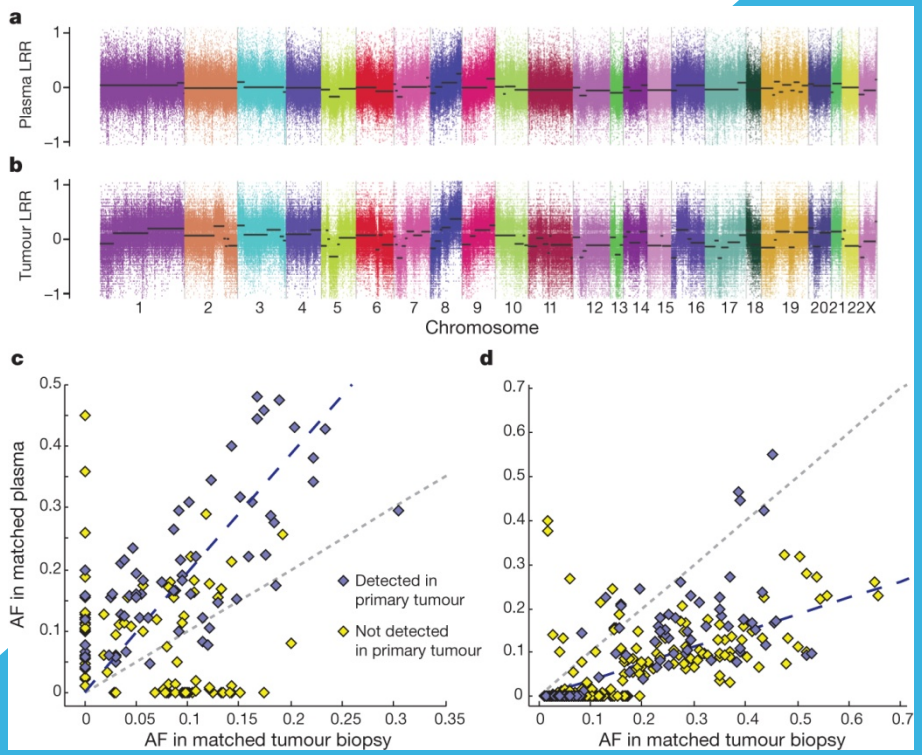
ctDNA CONCENTRATION CORRELATES WITH 2-YEAR SURVIVAL



DIFFERENT TUMOURS SHED DIFFERENT LEVELS OF ctDNA



Genome-wide concordance between plasma DNA and tumour DNA.



M Murtaza *et al. Nature* **000**, 1-5 (2013) doi:10.1038/nature12065

CIRCULATING TUMOR DNA (ctDNA) vs. TISSUE BASED TESTING

Safety and convenience

Minimally invasive, few risks

Accurate information

Archival tissue can be degraded and have different mutation profile

Accessibility

Tissue not always accessible; e.g. NSCLC

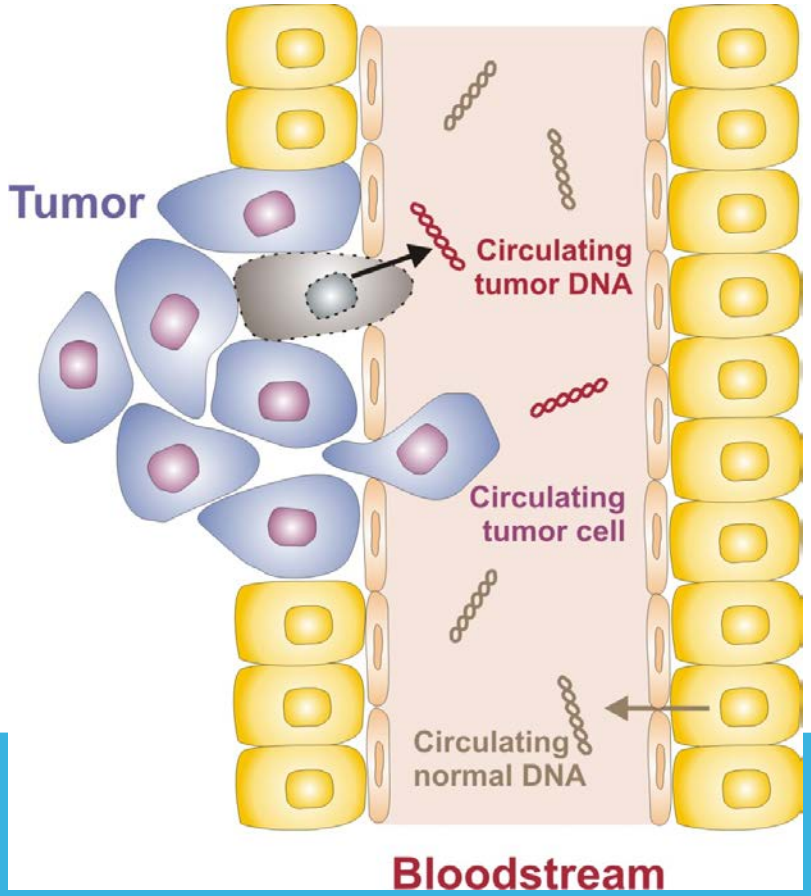
No selection bias

Evaluate primary tumor and metastases w/one sample

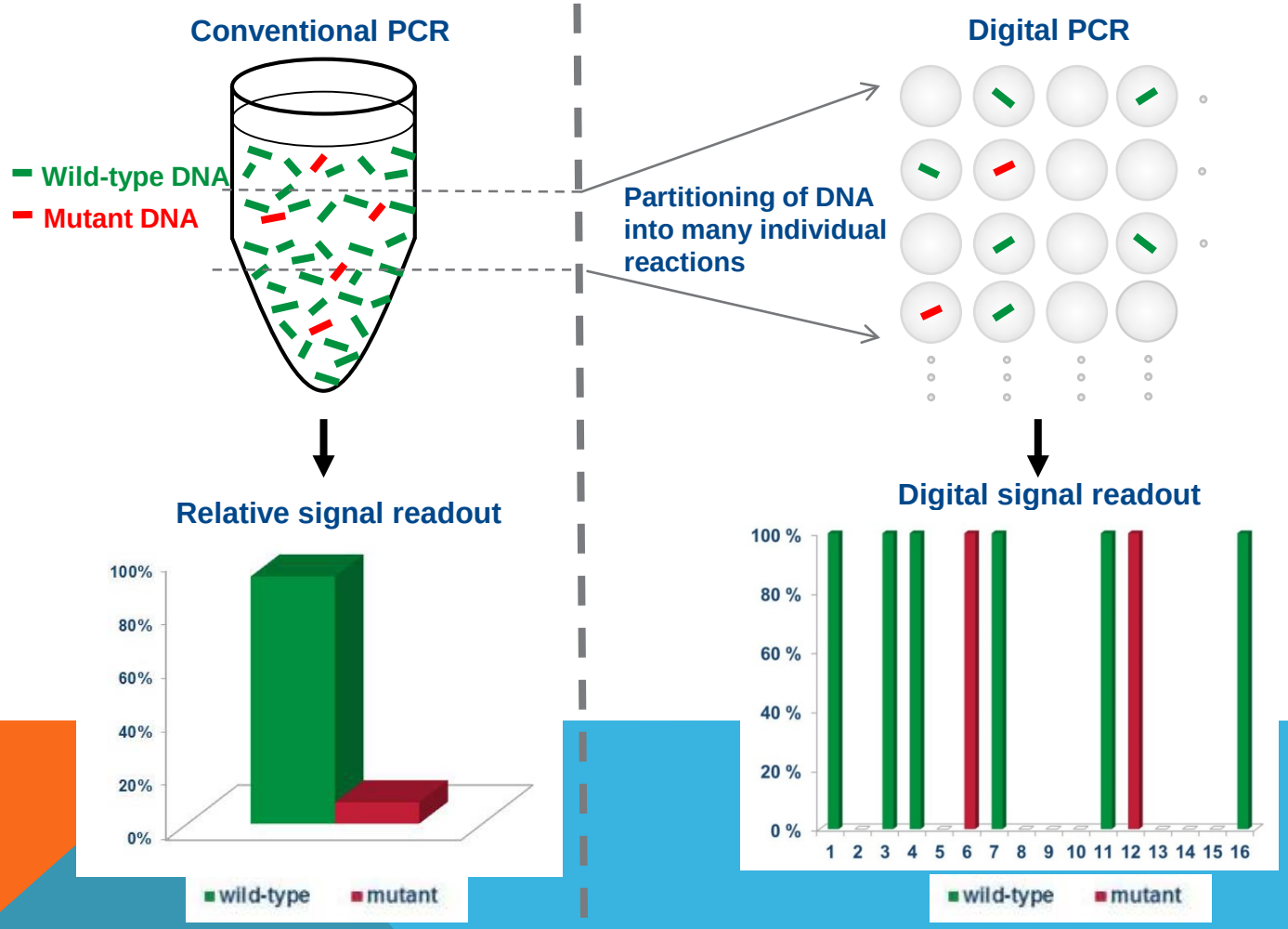
Tumor intra/inter-heterogeneity not a problem

Monitoring possible

Allows multiple measurements to assess drug response and resistance



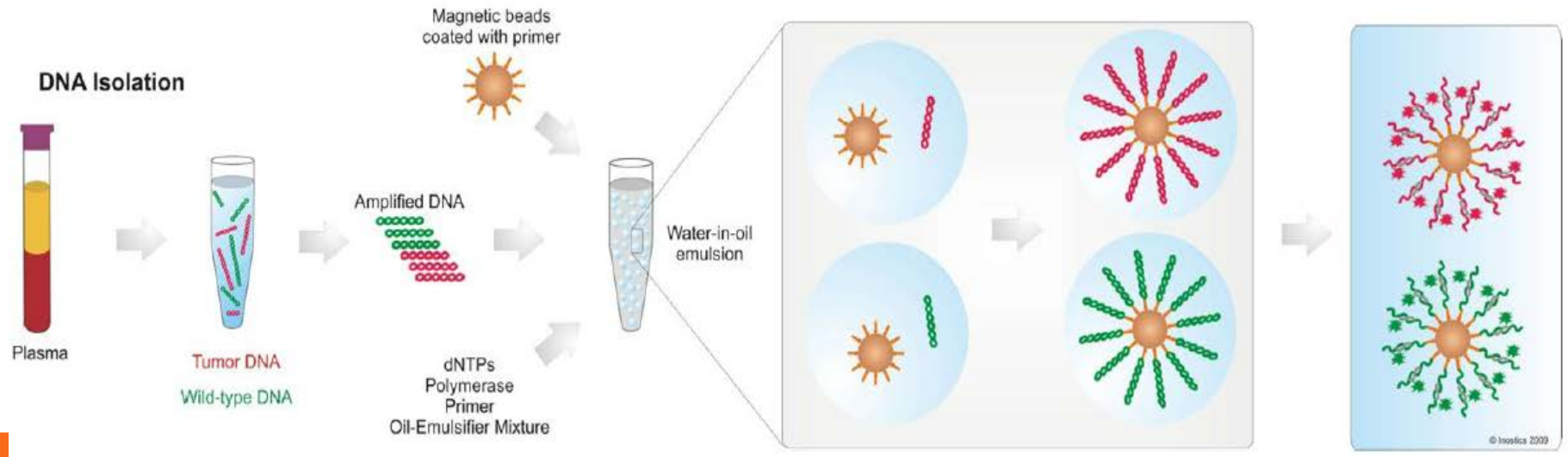
ADVANTAGE OF DIGITAL PCR VS CONVENTIONAL PCR FOR DETECTION OF CTDNA IN PLASMA



Vogelstein *et al.* PNAS 1999; mod. Diehl *et al* Curr Opin Oncol. 2007

BEAMing Digital PCR

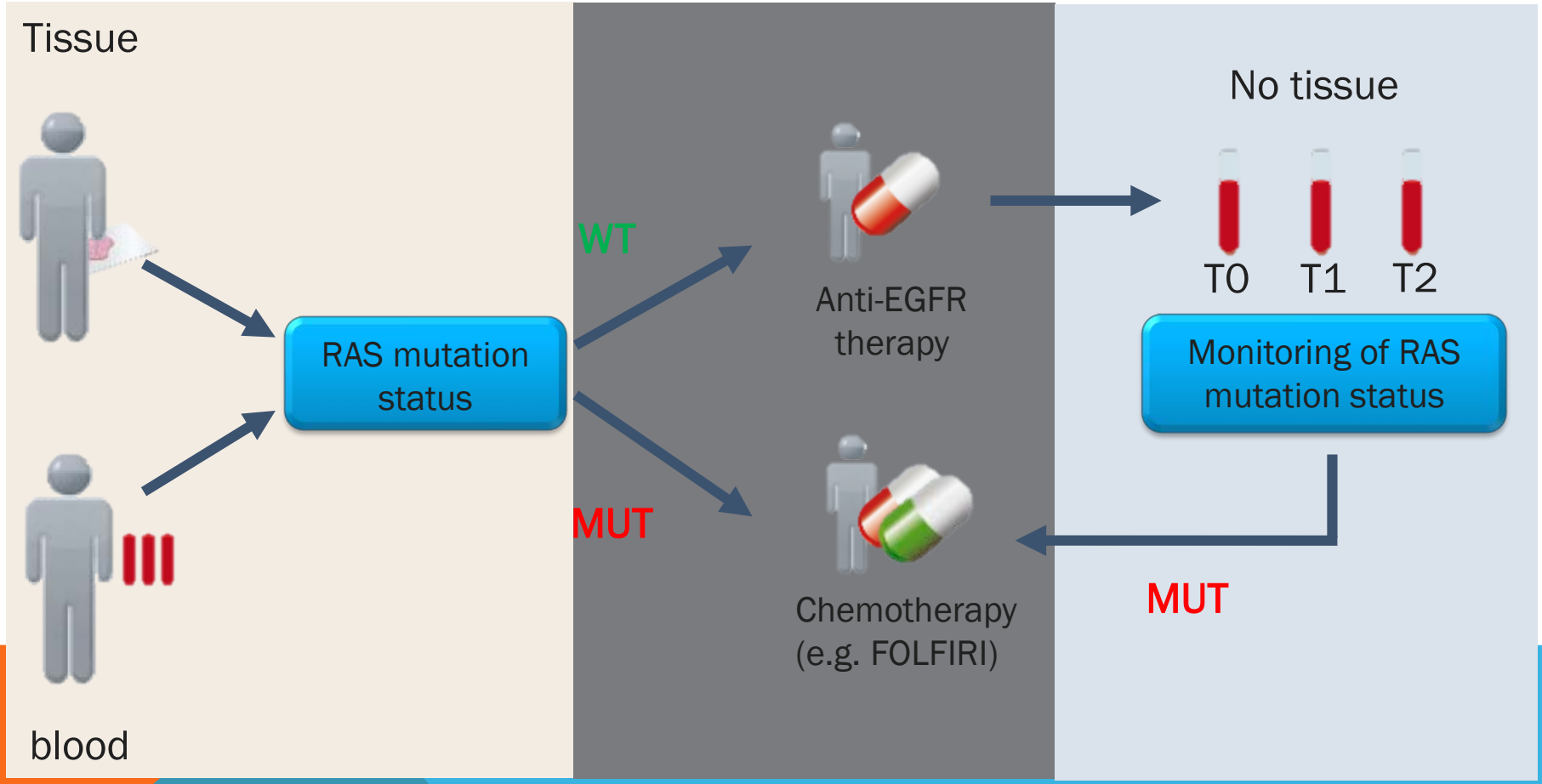
Pre-amplification | **Emulsion PCR** | **Hybridization & Flow Cytometry**



BEAMing (**B**eads, **E**mulsions, **A**mplification, **M**agnetics) has shown efficacy in several therapeutic clinical trials as well as in oncology patient testing applications.

RAS Mutation Testing in Colorectal Cancer anti-EGFR Therapy Selection

Blood-based RAS testing mCRC therapy selection and detection of resistance



Therapy Selection:

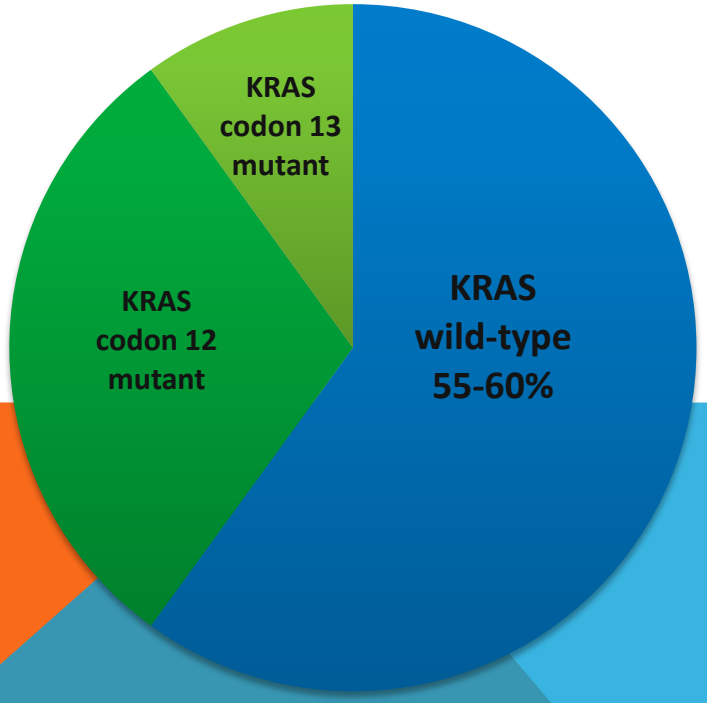
Concordance of RAS status in blood vs tissue

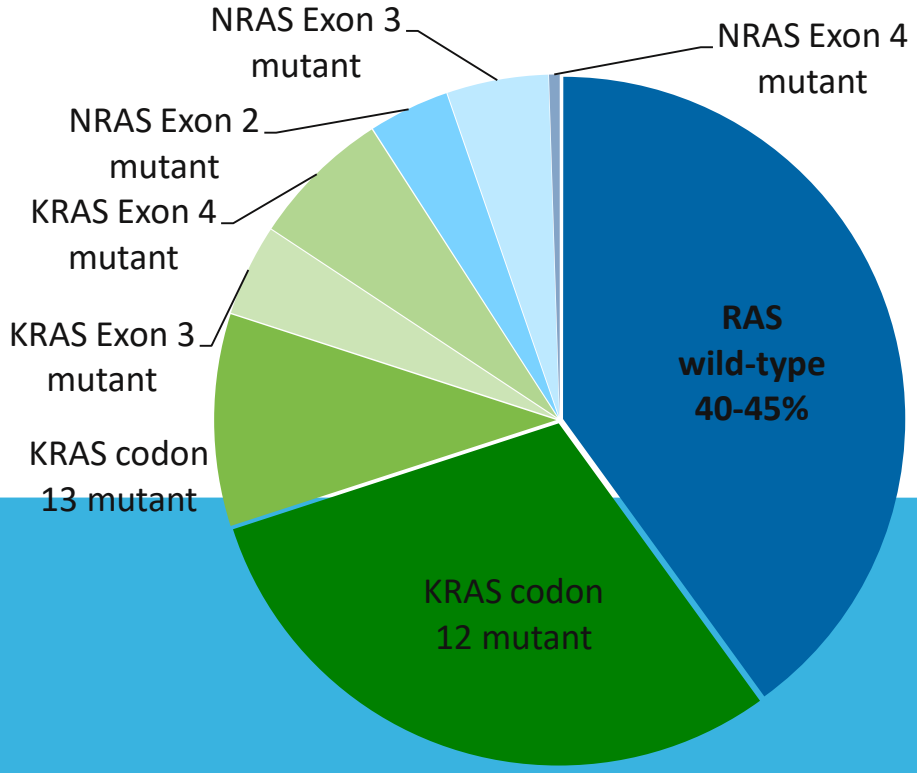
RAS Resistance Detection

Expanding the Understanding of RAS Mutations 2008 - 2015

KRAS exon 2 vs wild-type
(Testing in 2008)

Expanded RAS vs wild-type
(Testing today)





BEAMing RAS: Value in Therapy Selection and Resistance Detection

Expanded RAS mutation panel:

Validation in **OPUS** and **CRYSTAL** trials and utility in emerging RAS resistance detection studies:

1. van Cutsem et al. 2015, J. Clin Oncol 33, 692-700.
2. Bokemeyer et al. 2015, Eur J Cancer 51(10), 1243-52.
3. Diaz et al. 2012, Nature 486(7404), 537–54.0
4. Misale et al. 2012, Nature 486(7404), 532–536.
5. Morelli et al. 2015, Ann Oncol 26, 731-736.
6. Tabernero et al. 2015, Lancet Oncol July 13 online PMID: 26184520

KRAS		NRAS	
Exon	**Mutation**	**Exon**	**Mutation**
2	G12S	2	G12S
2	G12R	2	G12R
2	G12C	2	G12C
2	G12D	2	G12D
2	G12A	2	G12A
2	G12V	2	G12V
2	G13D	2	G13R
3	A59T	2	G13D
3	Q61L	2	G13V
3	Q61H	3	A59T
3	Q61H	3	Q61K
4	K117N	3	Q61R
4	K117N	3	Q61L
4	A146T	3	Q61H
4	A146V	3	Q61H
		4	K117N
		4	K117N
		4	A146T

CONCORDANCE OF RAS MUTATION STATUS IN COLORECTAL CANCER PATIENTS BY COMPARISON OF RESULTS FROM CIRCULATING TUMOUR DNA AND TISSUE-BASED TESTING

BACKGROUND

Value of Expanded RAS Testing in Colorectal Cancer anti-EGFR Therapy Selection

- Phase III trials showed superior overall survival
- In patients with RAS wild-type tumors, a significant benefit in OS, PFS and ORR was associated with addition of cetuximab to FOLFIRI vs FOLFIRI alone

Value of liquid biopsy RAS mutation testing to determine eligibility of mCRC patients for anti-EGFR therapy

- FFPE tumour tissue is currently used for RAS testing, which can cause delayed or inaccurate prescription of anti-EGFR therapy due to issues in availability, quality, or heterogeneity of tumour tissue samples
- Use of a blood-based RAS test is particularly advantageous in the metastatic setting, where the tumour sample may not represent the current RAS mutational status of the patient's disease
- A technology having high sensitivity is expected to be required to detect RAS mutations in circulating cell-free tumour DNA (ctDNA) in blood as compared to FFPE testing. The BEAMing RAS 33 mutation panel, already validated in the CRYSTAL study, provides an ideal method to compare performance of blood-based vs FFPE RAS testing for anti-EGFR therapy selection

OBJECTIVE

To determine whether blood-based RAS mutation testing is an appropriate surrogate for tissue-based RAS testing

Assess degree of concordance of plasma and tissue-based RAS testing in CRC patients having advanced disease (i.e. stage IV, or stage III disease with documented multiple lymph node involvement).

Combined RAS Concordance: Addition of data from study of first-line anti-EGFR therapy candidates (German & Australian study, Schmiegel et al. 2017)

Objective: Demonstrate that blood-based RAS mutation testing is an appropriate surrogate for tissue-based RAS testing for determining eligibility of CRC patients for anti-EGFR therapy.

Method: Compare RAS mutation status in plasma by BEAMing using Expanded RAS panel vs. RAS mutation status in FFPE tumor tissue using SOC. Determine overall/positive/negative agreement of RAS mutation results for **85 patients** with following attributes:

- Newly diagnosed stage IV CRC patients (N=50) - First-line EGFR antibody therapy candidates; (Plasma samples from blood drawn prior to tumor resection).
- Metastatic CRC patients showing recurrent disease (N=26) - later-line EGFR candidates; plasma sample may or may not be time-matched tissue obtained from resected tumor at metastatic site (N=21) vs. archival primary tumor (N=5)
- Advanced stage III CRC patients (N=9) - Primary tumor samples ; blood draw was taken prior to resection of tumor with no diagnosis of stage IV disease.

For all discordant cases, re-examine RAS mutation status by BEAMing in tissue sample, when available

Schmiedel et al. 2017 Mol. Oncol,

RAS Concordance Results: Combined data from AUS and GER Studies

		Tissue RAS result		
		Positive	Negative	Total
Plasma RAS result	Positive	41	2	43
	Negative	4	38	42
	Total	45	40	85

Overall Agreement : 79/85 = 93%

Positive Agreement : 41/45 = 91%

Negative Agreement : 38/40 = 95%

ANALYSIS OF PLASMA-/TISSUE+ DISCORDANT CASES

Tissue Source	Plasma RAS Result	Tissue RAS Sequencing Results	Tissue RAS BEAMing Results
Primary Tumor-IV	No Mutation Detected	KRAS Codon 12	tissue not available
Primary Tumor-IV	No Mutation Detected	KRAS Codon 12	tissue not available
Primary Tumor-III	No Mutation Detected	KRAS Codon 12	tissue not available
Lung	No Mutation Detected	KRAS Codon 12	KRAS Codon 12

- 3 cases (primary tumor) for which tissue was not available for re-examination by BEAMing
- 1 case (lung; mucinous adenocarcinoma) where tissue was re-examined by BEAMing; identical KRAS mutation was detected; discordant status was confirmed

Lack of RAS mutations in plasma may be attributed to biological factors that impact ctDNA release

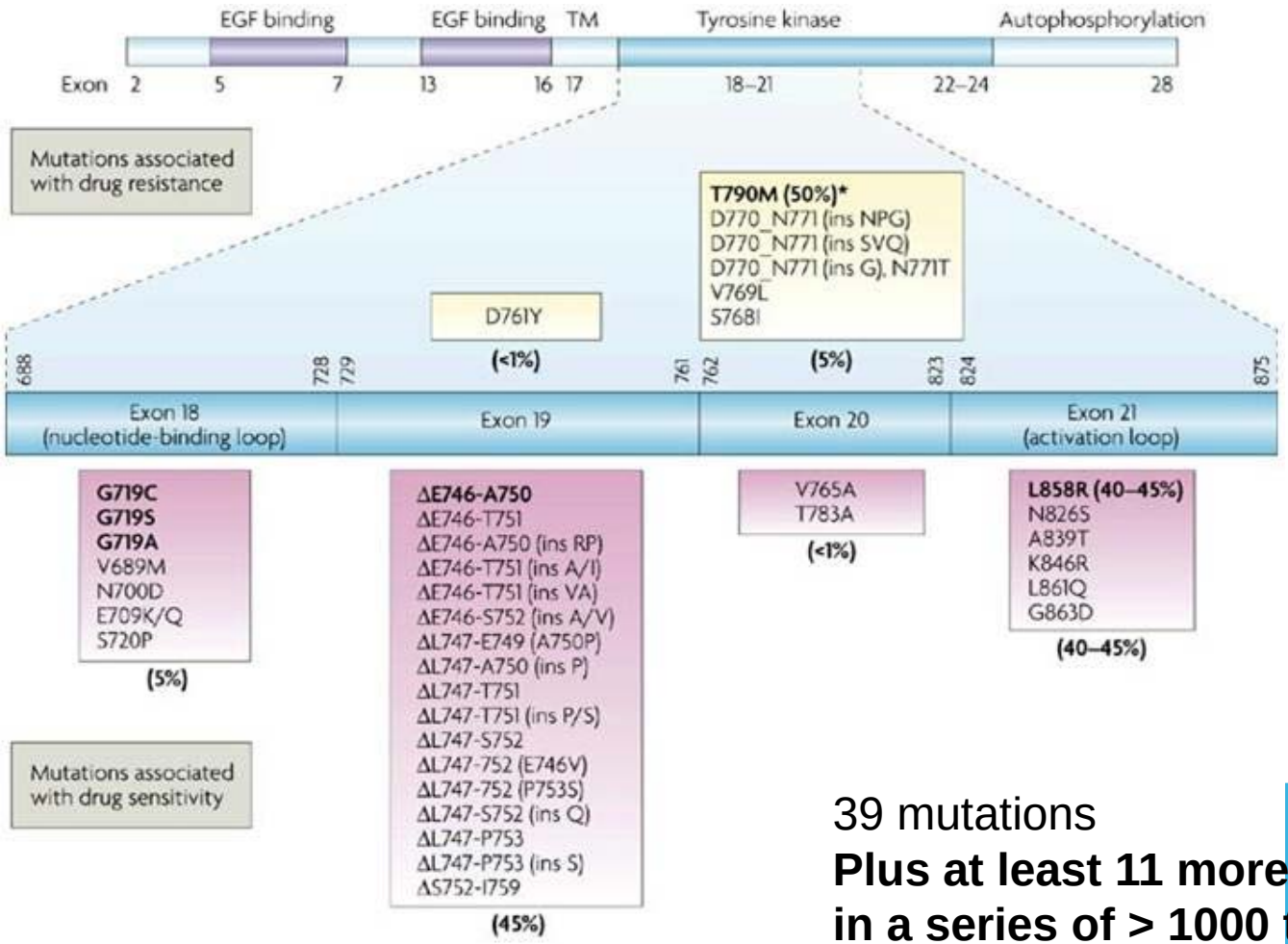
ANALYSIS OF PLASMA+/TISSUE- DISCORDANT CASES

Tissue Source	Plasma RAS Result	Tissue RAS Sequencing Results	Tissue RAS BEAMing Results
Primary Tumor	NRAS Codon 61	No Mutation Detected	tissue not available
Primary Tumor	KRAS Codon 12	No Mutation Detected	tissue not available
Primary Tumor	KRAS Codon 12	No Mutation Detected	KRAS Codon 12
Liver	KRAS Codon 146	No Result for KRAS Exons 3 & 4	tissue not available

- 2 cases where tissue was not available for re-examination by BEAMing and therefore results designated discordant; may represent patients with tumor molecular heterogeneity
- 1 case where tissue was re-examined with BEAMing; same KRAS mutation found in plasma was detected in tissue; highlights variability in current SOC tissue techniques.
- 1 case where a KRAS mutation was detected in plasma; however, tissue sequencing yielded an indeterminate result; tissue was not available for re-examination by BEAMing.

EGFR MUTATION TESTING IN LUNG CANCER
ANTI-EGFR THERAPY SELECTION

EGFR mutation frequency & Drug Response



39 mutations

Plus at least 11 more identified in a series of > 1000 tumours

Sharma SV, Bell DW, Settleman J, et al. Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer* 2007;7:169–181

CONCORDANCE

	cobas EGFR mutation test	therascreen EGFR ARMS-PCR	ddPCR	BEAMingdPCR
Exon 19 deletion				
Sensitivity	86%	82%	0	93%
	(24/28)	(23/28)		(26/28)
Specificity	100%	100%		100%
	(10/10)	(10/10)		(10/10)
Concordance	89%	87%		95%
L858R				
Sensitivity	90%	78%	90%	100%
	(9/10)	(7/9)	(9/1-)	(10/10)
Specificity	100%	100%	100%	93%
	(28/28)	(28/28)	(28/28)	(26/28)
Concordance	97%	95%	97%	95%
T790M				
Sensitivity	41%	29%	71%	71%
	(7/17)	(5/17)	(12/17)	(12/17)
Specificity	100%	100%	83%	67%
	(6/6)	(6/6)	(5/6)	(4/6)
Concordance	57%	48%	74%	70%

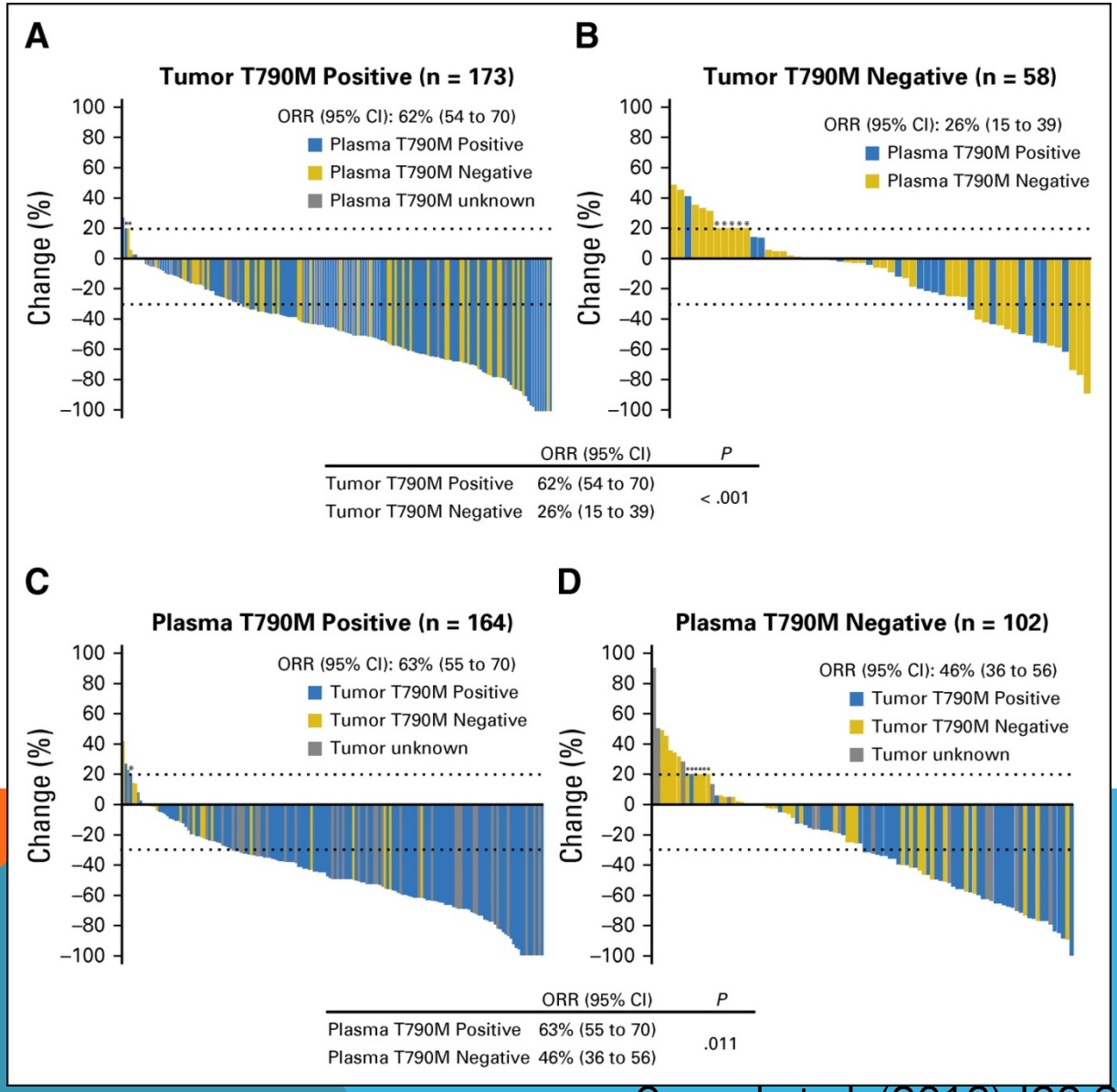
Taken from: Thress et al. EGFR mutation detection in ctDNA from NSCLC patient plasma:
A cross platform comparison of leading technologies to support the clinical development of AZD9291
Lung Cancer 90 (2015) 509-515

Sensitivity and Specificity of Plasma Genotyping Assays Compared With Tumor Genotype As a Reference Standard

Plasma Genotype (BEAMing)	Tumor Genotype (cobas central laboratory)	
	Exon 19 del+ (n=136)	**Exon 19 del –(n=80)**
Exon 19 del+ (n=114)	112 (82.3% sensitivity)	2
Exon 19 del- (n=102	24	78 (97.5% specificity)
	L858R+ (n=73)	**L858R- (n=143)**
L858R+ (n=68)	63 (86.3% sensitivity)	5
L858R- (n=148)	10	138 (96.5% specificity)
	T790M+ (n=158)	**T790M- (n=58)**
T790M+ (n=129)	111 (70.3% sensitivity)	18
T790M- (n=87)	47	40 (69,0% specificity)

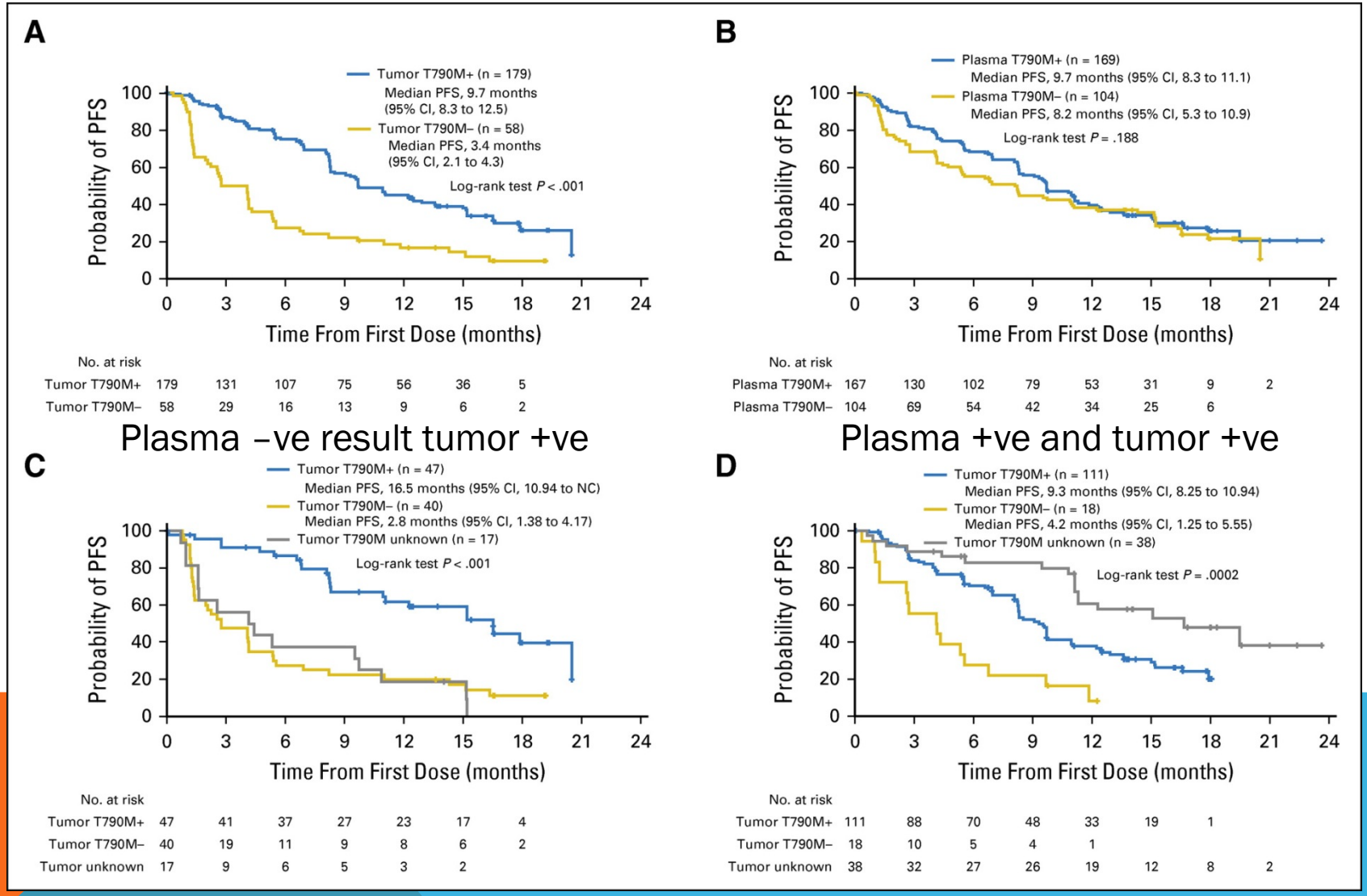
Oxnard et al. (2016) JCO 34(28):3375-82

WATERFALL PLOTS FOR BEST PERCENTAGE CHANGE IN TARGET LESION DIAMETER FOR EVALUABLE PATIENTS



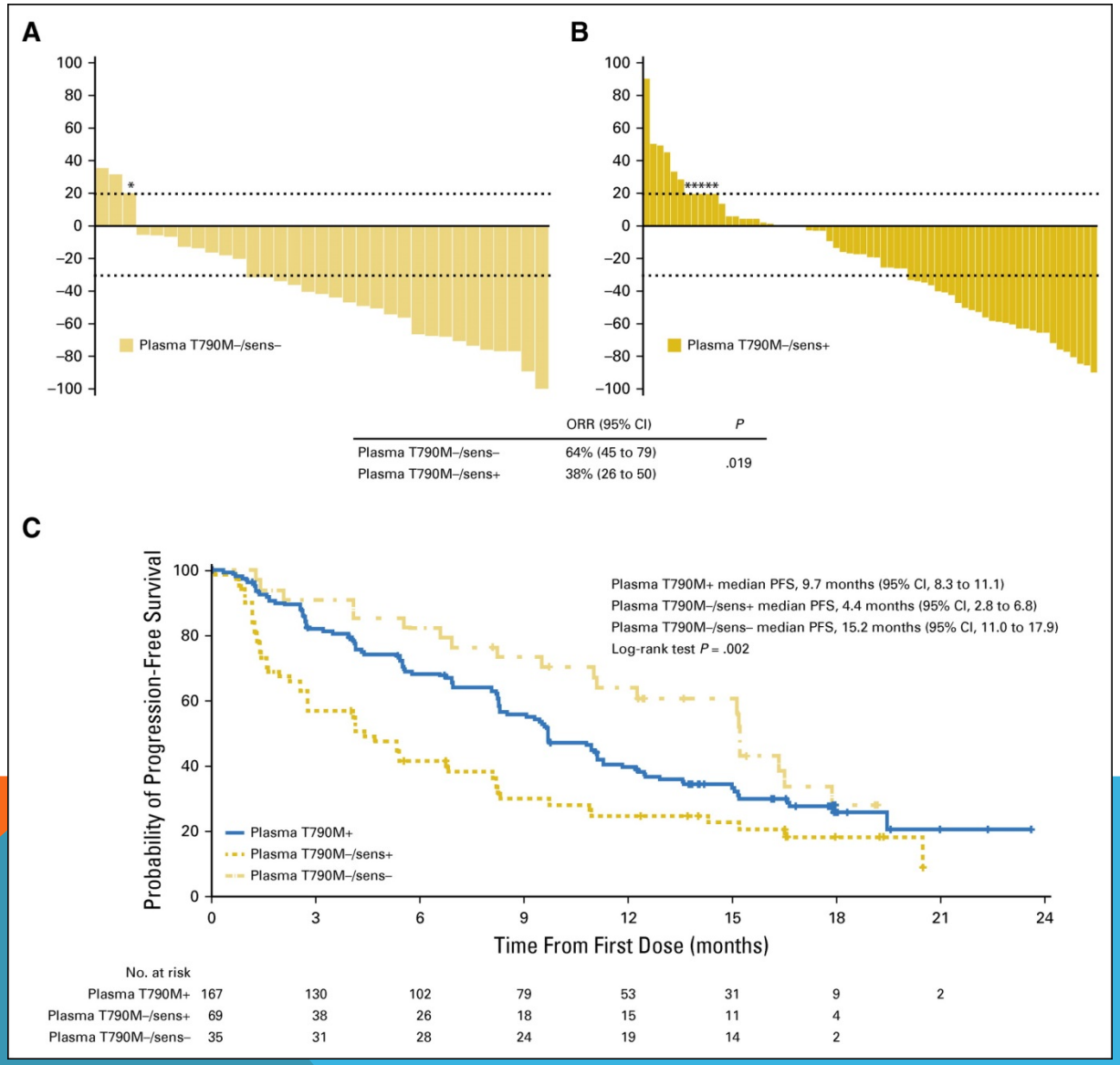
Oxnard et al. (2016) JCO 34(28):3375-82

PROGRESSION-FREE SURVIVAL (PFS) IN T790M-POSITIVE (T790M+) AND T790M-NEGATIVE (T790M−) SUBPOPULATIONS TREATED WITH OSIMERTINIB



Oxnard et al. (2016) JCO 34(28):3375-82

OBJECTIVE RESPONSE RATE (ORR) AND PROGRESSION-FREE SURVIVAL (PFS) OF THE PLASMA T790M NEGATIVE (T790M−) POPULATION USING DETECTION OF A PLASMA-SENSITIZING MUTATION (SENS) AS A QUALITY CONTROL



Oxnard et al. (2016) JCO 34(28):3375-82

Table A3. Genotyping and Treatment Results for 18 Patients Positive for T790M in Plasma but Negative for T790M in Tumor. *Subject 3 had discordant tumor and plasma genotyping. Plasma genotyping showed L858R and T790M while tumor genotyping showed G719X and no T790M.

Patient No.	Dose	Result of Central Tumor Genotyping for T790M	Result of Central Plasma BEAMing for T790M	T790M AF (BEAMing), %	EGFR Driver	EGFR Driver AF (BEAMing), %	Relative Prevalence Plasma T790M	T790M Detected With Alternative Plasma Assay	Alternative Plasma Assay Used	BOR	Best % Change From Baseline	PFS (month)
12	80 mg	Not detected	Detected	0.19	L858R	3.39	0.06	No	ddPCR	SD	−24.1	12.25*
11	80 mg	Not detected	Detected	0.34	19 del	5.08	0.07	Yes	Cobas	PR	−56.1	11.83
5	80 mg	Not detected	Detected	1.65	19 del	3.42	0.48	Yes	ddPCR	PR	−56.5	9.76*
18	80 mq	Not detected	Detected	0.06	19 del	ND	NA	Yes	ddPCR	SD	−34.1	9.66
1	80 mq	Not detected	Detected	7.05	19 del	34.75	0.2	Yes	ddPCR	PR	−50.6	6.74
14	80 mq	Not detected	Detected	0.09	19 del	11.09	0.01	Yes	ddPCR	SD	−22.2	5.55
7	160 mq	Not detected	Detected	0.64	19 del	ND	NA	Yes	ddPCR	SD	−21.3	5.32
6	80 mq	Not detected	Detected	1.11	L858R	34.14	0.03	Yes	Cobas	SD	−3.8	4.34
17	160 mq	Not detected	Detected	0.07	L858R	0.32	0.23	No	Cobas	PR	−43.4	4.17
4	80 mq	Not detected	Detected	2.04	19 del	18.14	0.11	Yes	Cobas	PR	−62	4.14
9	20 mg	Not detected	Detected	0.45	L858R	28.61	0.02	Yes	Cobas	SD	0	2.73
15	80 mg	Not detected	Detected	0.09	L858R	4.70	0.02	No	ddPCR	SD	−12.1	2.66
16	80 mg	Not detected	Detected	0.08	L858R	0.15	0.55	No	Cobas	SD	−1	2.6
3	40 mq	Not detected	Detected	2.24	L858R*	7.73	0.29	Yes	ddPCR	PD	41.3	1.25
13	40 mq	Not detected	Detected	0.12	L858R	0.37	0.33	Yes	Cobas	PD	−2.2	1.25
10	40 mq	Not detected	Detected	0.34	L858R	2.05	0.17	Yes	Cobas	PD	−20.4	1.05
2	80 mq	Not detected	Detected	3.45	19 del	20.49	0.17	Yes	ddPCR	PD	13.6	1.02
8	160 mq	Not detected	Detected	0.59	19 del	3.14	0.19	Yes	ddPCR	PD	13.3	0.36

Oxnard et al. (2016) JCO 34(28):3375-82

AURA3: T790M MUTATION IS DETECTED IN PLASMA OF ~50% OF PATIENTS WITH T790M IN TUMOUR TISSUE

Patients with tissue sample available at screening (n=756)

Plasma ctDNA test results, n	Tissue T790M positive (n=399)	Tissue Exon 19 deletion positive (n=427)	Tissue L858R positive (n=253)
Plasma positive	184	273	139
Plasma negative	175	60	67
No plasma test / invalid	37 / 3	91 / 3	47 / 0
Percent agreement using tissue test as reference, % (95% CI)*			
Positive percent agreement (sensitivity)	**51 (46, 57)**	82 (77, 86)	68 (61, 74)
Negative percent agreement (specificity)	**77 (71, 83)**	98 (96, 100)	99 (98, 100)
Overall concordance	61 (57, 65)	89 (86, 91)	88 (85, 90)

- 51% sensitivity and 77% specificity for T790M detection using cobas® tissue test as reference
- High sensitivity and specificity is observed for Exon 19 deletion and L858R

Data cut-off 15 April 2016.

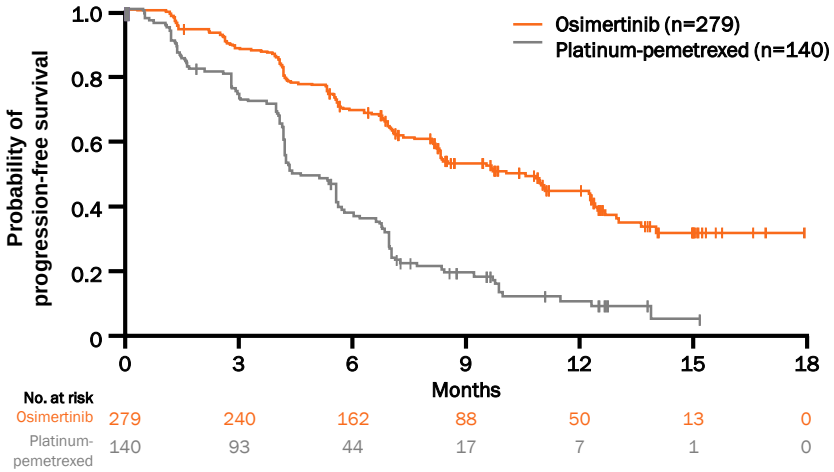
*Percent agreement of the cobas® plasma test with the cobas® tissue test. Positive percent agreement and negative percent agreement are used here as measures of test sensitivity and specificity, respectively, and calculated with invalid results excluded.

Wu YL, et al. *J Thorac Oncol*. 2017; 12(S1), abstract MA08.03.

AURA3: OSIMERTINIB BENEFIT IN PATIENTS WITH PLASMA T790M-POSITIVE STATUS IS SIMILAR TO PATIENTS WITH TUMOUR TISSUE T790M-POSITIVE STATUS[1,2]

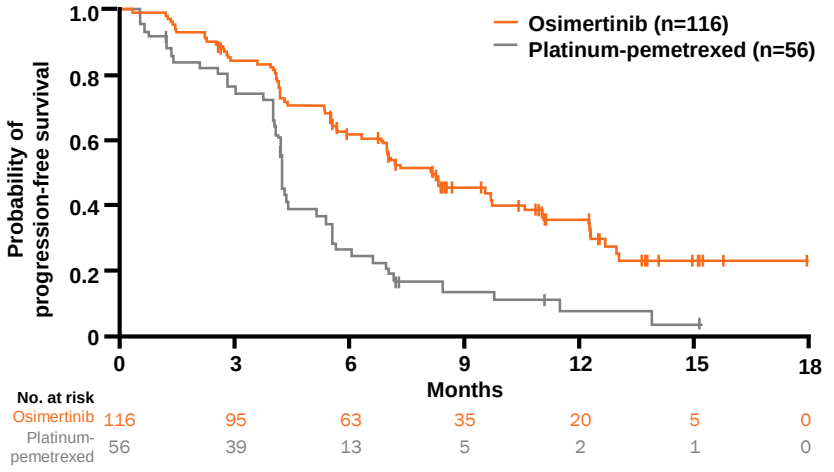
Tumour T790M-positive (intent-to-treat)*

	Osimertinib	Platinum-pemetrexed
Median PFS, months (95% CI)	10.1 (8.3, 12.3)	4.4 (4.2, 5.6)
PFS HR (95% CI)	0.30 (0.23, 0.41)*, p<0.001	
ORR†, % (95% CI)	71 (65, 76)	31 (24, 40)



Plasma T790M-positive status

	Osimertinib	Platinum-pemetrexed
Median PFS, months (95% CI)	8.2 (6.8, 9.7)	4.2 (4.1, 5.1)
PFS HR (95% CI)	0.42 (0.29, 0.61)	
ORR†, % (95% CI)	77 (68, 84)	39 (27, 53)



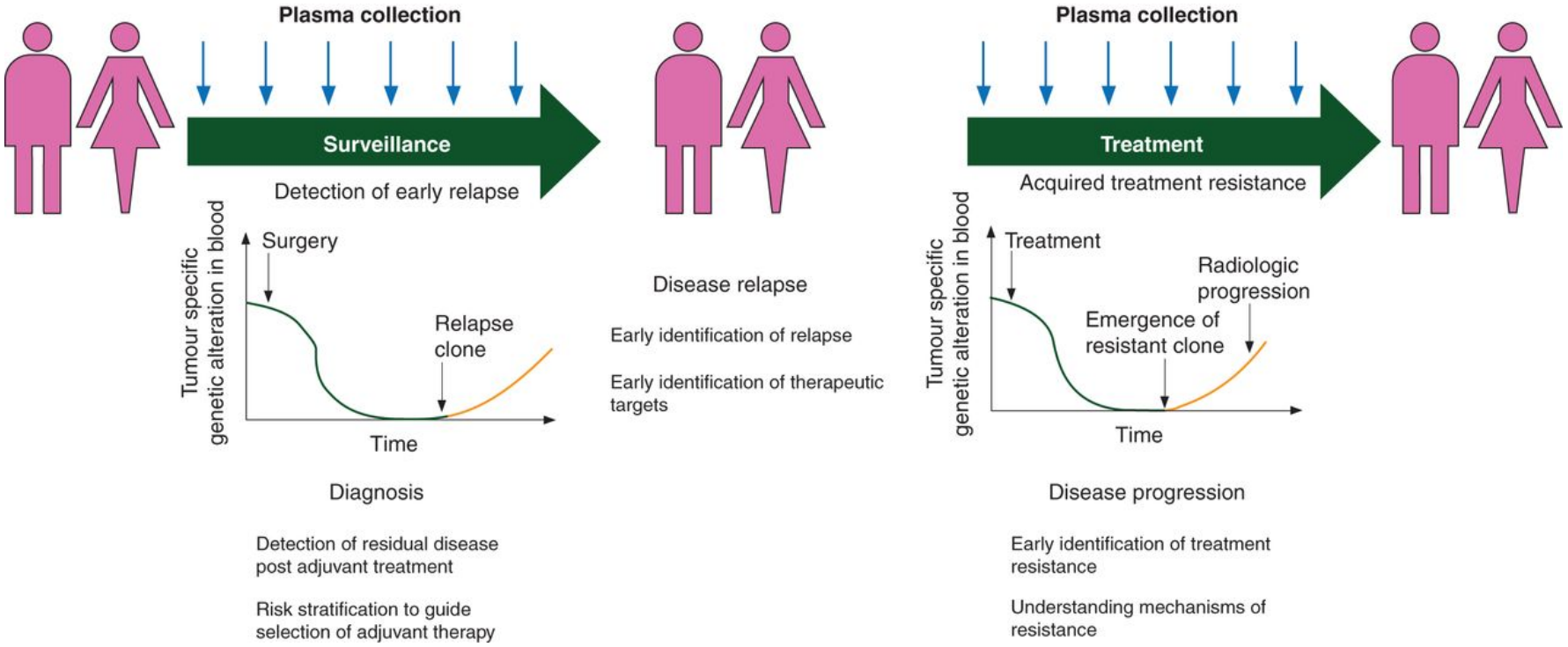
Data cut-off 15 April 2016. Tick marks indicate censored data.

PFS is defined as time from randomisation until date of objective disease progression or death. Progression included deaths in the absence of RECIST progression. Osimertinib administered 80 mg orally once daily. Platinum-pemetrexed group treatment consisted of: pemetrexed 500 mg/m^2 + carboplatin AUC5 or cisplatin 75 mg/m^2 Q3W for up to 6 cycles + optional maintenance pemetrexed for patients whose disease had not progressed after 4 cycles of platinum-pemetrexed. RECIST v1.1 assessments performed every 6 weeks until objective disease progression.

*PFS adjusted for ethnicity. All patients were selected using a tumour tissue test for EGFR T790M (by cobas® EGFR Mutation Test) from a biopsy after disease progression prior to study entry; †Response did not require confirmation per RECIST v1.1.

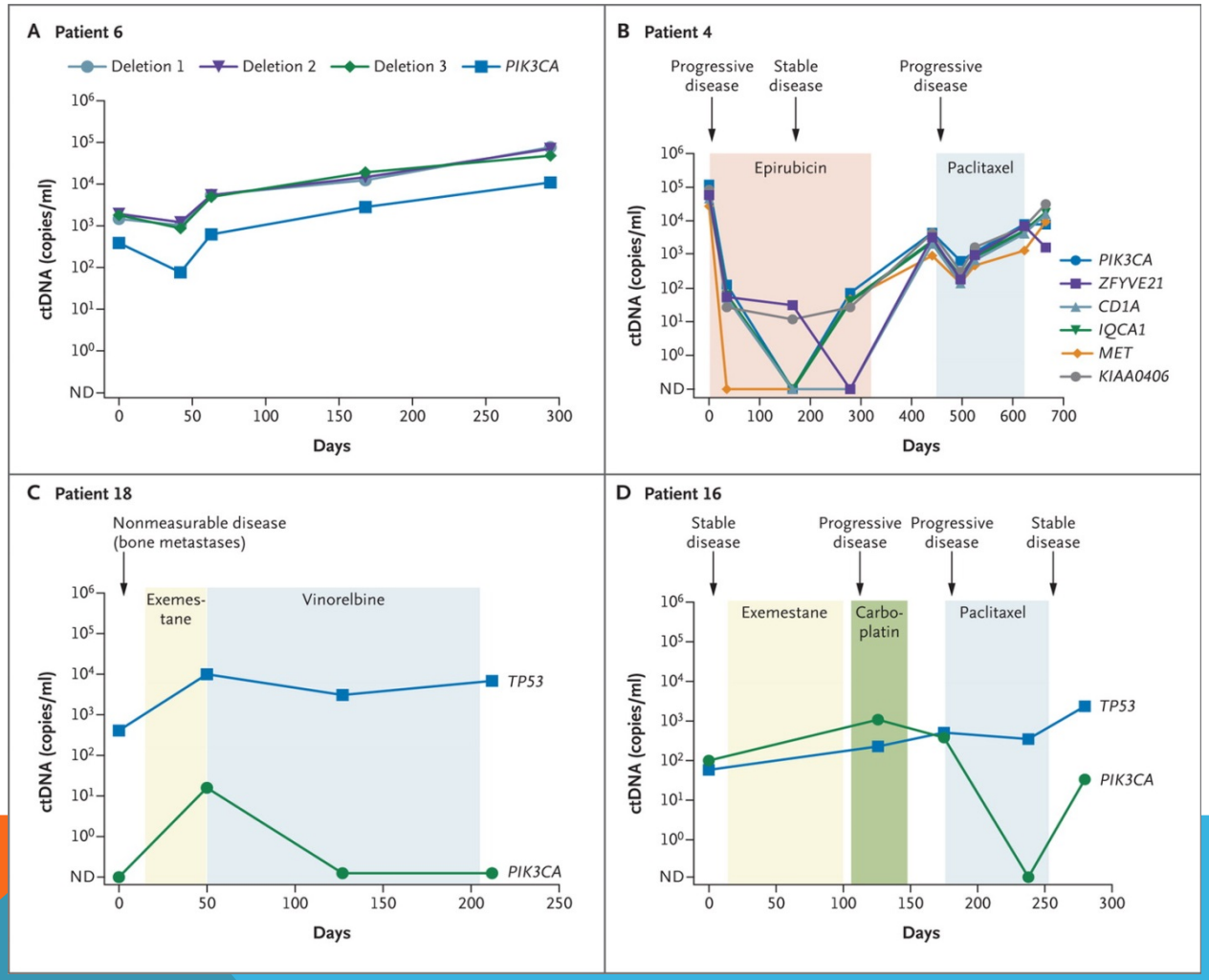
1. Mok TS, et al. *N Engl J Med*. December 6, 2016. DOI: 10.1056/NEJMoa1612674. [Epub ahead of print].
2. Suppl. Info for: Mok TS, et al. *N Engl J Med*. December 6, 2016. DOI: 10.1056/NEJMoa1612674. [Epub ahead of print].

POTENTIAL CLINICAL APPLICATIONS OF ctDNA ANALYSIS



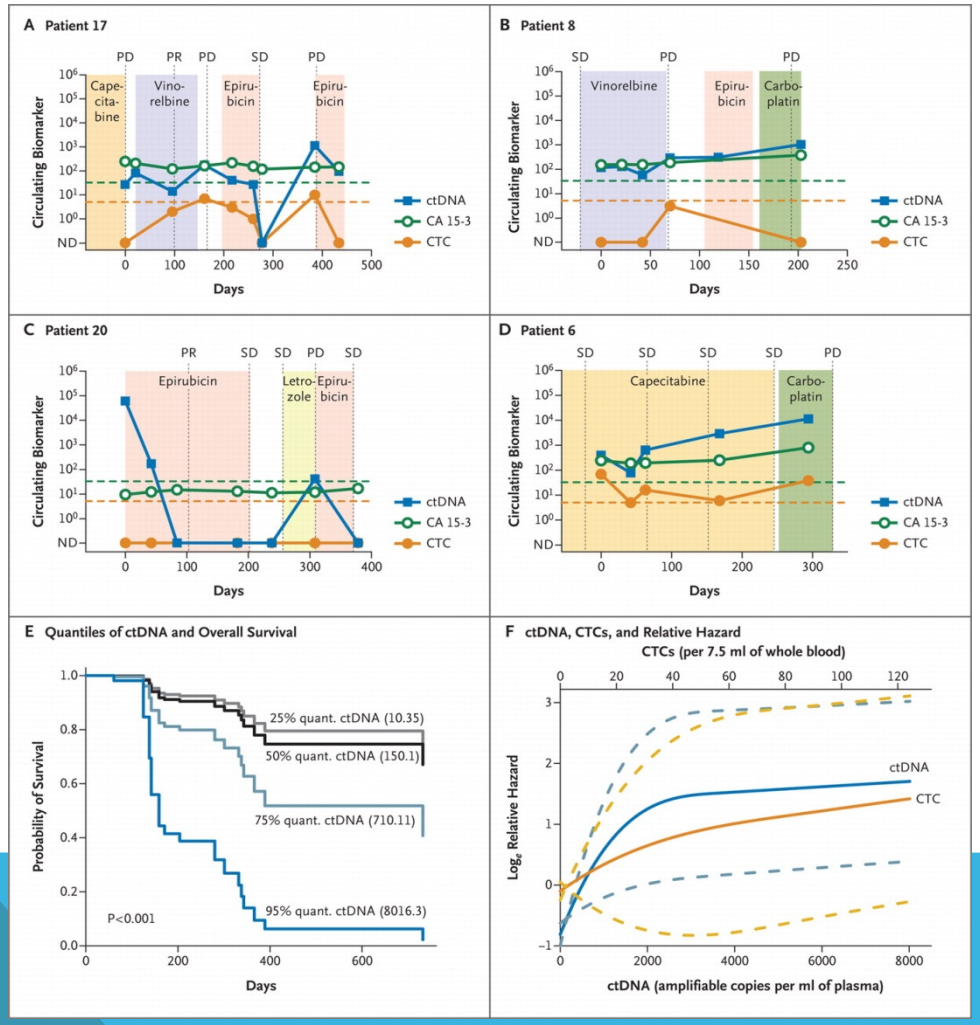
M. Ignatiadis, and S.-J. Dawson Ann Oncol 2014;25:2304-2313

Annals of Oncology



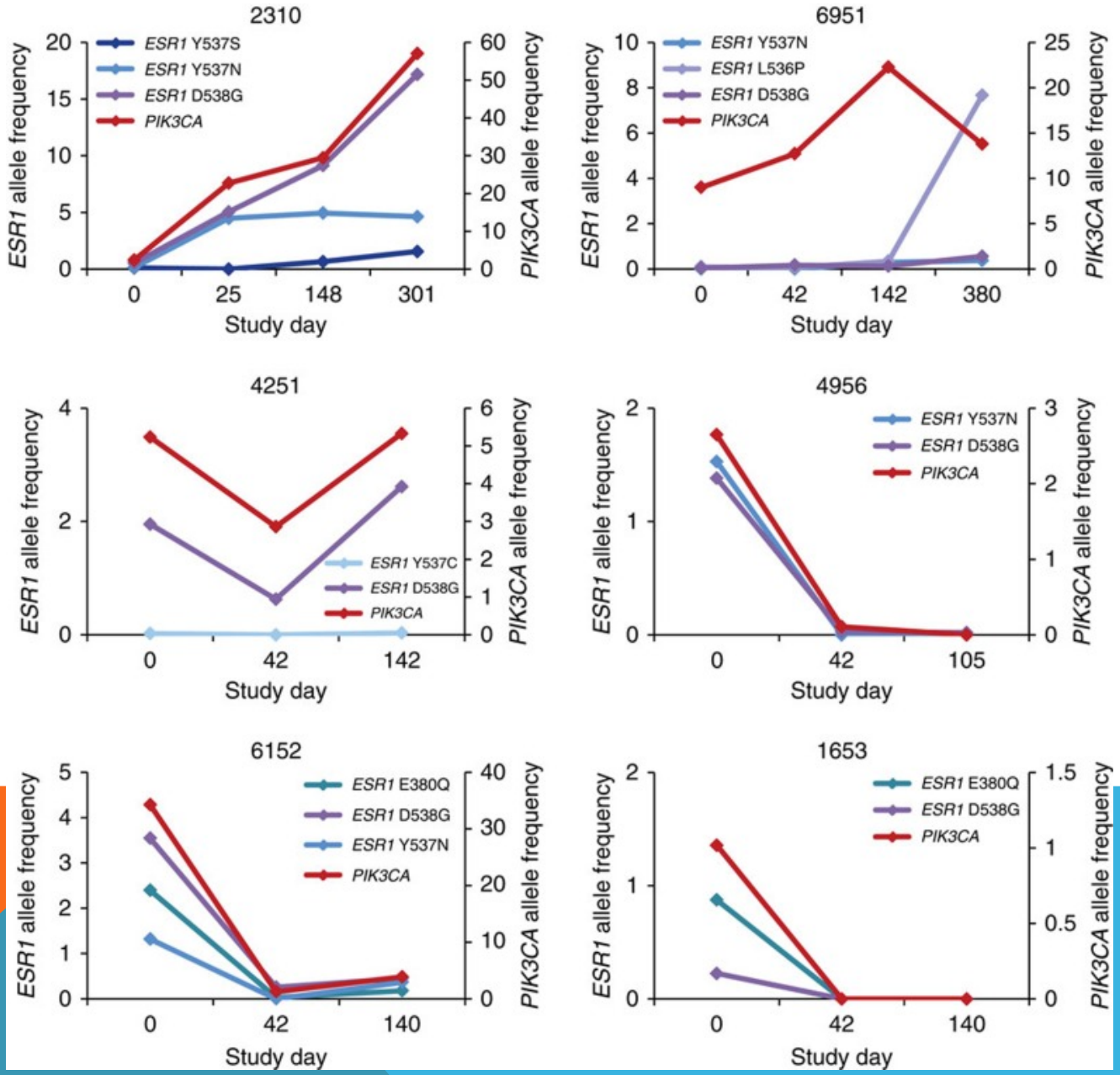
DAWSON S ET AL. N ENGL J MED 2013;368:1199-1209.

The NEW ENGLAND JOURNAL of MEDICINE

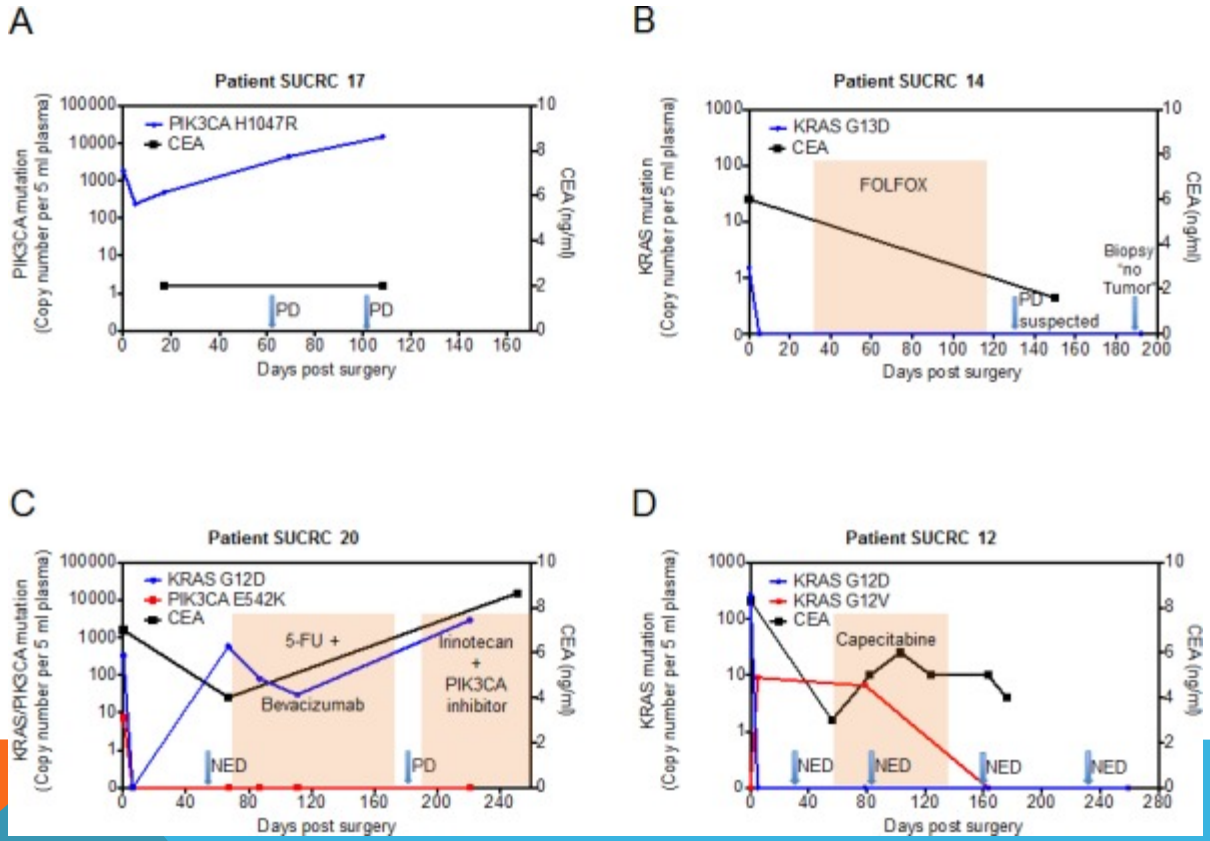


DAWSON S ET AL. N ENGL J MED 2013;368:1199-1209.

The NEW ENGLAND JOURNAL of MEDICINE


2310
ESR1 Y537S
ESR1 Y537N
ESR1 D538G
PIK3CA
ESR1 allele frequency
PIK3CA allele frequency
Study day
6951
ESR1 Y537N
ESR1 L536P
ESR1 D538G
PIK3CA
4251
ESR1 Y537C
ESR1 D538G
PIK3CA
4956
ESR1 Y537N
ESR1 D538G
PIK3CA
6152
ESR1 E380Q
ESR1 D538G
ESR1 Y537N
PIK3CA
1653
ESR1 E380Q
ESR1 D538G
PIK3CA

PERIOPERATIVE DYNAMICS OF PLASMA MUTATION LEVELS IN PATIENTS WITH STAGE IV COLORECTAL CANCER



CONCLUSION

BEAMing has high sensitivity for plasma based nucleic acid detection

ctDNA analysis is as good as, if not better than conventional tissue analysis

High degree of concordance of plasma and tissue testing results – BUT dependent on tumour type

Blood based RAS mutation testing may serve as a surrogate for tissue based testing in determining eligibility for anti-EGFR therapy