ISTITUTO NAZIONALE PER LO
STUDIO E LA CURA DEI TUMORI

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Clonal evolution in response to anti-EGFR therapies

Nicola Normanno

Tumor heterogenity and clonal evolution in NSCLC

- **The concept of inter- and intra-tumor heterogeneity**
- **Intra-tumor heterogeneity in EGFR mutant NSCLC**
- **Clonal evolution and resistance to EGFR targeting therapies**

Tumor heterogenity and clonal evolution in NSCLC

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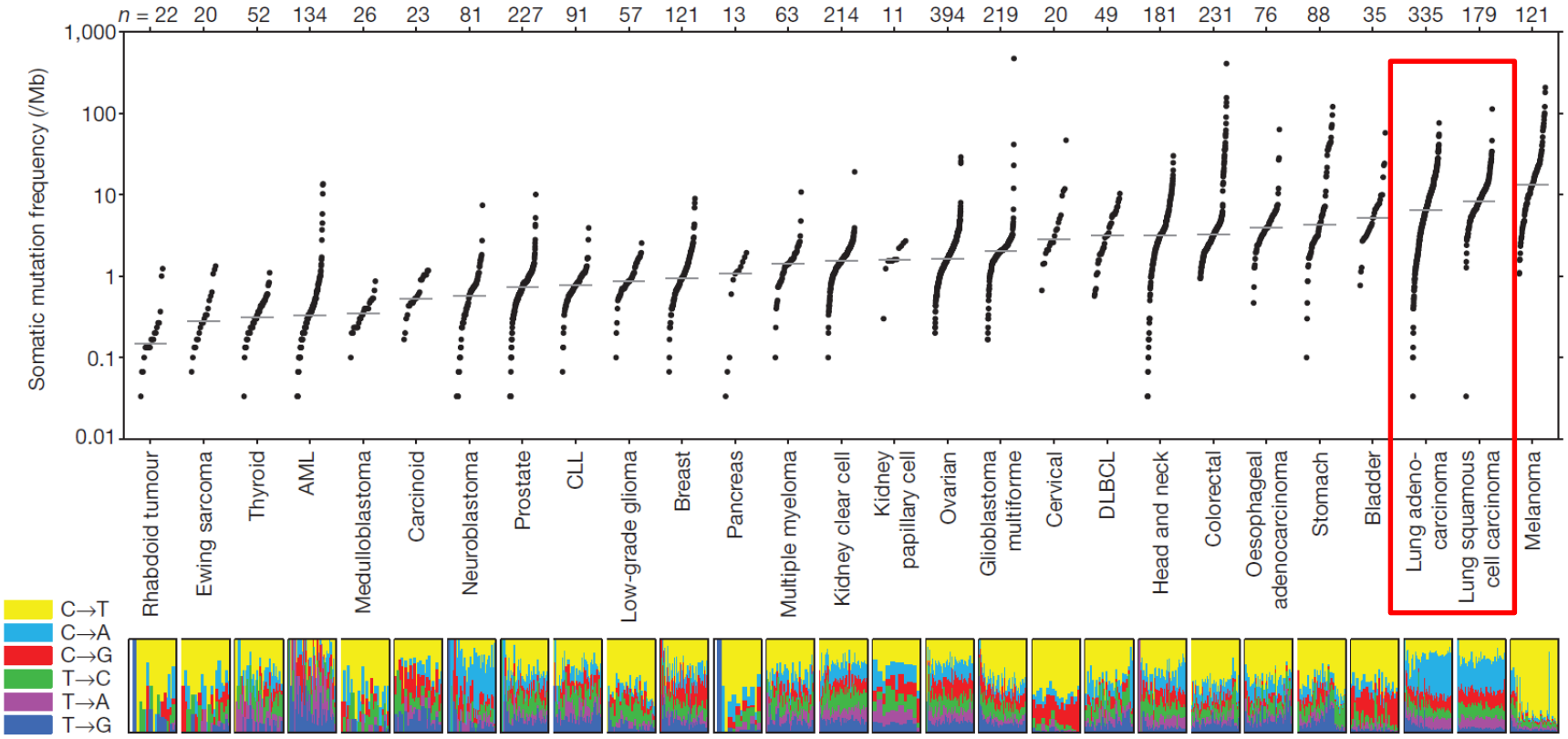
Somatic mutation frequencies in cancer



Lawrence Nature 2013

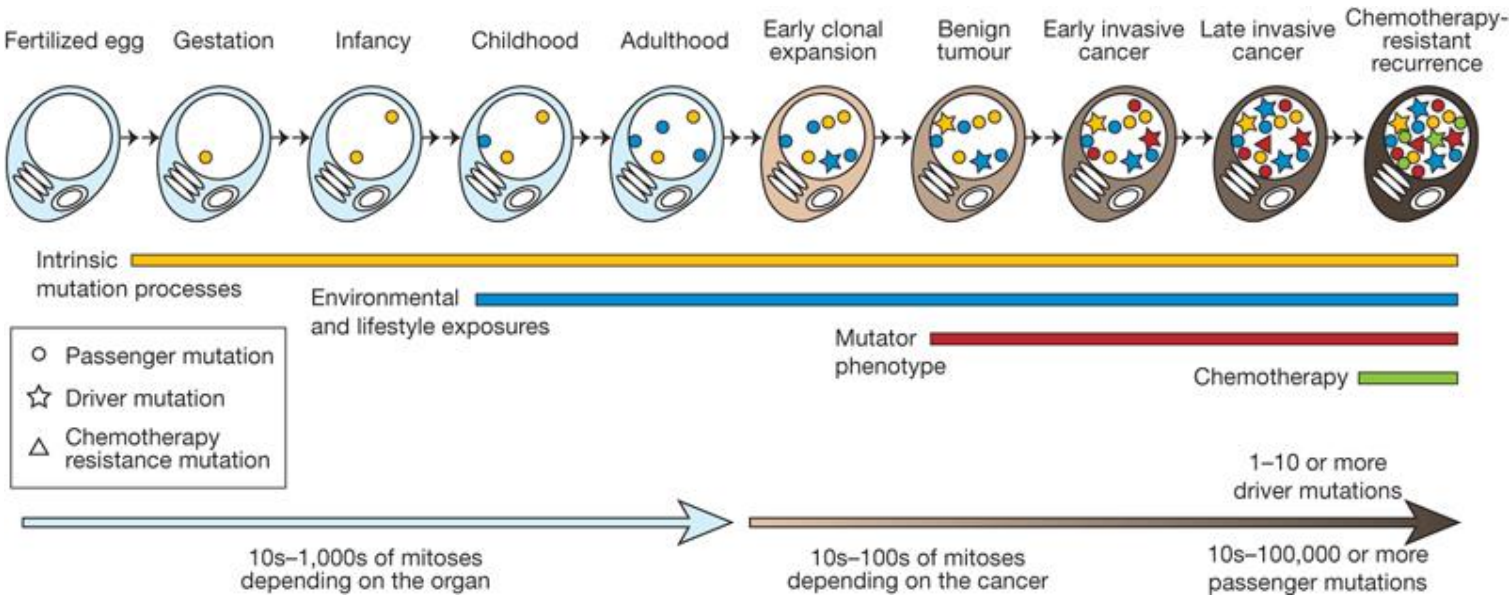
Tumor Mutation Burden in NSCLC

	Adeno (n=7,925)	SCC (n=1,324)	NSCLC NOS (n=1,773)	SCLC (n=640)	*EGFR* mutation (n=1,775)	*ALK* or *ROS1* fusion (n=489)	*MET*ex14 alteration (n=286)	*BRAF* mutation (n=493)	*KRAS* mutation (n=3,155)
Mean Mutations/Mb	9.1	11.3	11.0	10.3	4.5	3.1	6.2	9.7	10.3
TMB >10 (%)	2350 (30)	541 (41)	711 (40)	269 (42)	129 (7)	17 (3)	27 (9)	153 (31)	1,238 (39)
TMB >20 (%)	760 (10)	113 (9)	233 (13)	42 (7)	21 (1)	4 (1)	4 (1)	51 (10)	298 (9)
Wilcoxon signed-rank test vs *KRAS*					p<0.001	p<0.001	p<0.001	p<0.001	

The nuclear genome is 3.200 Mb!

Spigel ASCO 2016

The cancer genome

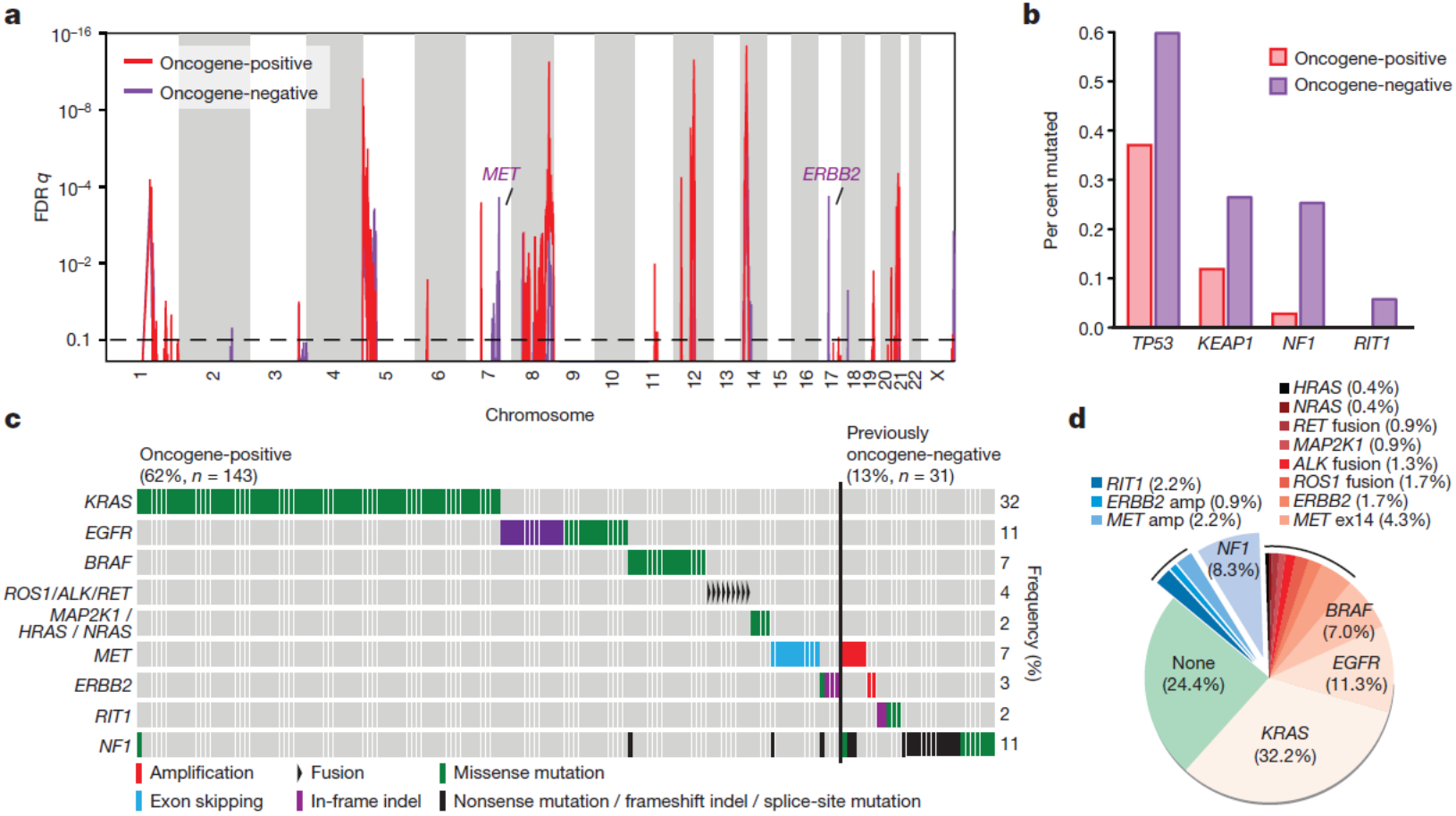


A **driver mutation** is causally implicated in oncogenesis. It has conferred growth advantage on the cancer cell and has been positively selected in the microenvironment of the tissue in which the cancer arises.

A **passenger mutation** has not been selected, has not conferred clonal growth advantage and has therefore not contributed to cancer development. Mutations without functional consequences often occur during cell division and will be carried along in the clonal expansion that follows.

MR Stratton *et al. Nature* **458**, 719-724 (2009) doi:10.1038/nature07943

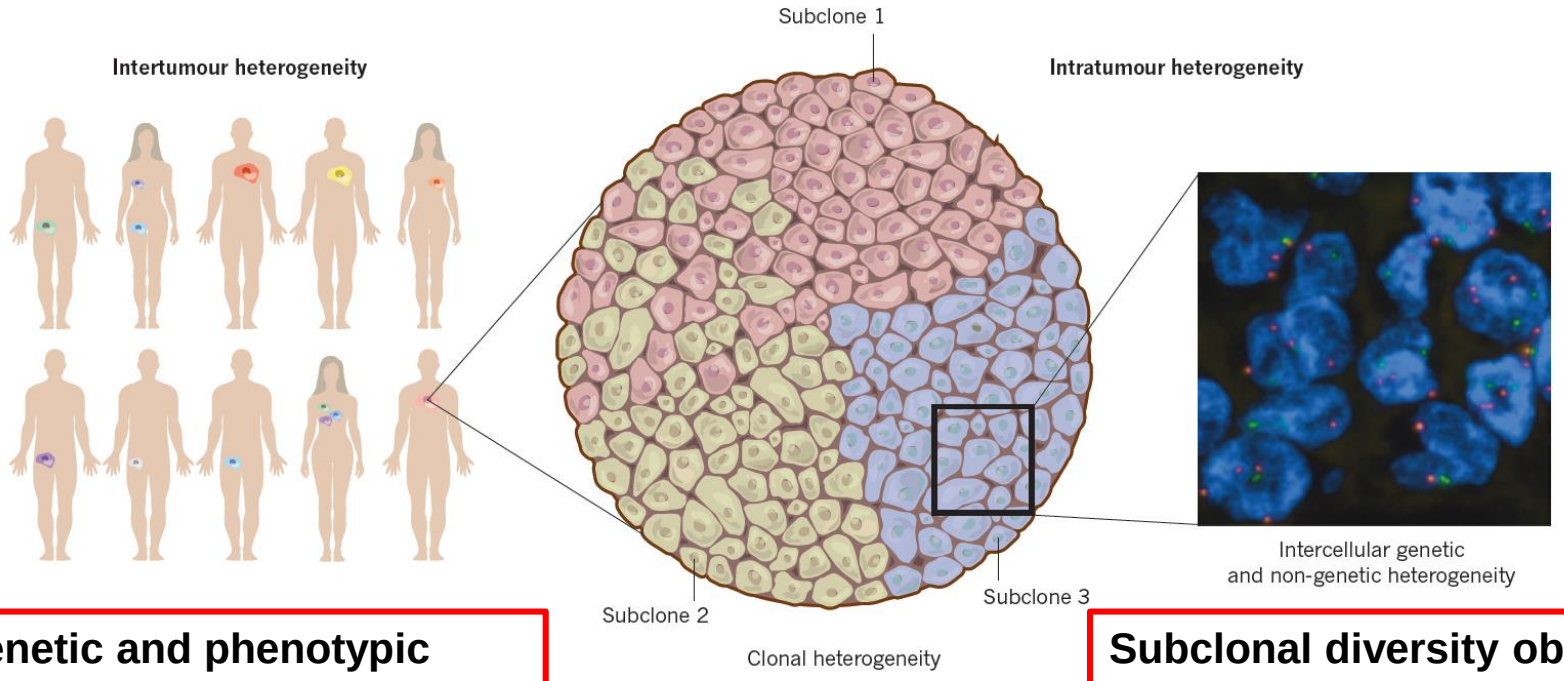
Identification of novel candidate driver genes in lung adenocarcinoma



TCGA Nature 2014

The efficacy of targeted therapy depends on

TUMOR HETEROGENEITY

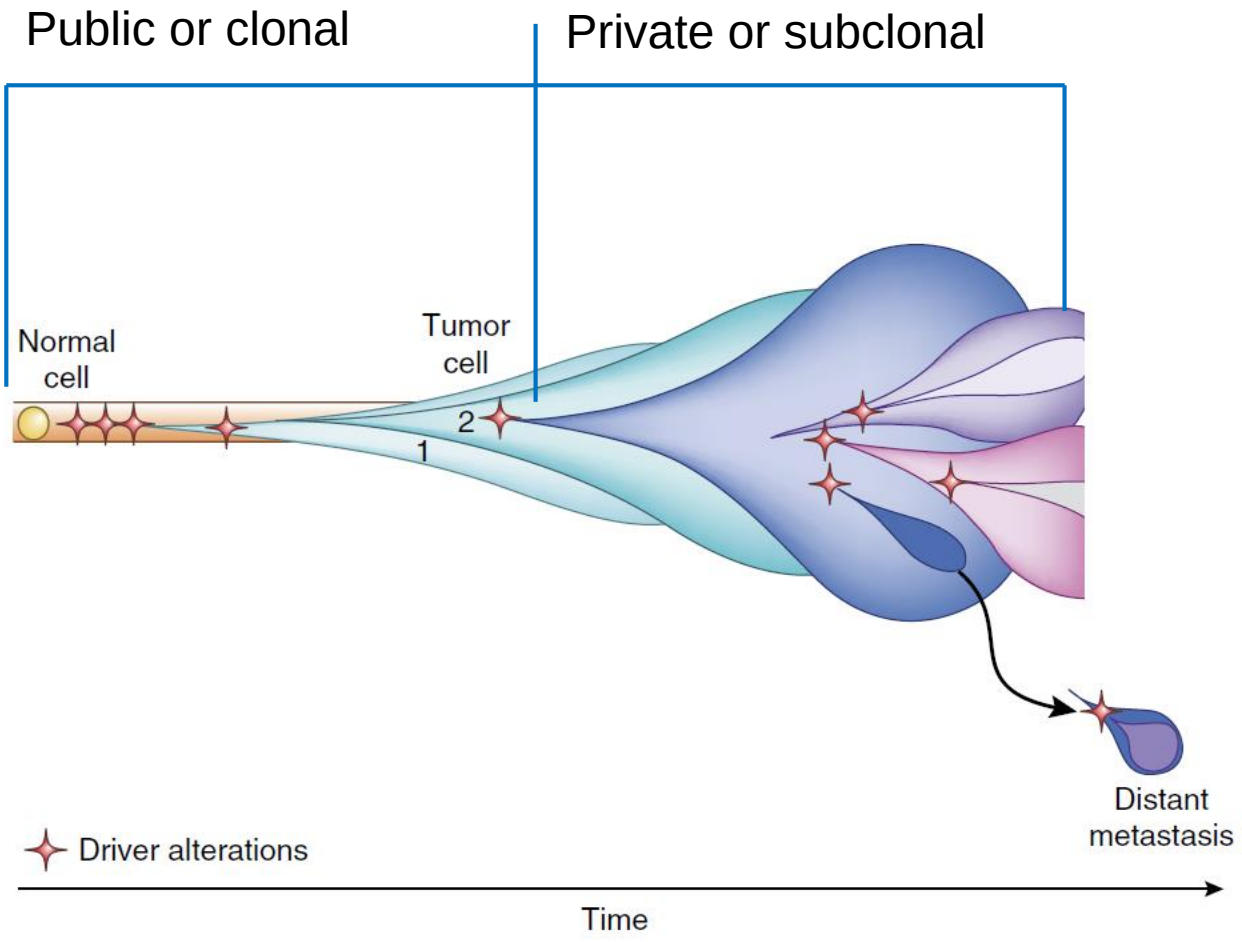


Genetic and phenotypic variation observed between tumors of different tissue and cell types, as well as between individuals with the same tumor type

Subclonal diversity observed within a tumor (tumors are formed of different clones with different genetic and molecular features)

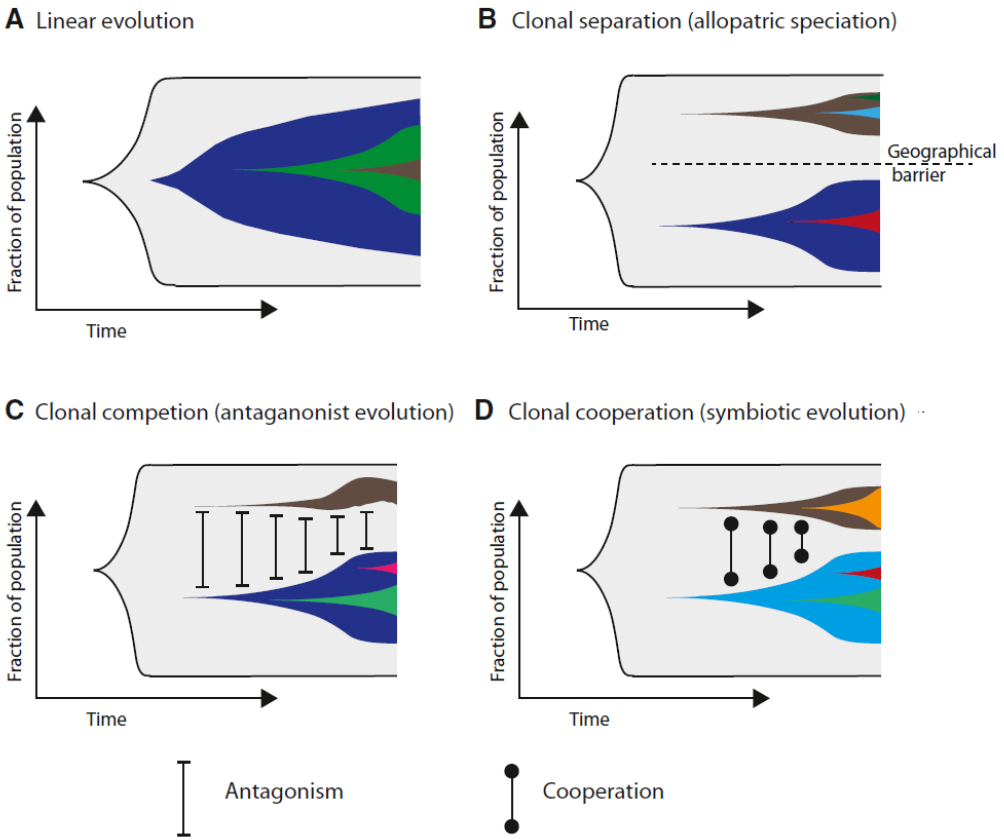
Burrell Nature 2013

The clonality of tumor evolution



Alizadeh Nat Med 2015

Modes of Tumor Evolution



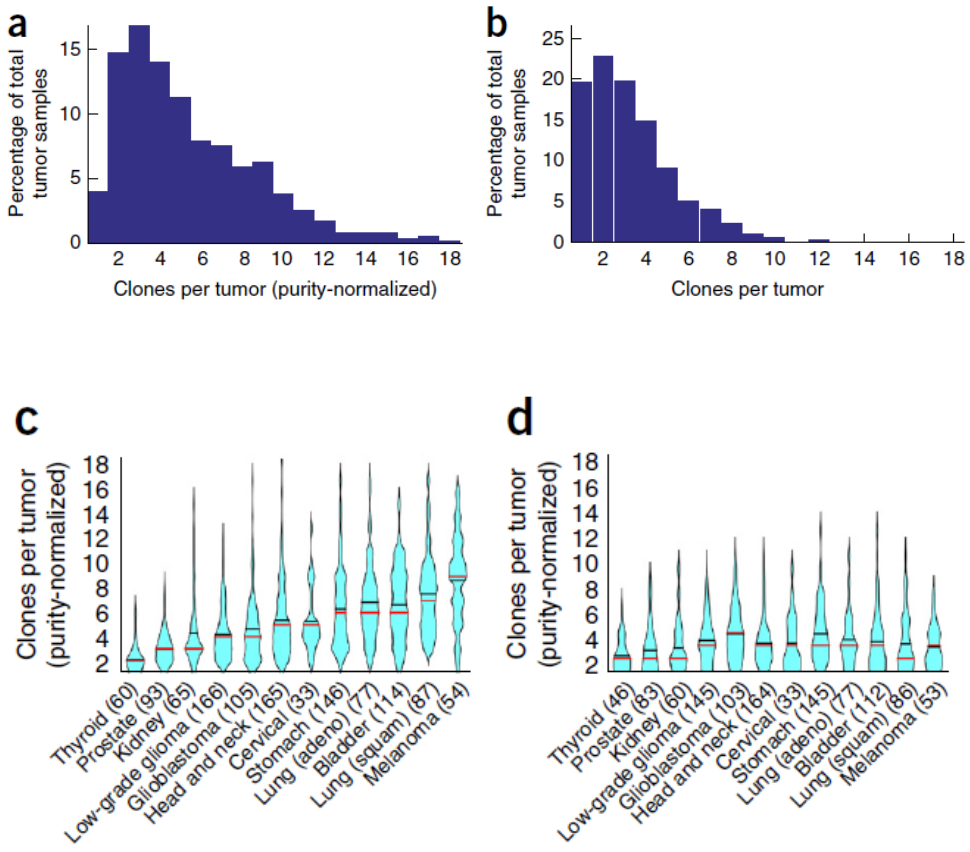
Tumor evolution is the result of genetic instability leading to accumulation of mutations that might provide growth advantage, and microenvironmental factors leading to clonal selection

McGranahan & Swanton Cancer Cell 2015

Tumor heterogenity and clonal evolution in NSCLC

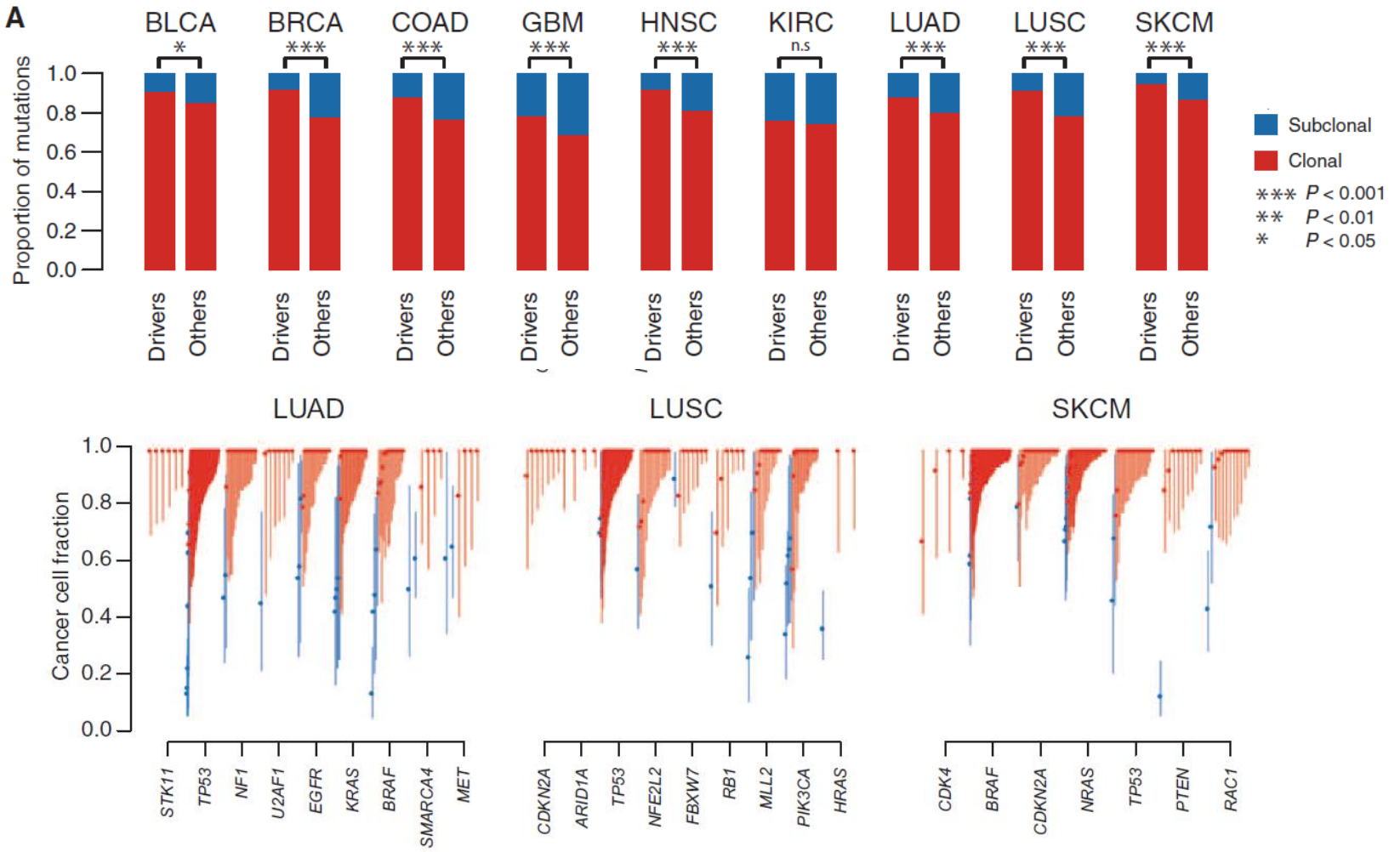
- The concept of inter- and intra-tumor heterogeneity
- **Intra-tumor heterogeneity in EGFR mutant NSCLC**
- Clonal evolution and resistance to EGFR targeting therapies

Intratumor genetic heterogeneity in 12 tumor types



Andor Nat Med 2016

Clonal and subclonal mutations in different cancer types



McGranahan Sci Transl Med 2015

NSCLC tumor and plasma samples analysis with the Oncomine Solid Tumour DNA

Tumor EGFR status	Plasma EGFR status	
	Wild Type	Mutant
Wild Type	20	2
Mutant	5	17

Specificity 90,1%
Sensitivity 77,3%

Rachiglio Oncotarget 2016

NSCLC tumor and plasma samples analysis with the Oncomine Solid Tumour DNA: discordant cases

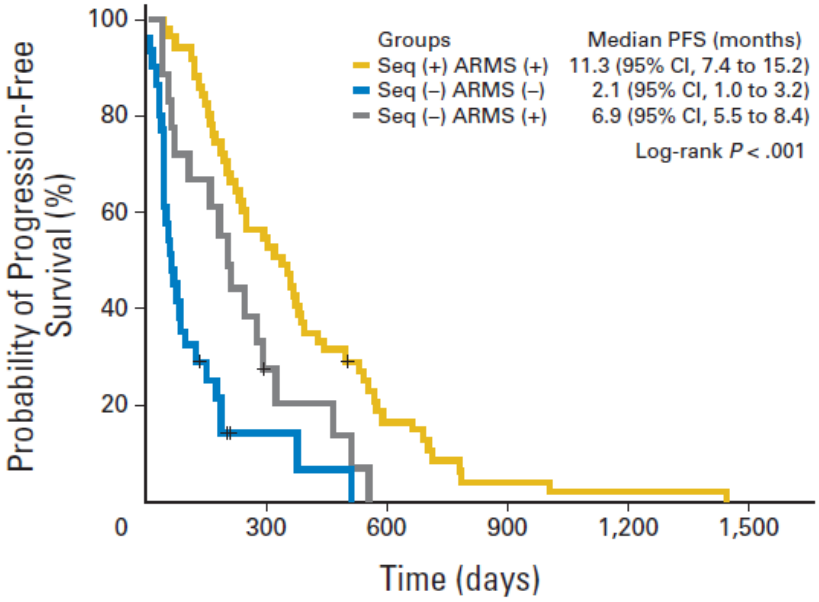
Case N.	Plasma analyses			Tissue analyses		
	NGS	Therascreen	ddPCR	NGS	Therascreen	ddPCR
L29	EGFR: p.E746_A750del (3,4%);	EGFR: wild type	EGFR: Del ex19 (4%)	-	EGFR: wild type	EGFR: Del ex19 (0.23%)
L33	EGFR: p.E746_A750del (1,6%); CTNNB1: p.S37C (12,3%)	EGFR: wild type	EGFR: Del ex19 (0.8%)	CTNNB1: p.S37C (13,3%)	EGFR: wild type	EGFR: Del ex19 (0.76%)

Rachiglio Oncotarget 2016

Detection of EGFR mutations in NSCLC Sequencing vs Therascreen

Table 3. Efficacy of Gefitinib Treatment

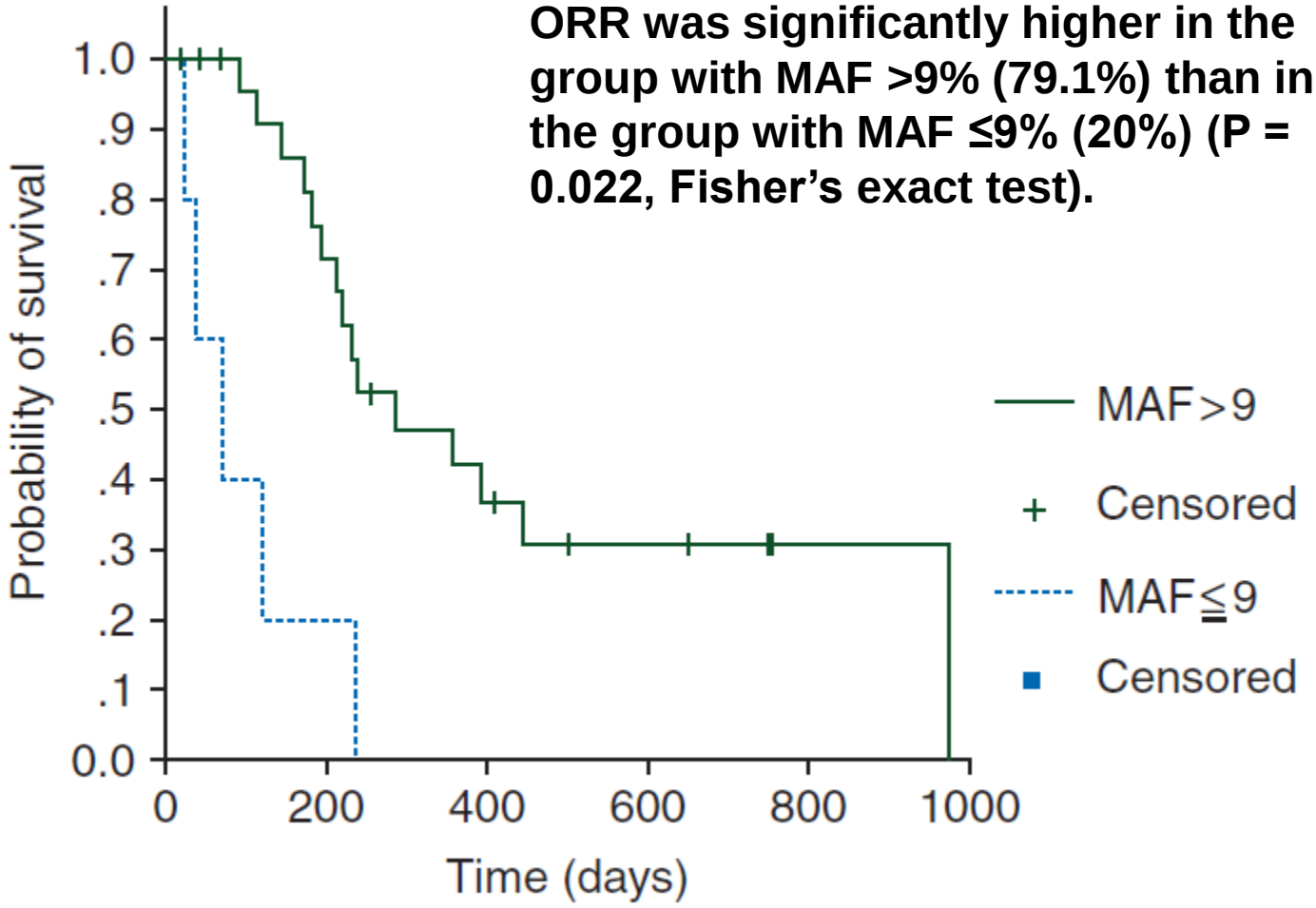
Efficacy	Group H* No.	Group H* %	Group L† No.	Group L† %	Group W‡ No.	Group W‡ %	P§	P‖
PFS, months							< .001	.014
Median	11.3		6.9		2.1			
95% CI	7.4 to 15.2		5.5 to 8.4		1.0 to 3.2			
OS, months							.011	.062
Median	15.9		10.9		8.7			
95% CI	13.4 to 18.3		2.7 to 19.1		4.6 to 12.7			
Tumor response							< .001	.176
CR	2	3.9	0	0	0	0		
PR	30	58.9	8	44.4	5	16.1		
SD	15	29.4	5	27.8	10	32.3		
PD	4	7.8	5	27.8	16	51.6		



Zhou JCO 2011

PFS stratified according to mutant allele frequency (MAF) of p.L858R EGFR mutation

ORR was significantly higher in the group with MAF >9% (79.1%) than in the group with MAF ≤9% (20%) (P = 0.022, Fisher's exact test).

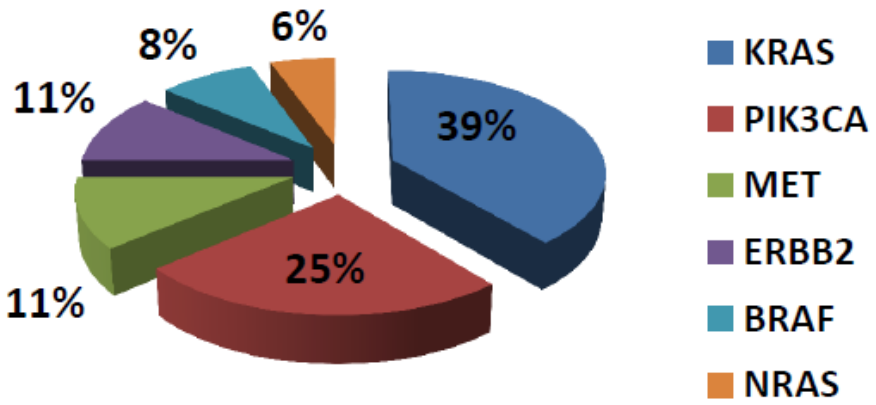


Ono Ann Onc 2014

Results: mutations in "other genes"

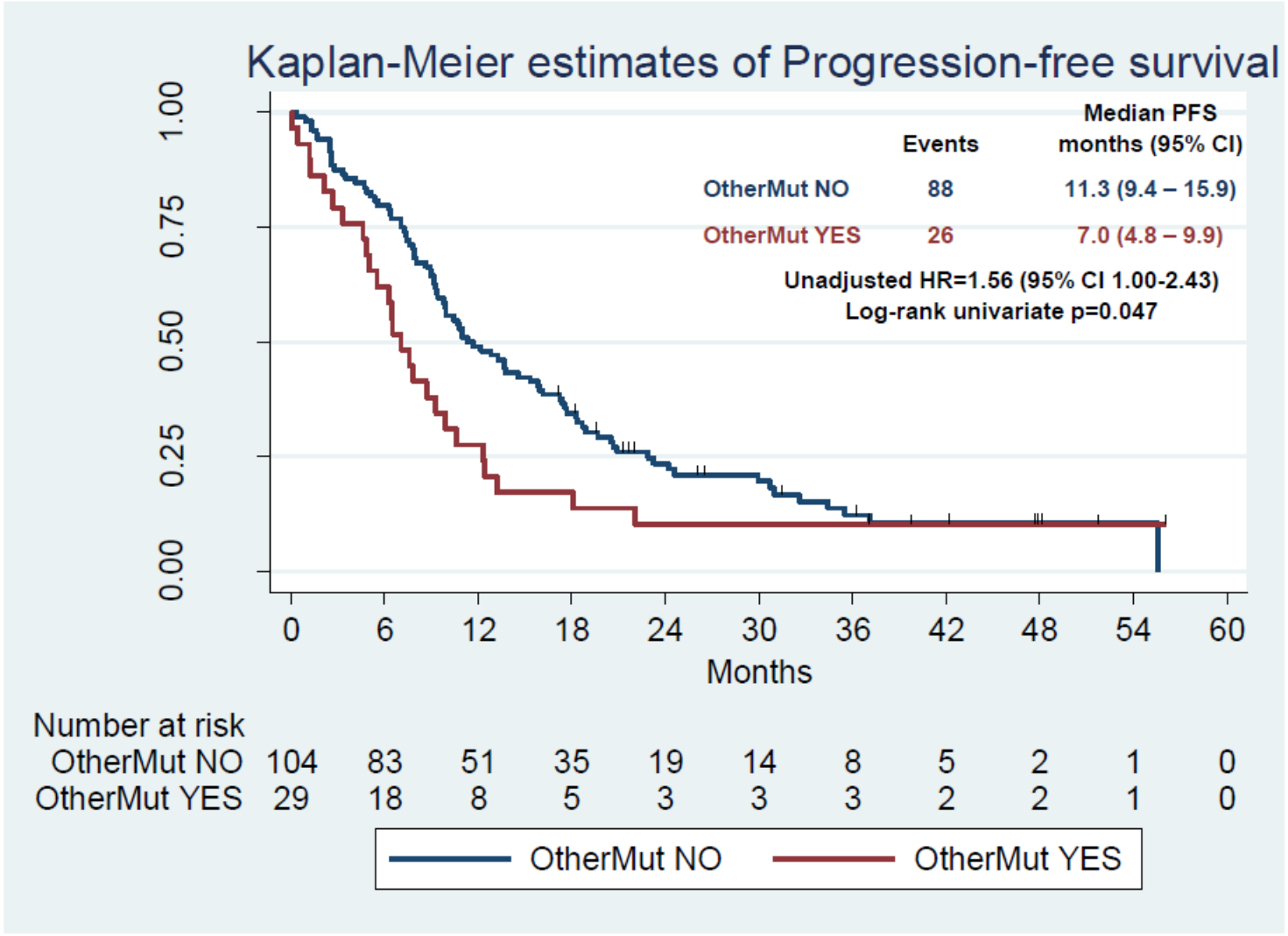
- **133 EGFR mutant advanced or metastatic NSCLC patients that received EGFR TKI treatment as first-line therapy were re-analyzed with NGS**

- Hotspot mutations in genes other than the EGFR, either KRAS, NRAS, BRAF, ERBB2, PIK3CA or MET, were found in 29/133 cases (21.8%).
- In most cases the allelic frequency of the other mutations was different as compared with EGFR mutations, suggesting intra-tumor heterogeneity.
- A T790M mutation was also found in 9/133 tumor samples (6.8%).



Normanno AIOM 2016

Results: PFS of patients with or without other mutations



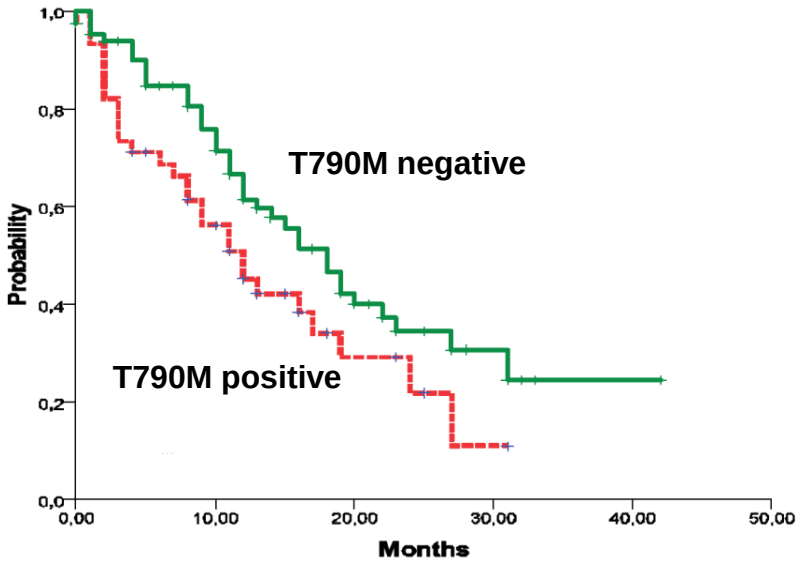
Normanno AIOM 2016

Results: Multivariate Cox regression model for PFS

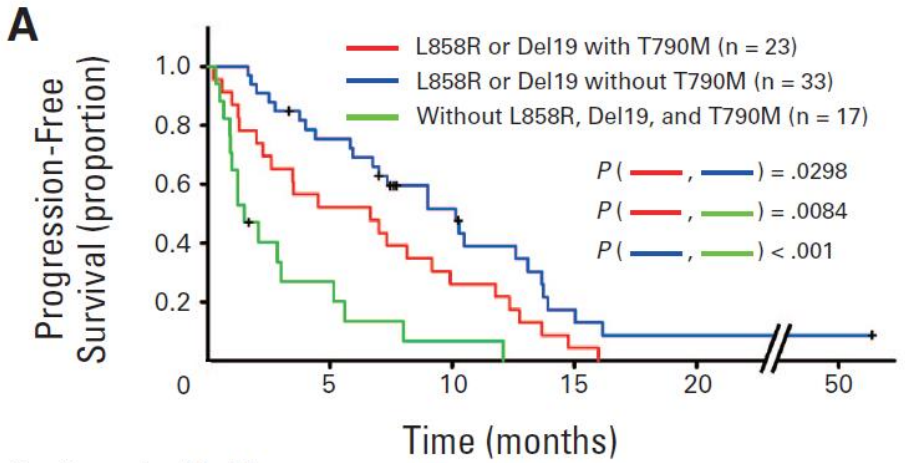
Variable	HR	95% CI	P
Other mutations	1.63	1.04-2.58	0.03
Sex	0.98	0.6-1.63	0.97
Age	1	0.98-1.02	0.70
Ever smoker	1.22	0.76-1.95	0.41
T790M	1.06	0.53-2.13	0.86

Normanno AIOM 2016

EGFR T790M mutation and outcome in NSCLC patients



Rosell CCR 2011



Cox Regression Model

Variable	Hazard Ratio	95% CI	*P*
L858R or Del19 without T790M	1.000		
L858R or Del19 with T790M	1.854	1.044 to 3.292	.035
Without L858R, Del19, and T790M	4.965	2.524 to 9.765	< .001

Su JCO 2012

EGFR Mutations Detected by Higly Sensitive Techniques

Table 1. *EGFR* Mutations Detected by Direct Sequencing, MALDI-TOF MS, and NGS in TKI-Naive and TKI-Treated Patients With NSCLC

						NGS Validation†			
	Direct Sequencing		MALDI-TOF MS			MALDI-TOF MS		NGS	
Patient Population	No.	%	No.	%	*P**	No.	%	No.	%
TKI-naive patients	107	100	107	100		38	100	38	100
EGFR wild type‡	67	62.6	59	55.1		19	50.0	19	50.0
EGFR-activating mutations§	40	37.4	48	44.9	.0196	19	50.0	19	50.0
EGFR-T790M	3	2.8	27‖	25.2	< .001	10	26.3	13	34.2
TKI-treated patients	88		88			16		16	
Pre-TKI	73¶	100	73¶	100		14	100	14	100
EGFR wild type‡	33	45.2	17	23.3		5	35.7	4	28.6
EGFR-activating mutations§	40	54.8	56	76.7	< .001	9	64.3	10	71.4
EGFR-T790M	2	2.7	23	31.5	< .001	1	7.1	2	14.3
Post-TKI	12	100	12	100		2	100	2	100
EGFR wild type‡	3	25.0	0	0.0		0	0.0	0	0.0
EGFR-activating mutations§	9	75.0	12	100		2	100	2	100
EGFR-T790M	4	33.3	10	83.3	.0143	2	100	2	100

Abbreviations: *EGFR*, epidermal growth factor receptor; MALDI-TOF MS, matrix-assisted laser desorption ionization–time of flight mass spectrometry; NGS, next-generation sequencing; NSCLC, non–small-cell lung cancer; TKI, tyrosine-kinase-inhibitor.

*McNemar test.

†Fifty-four DNA samples (38 for TKI-naive patients and 16 for TKI-treated patients) were available and qualified for NGS validation.

‡Patients without *EGFR* L858R or Del19 mutations.

§Patients with *EGFR* L858R or Del19 mutations.

‖Twelve T790M patients without *EGFR* L858R or Del19 mutations in MALDI-TOF MS analysis.

¶Three patients with *EGFR* mutations except L858R and Del19 were excluded from the analysis.

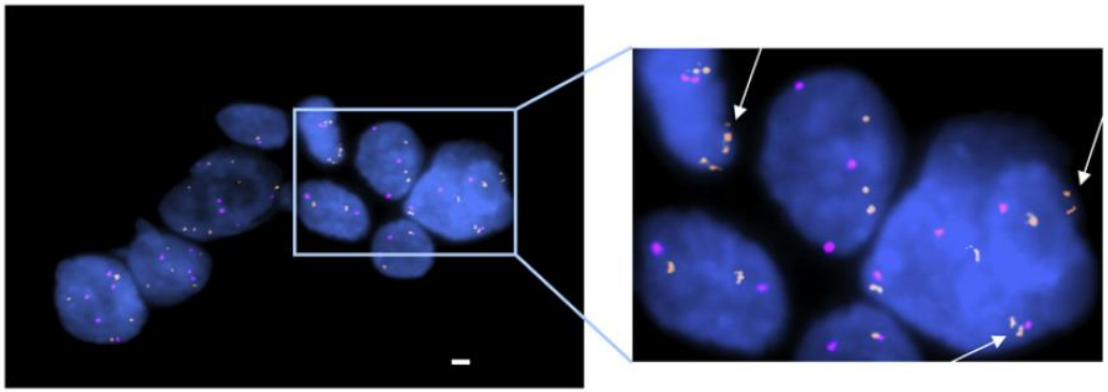
Su JCO 2012

Preexistence of MET Amplification in EGFR Mutant NSCLC

A

	Paired Specimens						
	Pre-Treatment			*Drug Resistant*			
#	**EGFR mutation**	**MET Amp**	**HGF Score**	**EGFR mutation**	**T790M**	**MET Amp**	**HGF Score**
1	Exon 19 del	No	30	Exon 19 del	No	No	200
2	Exon 19 del	No	50	Exon 19 del	No	No	120
3	Exon 19 del	No	N/A	Exon 19 del	Yes	No	300
				Exon 19 del	Yes	No	200
4	Exon 19 del	Yes (< 1%)	200	Exon 19 del	No	Yes	300
5	Exon 19 del	Yes (< 1%)	120	Exon 19 del	Yes	No	200
6	Exon 19 del	No	200	Exon 19 del	No	No	200
7	Exon 19 del	N/A	400	Exon 19 del	Yes	No	350
				Exon 19 del	Yes	No	350
8	L858R	N/A	N/A	L858R	Yes	No	90
9	G719S, S768I	No	95	None*	N/A	No	60
10	L858R	Yes (< 1%)	60	L858R	No	Yes	400
11	Exon 19 deletion	N/A	70	Exon 19 del	Yes	No	300
12	L858R	N/A	100	L858R	No	No	50
13	Exon 19 del	No	300	Exon 19 del	Yes	No	145
14	L858R	No	40	L858R	No	No	180
15	Exon 19 del	Yes (< 1%)	0	Exon 19 del	Yes	Yes	100
16	Exon 19 del	Yes (< 1%)	100	Exon 19 del	Yes	Yes**	150
	Resistant Only						
17				Exon 19 del	Yes	No	180
18				Exon 19 del	Yes	No	200
19				L858R	Yes	No	200
20				Exon 19 del	Yes	No	300
21				Exon 19 del	Yes	No	200
22				Exon 19 del	Yes	No	80
23				Exon 19 del	No	No	30
24				L858R	No	No	400
25				Exon 19 del	No	No	N/A
26				Exon 19 del	No	No	N/A
27				Exon 19 del	Yes	No	0

B



Turke Cancer Cell 2010

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- The concept of inter- and intra-tumor heterogeneity
- Intra-tumor heterogeneity in EGFR mutant NSCLC
- **Clonal evolution and resistance to EGFR targeting therapies**

Resistance to anti-EGFR agents

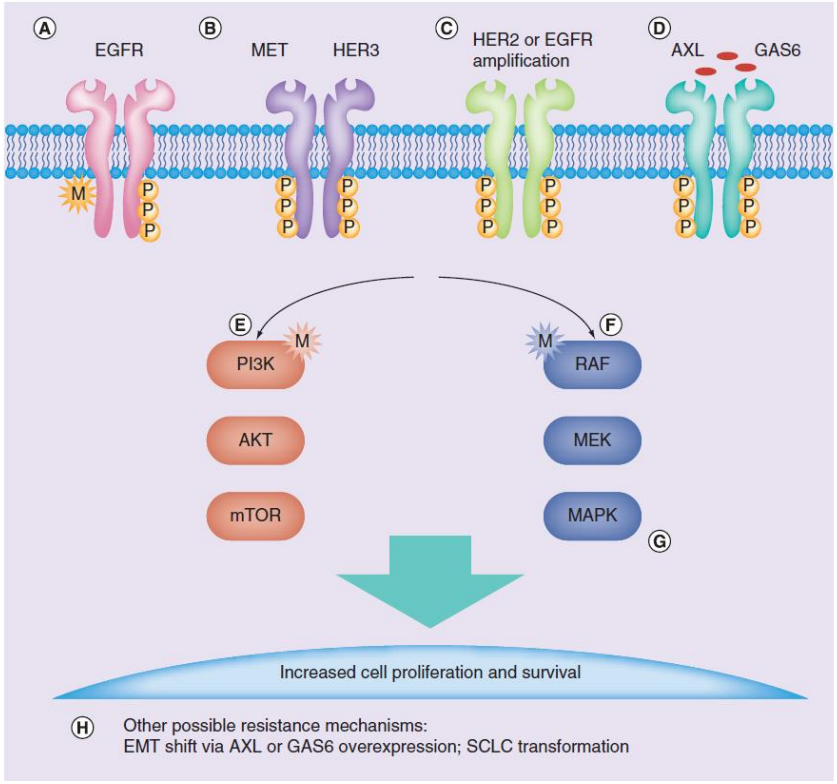


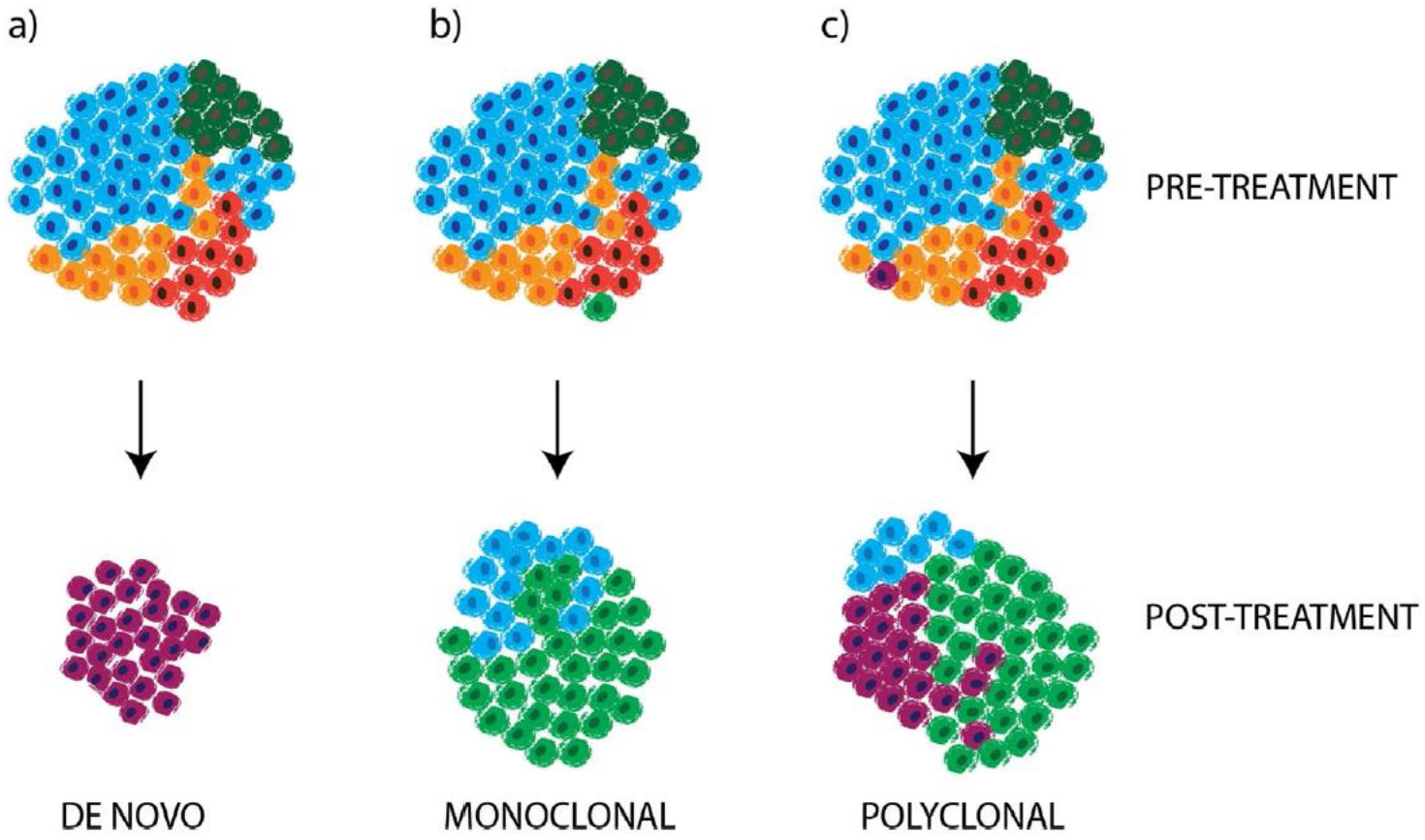
Table 1. Main mechanisms involved in acquired resistance to EGF receptor-tyrosine kinase inhibitors.

Molecular alteration	Frequency (%)†
T790M mutation	~50
MET amplification	5–20
EGFR amplification	8‡
HER2 amplification	5–13
MAPK1 amplification	4.8
PIK3CA mutations	5
BRAF mutations	1
AXL overexpression	20
GAS6 overexpression	25
EMT	1–2
SCLC transformation	5–14

†Frequencies are derived from different studies [5,9,22,37–41].
‡*EGFR* amplification + T790M mutation [37].
EMT: Epithelial-to-mesenchymal transition; SCLC: Small-cell lung carcinoma.

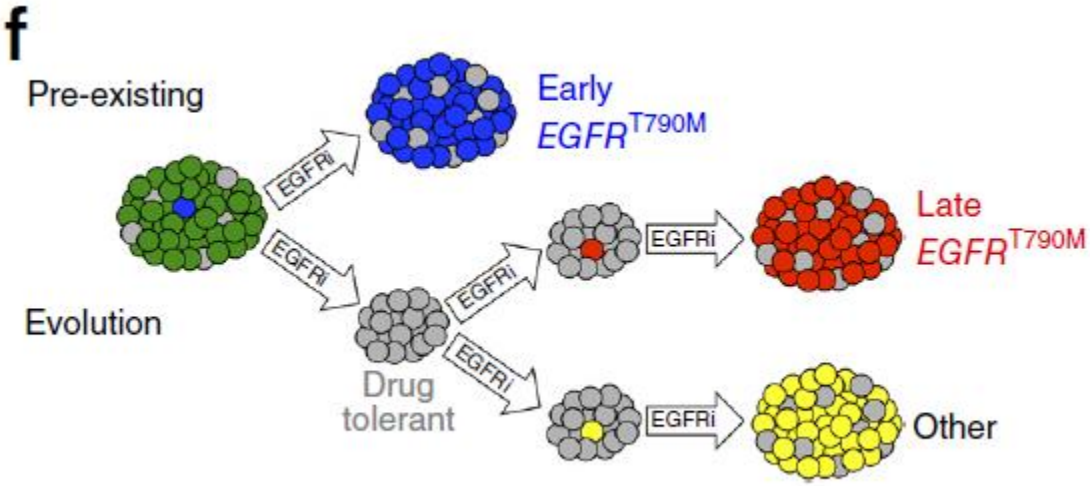
Fenizia Future Oncology 2015

Clonal Evolution and Drug Resistance



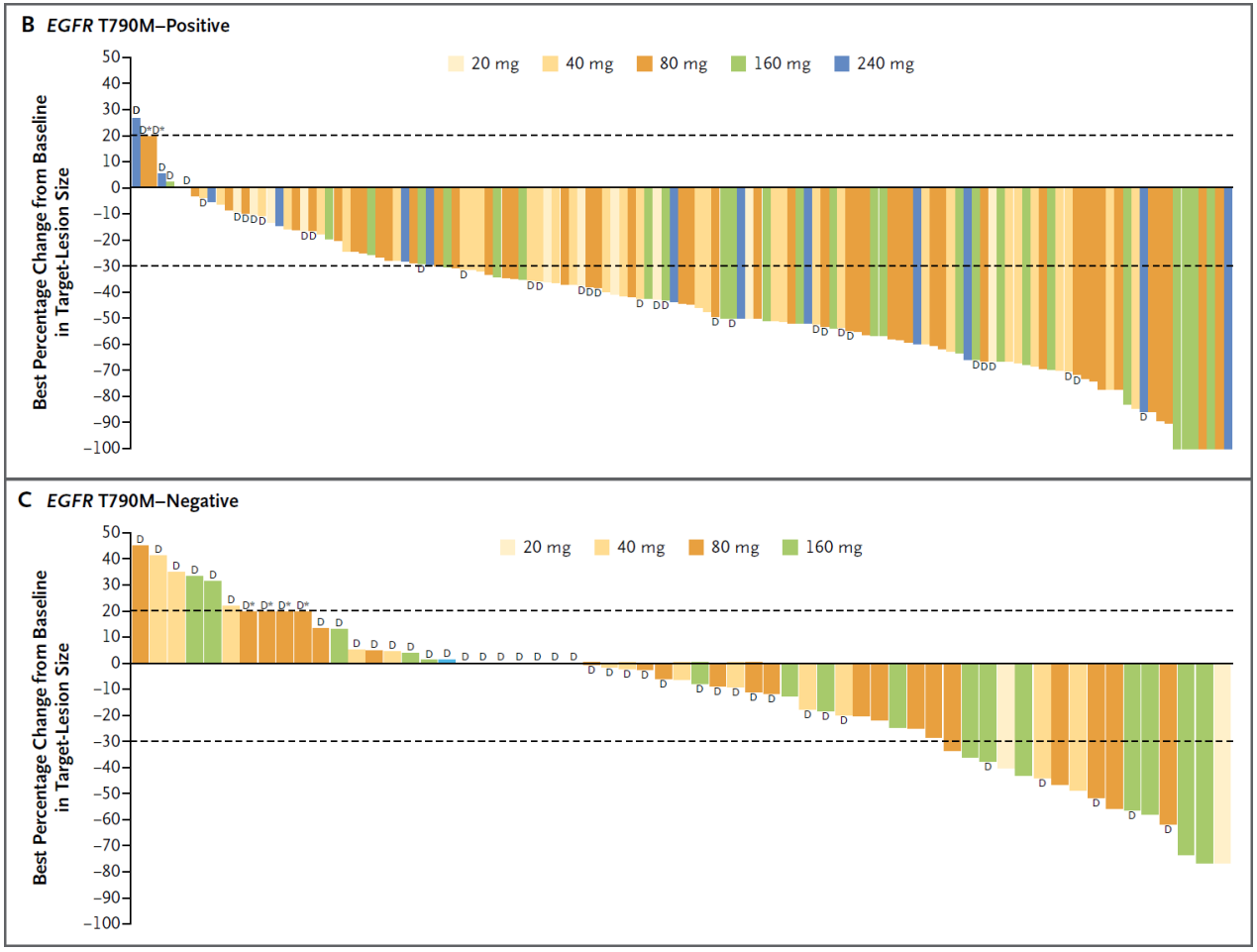
Burrell & Swanton Mol Oncol 2014

Model for the development of EGFR T790M-determined acquired resistance



Hata Nat Med 2016

Response to AZD9291 in NSCLC patients



ORR* = 64% (69/107; 95% CI 55%, 73%)
Overall disease control rate (CR+PR+SD) = 94% (101/107; 95% CI 88%, 98%)

ORR* = 22% (11/50; 95% CI 12%, 36%)
Overall disease control rate (CR+PR+SD) = 56% (28/50; 95% CI 41%, 70%)

Janne NEJM 2015

EGFR T790M testing on patient progression: tissue or liquid biopsy?

Tissue biopsy

- Techniques for tissue testing are well established
- Re-biopsy at progression is not a common practice in many countries
- Invasive procedure with potential risks for the patient
- Sampling limited to a single disease site

Liquid biopsy

- Liquid biopsy is a non-invasive procedure
- Analysis is more rapid as compared with tissue biopsy
- Liquid biopsy may provide a more complete picture of the tumor molecular portrait
- Methods for analysis of liquid biopsy have not been standardized yet and have some limitations

Performance of four different plasma assays (72 plasma samples from the AURA trial)

	cobas® EGFR Mutation Test	BEAMing dPCR
Exon 19 deletion		
Sensitivity	82% (23/28)	82% (23/28)
Specificity	97% (30/31)	97% (30/31)
L858R		
Sensitivity	87% (20/23)	87% (20/23)
Specificity	97% (35/36)	97% (35/36)
T790M		
Sensitivity	73% (30/41)	81% (33/41)
Specificity	67% (16/24)	58% (14/24)

Thress Lung Cancer 2015

cobas plasma test versus cobas tissue test as a reference method

cobas plasma test performance	Pooled AURA Phase II studies (AURA extension and AURA2)		
	L858R	Exon 19 deletion	T790M
Using cobas tissue test as reference			
PPA / sensitivity	75.6%	85.1%	61.4%
NPA / specificity	98.1%	98.0%	78.6%
OPA / concordance	90.9%	90.0%	65.4%

Differences in detection of T790M using tissue and plasma are thought to reflect tumour biology and molecular heterogeneity in the resistance setting

The **cobas**® EGFR Mutation Test is not available for use with plasma samples in U.S.

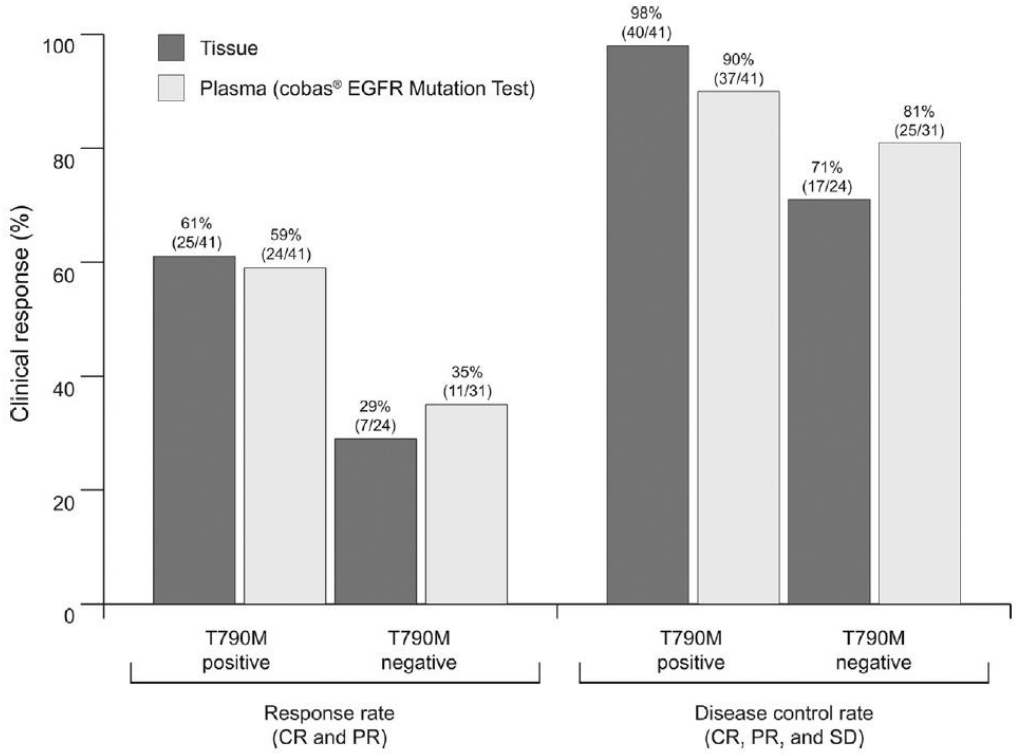
EUROPEAN LUNG CANCER CONFERENCE 2016
Presented by James C-H Yang at the 6th IASLC/ESMO European Lung Cancer Conference, 13–16 April 2016, Geneva, Switzerland; Abstract 134O_PR.

Patient	Tissue[a]	Plasma		
	cobas® EGFR Mutation Test	BEAMing dPCR (% mutant)	cobas® EGFR Mutation Test	
1	**Positive**	**Positive (0.021%)**	Negative	'False' negatives by cobas® EGFR Mutation Test
2	**Positive**	**Positive (0.048%)**	Negative	
3	**Positive**	**Positive (0.064%)**	Negative	
4	**Positive**	**Positive (0.202%)**	Negative	
5	**Positive**	Negative	Negative	
6	**Positive**	Negative	Negative	
7	**Positive**	Negative	Negative	
8	**Positive**	Negative	Negative	
9	**Positive**	Negative	Negative	
10	**Positive**	Negative	Negative	
11	**Positive**	Negative	Negative	
12	Negative	**Positive (0.026%)**	Negative	'False' positives by BEAMing dPCR
13	Negative	**Positive (0.027%)**	**Positive**	
14	Negative	**Positive (0.054%)**	**Positive**	
15	Negative	**Positive (0.080%)**	Negative	
16	Negative	**Positive (0.283%)**	**Positive**	
17	Negative	**Positive (0.340%)**	**Positive**	
18	Negative	**Positive (0.344%)**	**Positive**	
19	Negative	**Positive (0.491%)**	**Positive**	
20	Negative	**Positive (1.113%)**	**Positive**	

Discordant results with two different plasma assays for detection of the EGFR T790M mutation from circulating tumor DNA

Thress Lung Cancer 2015

Clinical response to AZD9291 according to EGFR T790M mutation at baseline



In patients with plasma positive but tumor negative for T790M, the clinical ORR was 38% (3/8 patients) and the disease control rate was 75% (6/8 patients).

Thress Lung Cancer 2015

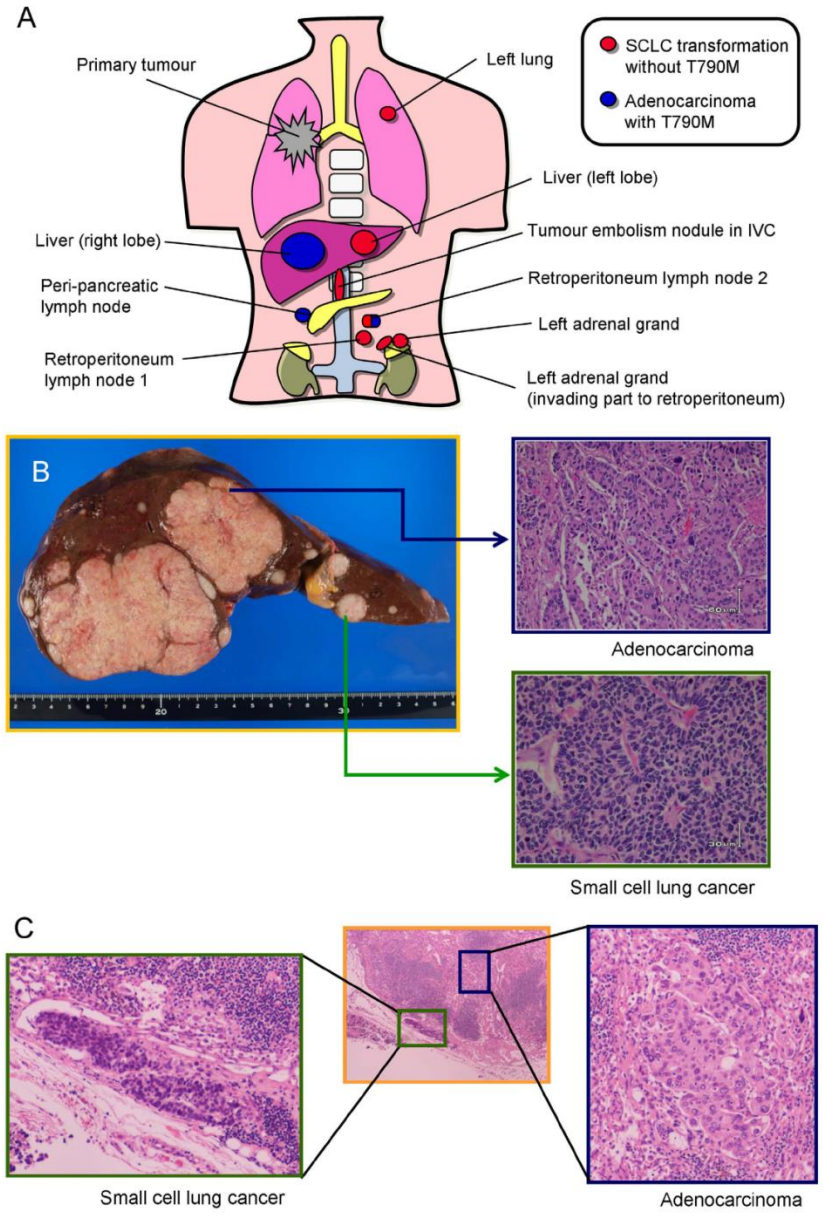
T790M Plasma Testing is a Viable Alternative to Tissue Testing

Objective response rate for 188 evaluable patients with both central T790M tissue test result and plasma T790M result

		Plasma T790M		
		+	-	
Tissue T790M	+	55% (72/130)	43% (13/30)	53% (85/160)
	-	35% (6/17)	27% (3/11)	32% (9/28)
		53% (78/147)	39% (16/41)	

- Similar ORR observed when detecting T790M in either tissue or plasma
- Not all patients with progression on first-line TKI are candidates for tissue re-biopsy

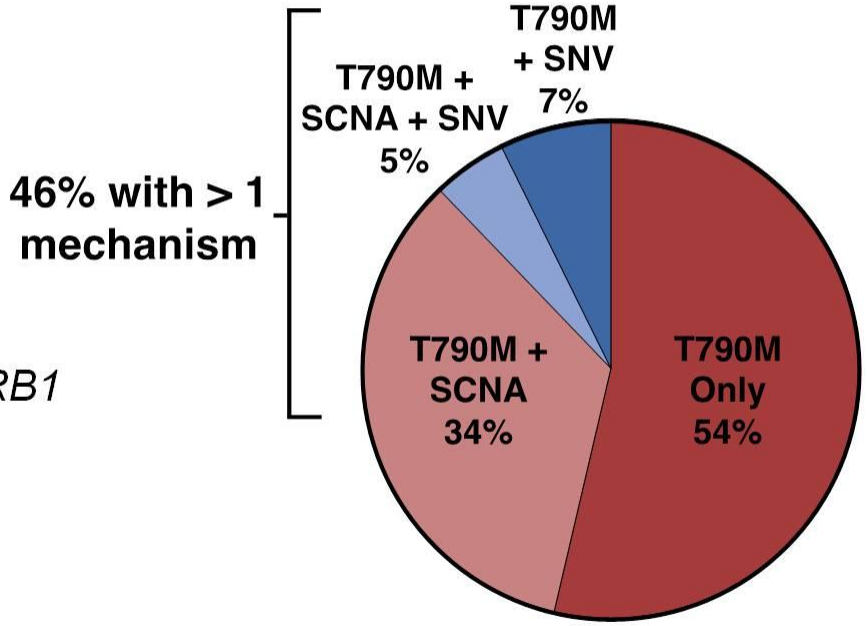
T790M Mutation Heterogeneity



Suda Sci Rep 2015

Intra-patient Heterogeneity of Resistance Mechanisms to First-line EGFR TKIs

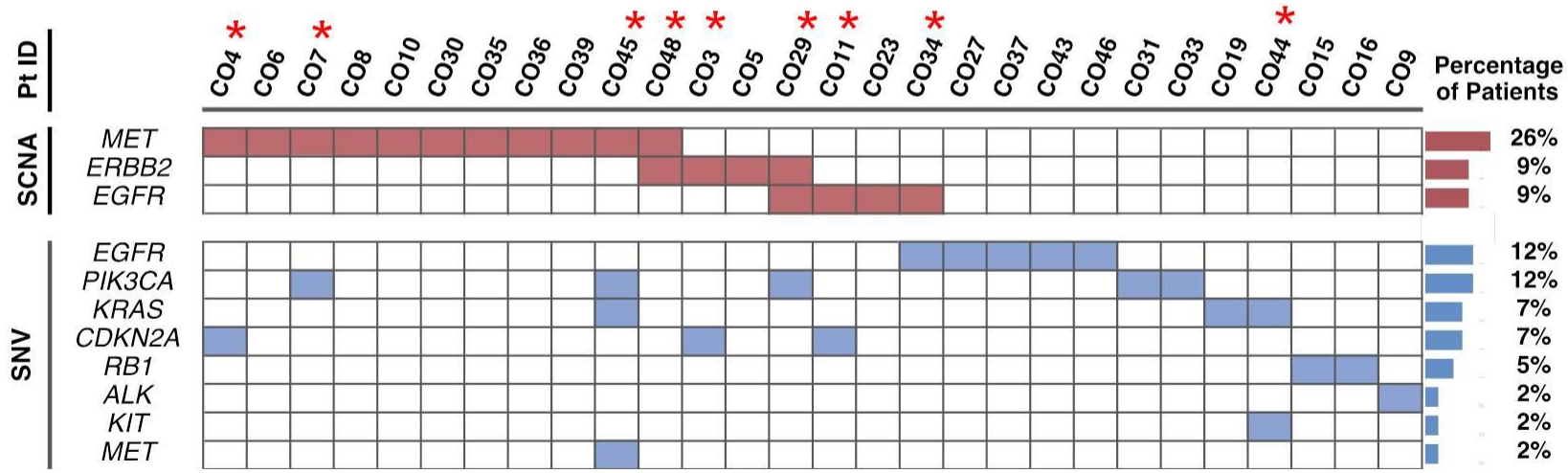
- Baseline rociletinib plasma
  - *n* = 41 patients with detectable T790M
- 34% T790M+SCNA (copy number gain)
  - *MET* or *ERBB2*
- 7% T790M+SNV(s)
  - *EGFR*, *PIK3CA* or *RB1*
- 5% T790M+SCNA+SNV
  - SCNA in *MET* and SNV in *PIK3CA* or *RB1*



SNV=single nucleotide variant, SCNA=somatic copy number alteration

Inter- and Intra-patient Heterogeneity of Resistance to Rociletinib

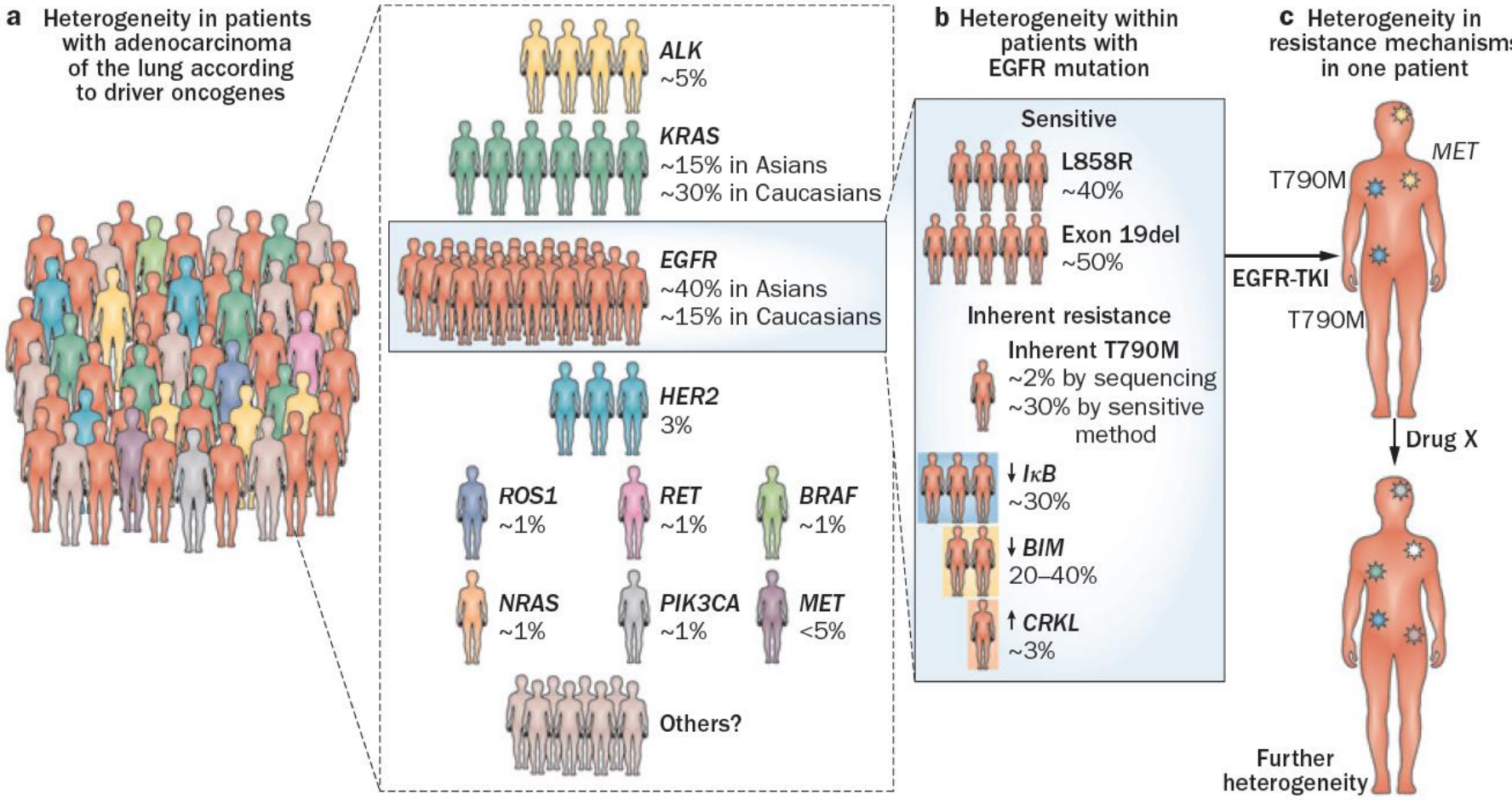
- Putative resistance mechanism criteria:
  - Emerged at progression
  - Increased from baseline to progression
- Mechanism(s) identified in 65% of patients
  - 9 genes involved
  - 21% of patients develop multiple resistance mechanisms (*)



SNV=single nucleotide variant, SCNA=somatic copy number alteration, * =patient with > 1 mechanism identified

Presented By Jacob Chabon at 2016 ASCO Annual Meeting

TREATMENT OF NSCLC WITH TARGET BASED AGENTS INCREASES TUMOR HETEROGENEITY



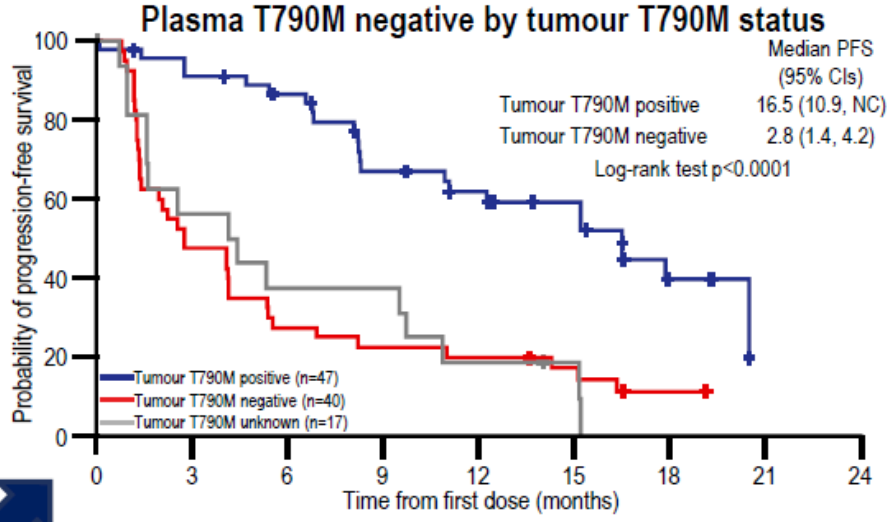
Mitsudomi Nat Rev Clin Oncol 2013

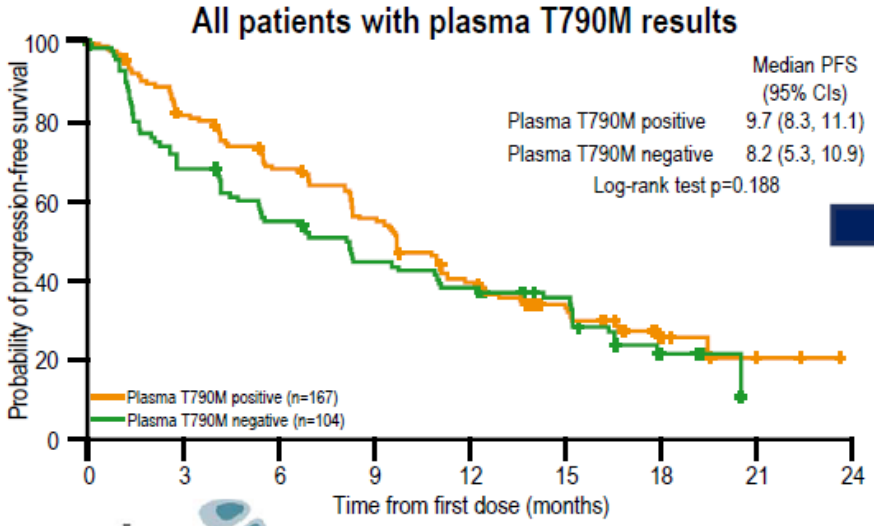
EGFR T790M testing on patient progression: tissue or liquid biopsy?

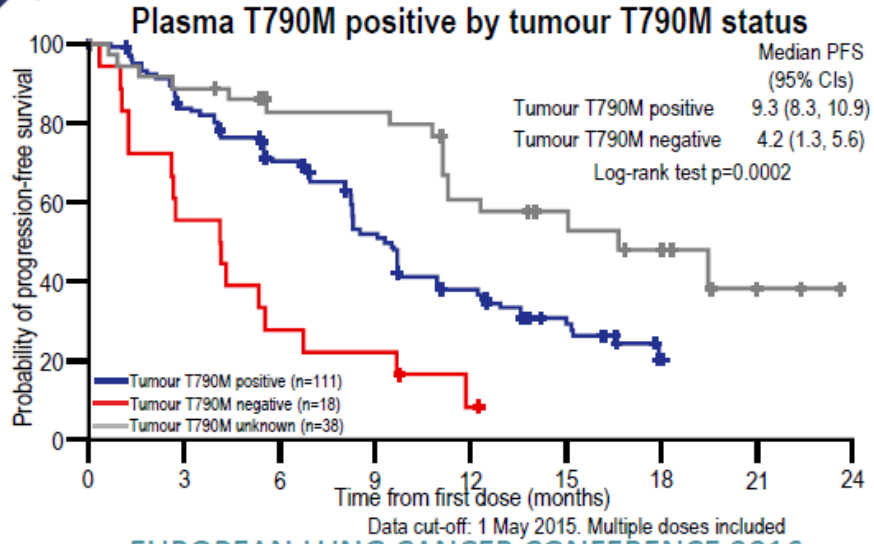
- **Some tumors are heterogenous with regard to the presence of the T790M mutation**
- **Liquid biopsy will allow to identify T790M mutation in heterogenoeus tumor that might be negative at tissue biopsy**
- **However, liquid biopsy still suffers from a relative low sensitivity: a fraction of cases that are positive on tissue might result negative on plasma**
- **Liquid biopsy and tissue biopsy are complementary in providing information on T790M status of patients at progression following EGFR TKI treatment**

PFS by tumour and plasma T790M status

- In plasma T790M negative patients, tumour genotyping can distinguish those patients with better and worse outcomes
- Interestingly, a difference based on tumour genotype is also seen in plasma T790M positive cases





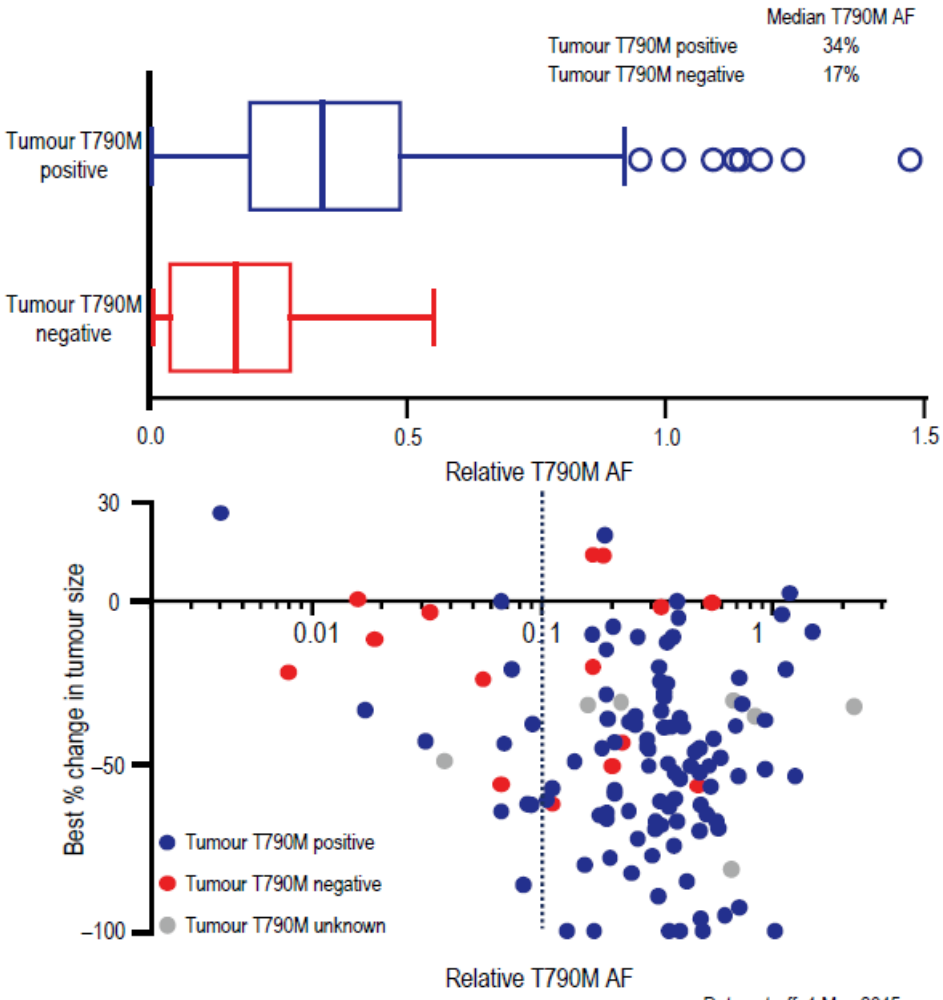


Data cut-off: 1 May 2015. Multiple doses included

Presented by Geoffrey R Oxnard at the 6th IASLC/ESMO European Lung Cancer Conference, 13–16 April 2016, Geneva, Switzerland; Abstract 135O_PR.

T790M heterogeneity in plasma "false positives"

- We hypothesized that cases T790M negative in tumour and T790M positive in plasma might have heterogeneous presence of T790M
- Relative T790M AF was calculated as a proportion of EGFR sensitising AF:
  - T790M AF / sensitising AF
- Relative T790M AF was lower in cases with T790M negative in tumour, suggesting T790M may be present as a minor clone
- There was a trend toward lower response magnitude in the group with relative T790M AF <10% (p=0.08)

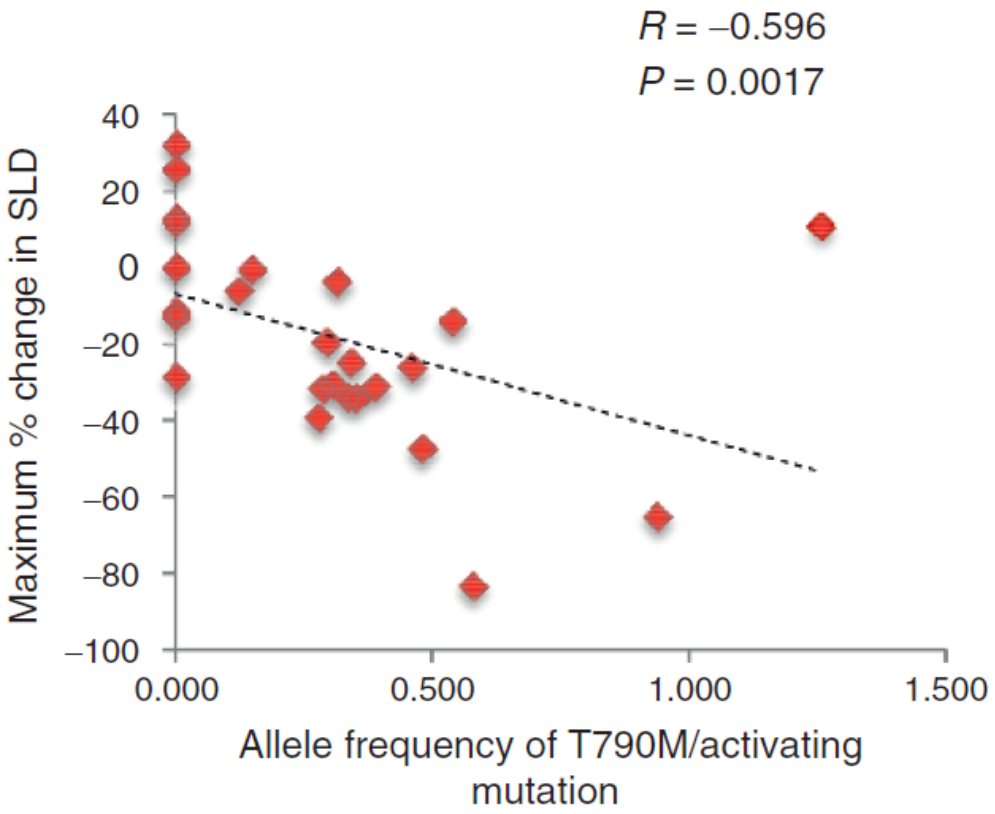


Data cut-off: 1 May 2015

Relative T790M AF was calculated as a proportion of EGFR sensitising AF

Presented by Geoffrey R Oxnard at the 6th IASLC/ESMO European Lung Cancer Conference, 13–16 April 2016, Geneva, Switzerland; Abstract 135O_PR.

The relationship between the allelic fraction of T790M in the pre-rociletinib biopsy and the maximum reduction in tumor volume



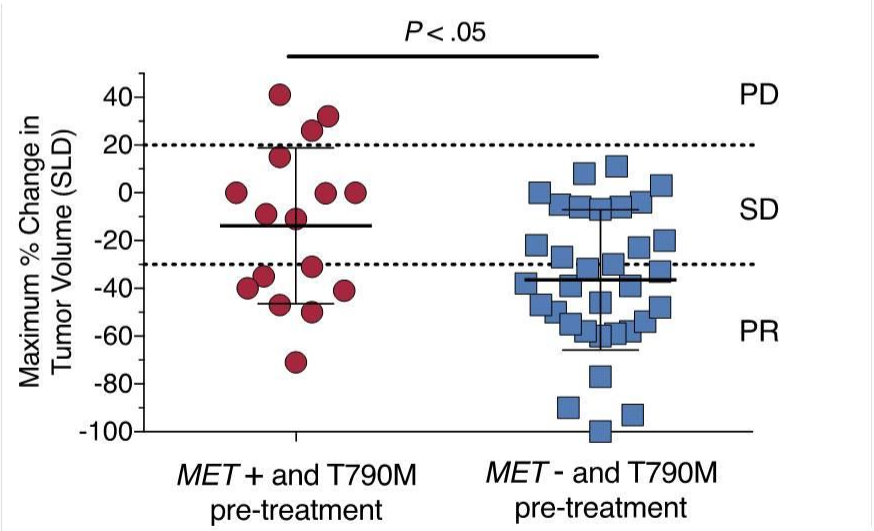
Piotrowska Cancer Discov 2015

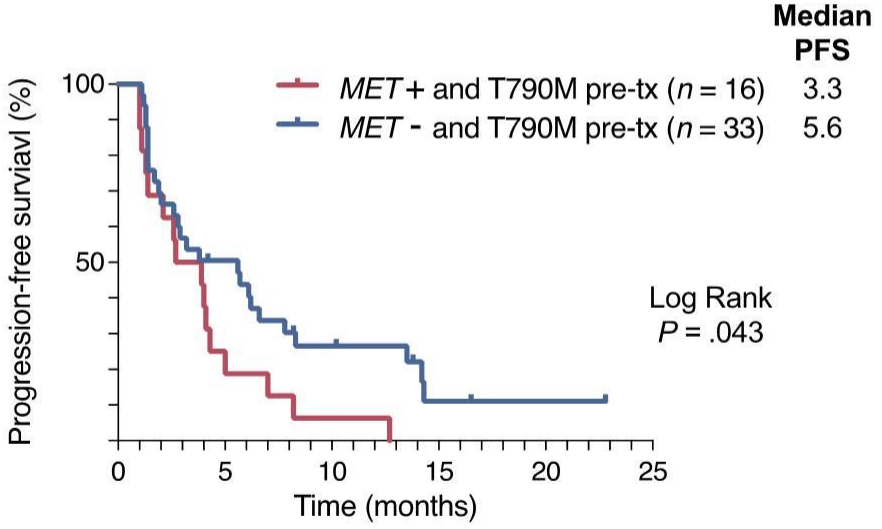
Presence of Multiple Resistance Mechanisms is Associated with Poor Outcome

Expanded cohort with pre-treatment *MET* assessment[1]

Group A: *MET*+ & T790M+ Patients (*n* = 16)

Group B: *MET*- & T790M+ Patients (*n* = 33)

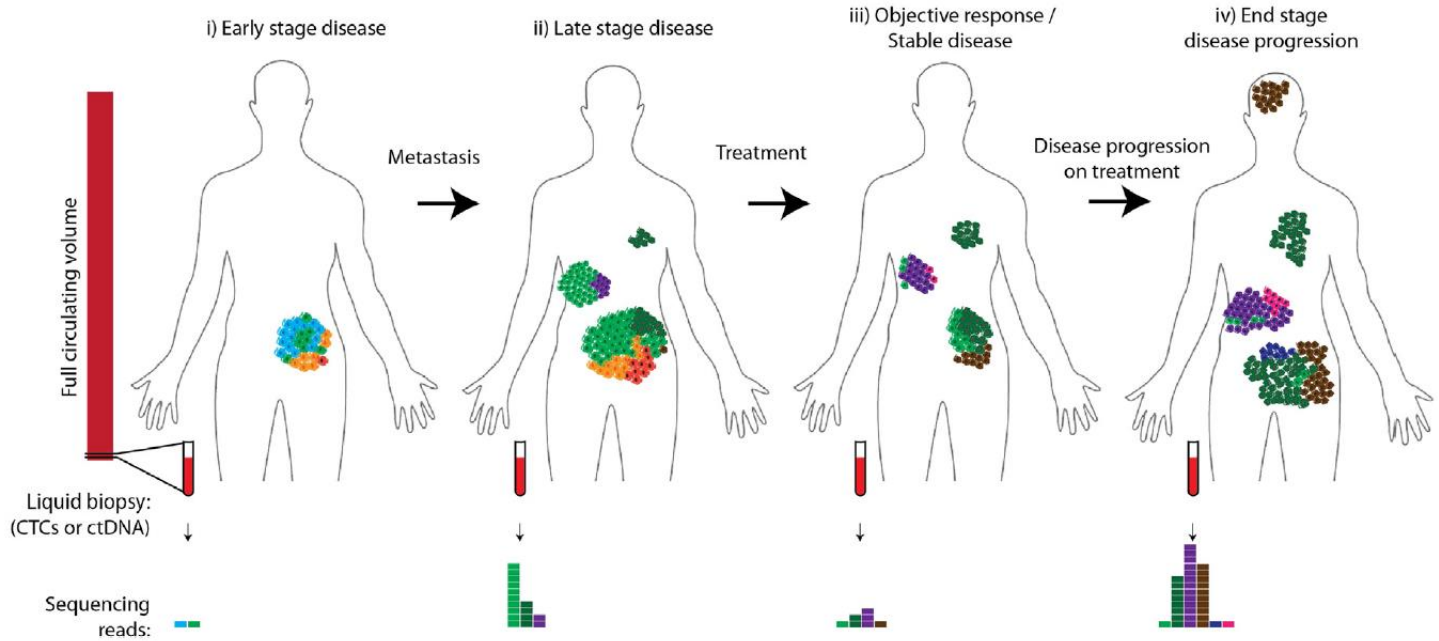




[1]*MET* status was determined by CAPP-Seq ctDNA analysis, FISH on tumor biopsy, or prior patient history

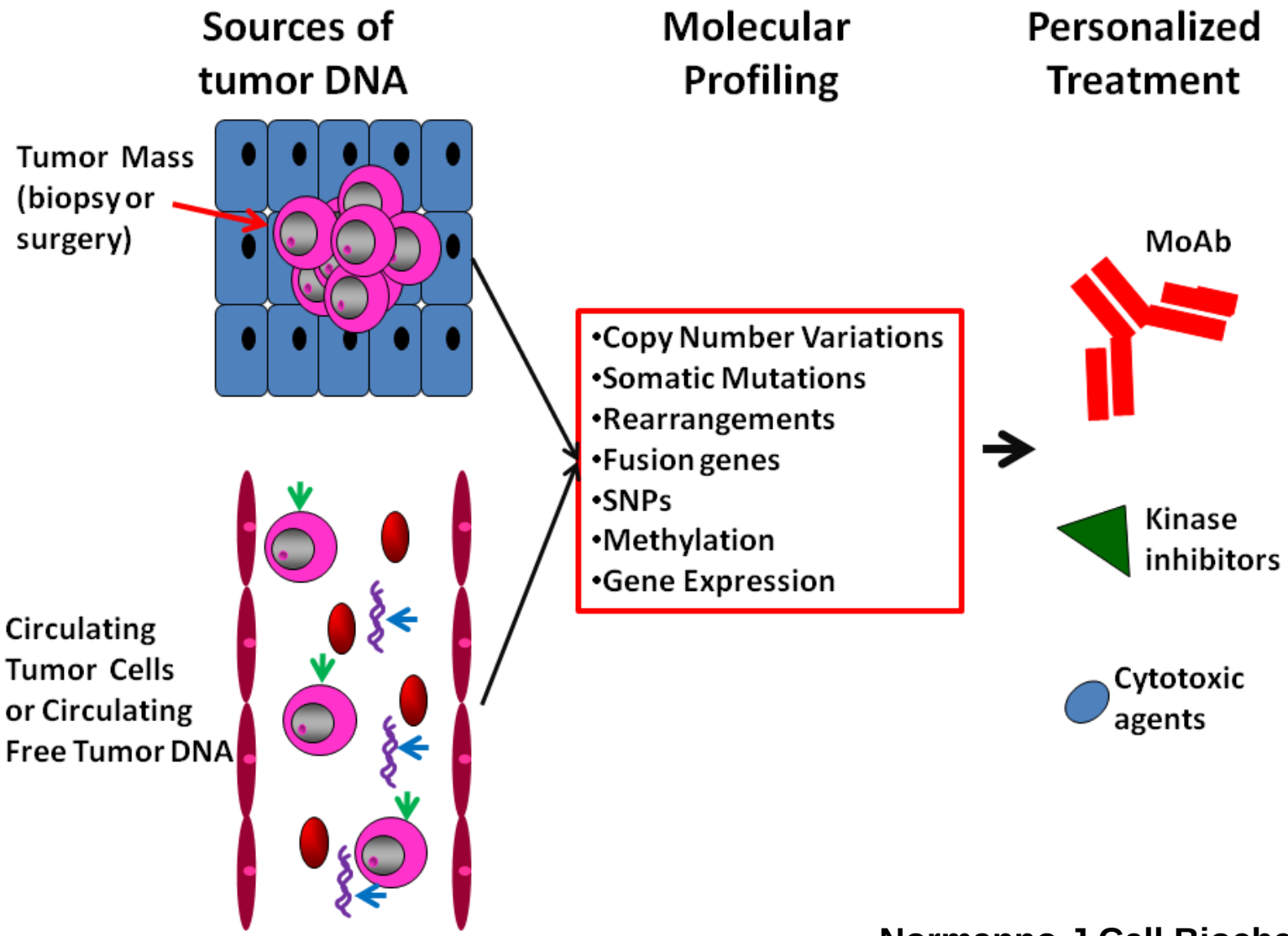
Presented By Jacob Chabon at 2016 ASCO Annual Meeting

Liquid biopsy can represent temporal and spatial heterogeneity in cancer progression



Burrell & Swanton Mol Oncol 2014

The future of biomarker testing



Normanno J Cell Biochem 2013

ISTITUTO NAZIONALE PER LO STUDIO
E LA CURA DEI TUMORI
FONDAZIONE G. Pascale – NAPOLI

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