How Liquid Biopsy Technology is Delivering Personalized Therapy in NSCLC

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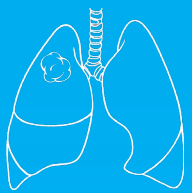

Center for Thoracic Oncology

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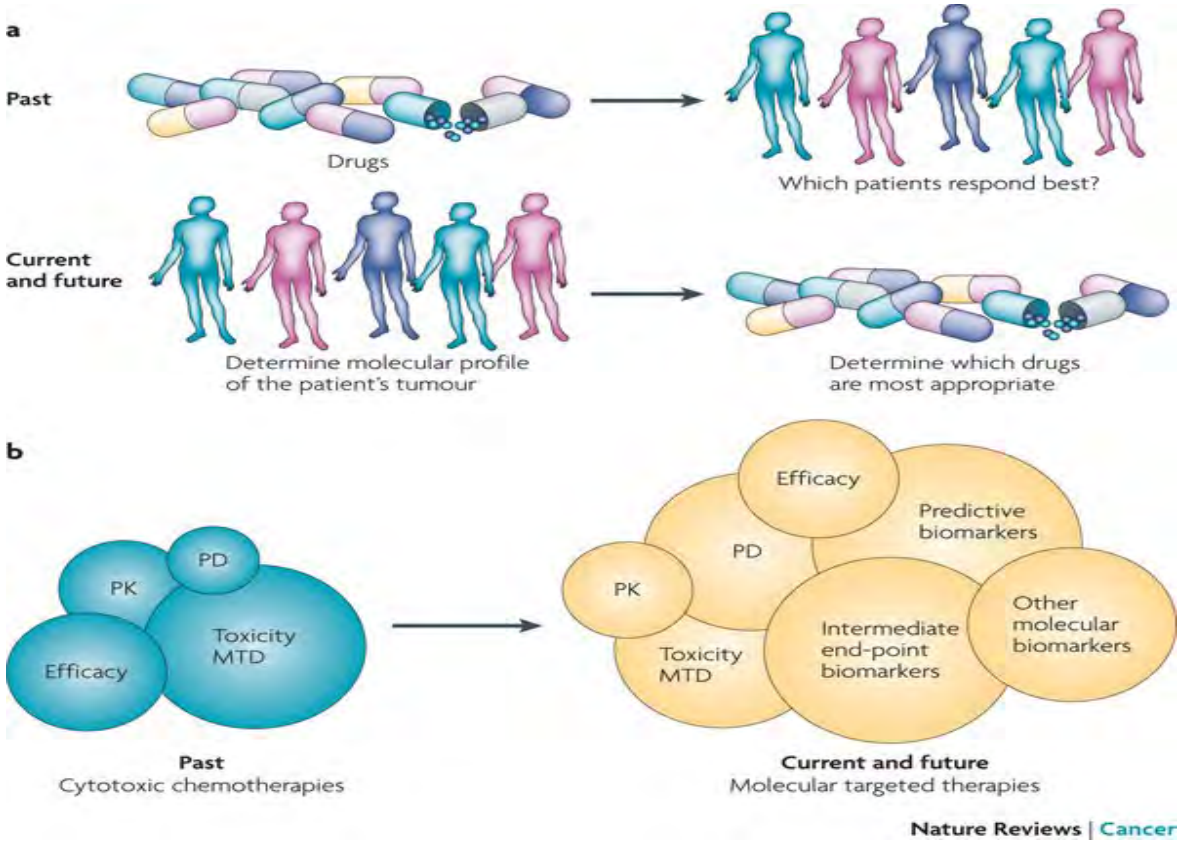
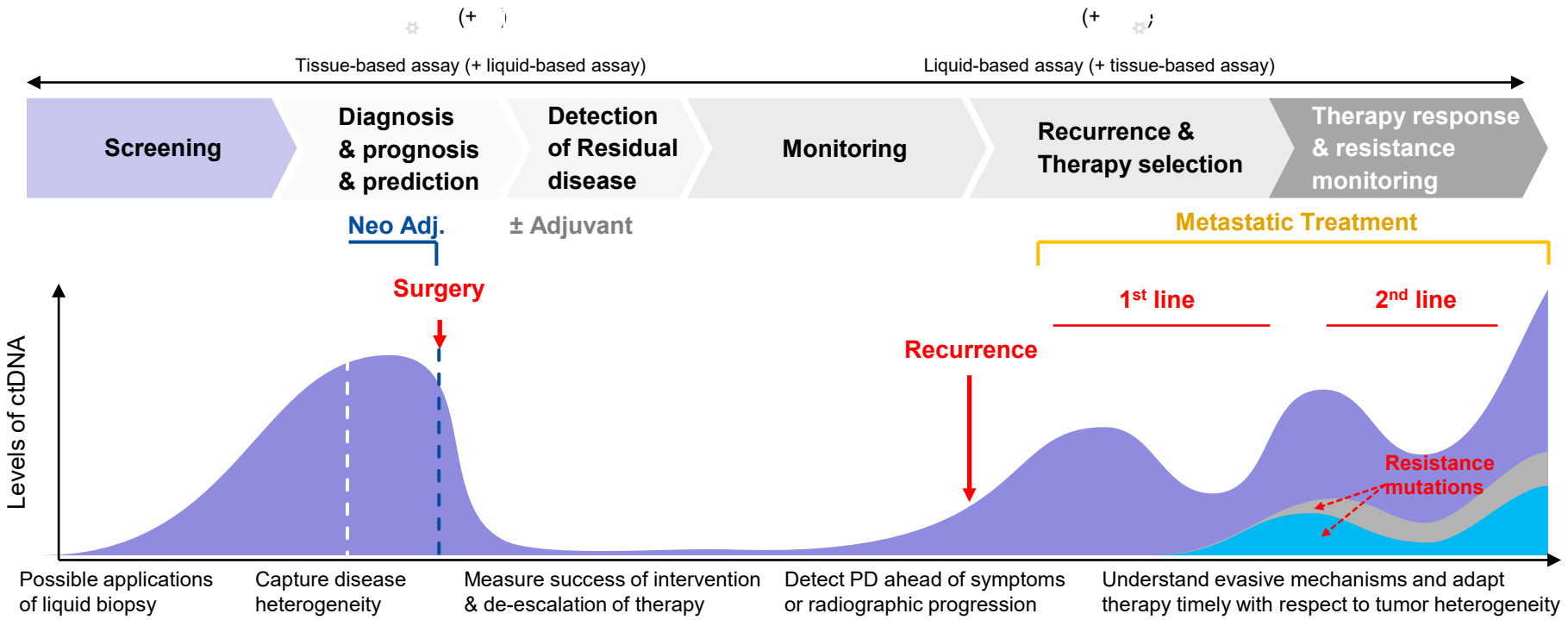
Current Treatment and Trial Paradigm



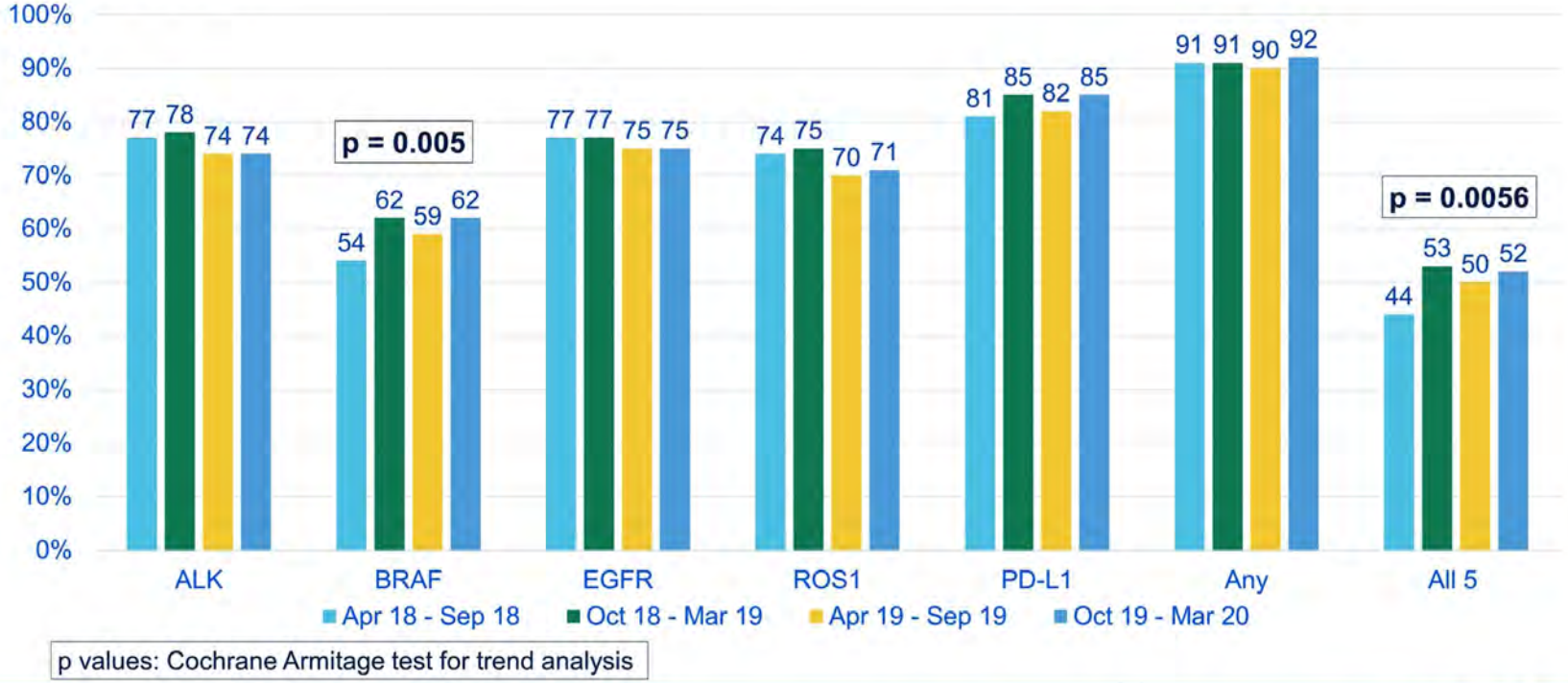
Liquid biopsy can provide clinically-valuable information along the whole patient journey



PD: progressive disease.

Adapted from Wan, J.C.M., et al., (2017) *Nat Rev Cancer* 17:223-38.

Biomarker testing rates over time for overall study population



p values: Cochrane Armitage test for trend analysis

Presented By: **Nicholas J. Robert, MD**
On behalf of the MYLUNG Consortium™

NGS testing rates over time for the overall population

p value: Cochrane Armitage test for trend analysis

Presented By: **Nicholas J. Robert, MD**
On behalf of the MYLUNG Consortium™

2021 ASCO ANNUAL MEETING

Tissue biopsy may not capture the genomic landscape of a patient's entire tumour burden

Intratumour heterogeneity

The genomic landscape **within a single tumour manifestation** may not be uniform

Tissue biopsy may not capture subclonal populations of tumour cells with distinct alterations

Intrapatient heterogeneity

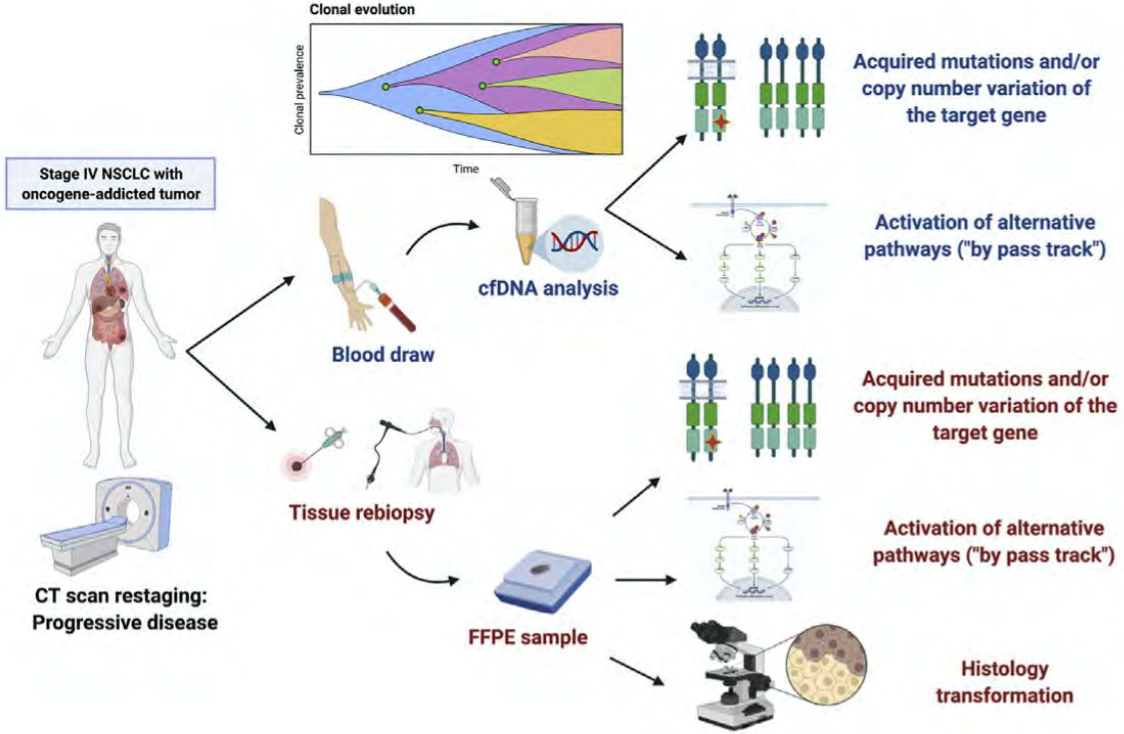
The genomic landscape may **differ between tumour sites** within a patient

Tissue biopsy from a single lesion will miss alterations unique to other lesions

As well as spatial heterogeneity, as the genomic landscape of a cancer evolves over time, temporal heterogeneity should also be considered
Therefore archival tissue may not fully represent the tumour genotype at progression

Scherer, F. (2020) in *Recent Results in Cancer Research: Tumor Liquid Biopsies*. Springer.

Liquid biopsy and tissue rebiopsy after acquired resistance to TKI



Main liquid biopsy techniques used

NGS-based approaches:
- ✓High sensitivity
- ✓Multiplex
- ✓Gene rearrangements
- ✓Gene amplifications

PCR-based approaches:
- ✓Variable sensitivity
- ✓Single gene testing
- ✓Only for mutations

Main techniques used for tumor tissue

NGS-based approaches:
- ✓High sensitivity
- ✓Multiplex
- ✓Gene rearrangements
- ✓Gene amplifications

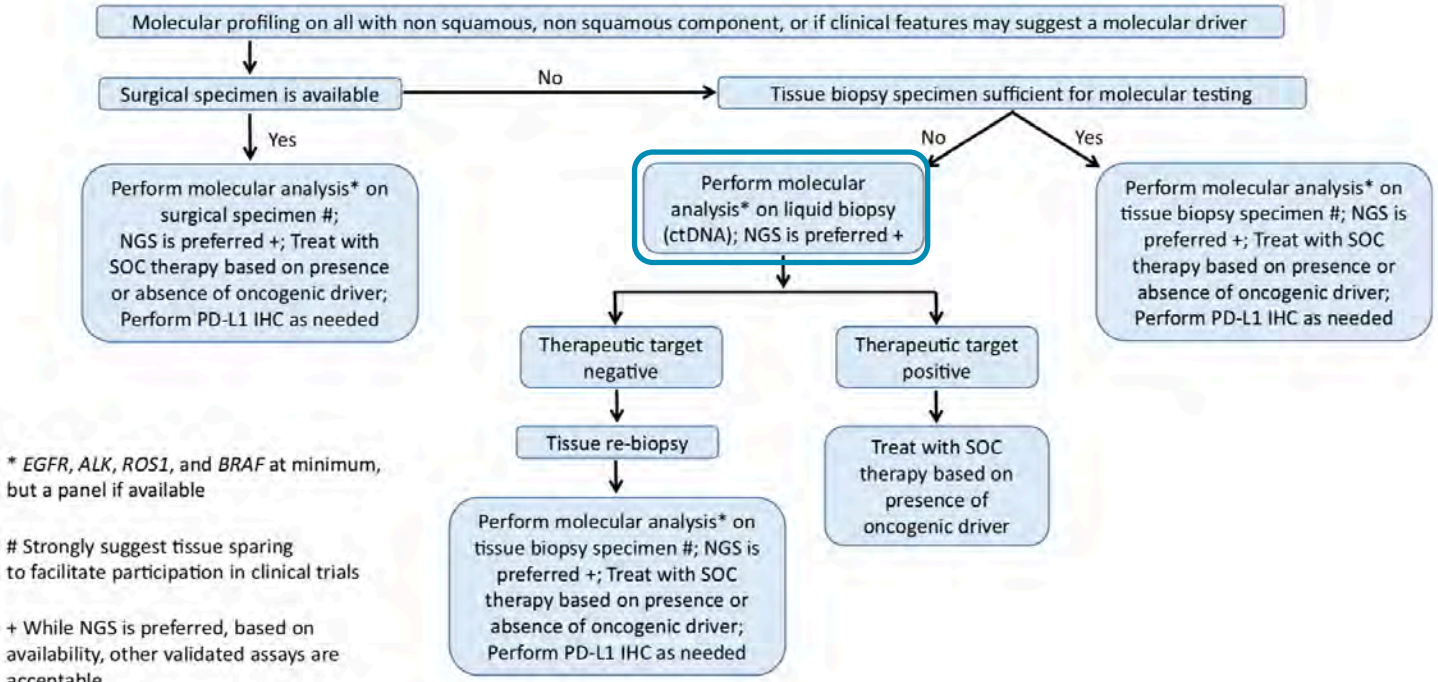
FISH:
- ✓Gene rearrangements & amplifications

PCR-based approaches:
- ✓Variable sensitivity
- ✓Single/Multiplex gene testing
- ✓Only for mutations

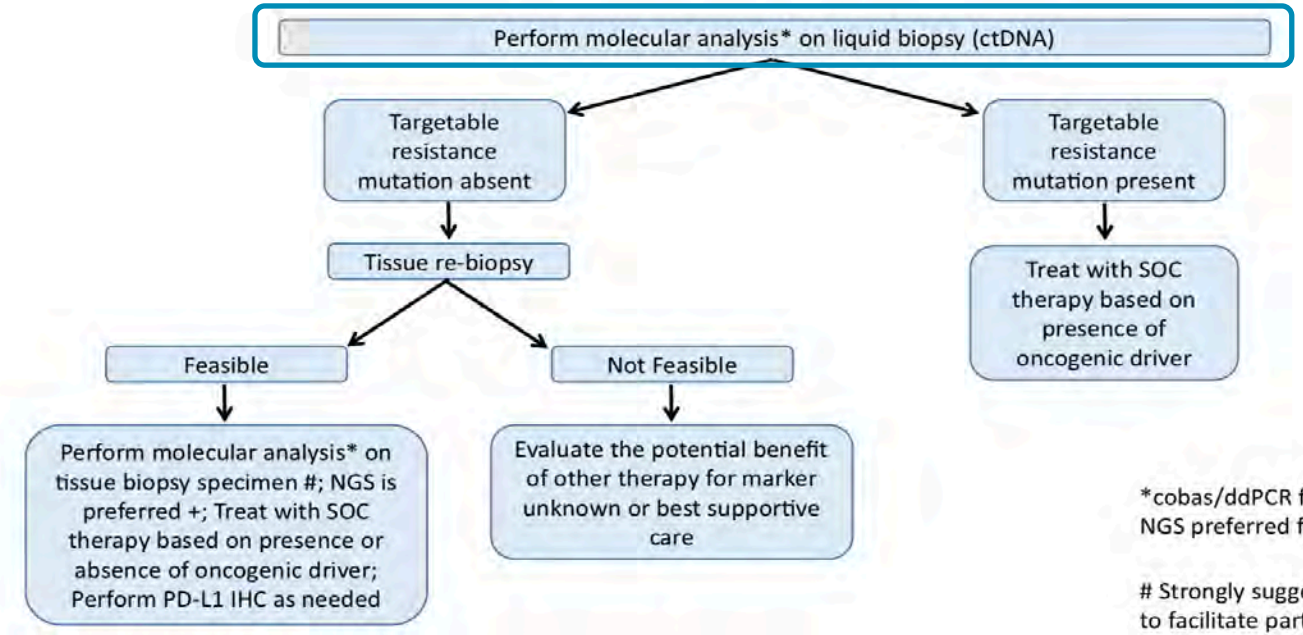
IHC:
- ✓Protein expression

Rolfo et al, JTO 2021 Oct;16(10):1647-1662

Patients with advanced treatment-naive NSCLC



Patients with progressive or recurrent NSCLC during treatment with TKI



*cobas/ddPCR for *EGFR* mutation
NGS preferred for *ALK* and *ROS1*

Strongly suggest tissue sparing to facilitate participation in clinical trials

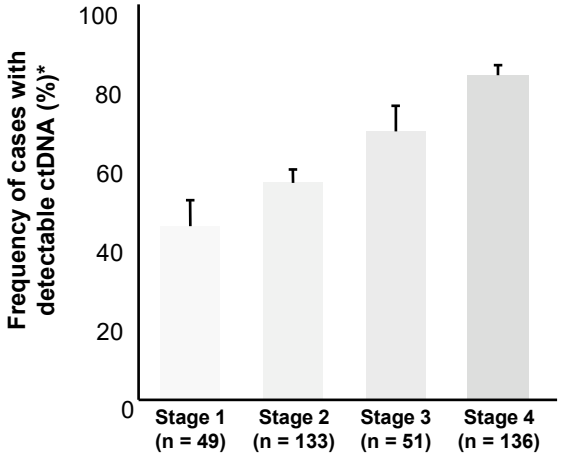
+ While NGS is preferred, based on availability, other validated assays are acceptable

Analysis of circulating tumour DNA (ctDNA) poses distinct challenges

ctDNA

- constitutes a **highly variable fraction** of the total plasma cfDNA **from < 0.1% to > 90%**[1,2]
  - if ctDNA fraction is low, detection of **alterations is more challenging**[2,3]
  - need to be able to **detect mutations down to ≤ 0.1%** MAF (particularly for detection of MRD)[3,4]
- is more **fragmented at 134 - 144 bp**, compared with ~166 bp fragments of 'normal' plasma cfDNA[5]
- has a very short **half-life of less than one hour** in circulation[2,6]

Amount of shedded, or detectable, ctDNA is variable depending on factors such as tumour stage, histology, vascularity and treatment[1,5-8]



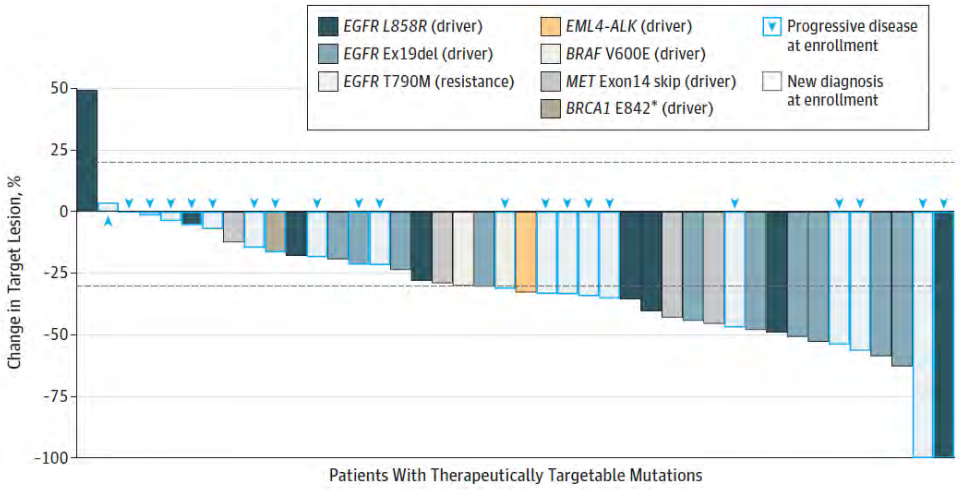
Somatic cfDNA alterations were detected in 85% (18,503 / 21,807) of patients across various cancer types[9]

* Figure adapted from reference 5. cfDNA: cell-free DNA; ctDNA: circulating tumour DNA; MAF: mutant allele frequency; MRD: minimal residual disease.

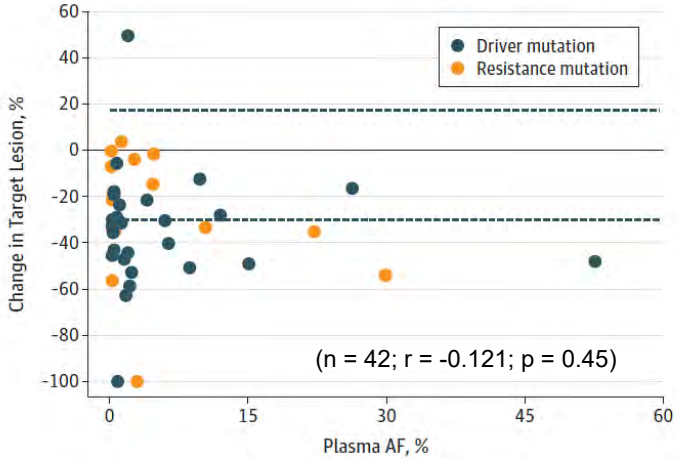
1. Hinrichsen, T., et al. (2016) *J Lab Med* 40:313-22; 2. Corcoran, R.B. and Chabner, B.A. (2018) *N Engl J Med* 379:1754-65; 3. Johansson, G., et al. (2019) *Biomol Detect Quantif* 17:100078;
4. Jennings, L. et al. (2017) *J Mol Diagn* 19:341-65 5. Wan, J.C.M., et al., (2017) *Nat Rev Cancer* 17:223-38; 6. Mattox, A. K., et al (2019) *Sci Transl Med* 11:eaay1984;
7. Bettegowda, C., et al. (2014) *Sci Transl Med* 6:224ra24; 8. Diaz, L.A. and Bardelli, A. (2014) *J Clin Oncol* 32:579-86; 9. Zill, O.A., et al. (2018) *Clin Cancer Res* 24:3528-38.

Plasma-based biomarkers with low allele frequency may still respond to targeted therapy

Responses to plasma-indicated targeted therapy by RECIST



Correlation of RECIST and allele frequency



AF: allele frequency; RECIST: Response Evaluation Criteria in Solid Tumours.

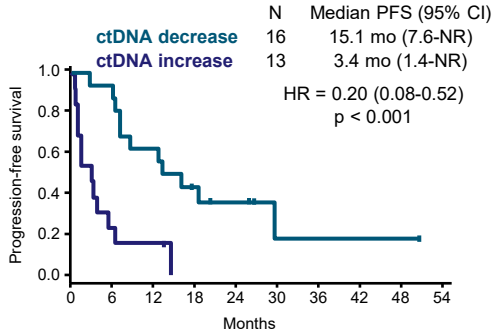
Aggarwal, C., et al. (2019) *JAMA Oncol* 5:173-80.

ctDNA kinetics as predictive marker for treatment response or resistance

Identification of early plasma ctDNA changes to predict response to first-line pembrolizumab +/- chemotherapy in aNSCLC patients[1]

Blood samples were collected on 1st day of treatment and at each subsequent cycle

A 36-gene panel NGS* **detected early quantitative changes** across a wide range of variants

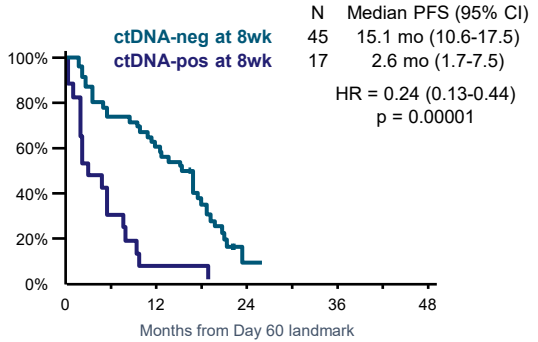


Rapid **decrease of ctDNA** correlated with **clinical benefit**, while **increase** correlated with **PD**

Residual ctDNA to predict PFS and OS in *EGFR*mut NSCLC patients treated with afatinib +/- cetuximab[2]

Blood samples were collected at baseline, cycle 3-day 1 and at progression

A 73-gene panel NGS† **detected quantitative changes in *EGFR*mut ctDNA** (primary activating mutations E19del or L858R)



Clearance of *EGFR* ctDNA after 60 days of therapy correlated with substantial **improvement in PFS and OS**

In both studies PFS is significantly longer in NSCLC patients with early ctDNA decrease / clearance

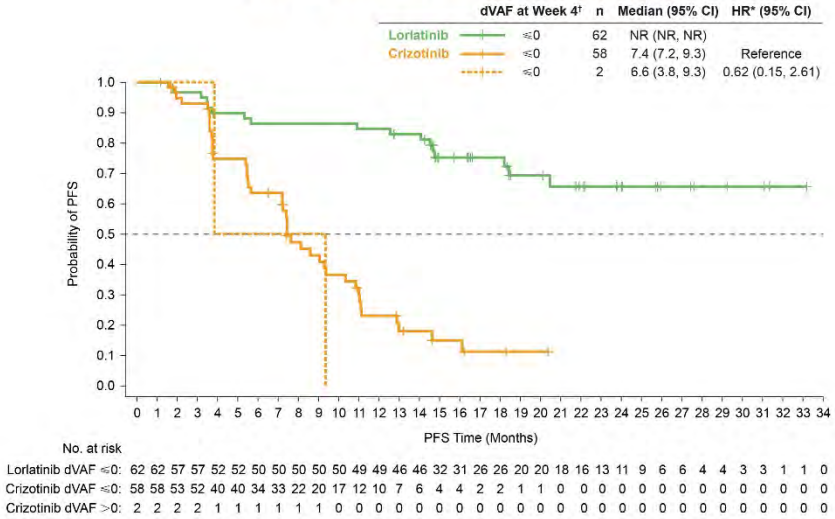
These results suggest a potential role for ctDNA NGS analysis to detect pharmacodynamic biomarkers of response or resistance to targeted therapies and immunotherapies

*Samples were analysed in the Inivata CLIA-accredited laboratory (Research Triangle Park, NC) for InVision ctDNA analysis. †Tested by Guardant Health, Inc. using G360 panel. aNSCLC: advanced non-small cell lung cancer; ctDNA: circulating tumour DNA; mo: months; NGS: next-generation sequencing; OS: overall survival; PD: progressive disease; PFS: progression-free survival; pts: patients; wk: weeks.

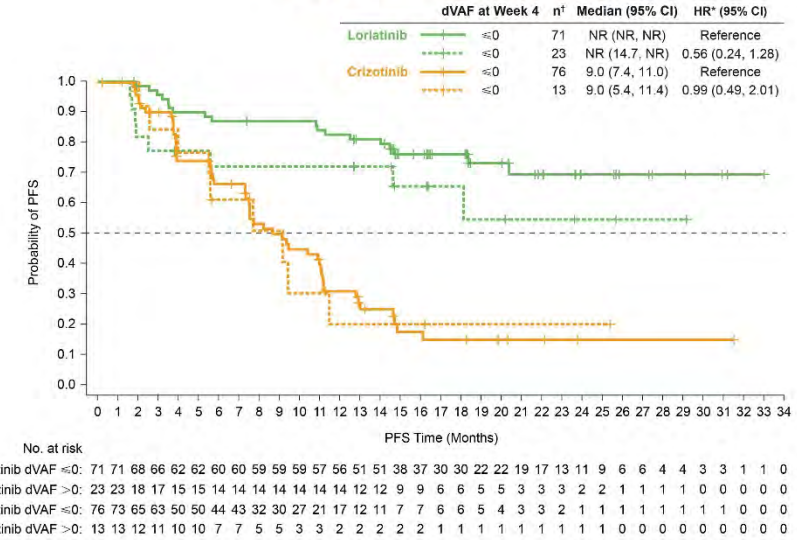
1. Ricciuti, P.C., et al. (2020) ASCO poster 3518; 2. Mack, P.C., et al. (2020) ASCO poster 9532.

Results: Reduction in dVAF at Week 4 Was Associated With Longer PFS With Lorlatinib

ALK Fusion and/or Mutation



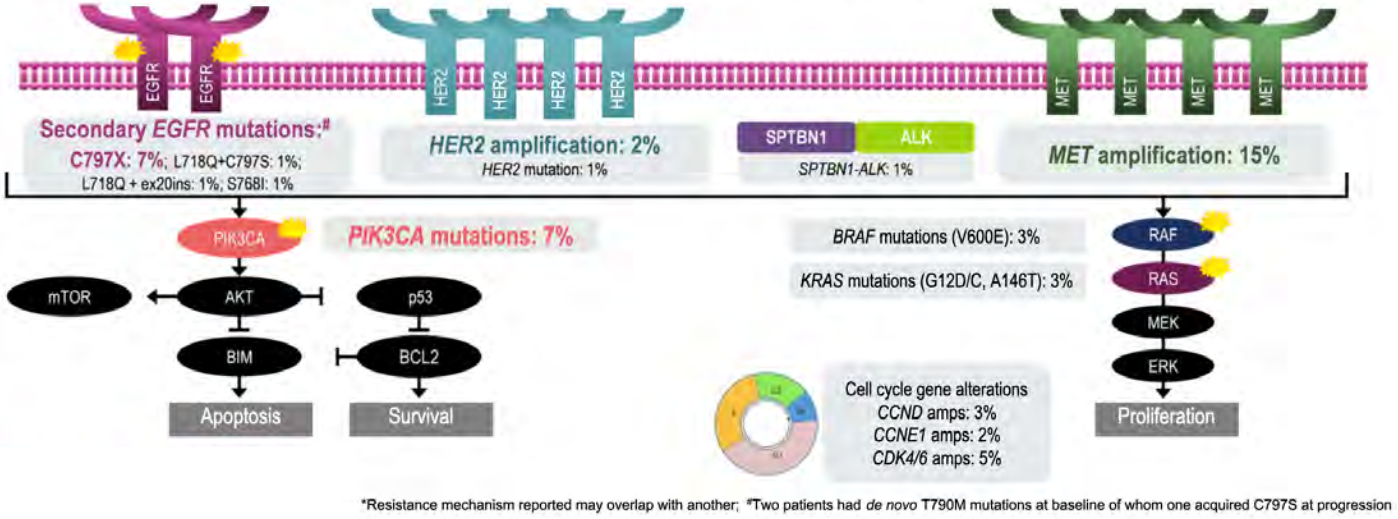
Any Somatic Alteration



*HR <1.00 favors dVAF ≤0.
†No patients in the lorlatinib group had a dVAF >0 (*ALK* fusion and/or mutation) at week 4.
HR, hazard ratio; NR, not reached.

Acquired mechanisms of resistance to 1st line Osimertinib: The FLAURA analysis (LB)

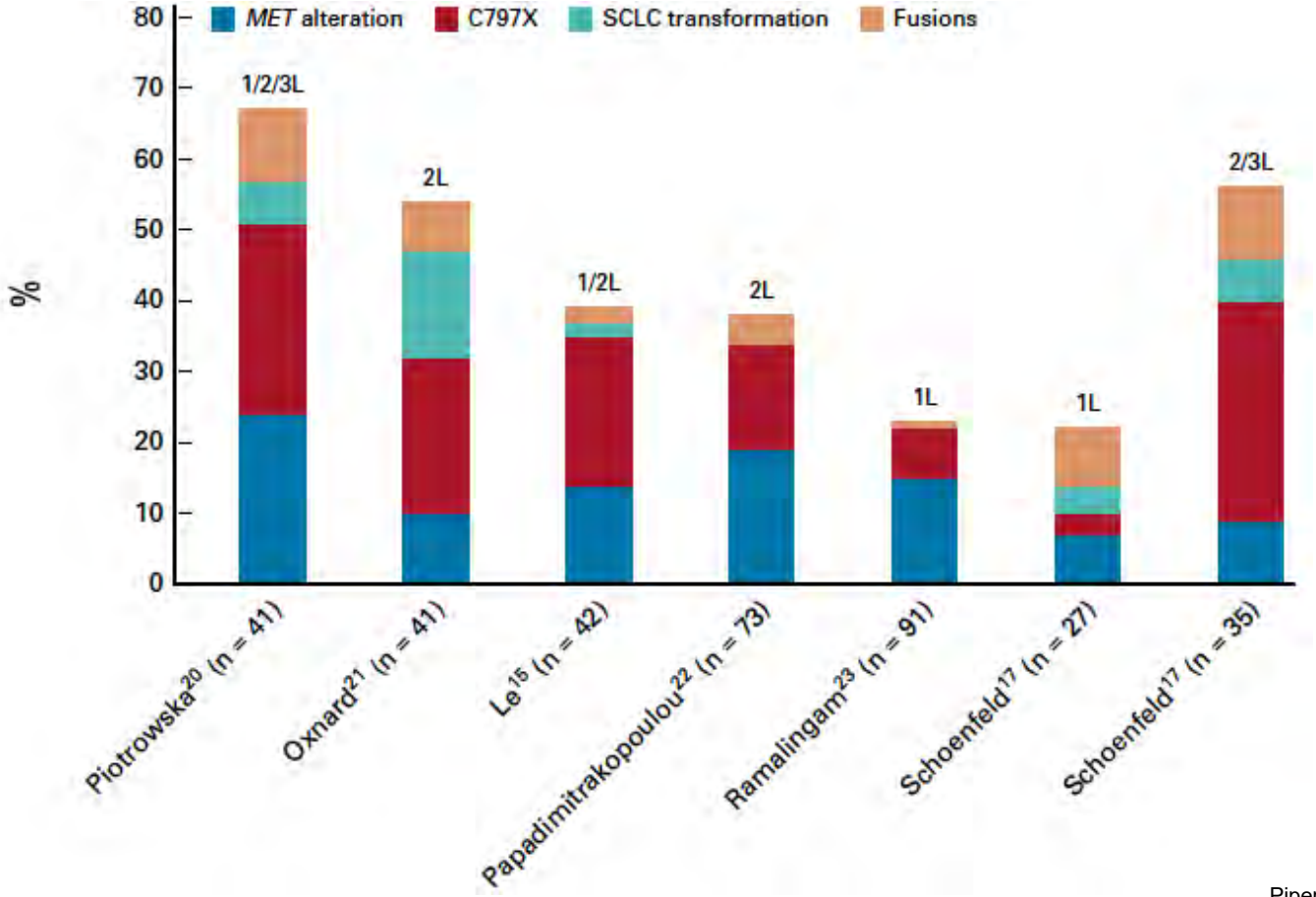
- No evidence of acquired EGFR T790M
- The most common resistance mechanisms were *MET* amplification and EGFR C797S mutation
  - Other mechanisms included *HER2* amplification, *PIK3CA* and *RAS* mutations



*Resistance mechanism reported may overlap with another; #Two patients had *de novo* T790M mutations at baseline of whom one acquired C797S at progression

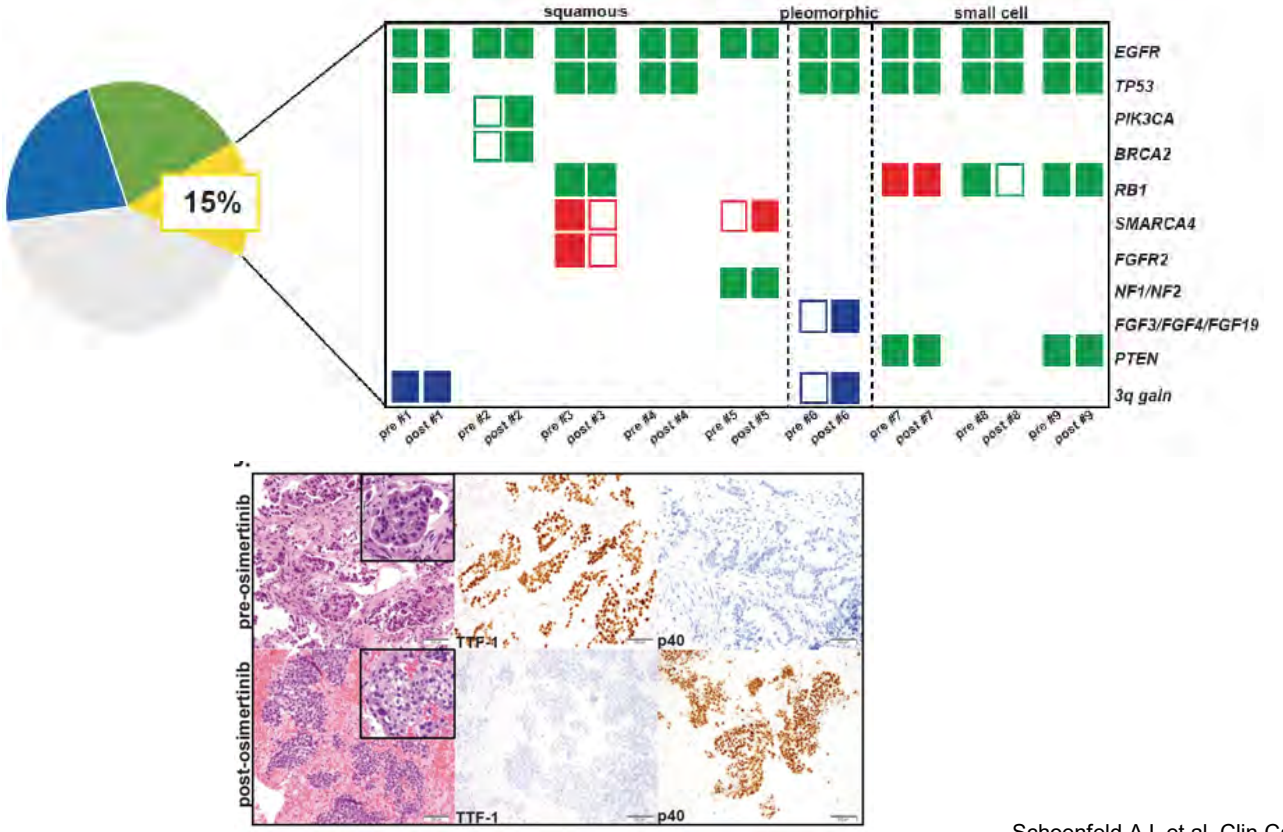
Ramalingam SS, et al. ESMO 2018

Osimertinib resistance series



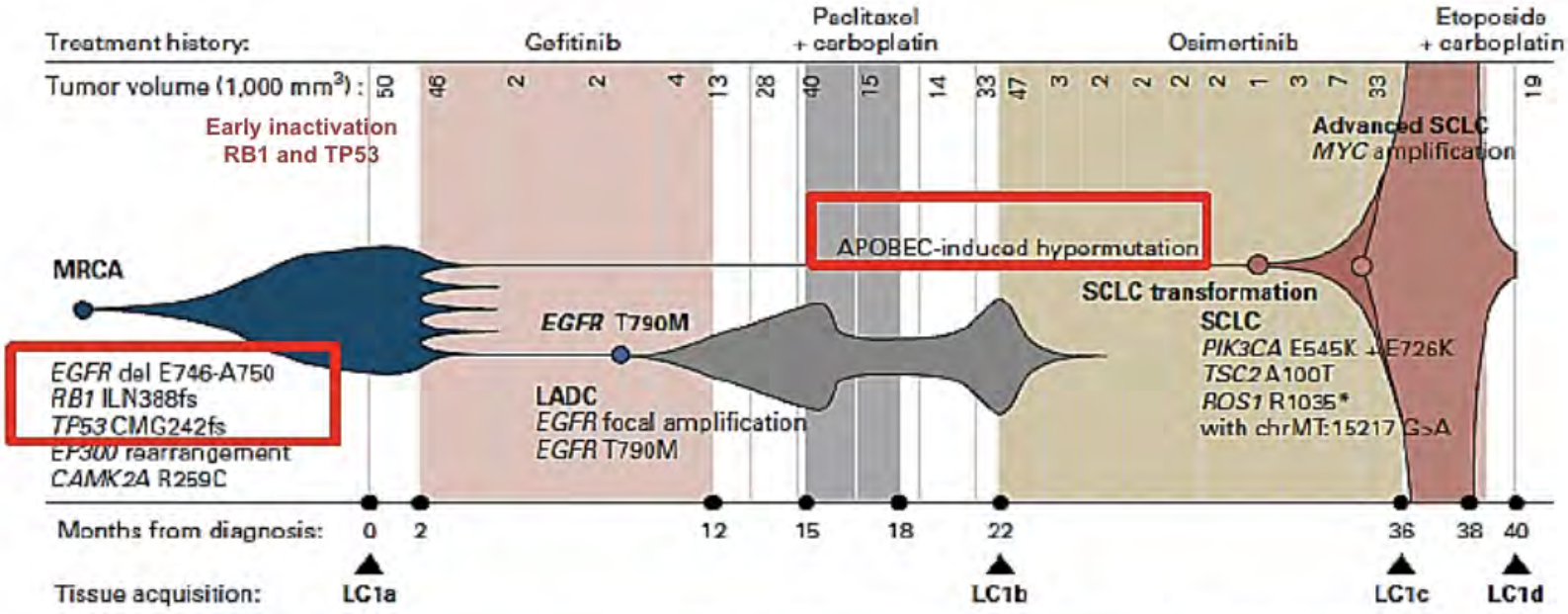
Piper-Vallillo AJ, et al. JCO 2020

The genomic profile of patients with histologic transformation subtype



Schoenfeld AJ, et al. Clin Cancer Res 2020

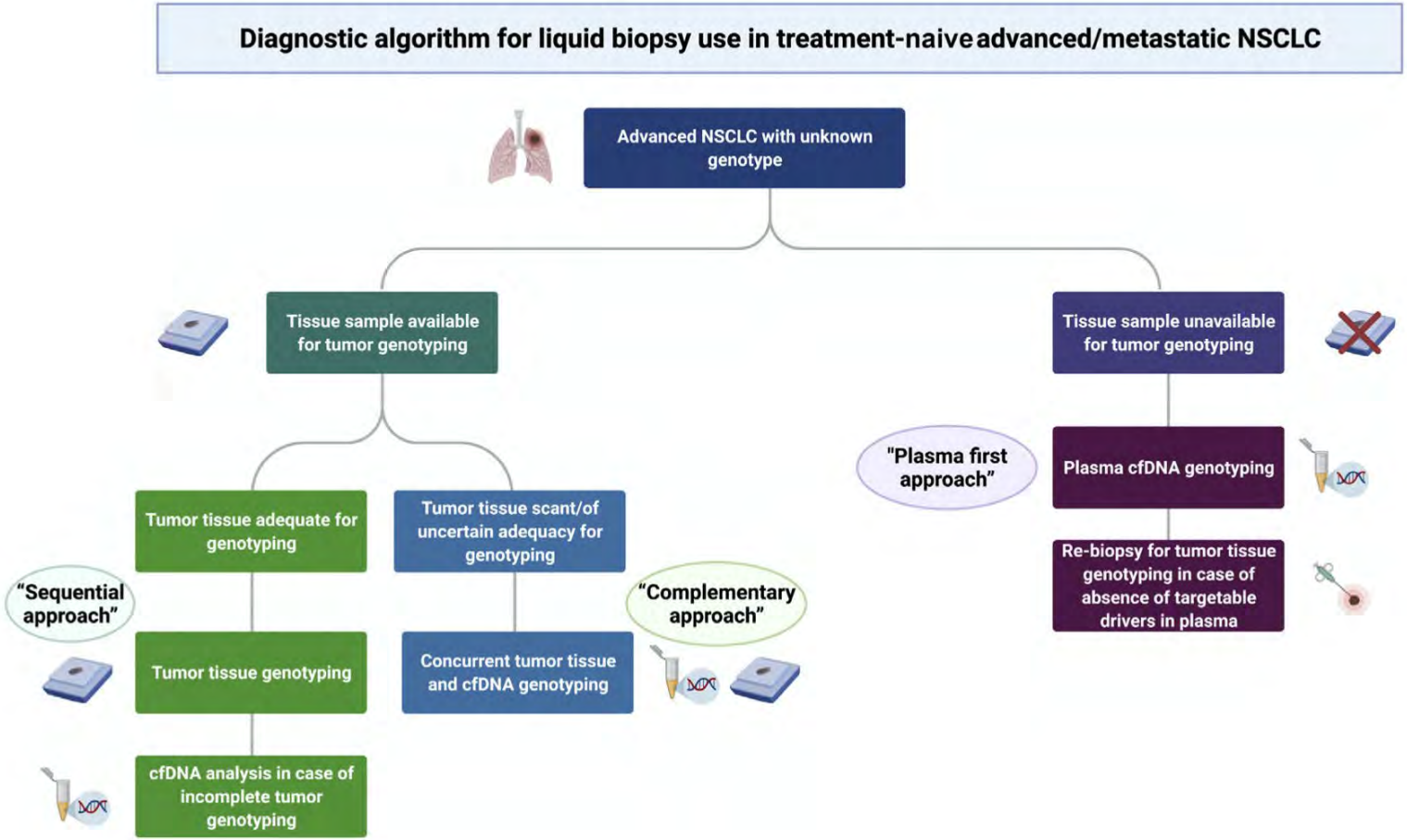
Early Clonal loss od Rb1 and Tp53 predict SCLC transformation



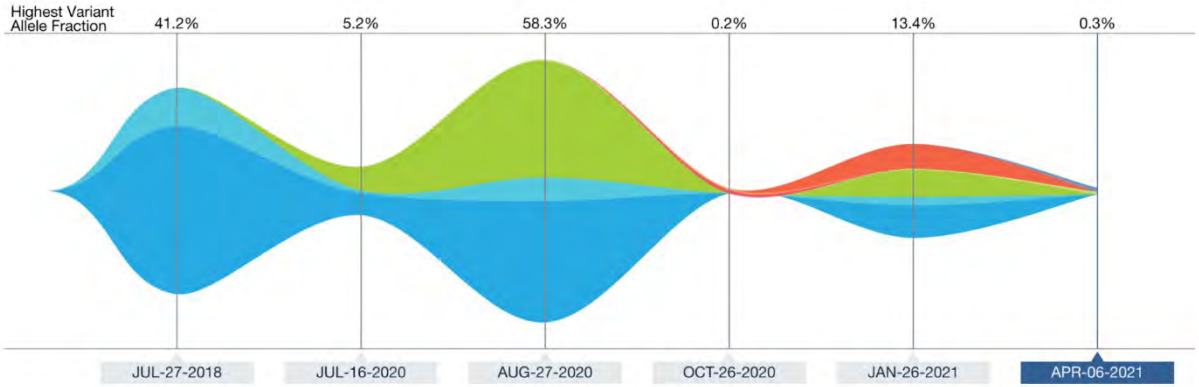
EGFR-mutant + inactivated Rb/p53 have 43 times greater risk of SCLC transformation (n=75)

Courtesy Andrés Cardona

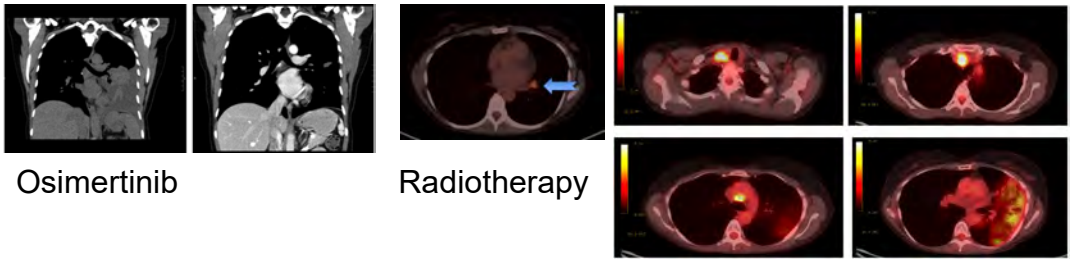
J.K. Lee,JCO 2017



Rolfo et al, JTO 2021 Oct;16(10):1647-1662



EGFR Amplification	ND	
EGFR C797S	ND	
EGFR E746_A750del (Exon 19 deletion)	ND	
NTRK2 L699L	ND	
EGFR N338N	ND	
TP53 S127F	ND	
EGFR T790M	ND	



Osimertinib

Radiotherapy

Erlotinib

PFS 24 months

PR 2 months

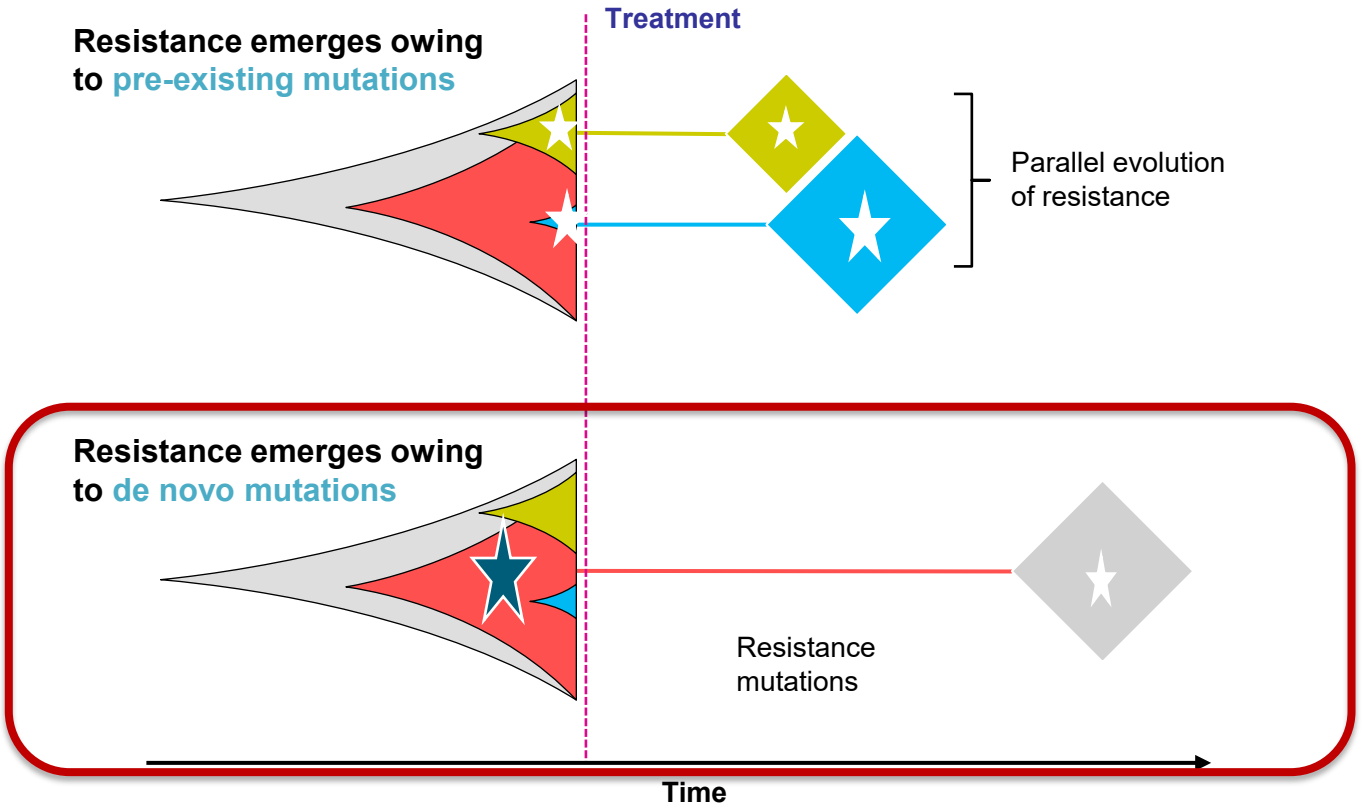
PFS 5 months

Multiple nodules

Chemotherapy

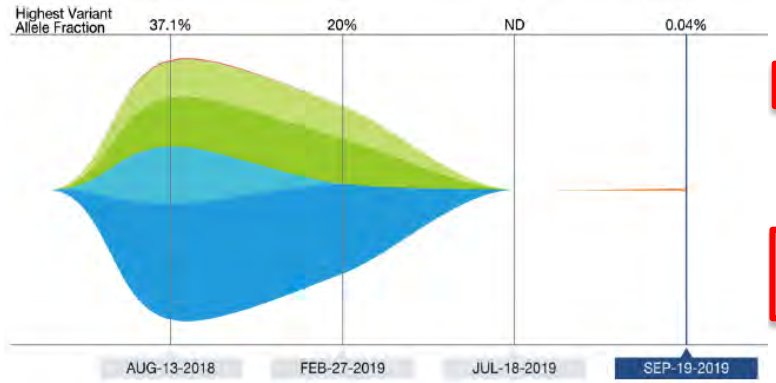
Complete Response

Clonal evolution of treatment resistance



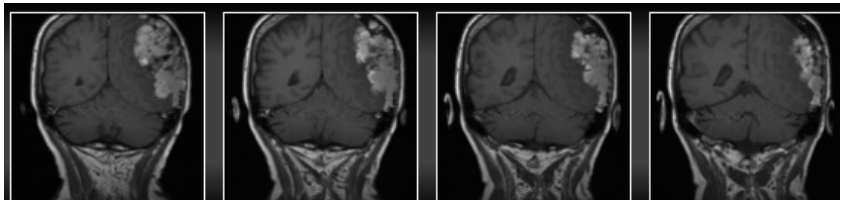
Turajlic S, et al. (2019) *Nat Rev Genet* 20:404-16.

Case #2: 71 year old NSCLC patient



	Aug 18	Feb 19	Jul 19	Sep 19
EGFR L858R	37.1%	20.0%	ND	ND
EGFR T790M	15.8%	ND	ND	ND
TP53 Splice Site SNV	12.5%	8.4%	ND	ND
BRCA1 D2N	8.9%	5.7%	ND	ND
TP53 V272M	0.1%	ND	ND	ND
EGFR Amplification	++	+	ND	ND
MET Amplification	++	+++	ND	ND
AR Amplification	+	ND	ND	ND

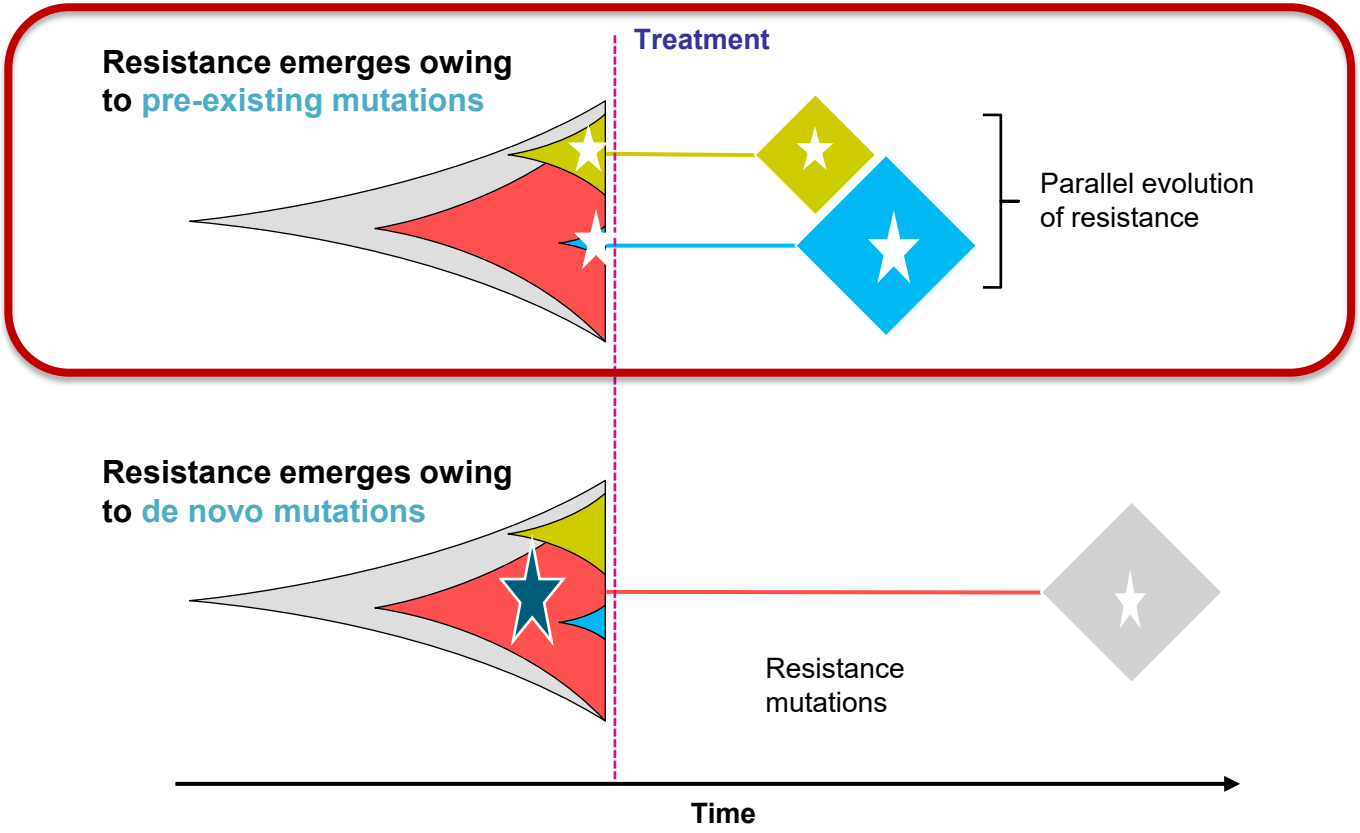
osimertinib start → PFS 6 months → osimertinib + crizotinib start



NSCLC: non-small cell lung cancer; PFS: progression-free survival.

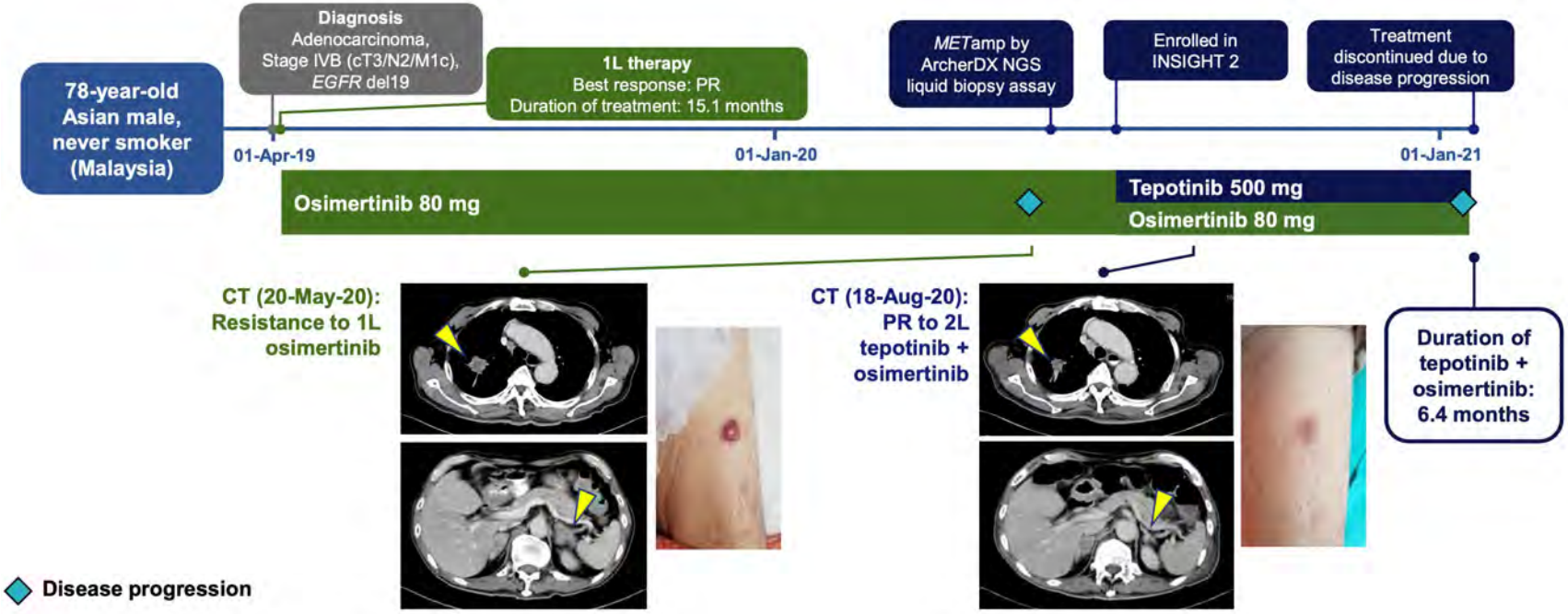
Case courtesy of Dr Rolfo, University of Maryland School of Medicine.

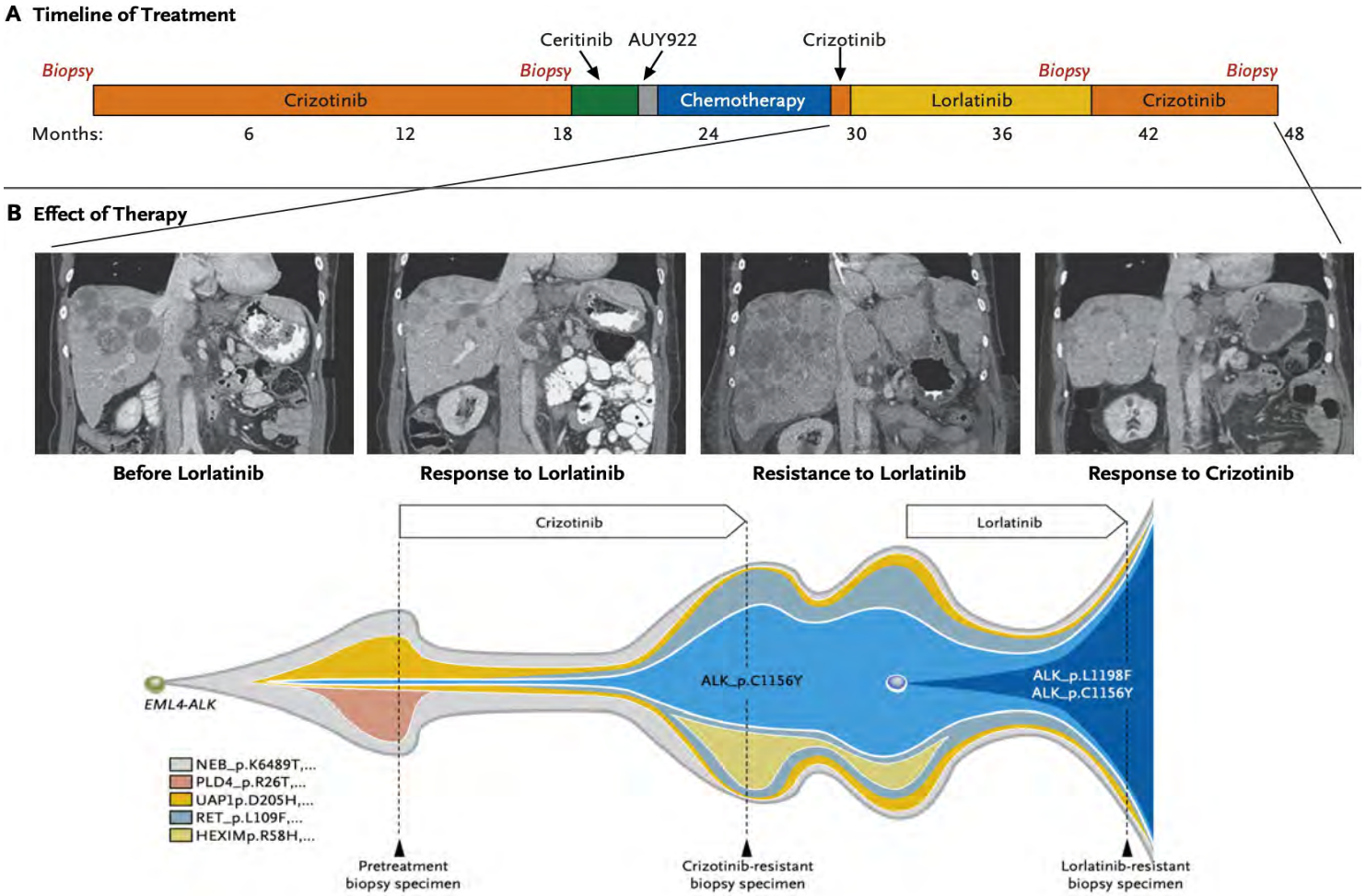
Clonal evolution of treatment resistance



Turajlic S, et al. (2019) *Nat Rev Genet* 20:404-16.

Prolonged duration of tepotinib + gefitinib after progression on erlotinib in a patient with *MET*amp





Shaw et al, NEJM , Dec 2015


NEW KIDS ON THE BLOCK

VISION
Phase 2 trial

Single-arm study of tepotinib in stage IIIB/IV NSCLC (all histologies) with *MET* ex14 skipping mutations (Cohort A)

First-, second- and third-line therapy patients included, unless prior anti-MET therapy was used

Patients with active brain metastases excluded

Tepotinib in NSCLC with *MET* exon 14 skipping mutations (*MET* ex14)

***MET* ex14 positive by:**	**Liquid biopsy** (n=48)	**Tissue biopsy** (n=51)
Best overall response by RECIST 1.1 (independent review committee), n (%)		
Complete response	0 (0)	0 (0)
Partial response	24 (50.0)	23 (45.1)
Stable disease	8 (16.7)	14 (27.5)
Progressive disease	7 (14.6)	8 (15.7)
Not evaluable	9 (18.8)	6 (11.8)
ORR* n (%) [95% CI]	24 (**50.0**) [35.2, 64.8]	23 (**45.1**) [31.1, 59.7]

*ORR = Complete response + partial response

CI: confidence interval;
NSCLC: non-small cell lung cancer; ORR: overall response rate.
Paik, P.K., et al. (2019) Slide presentation at ASCO 2019:abstract 9005.

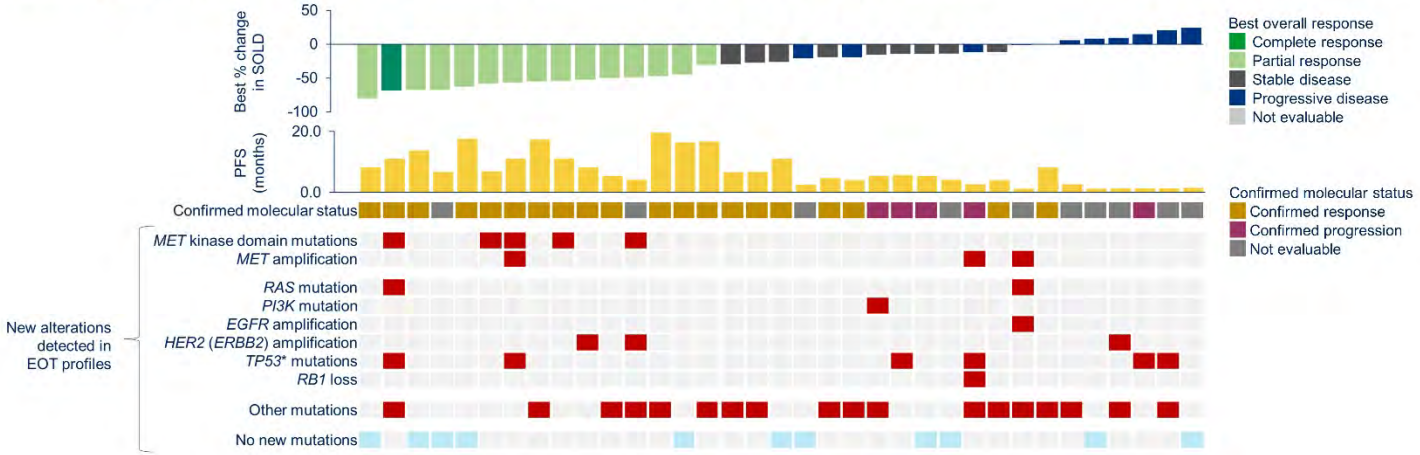
Courtesy G. Oxnard

@ChristianRolfo

*MET*ex14 ctDNA dynamics & resistance mechanisms detected in liquid biopsy (LBx)

End-of-treatment [16]

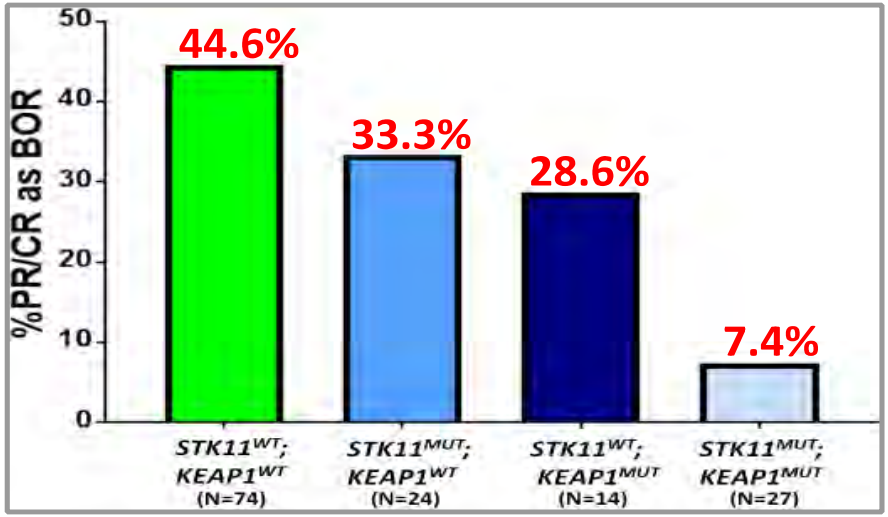
Emerging resistance mechanisms were detected in samples taken at the time of disease progression

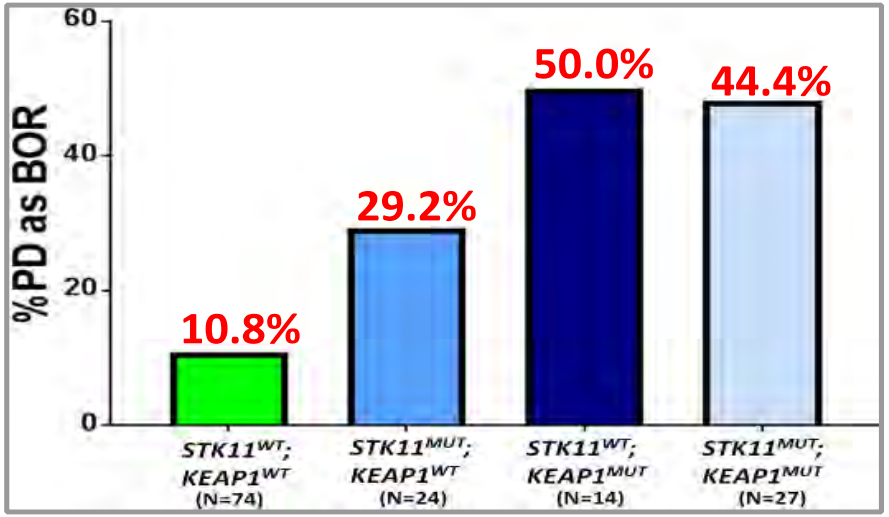


- New alterations detected included *MET* mutation/amplification (n=7/35), *TP53* mutation/*RB1* loss (n=6/35), *EGFR/HER2* amplification (n=4/35), and *RAS/PI3K* mutations (n=3/35)
  - No high frequency bypass pathway alterations were detected
- Previously reported resistance mechanisms to MET inhibitors include mutations and amplifications in *RAS* and *EGFR* genes[1–4]

All efficacy outcomes were investigator-assessed.
*All non-silent *TP53* mutations included.
1. Guo R, et al. *J Clin Oncol.* 2019;37(15):9006. 2. Awad MM, et al. *J Clin Oncol.* 2018;36(15):9069; 3. Salgia R, et al. *Can Treat Rev.* 2020;87:102022; 4. Hong L, et al. *Ther Adv Med Oncol.* 2021,13:1–16.

ORR with PCP in *STK11* and *KEAP1*-defined subgroups





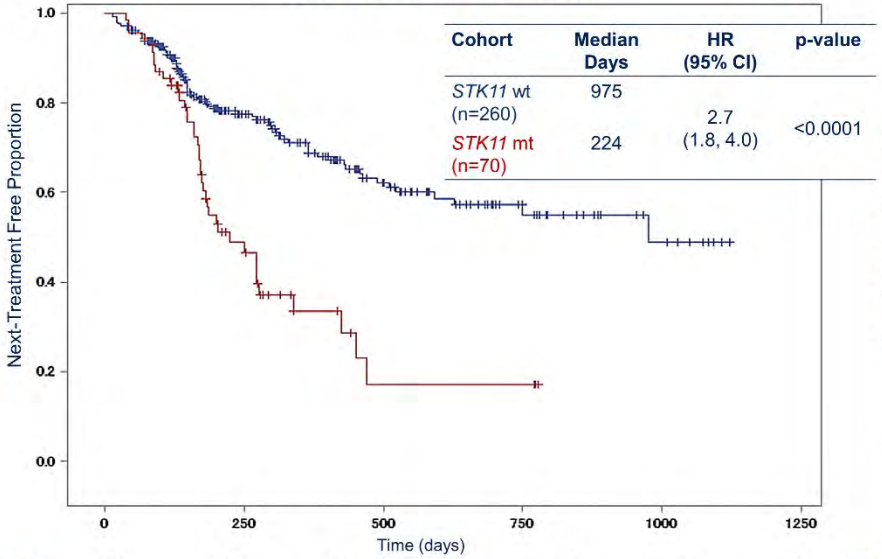
***STK11*^{MUT} and/or *KEAP1*^{MUT} PR/CR: 21.5%, SD: 38.5%, PD 40%**

- **26/34 (76.5%) patients with primary refractory disease had *STK11* and/or *KEAP1* mutations**

Skoulidis F, et al. WCLC 2019

Patients with *KRAS*^{G12C} and co-occurring *STK11* mutation have worse real-world outcomes with 1L ICI than those with *KRAS*^{G12C} and no *STK11* mutation

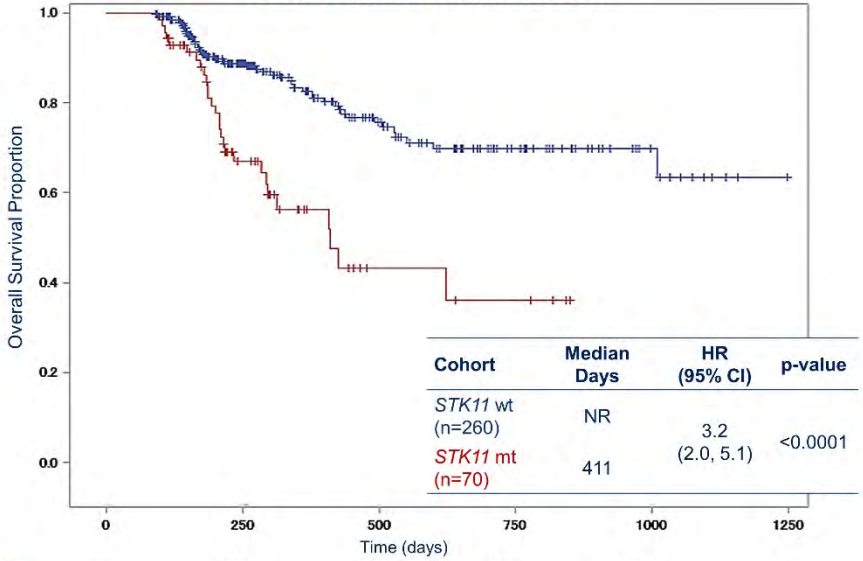
Time to Next Treatment



Cohort	Median Days	HR (95% CI)	p-value
STK11 wt (n=260)	975	2.7 (1.8, 4.0)	<0.0001
STK11 mt (n=70)	224		

STK11 wt	260	146	81	41	19	8	0
STK11 mt	70	30	8	3	0		

Real-World Overall Survival



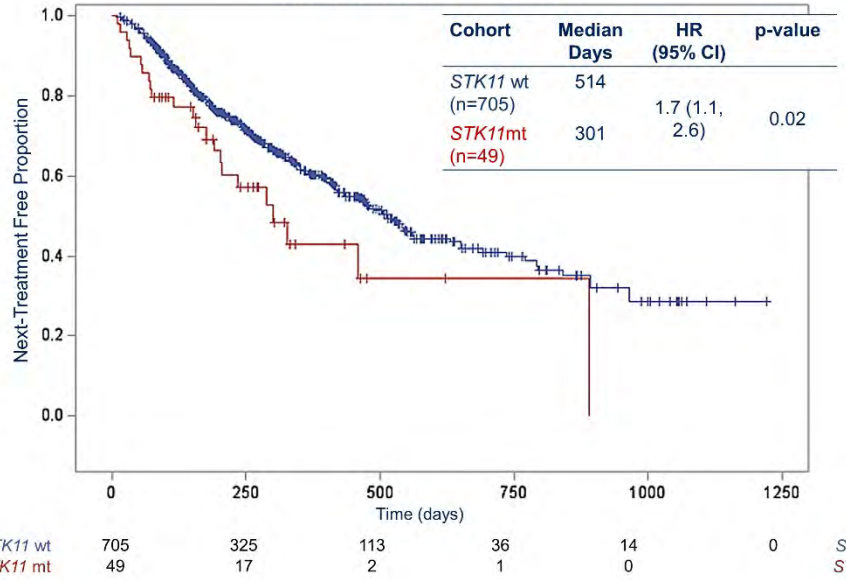
Cohort	Median Days	HR (95% CI)	p-value
STK11 wt (n=260)	NR	3.2 (2.0, 5.1)	<0.0001
STK11 mt (n=70)	411		

STK11 wt	260	154	71	32	11	0	
STK11 mt	70	32	6	4	0		

wt, wild type; mt, mutation; NR, not reached

Shorter TTNT, but no significant difference in RW-OS with *STK11* mutation in patients with *KRAS* wild-type lung adenocarcinoma

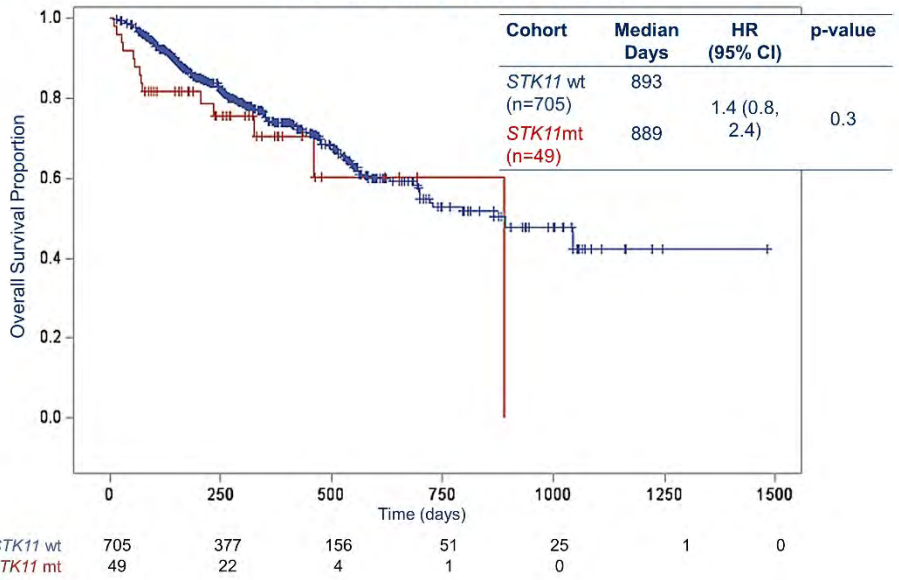
Time to Next Treatment



Cohort	Median Days	HR (95% CI)	p-value
STK11 wt (n=705)	514	1.7 (1.1, 2.6)	0.02
*STK11*mt (n=49)	301		

STK11 wt	705	325	113	36	14	0
STK11 mt	49	17	2	1	0	

Real-World Overall Survival

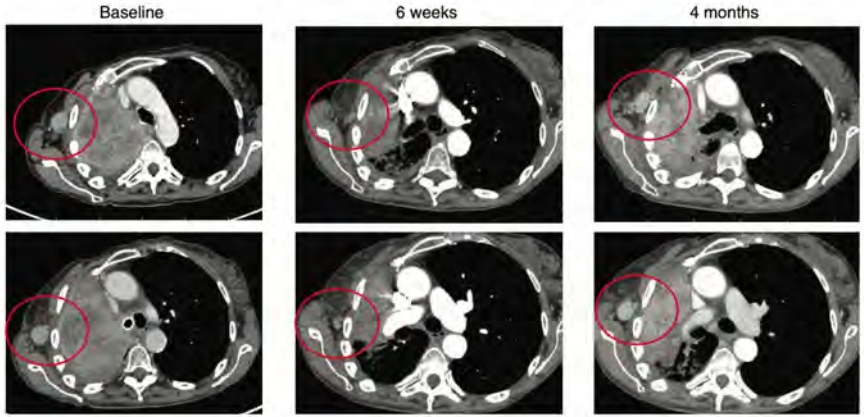


Cohort	Median Days	HR (95% CI)	p-value
STK11 wt (n=705)	893	1.4 (0.8, 2.4)	0.3
*STK11*mt (n=49)	889		

STK11 wt	705	377	156	51	25	1	0
STK11 mt	49	22	4	1	0		

wt, wild type; mt, mutation; NR, not reached

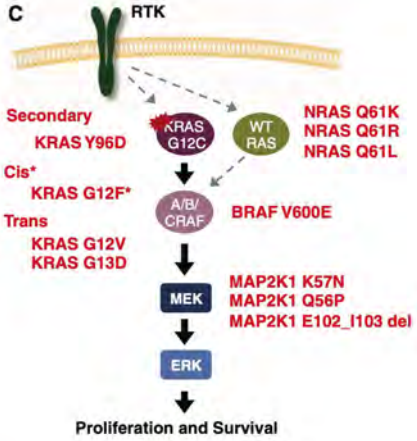
Acquired resistance to KRASG12C inhibitor MRTX849 (adagrasib)



B

	Tumor	cfDNA			
	Pre-MRTX849	Pre-MRTX849	Days post-MRTX849 discontinuation: 0	9	51
TP53 F338fs	36.8%	0.22%	8.8%	10.1%	14.3%
KRAS G12C	21.3%	0.12%	31.7%	47.1%	24.9%
KRAS G12V	-	-	-	-	0.09%
KRAS G13D	-	-	-	0.13%†	0.04%
KRAS Y96D	-	-	0.4%	0.2%	-
NRAS Q61L	-	-	-	0.2%	-
NRAS Q61R	-	-	-	-	0.02%
NRAS Q61K	-	-	0.6%	0.6%	0.9%
BRAF V600E	-	-	0.1%	0.1%	0.5%
MAP2K1 K57N	-	-	0.05%†	-	0.3%
MAP2K1 Q56P	-	-	-	-	0.1%
MAP2K1 E102_I103del	-	-	-	0.12%†	0.2%

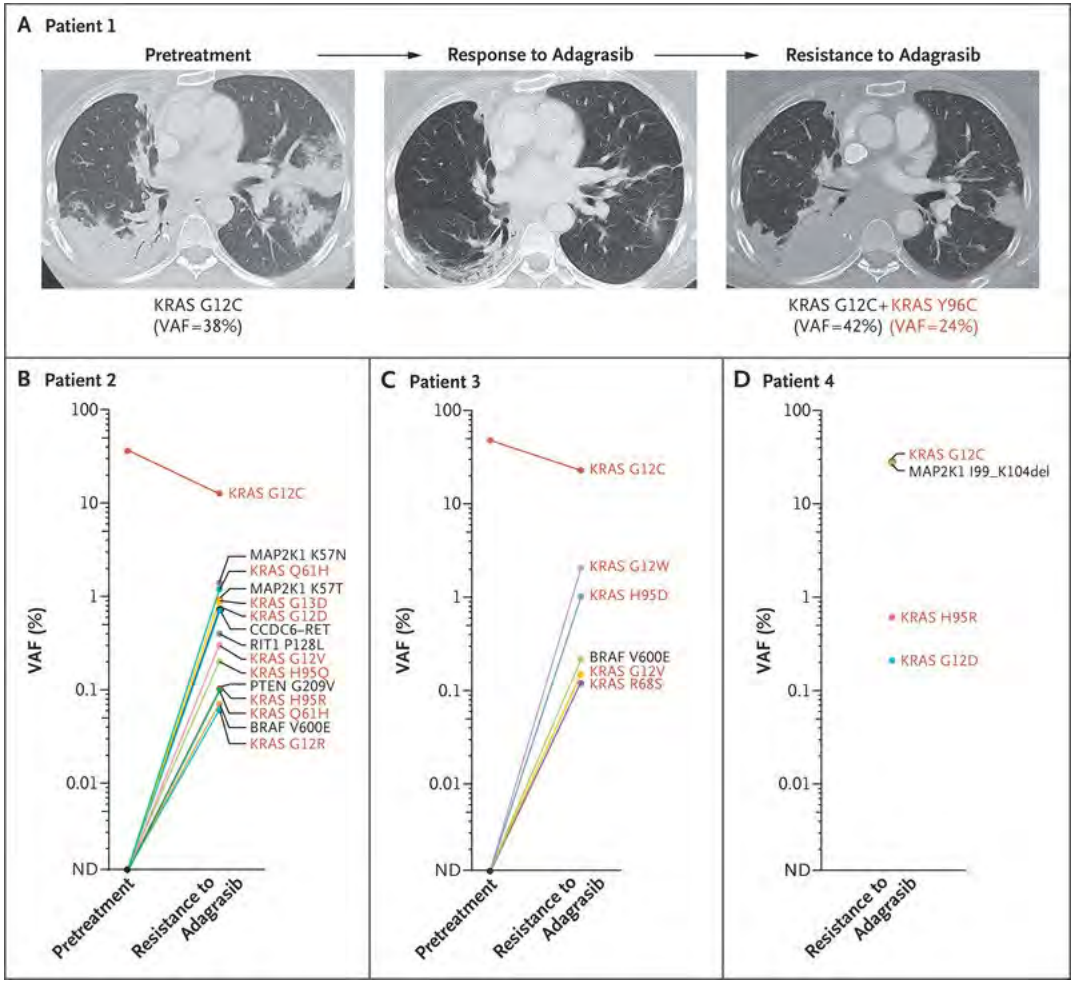
C



Patient with *KRAS*G12C NSCLC who developed polyclonal acquired resistance to MRTX849 with the emergence of 10 heterogeneous resistance alterations in serial cell-free DNA spanning **four genes (*KRAS, NRAS, BRAF, MAP2K1*),** all of which converge to reactivate RAS–MAPK signaling.

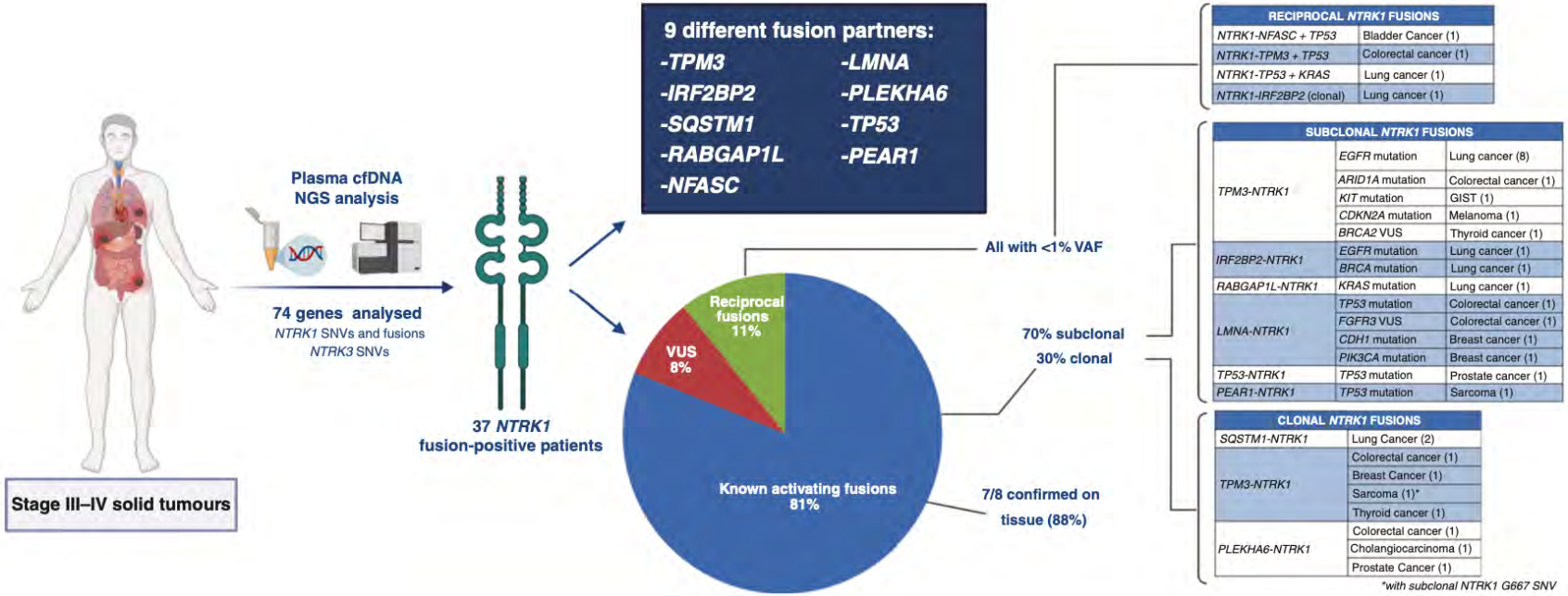
Variant allele fractions of mutations detected in the patient's serial plasma samples. †, indicates the mutations were detected by ddPCR but not by plasma next-generation sequencing

Tanaka et al, Cancer Discovery,Aug 2021



Awad et al, NEJM, 2021

NTRK1 Fusions identified by non-invasive plasma next-generation sequencing (NGS) across 9 cancer types



RECIPROCAL *NTRK1* FUSIONS	
NTRK1-NFASC + TP53	Bladder Cancer (1)
NTRK1-TPM3 + TP53	Colorectal cancer (1)
NTRK1-TP53 + KRAS	Lung cancer (1)
NTRK1-IRF2BP2 (clonal)	Lung cancer (1)

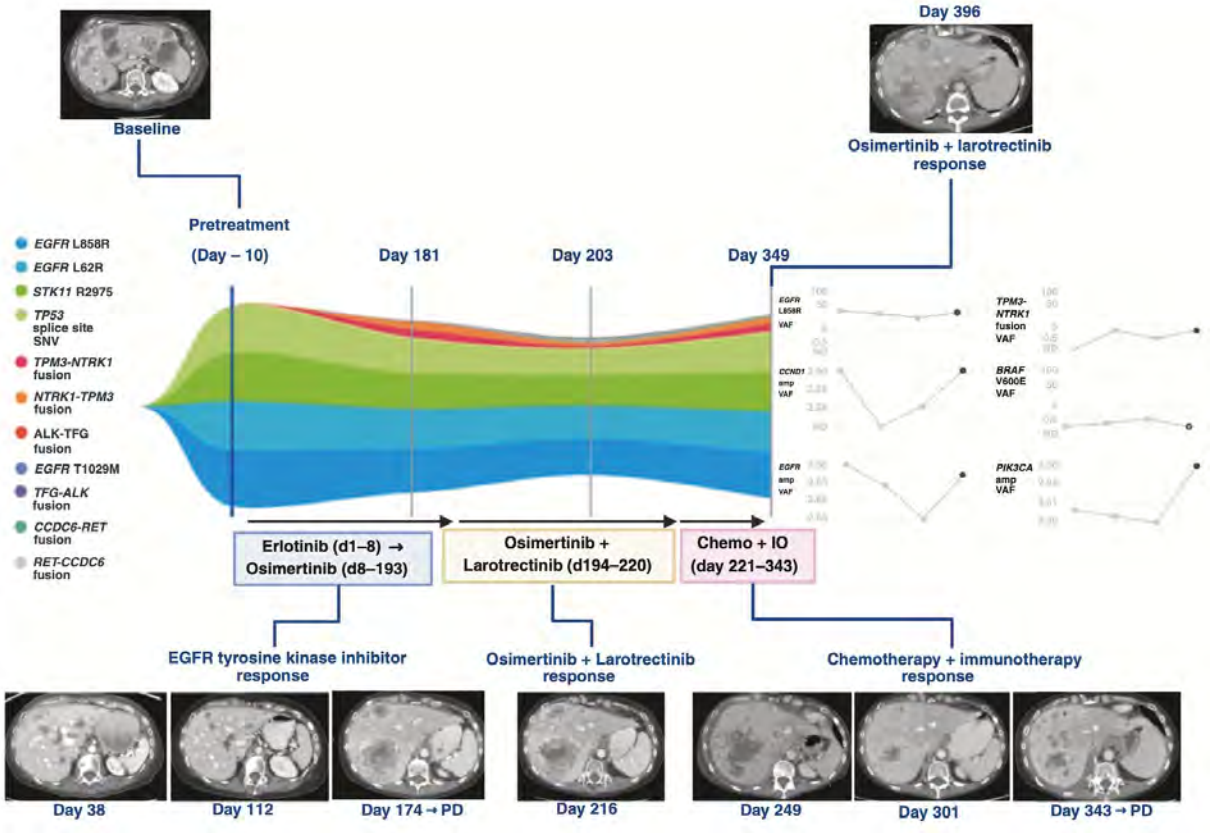
SUBCLONAL *NTRK1* FUSIONS		
TPM3-NTRK1	*EGFR* mutation	Lung cancer (8)
	ARID1A mutation	Colorectal cancer (1)
	KIT mutation	GIST (1)
	CDKN2A mutation	Melanoma (1)
	BRCA2 VUS	Thyroid cancer (1)
IRF2BP2-NTRK1	*EGFR* mutation	Lung cancer (1)
	BRCA mutation	Lung cancer (1)
RABGAP1L-NTRK1	*KRAS* mutation	Lung cancer (1)
LMNA-NTRK1	*TP53* mutation	Colorectal cancer (1)
	FGFR3 VUS	Colorectal cancer (1)
	CDH1 mutation	Breast cancer (1)
	PIK3CA mutation	Breast cancer (1)
TP53-NTRK1	*TP53* mutation	Prostate cancer (1)
PEAR1-NTRK1	*TP53* mutation	Sarcoma (1)

CLONAL *NTRK1* FUSIONS	
SQSTM1-NTRK1	Lung Cancer (2)
TPM3-NTRK1	Colorectal cancer (1)
	Breast Cancer (1)
	Sarcoma (1)*
	Thyroid cancer (1)
PLEKHA6-NTRK1	Colorectal cancer (1)
	Cholangiocarcinoma (1)
	Prostate Cancer (1)

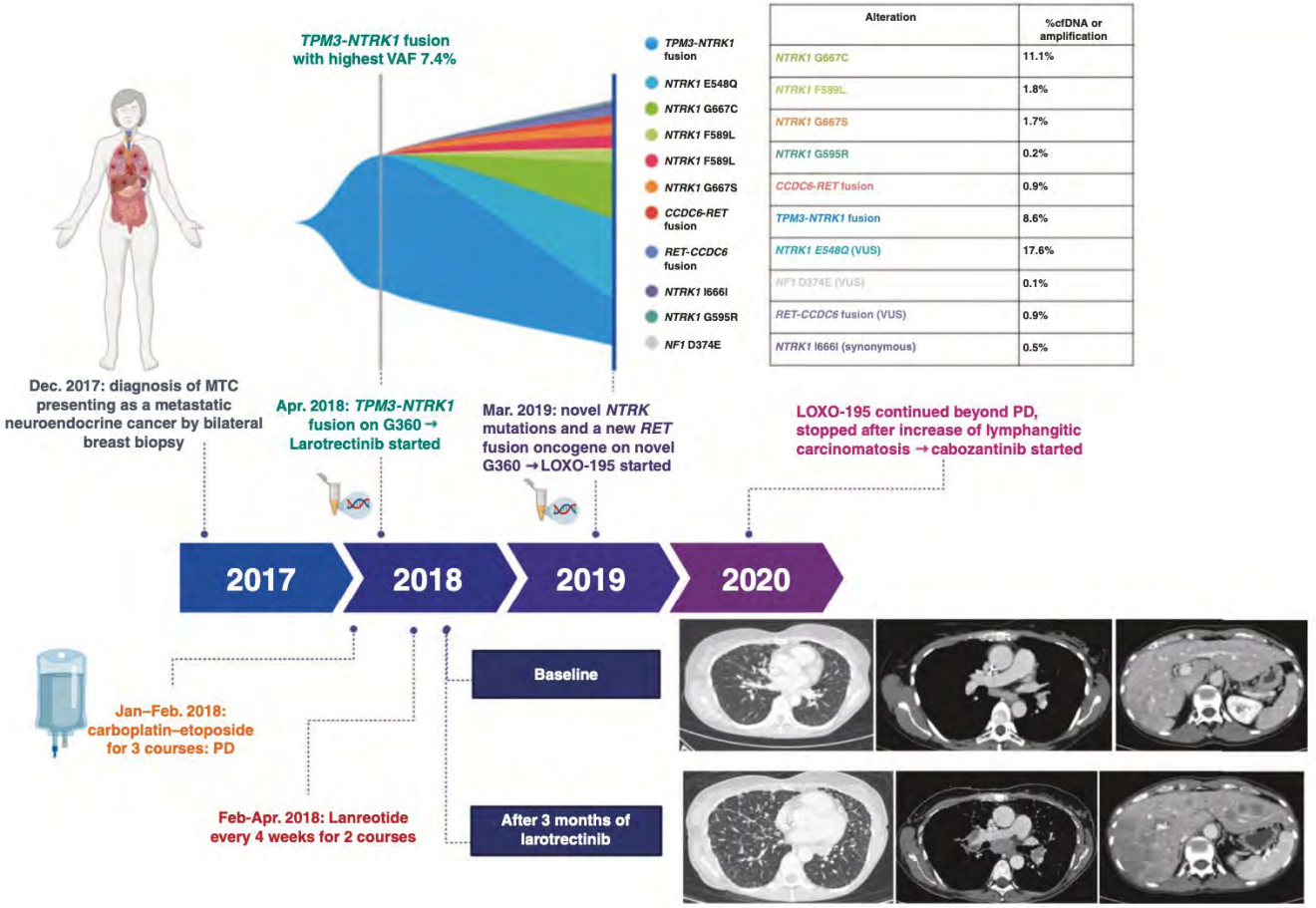
*with subclonal NTRK1 G667 SNV

Rolfo C et al, British Journal of Cancer, Sep 2021

NTRK in Lung Cancer as a mechanism of resistance of EGFR



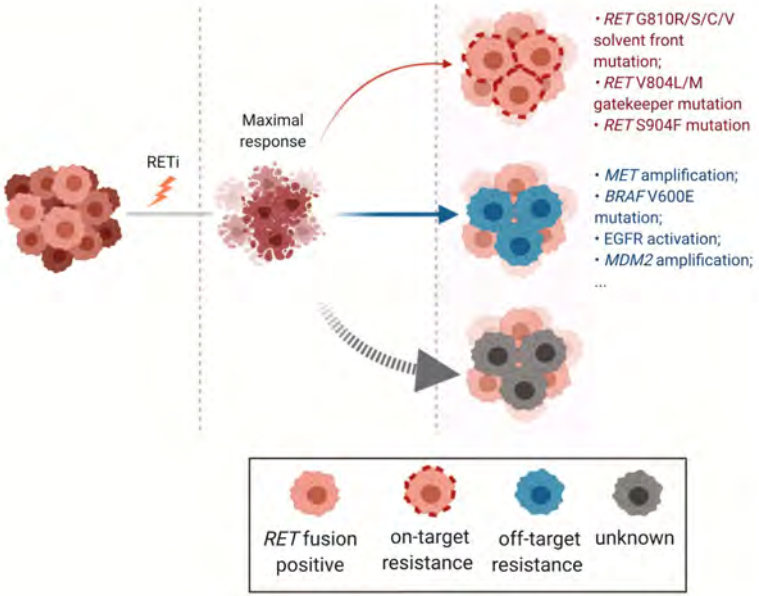
Rolfo C et al, British Journal of Cancer, Sep 2021



Alteration	%cfDNA or amplification
NTRK1 G667C	11.1%
NTRK1 F589L	1.8%
NTRK1 G667S	1.7%
NTRK1 G595R	0.2%
CCDC6-RET fusion	0.9%
TPM3-NTRK1 fusion	8.6%
NTRK1 E548Q (VUS)	17.6%
NF1 D374E (VUS)	0.1%
RET-CCDC6 fusion (VUS)	0.9%
NTRK1 I666I (synonymous)	0.5%

Rolfo C et al, British Journal of Cancer, Sep 2021

Resistance to Novel Selective RET Inhibitors in Lung Cancer.

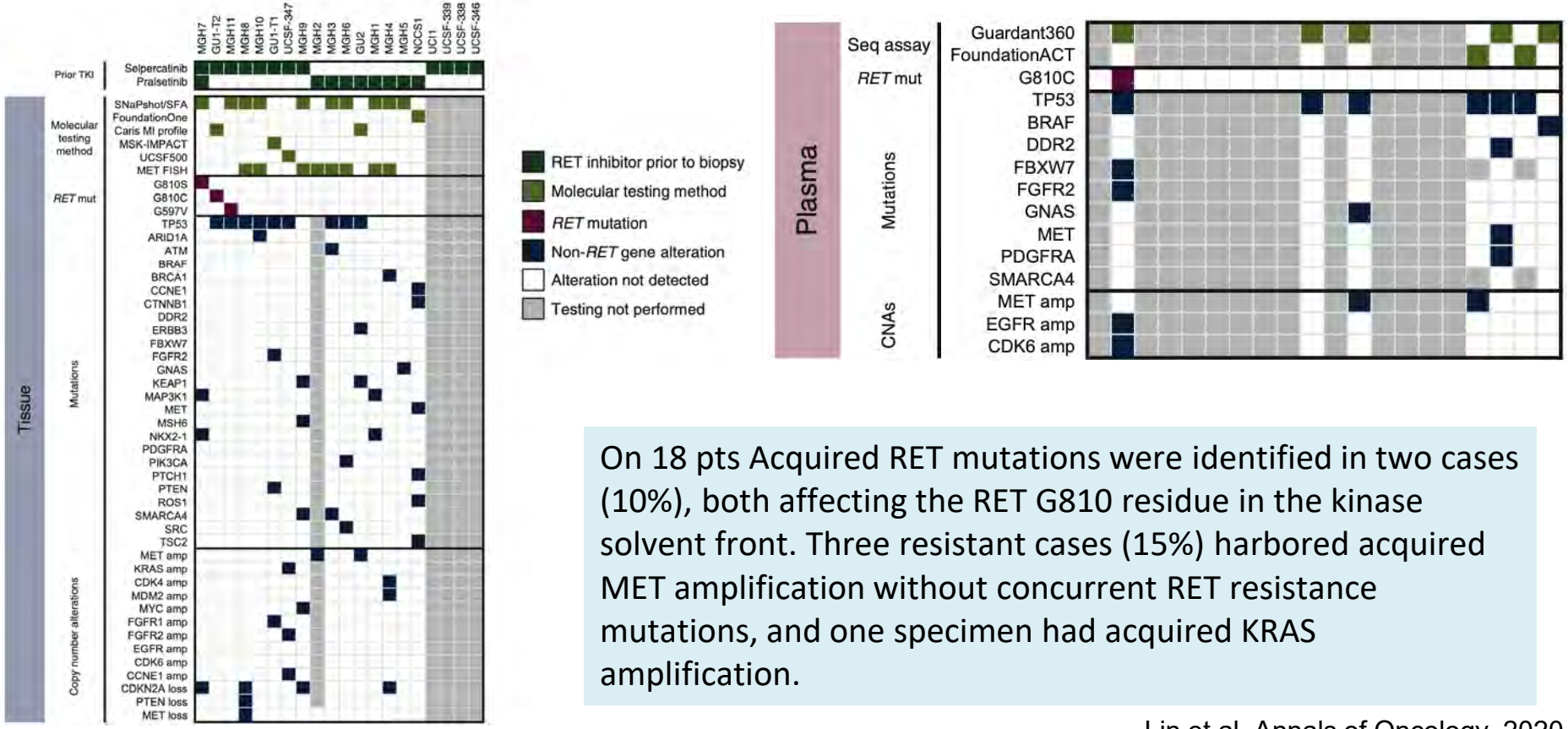


Mutation Status		Cabozantinib [112]	Vandetanib [112]	Lenvatinib [112]	Ponatinib [112]	Selpercatinib [109]	Pralsetinib [109]
Gatekeeper	V804M	4.26	5.83	5.42	0.0339	0.0559	0.0168
	V804L	3.22	6.10	10.60	0.43 [60]	0.0172	0.0018
Solvent front	G810A	0.22	2.76	0.11	0.008 [60]	-	-
	G810R	-	-	-	-	2.744	2.650
	G810S	1.05	5.47	0.67	-	0.8802	0.3906
	G810C	-	-	-	-	1.227	0.6417
Other	S904F	-	0.908 [98]	-	-	-	-
	Y806C	-	0.933 [113]	-	-	0.1744	0.2958
	Y806N	4.76	5.86	1.93	-	0.1498	0.2925
	V738A	1.20	1.05	2.35	-	0.2388	0.1775

Lu a &Zhou , Cancer Treatment Reviews 96 (2021) 102153

Fancelli et al, Cancers 2021, *13*, 1091

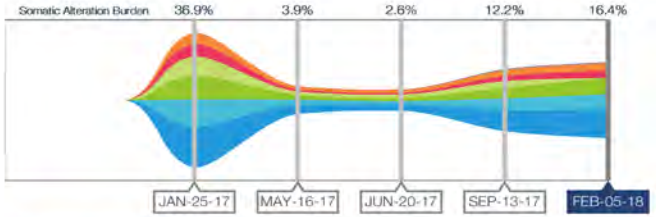
RET fusions



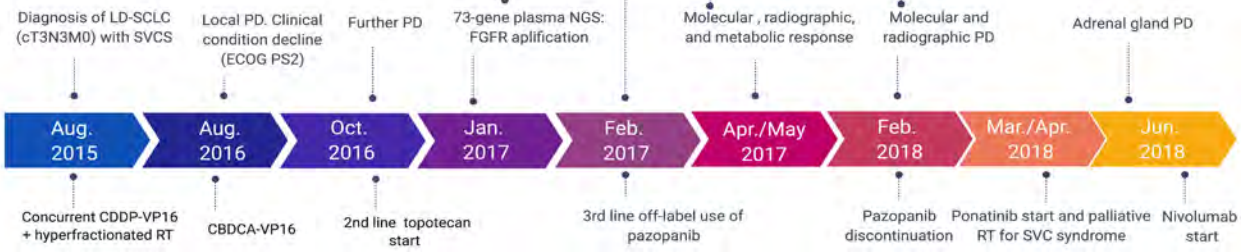
On 18 pts Acquired RET mutations were identified in two cases (10%), both affecting the RET G810 residue in the kinase solvent front. Three resistant cases (15%) harbored acquired MET amplification without concurrent RET resistance mutations, and one specimen had acquired KRAS amplification.

Lin et al, Annals of Oncology, 2020

Thinking outside the box



Genetic alterations	% cfDNA or amplification				
TP53 C176F	36.9%	3.9%	2.6%	12.2%	16.4%
RB1 R763*	22.7%	2.2%	1.3%	6.0%	7.7%
CDKN2A A57S	19.0%	2.0%	1.2%	5.3%	8.2%
PTEN S287*	8.6%	0.6%	0.4%	2.0%	2.9%
MET D859Y	14.7%	1.1%	1.1%	2.9%	ND
MEF Q714E	7.1%	1.1%	0.9%	2.7%	3.2%
AR S29R	-	-	0.1%	-	-
NOTCH1	-	-	-	0.2%	ND
FGFR1 AMP	+++	ND	ND	++	++
MYC AMP	++	ND	ND	ND	ND
RAF1 AMP	++	ND	ND	ND	+
BRAF AMP	+	ND	ND	ND	ND
CDK6 AMP	ND	ND	ND	ND	+



Russo A (Rolfo C). JCO Precis Oncol 2019

Liquid Biopsy in Personalize Treatment

- cfDNA is a good tool fo all the patient´s journey
- Make sure you use a validated platform, comprehensive
- Report every acencdotical case!
- Test your patients! At least to know the ones will not response to Immunotherapy


Don't rush Marty! Still there are people who is not testing!
So Doc ?? Is the future of liquid biopsy really cool?
Exosomes, PLTs, CTS, multomics, all over the place!!
I can't wait to be there!!

Thanks

ISLB
INTERNATIONAL SOCIETY
OF LIQUID BIOPSY

@isliquidbiopsy

Thanks

Christian.Rolfo@mssm.edu

@ChristianRolfo