UNIVERSITY of MARYLAND
SCHOOL OF MEDICINE

Liquid Biopsy in Non-Small Cell Lung Cancer: From Target to Immunotherapy

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UNIVERSITY of MARYLAND
MARLENE AND STEWART GREENEBAUM
COMPREHENSIVE CANCER CENTER

NCI Comprehensive Cancer Center
A Cancer Center Designated by the National Cancer Institute

DISCLOSURE INFORMATION

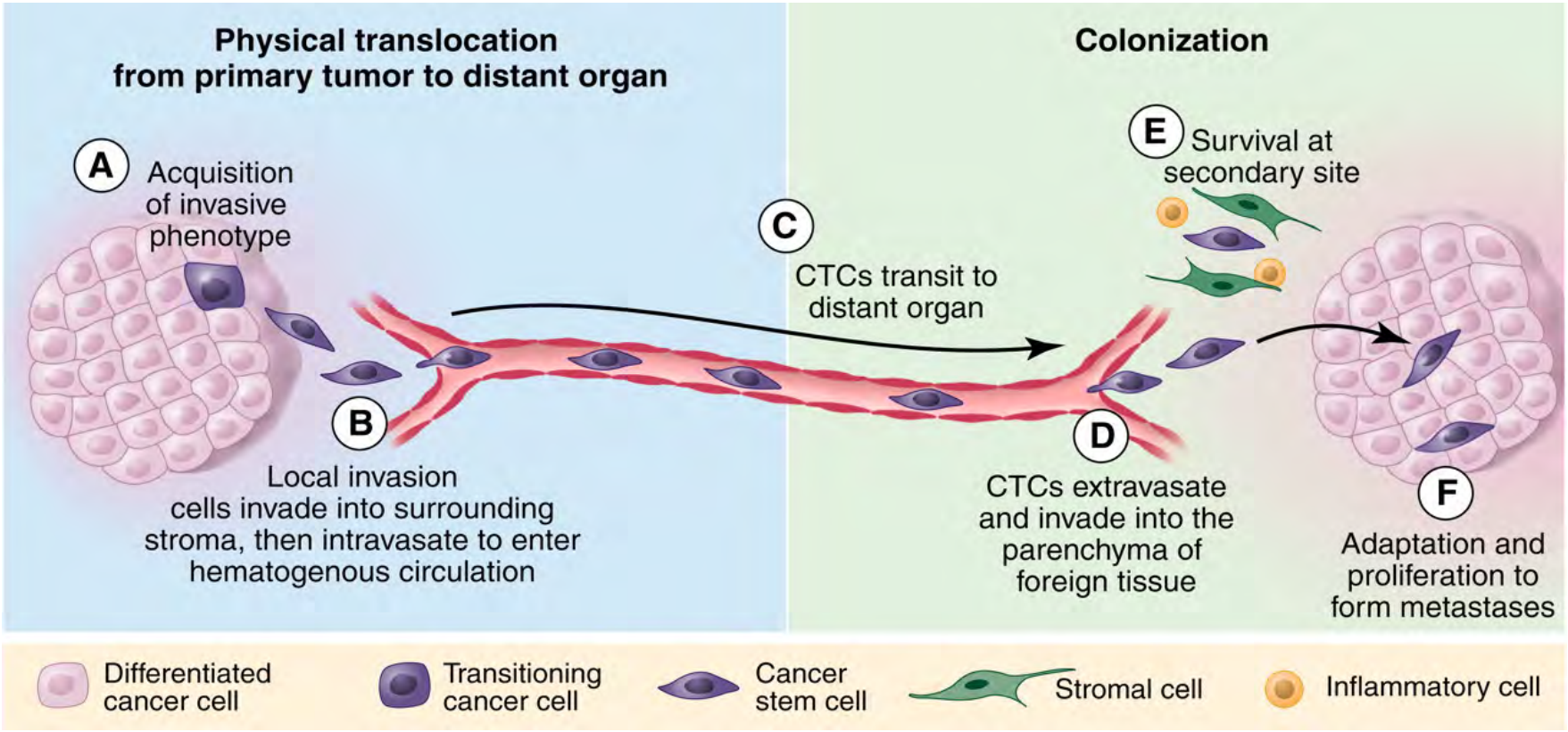
- **Personal financial interests**
  Speaker bureau: MSD, Novartis, GuardantHealth; Scientific advisor: Mylan

- **Institutional financial interests**
  Research grant at Antwerp University Hospital, Belgium: Novartis, Sanofi

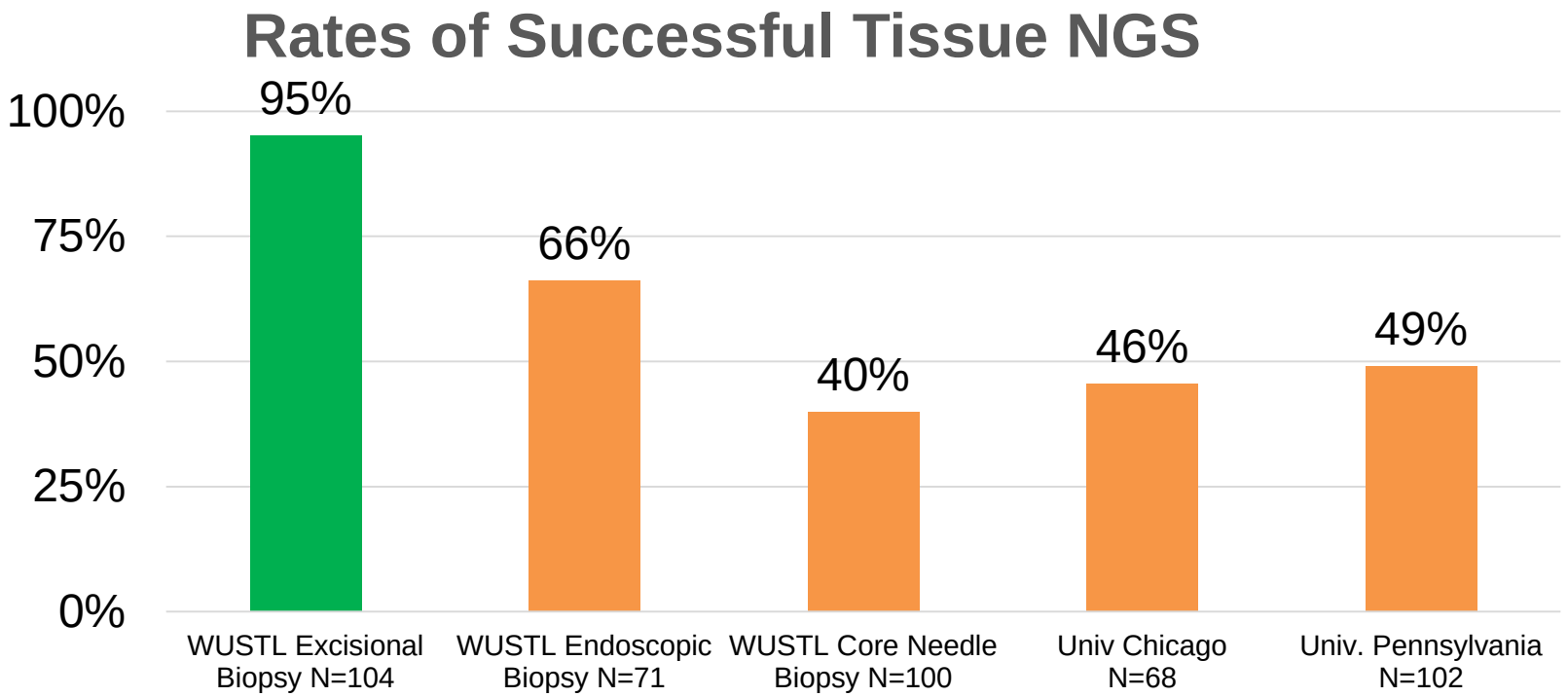
- **Non-financial interests**: Oncompass Steering scientific committee;
  OncoDNA: Research collaboration no remunerated for Exosomes (2017)

- **Leadership roles:**
  Educational Committee Member: IALSC - Vice President : ISLB (International Society of Liquid Biopsy) -
  Educational Chair: OLA Oncology Latin American Association - Faculty for ASCO International
  Scientific Committee Member at ESO (European School of Oncology).

Beginning of Concept of Liquid Biopsy

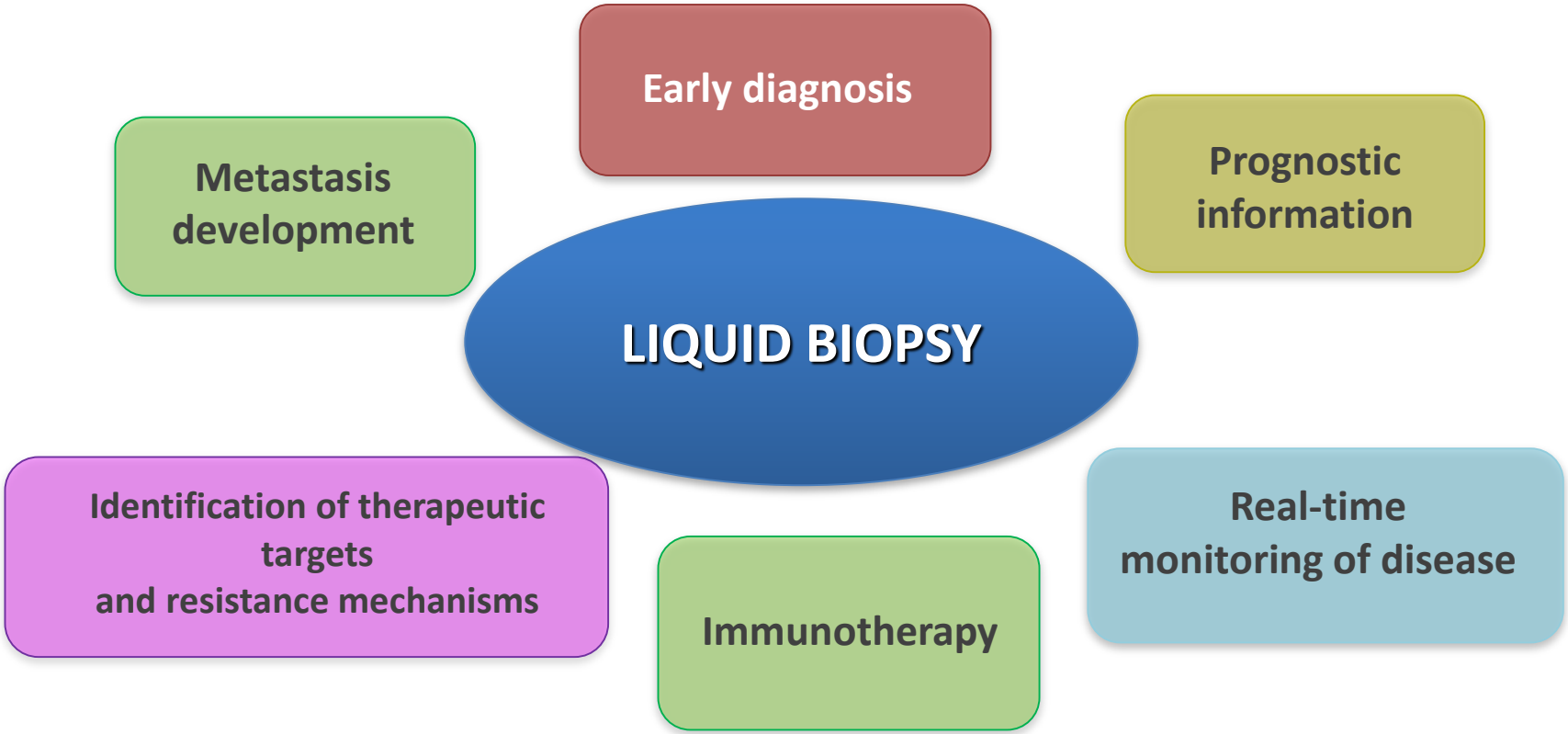


In **NSCLC Tissue is still an Issue…**
…**Insufficient for Genotyping**

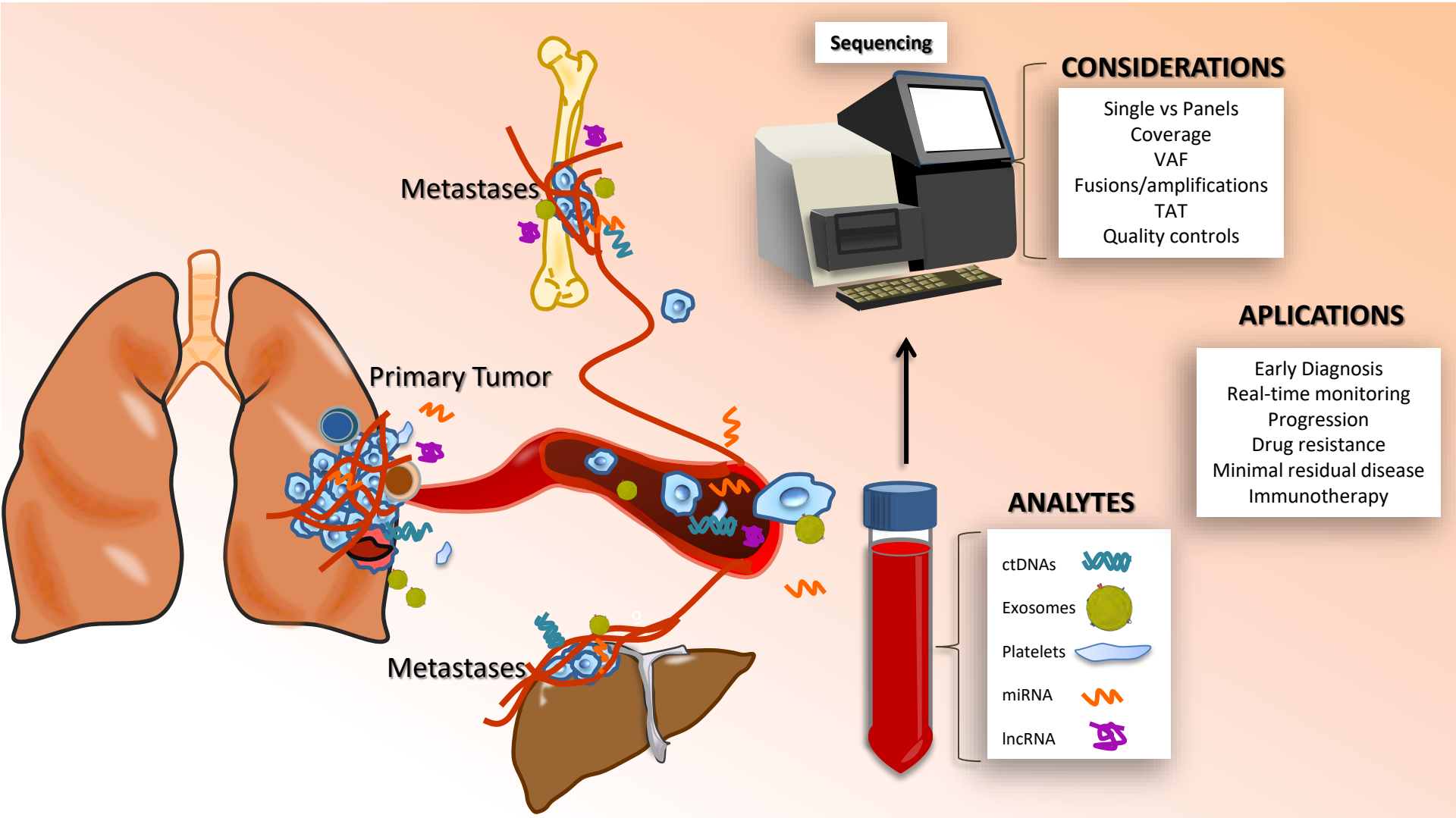


- [1]Hagemann (Govindan) et al. 2015 *Cancer*
- [2]Villaflor (Salgia) et al. 2016 *Oncotarget*
- [3]Thompson (Carpenter) et al. 2016 *Clin Canc Res*

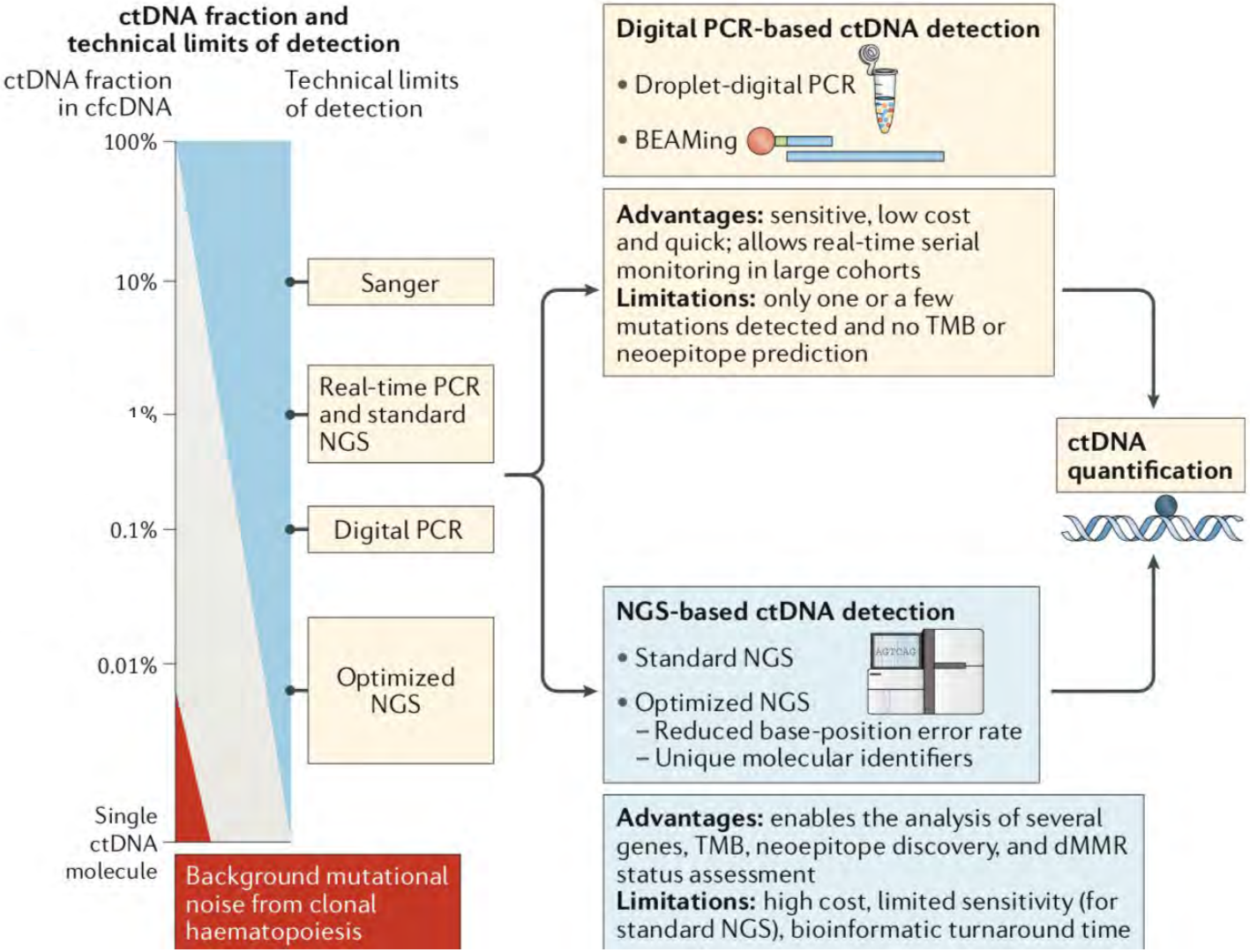
Liquid Biopsy: clinical application



Rolfo, Castiglia, Russo et al. Biochim Biophys Acta. 2014 Dec;1846(2):539-46



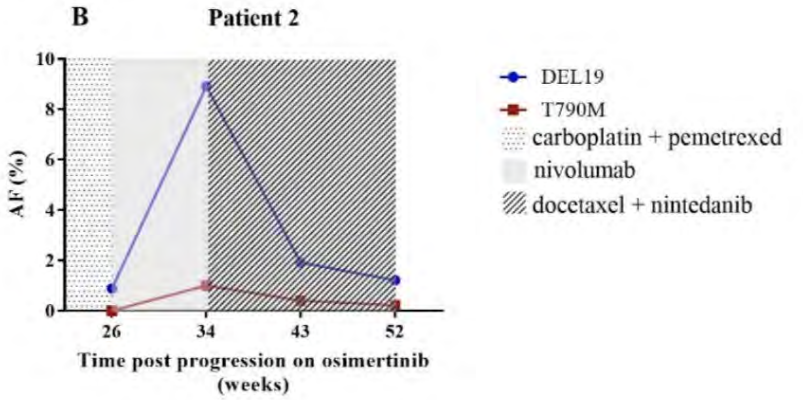
Scilla & Rolfo, Curr Treat Options in Oncology, in press 2019



Cabel et al, Nature Rev Clin Onc, Oct 2018

A Multicenter Study to Assess *EGFR* Mutational Status in Plasma: Focus on an Optimized Workflow for Liquid Biopsy in a Clinical Setting

Laure Sorber

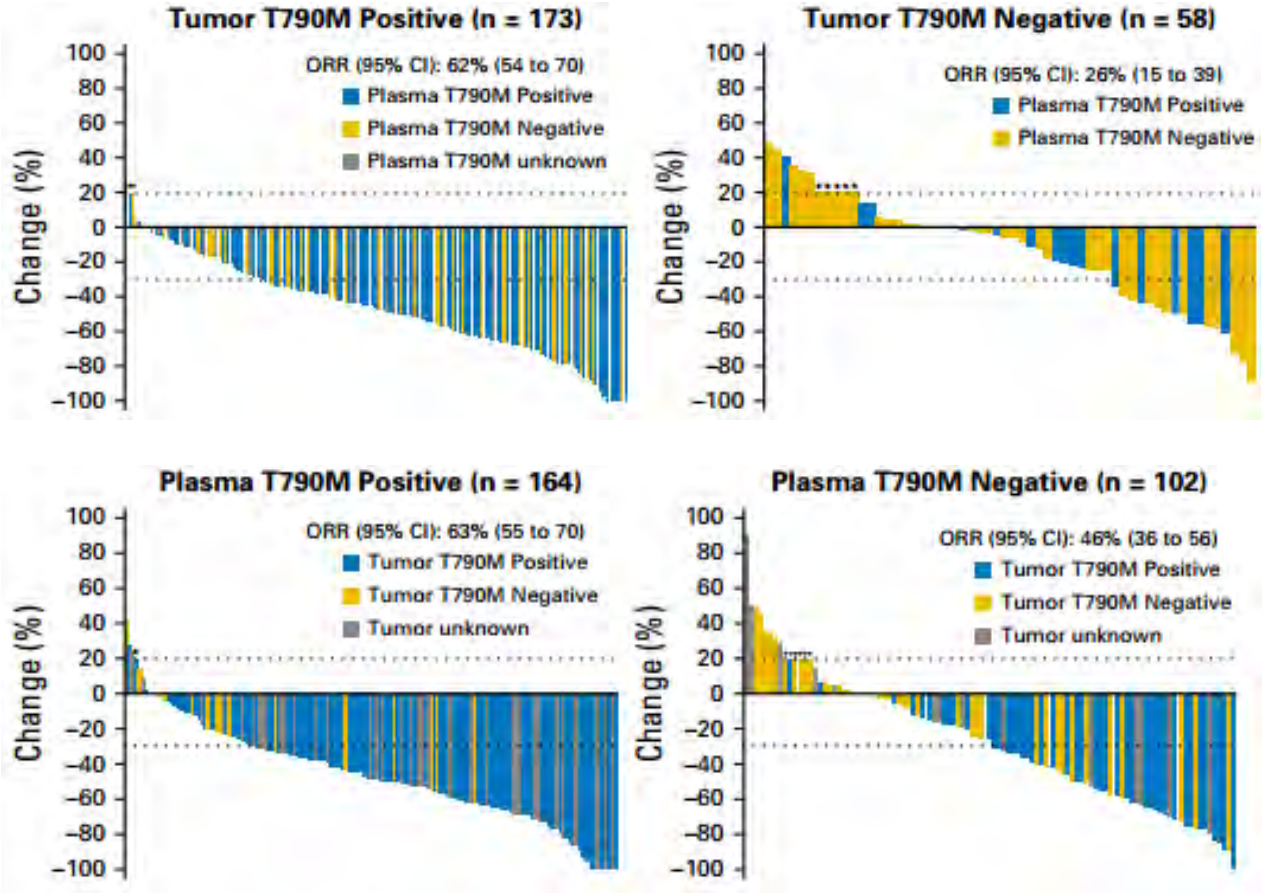


549 plasma samples from 234 non-small cell lung cancer (NSCLC) patients were collected. Epidermal Growth Factor Receptor (*EGFR)* circulating cell-free tumor DNA (ctDNA) mutational analysis was performed using digital droplet PCR (ddPCR).

- Longer transit time increased the risk of hemolysis
- Low temperatures were shown to have a negative effect.
- Metastatic sites were found to be strongly associated with ctDNA detection ($p < 0.001$), as well as allele frequency ($p = 0.034$).
- Activating mutations were detected in a higher concentration
- and allele frequency compared to the T790M mutation ($p = 0.003$, and $p = 0.002$, respectively)

Sorber (Rolfo & Pauwels) et al, *Cancers* 2018, *10*, 290

RR to Osimerinib according to T790M in plasma or tumor tissue



Tumor tissue
ORR: 62% vs 26%

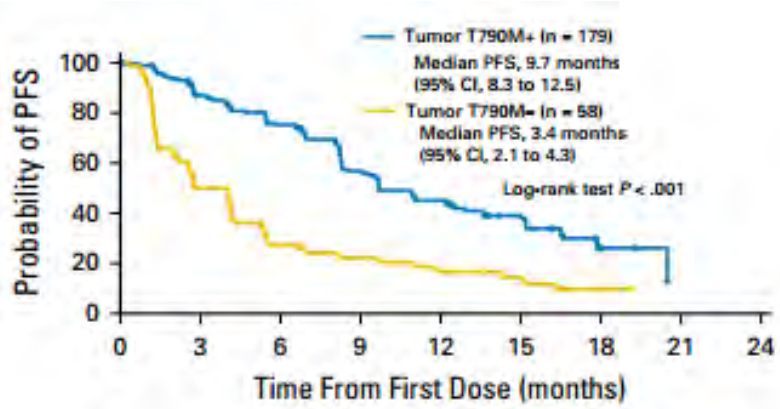
Plasma
ORR: 63% vs 46%

Tissue vs Plasma
ORR (T790M+): 62% vs 63% / ORR (T790M-): 26% vs 46%

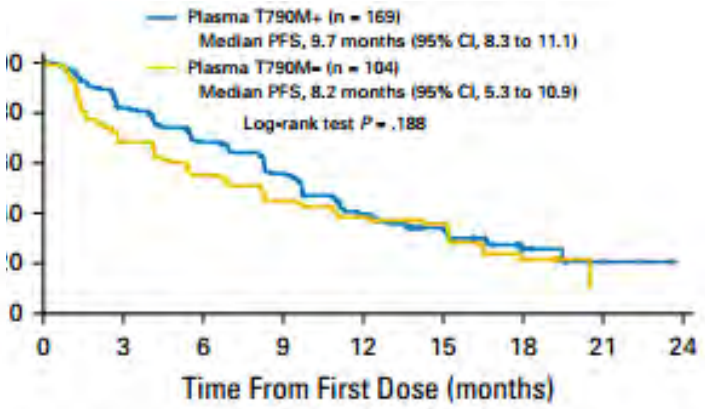
Oxnard, JCO 2016

PFS to Osimerinib according to T790M in plasma or tumor tissue

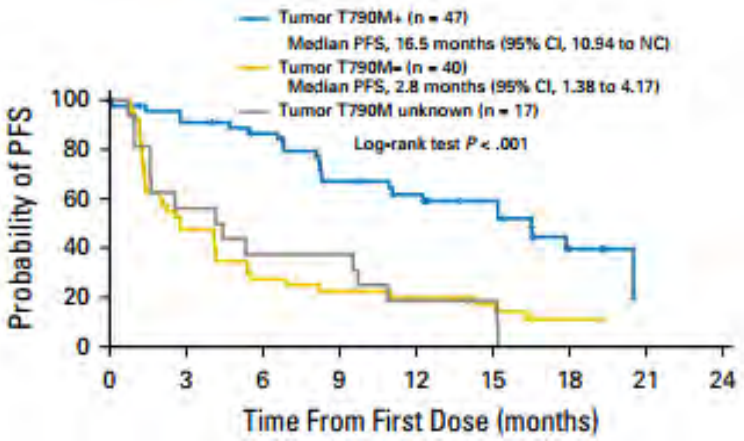
Tumor T790M+ vs T790M-



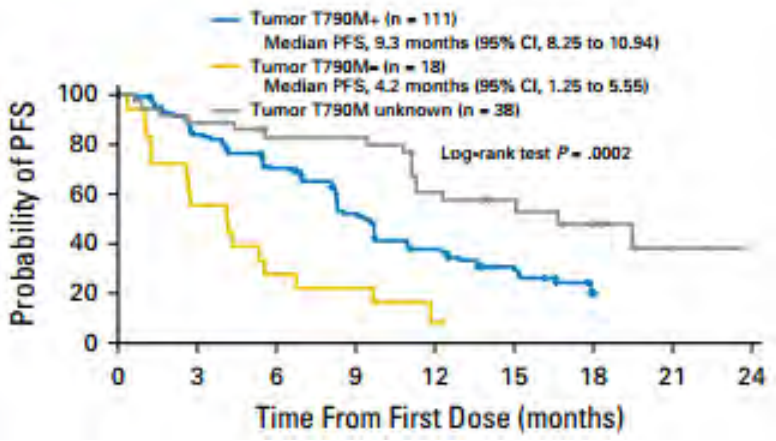
Plasma T790M+ vs T790M-



PlasmaT790M- by tissue status



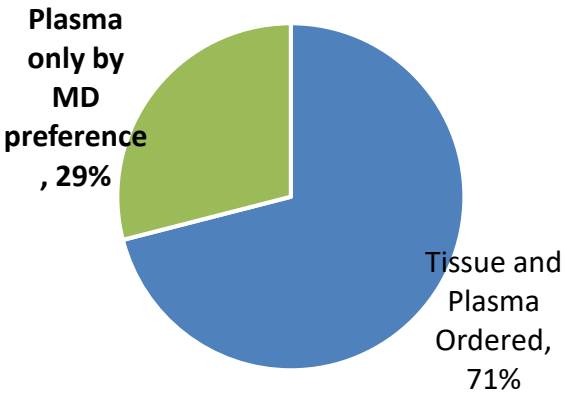
PlasmaT790M+ by tissue status



Oxnard, JCO 2016

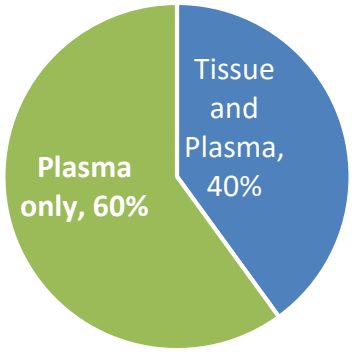
Prospective Study of 323 Advanced NSCLC Patients with Guardant360 Ordered as Standard of Care

NGS Orders



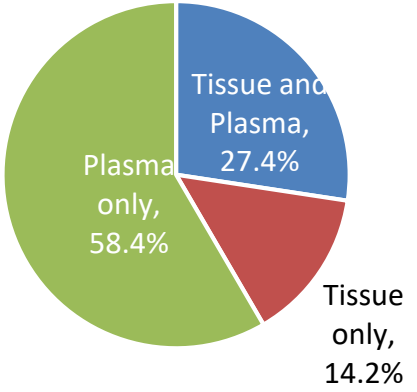
Tissue NGS Order Outcomes
- 10%: biopsy not possible
- **34%: tissue QNS**
- **56%: completed**

Completed NGS Profiling



Tissue-Plasma Concordance
- **89% at initial diagnosis**
- 70% at disease progression (reflection of heterogeneity)

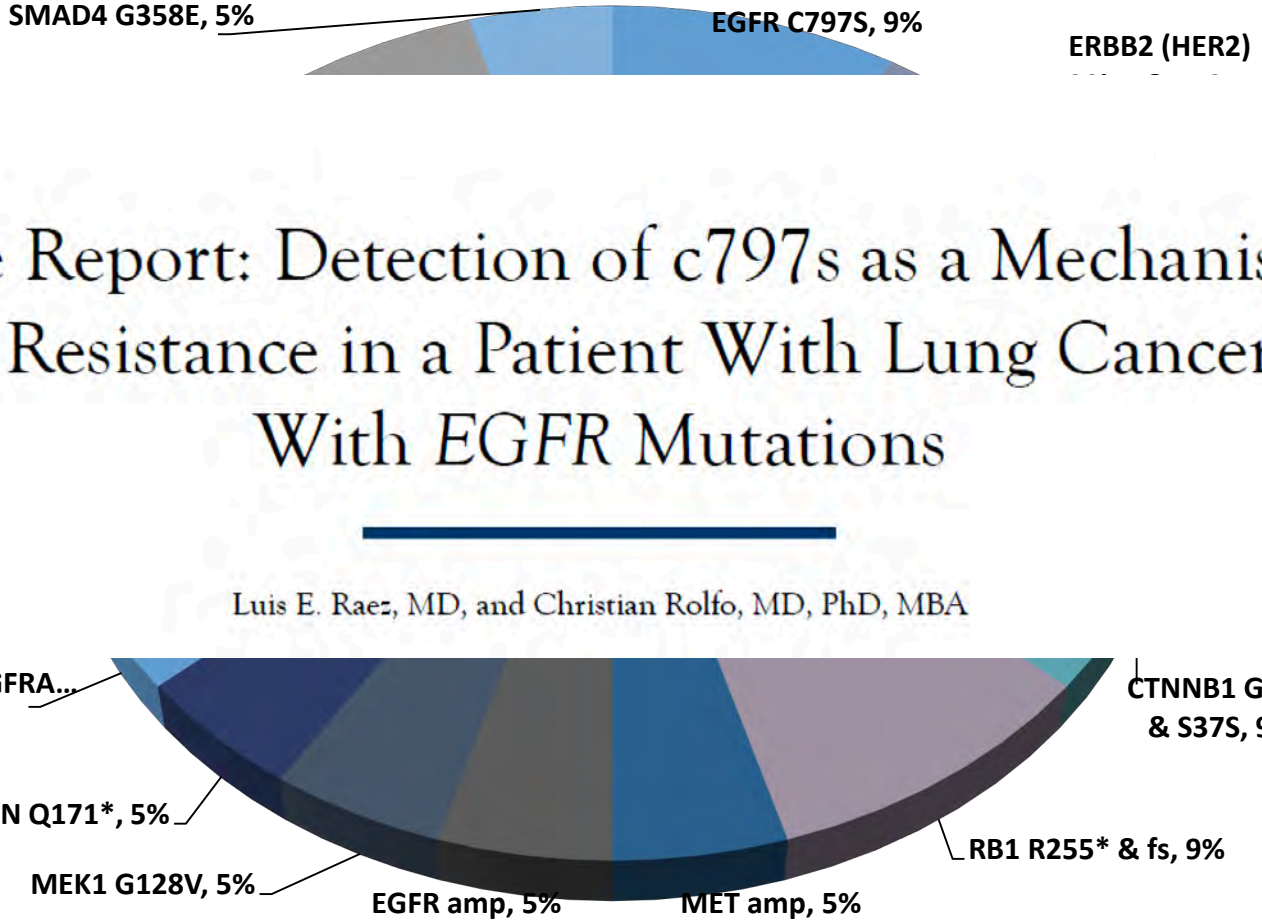
Therapeutic Target Identification



Plasma Performance
- Detected 86% of targetable mutations
- **Only source of targetable mutation detection for 58% of patients**

Aggarwal (Carpenter) et al. 2018 *JAMA Oncology*

When Osimertinib Moves to First Line – Comprehensive Genomic Testing Reveals Acquired Resistance Mechanisms

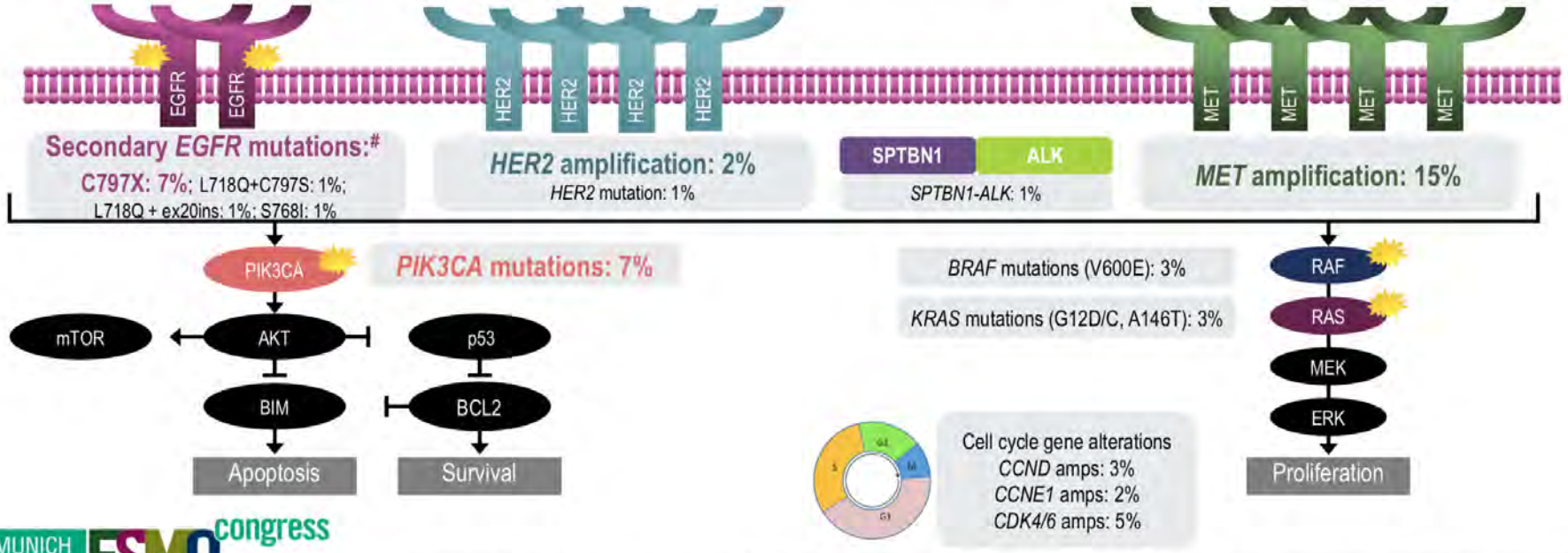


Ramalingam (Jänne) et al. 2017 *Journal of Clinical Oncology*

Raez & Rolfo AJHO. 2017;13(9):19-22

RESULTS: CANDIDATE ACQUIRED RESISTANCE MECHANISMS WITH OSIMERTINIB (n=91)*

- 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



MUNICH 2018 ESMO congress

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

Ramalingam S, ESMO 2018

Liquid Biopsy: Guidelines & Recommendations

"If repeat biopsy is not feasible, plasma biopsy should be considered"
"Testing should be conducted as part of broad molecular profiling"

NCCN 2017 NSCLC Practice Guidelines[1]

"Key new recommendations include the inclusion of additional genes (*ERBB2*, *MET*, *BRAF*, *KRAS*, and *RET*)…and the use of cell-free DNA to "rule in" targetable mutations when tissue is limited or hard to obtain.

AMP/CAP/IASLC 2018 Molecular Testing Guidelines for Lung Cancer[2]

"Even for patients who are able to undergo a traditional tissue biopsy, a liquid biopsy may be safer, quicker, and more convenient—and perhaps even more informative."

2017 ASCO Clinical Cancer Advances[3]

[1]Ettinger (Hughes) et al. 2017 *JNCCN* [2]Lindemann (Yatabe) et al. 2018 *J Thor Onc* [3]Burstein (Dizon) et al. 2017 *J Clin Onc*

Liquid Biopsy for Advanced Non-Small Cell Lung Cancer (NSCLC): A Statement Paper from the IASLC

Christian Rolfo, MD, PhD, MBA,[a] Philip C. Mack, PhD,[b]
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Trever G. Bivona, MD, PhD,[f] Roy S. Herbst, MD, PhD,[g] Tony S. Mok, MD,[h]
Nir Peled, MD, PhD,[i] Robert Pirker, MD,[j] Luis E. Raez, MD,[k] Martin Reck, MD, PhD,[l]
Jonathan W. Riess, MD,[b] Lecia V. Sequist, MD, MPH,[m] Frances A. Shepherd, MD,[n]
Lynette M. Sholl, MD,[o] Daniel S. W. Tan, MBBS, PhD,[p] Heather A. Wakelee, MD,[q]
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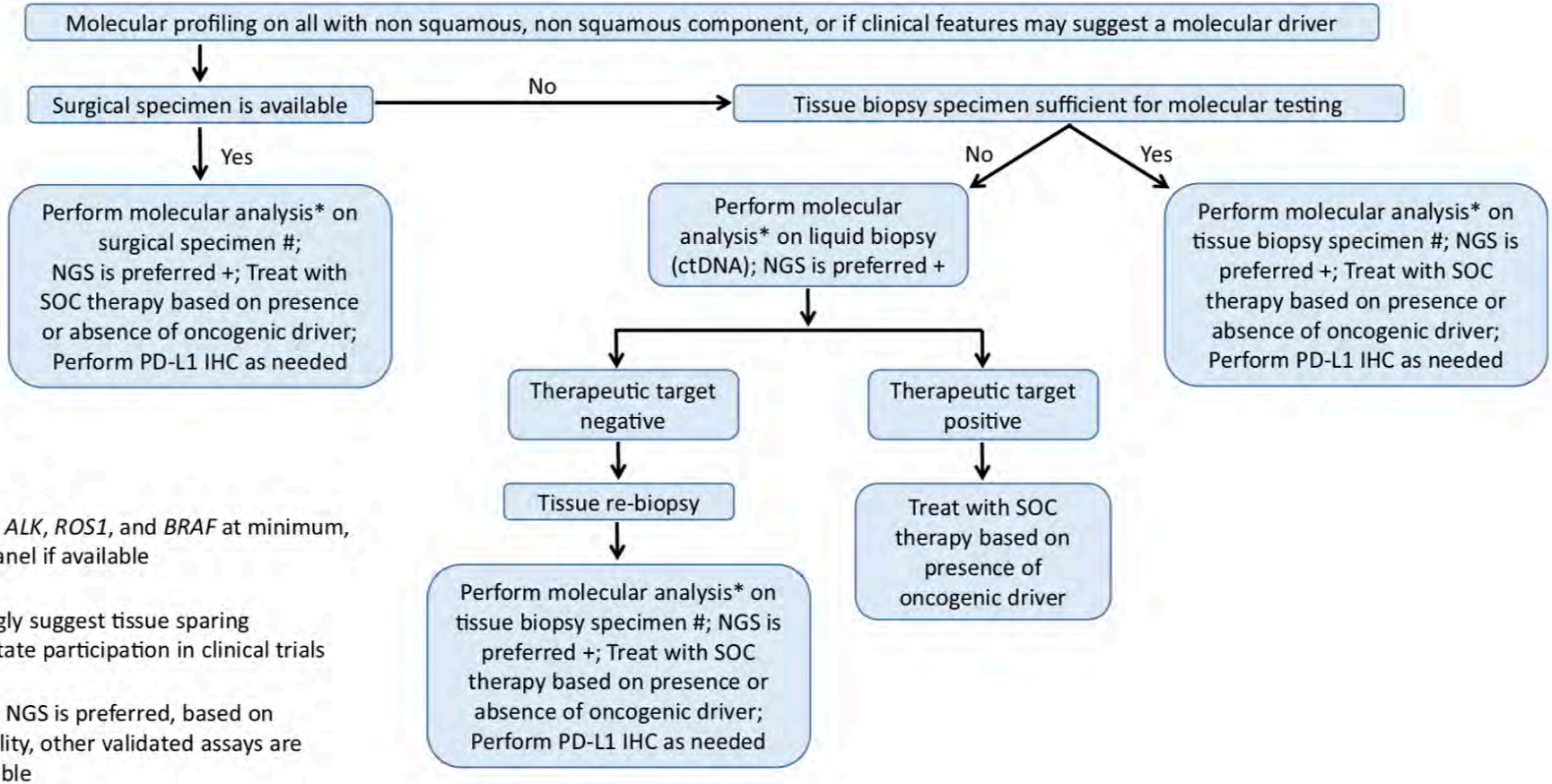
Rolfo et al, J Thorac Oncol. 2018 Sep;13(9):1248-1268

Patient with advanced treatment naive NSCLC



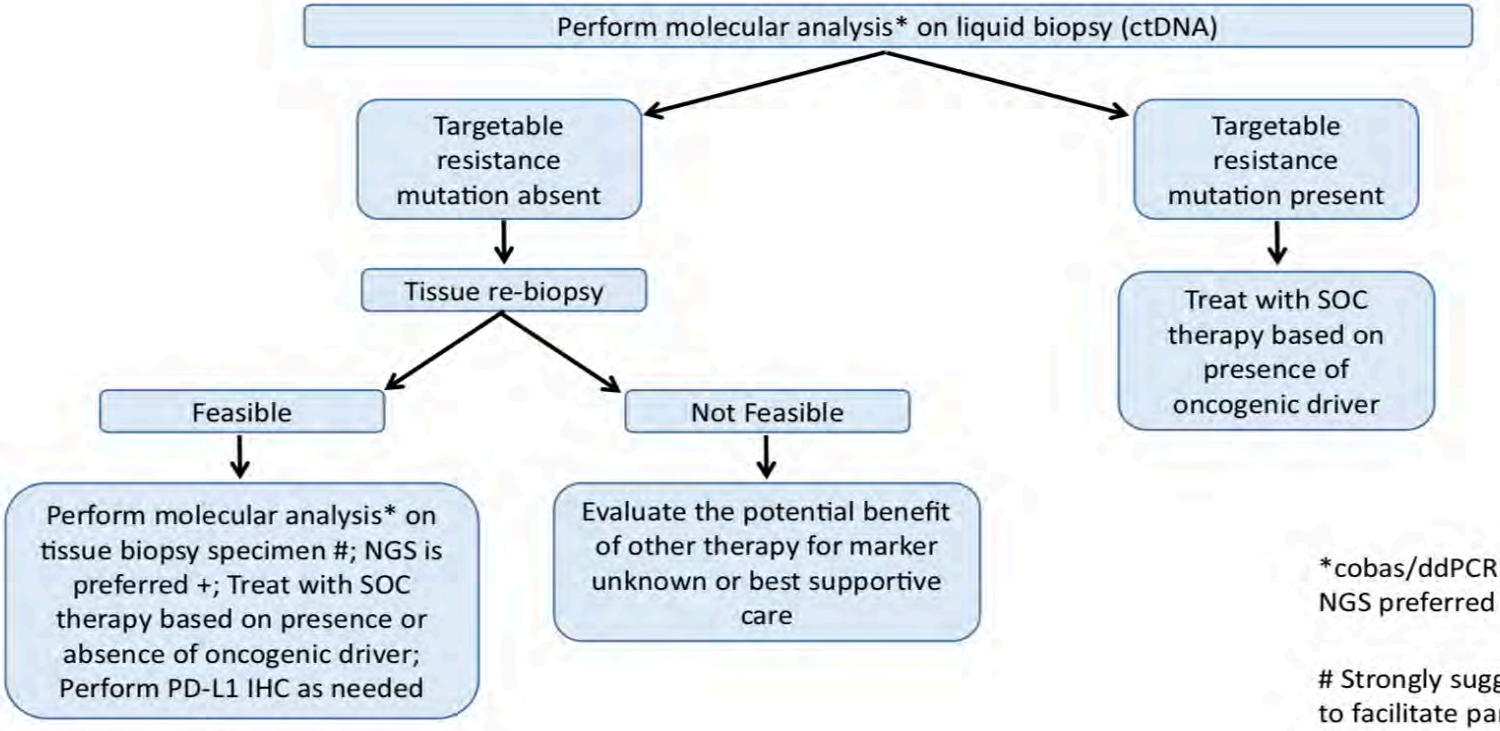
* *EGFR*, *ALK*, *ROS1*, and *BRAF* at minimum, but a panel if available

Strongly suggest tissue sparing to facilitate participation in clinical trials

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

Rolfo et al, J Thorac Oncol. 2018 Sep;13(9):1248-1268

Patient with NSCLC progressive or recurrent disease 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

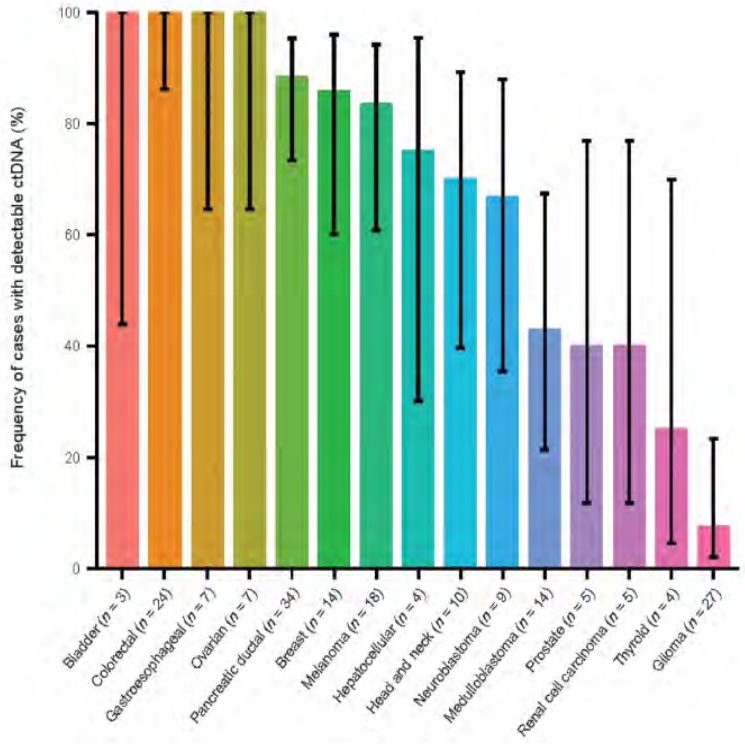
Pre-analytical factors for optimal ctDNA testing!

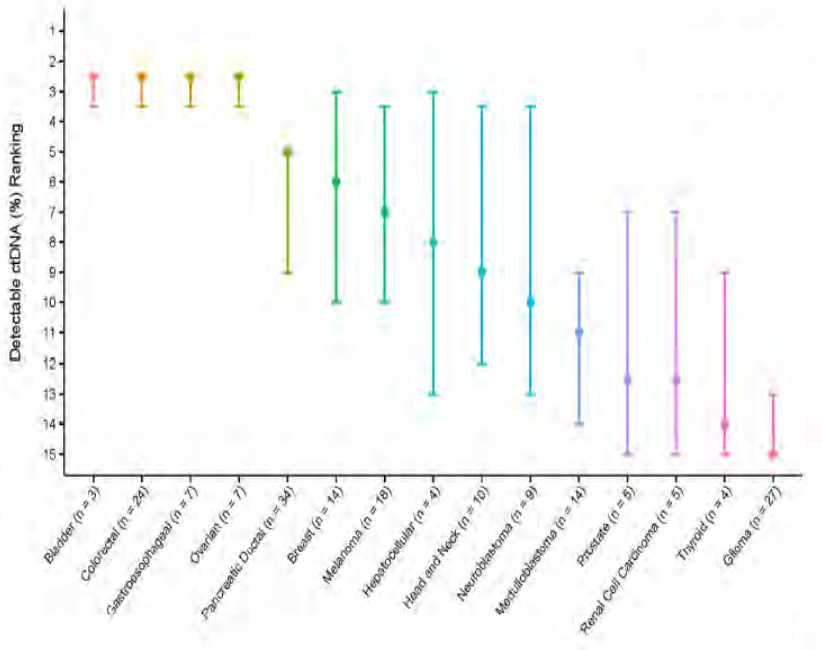
Rolfo et al, J Thorac Oncol. 2018 Sep;13(9):1248-1268

SPECIAL CONSIDERATIONS...

Liquid biopsy: ctDNA

Does different tumor types release the same amount of DNA in the blood?

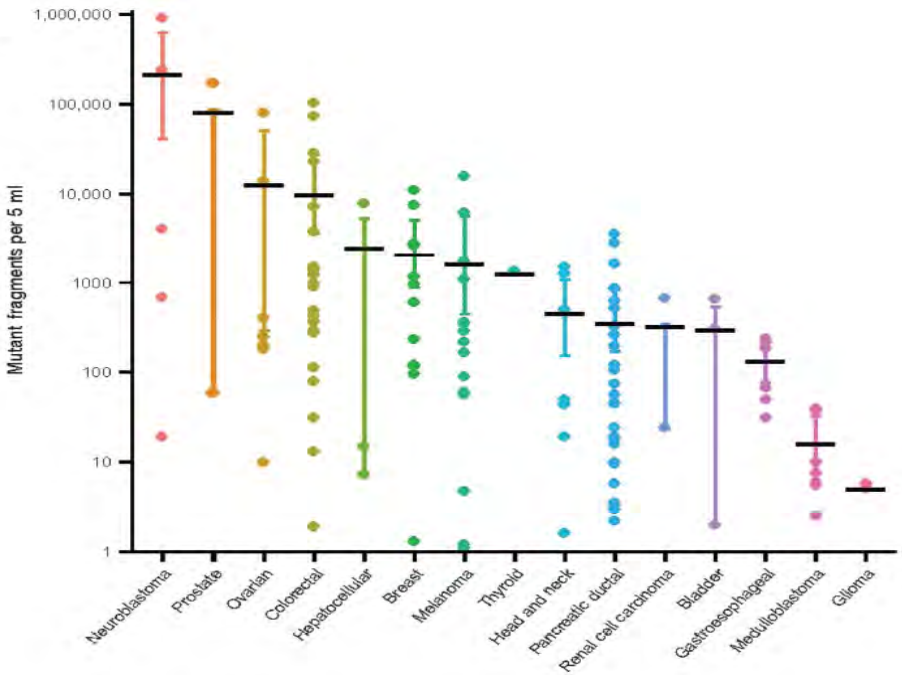


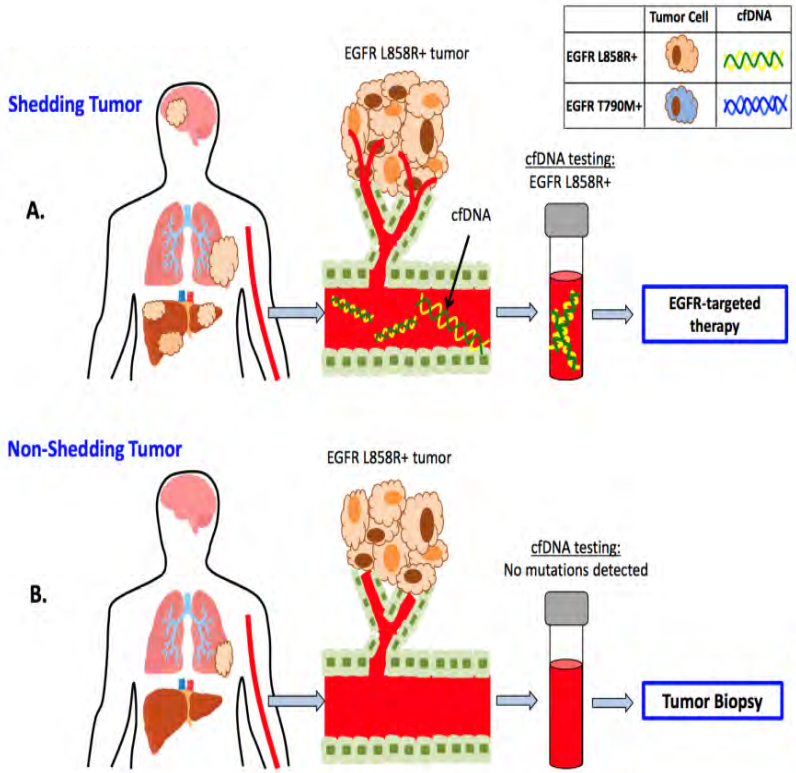


Bettegowda et al., Sci Trans Med, 2014

Liquid biopsy: ctDNA

Does ctDNA concentration is the same among patients with the same tumor?

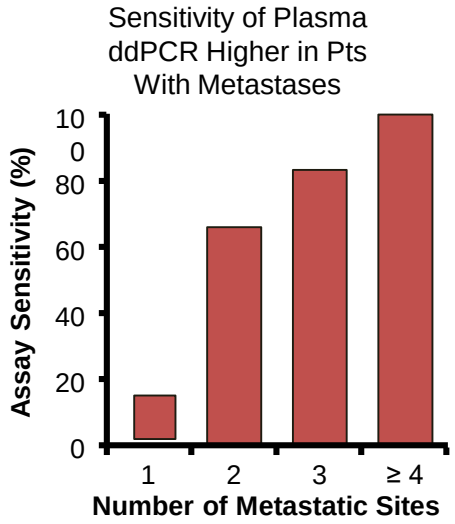




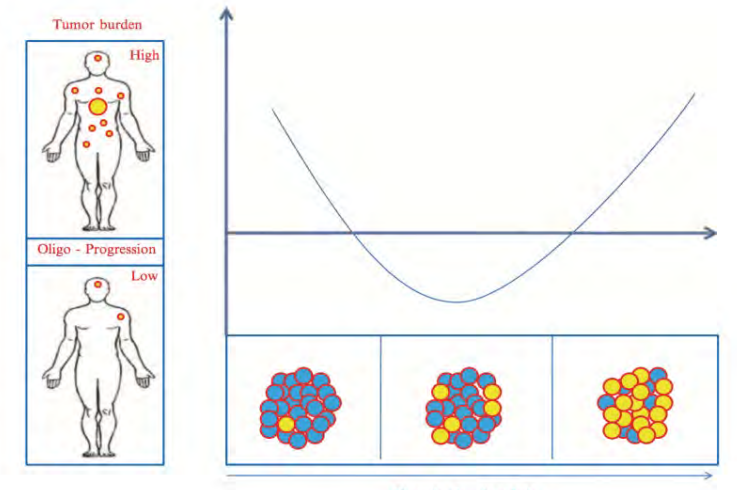
Bettegowda et al., Sci Trans Med, 2014

Sacher, Komatsubara,Oxnard J Thorac Oncol. 2017 Sep;12(9):1344-1356

Some considerations



Correlation between tumor burden (*y*-axis) and dynamic clonal evolution of the tumor



Increasing number of metastatic sites (*P* = .001) and presence of bone (*P* = .007), hepatic (*P* = .001) metastases significantly associated with assay sensitivity

Sacher AG, et al. JAMA Oncol. 2016

Pisapia, Malapelle, Troncone, Springer Book 2017

Metastatic site influences accurancy in EGFR mut

- Pooled analysis

Study (Reference)	EGFR Mutation (ctDNA)	Sensitivity (M1b) n.(%)	Sensitivity (M1a) n.(%)	Odds Ratio (95% CI)
Oxnard *et al.* 2016 *(15)*	Del19/L858R	139/161 (86%)	36/48 (75%)	2.11 (0.95-4.66)
Normanno *et al.* 2016 *(18)*	Del19/L858R	52/82 (63%)	13/57 (23%)	5.87 (2.73-12.6)
Yi-Long Wu *et al.* 2016 *(19)*	Del19/L858R	180/234 (77%)	63/105 (60%)	2.22 (1.35-3.65)
Tseng *et al.* 2015 *(20)*	Del19/L858R	32/41 (78%)	5/21 (24%)	11.28 (3.27-39.6)
Kumar *et al.* 2017 *(22)*	Del19/L858R	21/28 (75%)	15/27 (55%)	2.40 (0.76-7.53)
Kasahara *et al.* 2017 *(23)*	Del19/L858R	26/33 (79%)	8/16 (50%)	3.71 (1.03-13.46)
Karlovich *et al.* 2016 *(17)*	Del19/L858R	52/55 (95%)	7/18 (39%)	27.24 (6.07-122.17)
Karlovich *et al.** 2016 *(17)*	T790M	47/49 (96%)	4/15 (27%)	64.63 (10.47-398.8)
Thress *et al.* 2015 *(21)*	T790M	21/27 (78%)	2/11 (18%)	15.75 (2.65-93.46)
Jenkins *et al.* 2017 *(24)*	T790M	111/154 (72%)	123/243 (51%)	2.52 (1.63-3.88)

A significant association was observed for both **EGFR-activating** (OR: 4.30, 95% CI: 2.35-7.88) **and resistant T790M mutations** (OR: 11.89, 95% CI: 1.45-97.22), regardless of the use of digital-PCR (OR: 5.85, 95% CI: 3.56-9.60) or non-digital PCR technologies (OR: 2.96, 95% CI: 2.24-3.91).

Passiglia,,Rizzo, Rolfo et al (Russo) Current Cancer Drug Target, Sept 2018

Important considerations

NEXT GENERATION SEQUENCING PLATFORMS

- **Assay:** laboratory developed *vs.* commercial
- **Commercial tests:** test panel *vs.* central CLIA-lab
- **Coverage:** number of bases, genes, exons, VAF
- **Validation and Quality Controls**
- **Enrichment technology:** multiplex PCR, Hybrid capture
- **Limit of detection:** % mutant allele / wild type allele
- **Sensitivity & specificity:** samples with known mutant allele frequency
- **Bioinformatics:** variant calling and error correction methods
- **Interpretation and reporting**
- **TAT and costs!**

Adapted from Ming S. Tsao, WCLC 2018

Guardant360 – All NCCN Targets in a Single Blood Test

Critical exons completely sequenced and all four major classes of alterations

Point Mutations – 73 Genes

AKT1	*ALK*	***APC***	***AR***	*ARAF*	*ARID1A*	*ATM*	***BRAF***	***BRCA1***	***BRCA2***
CCND1	*CCND2*	*CCNE1*	*CDH1*	*CDK4*	*CDK6*	*CDKN2A*	*CTNNB1*	*DDR2*	***EGFR***
***ERBB2* (HER2)**	*ESR1*	*EZH2*	*FBXW7*	*FGFR1*	*FGFR2*	*FGFR3*	*GATA3*	*GNA11*	*GNAQ*
GNAS	*HNF1A*	***HRAS***	*IDH1*	*IDH2*	*JAK2*	*JAK3*	***KIT***	***KRAS***	*MAP2K1 (MEK1)*
MAP2K2 (MEK2)	***MAPK1 (ERK2)***	***MAPK3 (ERK1)***	***MET***	*MLH1*	*MPL*	*MTOR*	***MYC***	*NF1*	*NFE2L2*
NOTCH1	*NPM1*	***NRAS***	*NTRK1*	*NTRK3*	*PDGFRA*	***PIK3CA***	*PTEN*	*PTPN11*	*RAF1*
RB1	*RET*	*RHEB*	*RHOA*	*RIT1*	*ROS1*	*SMAD4*	*SMO*	***STK11***	*TERT***
TP53	*TSC1*	*VHL*					** Includes *TERT* promoter region		

Indels – 23 Genes

ATM	*APC*	*ARID1A*	*BRCA1*	*BRCA2*	*CDH1*	*CDKN2A*	*EGFR*	*ERBB2*	*GATA3*
KIT	*MET ex14*	*MLH1*	*MTOR*	*NF1*	*PDGFRA*	*PTEN*	*RB1*	*SMAD4*	*STK11*
TP53	*TSC1*	*VHL*							

Amplifications – 18 Genes

AR	*BRAF*	*CCND1*	*CCND2*	*CCNE1*	*CDK4*	*CDK6*	*EGFR*	*ERBB2*
FGFR1	*FGFR2*	*KIT*	*KRAS*	*MET*	*MYC*	*PDGFRA*	*PIK3CA*	*RAF1*

Fusions – 6 Genes

ALK	*FGFR2*	*FGFR3*	*RET*	*ROS1*	*NTRK1*

Oncomine™ Pan-Cancer Cell-Free Assay | *Gene Content*

Assay	Configuration	Unique Genes	DNA	RNA
Pan Cancer	TNA (DNA + RNA)	52	50	12

Hotspot Genes		Tumor Suppressor Genes	Copy Number Genes	Gene Fusions
AKT1	HRAS	APC	CCND1	ALK
ALK	IDH1	FBXW7	CCND2	BRAF
AR	IDH2	PTEN	CCND3	ERG
ARAF	KIT	TP53	CDK4	ETV1
BRAF	KRAS		CDK6	FGFR1
CHEK2	MAP2K1		EGFR	FGFR2
CTNNB1	MAP2K2		ERBB2	FGFR3
DDR2	MET		FGFR1	MET
EGFR	MTOR		FGFR2	NTRK1
ERBB2	NRAS		FGFR3	NTRK3
ERBB3	NTRK1		MET	RET
ESR1	NTRK3		MYC	ROS1
FGFR1	PDGFRA			
FGFR2	PIK3CA			
FGFR3	RAF1			
FGFR4	RET			
FLT3	ROS1			
GNA11	SF3B1			
GNAQ	SMAD4			
GNAS	SMO			

Variant Type	Total Variants
SNV	> 900
CNV	12
Fusion/MET Exon Skipping	99

Single Pool design (DNA & RNA)

Performance Specs:

Hotspot SNV/Indel
- 0.1% AF LOD with 20 ng input

Whole target SNV/Indel
- 1.0% AF

CNV detection
- 1.4x fold change

Fusion detection & MET exon 14 skipping
- 1% RNA fusions in cfTNA

Sample Plexy
- 4 libraries on a 540 chip
- 8 libraries on a 550 chip

Oncomine data.

Turnaround Time Shorter for Plasma ddPCR vs Tissue Genotyping

- Turnaround time shorter for plasma genotyping vs tissue genotyping ($P < .001$ for cohort 1)

Turnaround Time, Median Days (Range)	Cohort 1, Newly Diagnosed (n = 115)	Cohort 2, Acquired Resistance (n = 59)
Plasma genotyping*	3 (1-7)	2 (1-4)
Tissue genotyping†	12 (1-54)	27 (1-146)

- Plasma genotyping completed for all pts

*Plasma turnaround time: business days from blood sampling to reporting.
†Tissue turnaround time: date of initial order to date of first report; includes time for repeat biopsies.

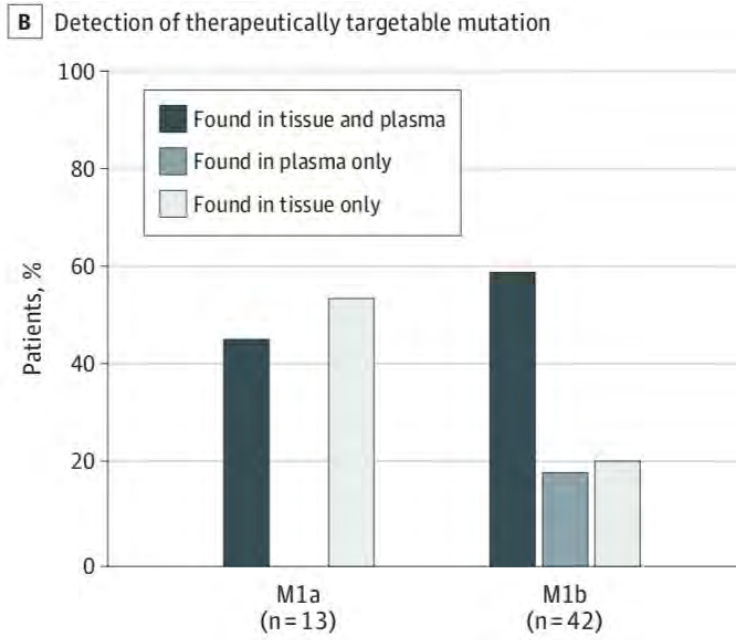
- Repeat biopsies required for 19% of newly diagnosed pts and 21% of pts with acquired resistance

Sacher AG, et al. JAMA Oncol. 2016;

The importance of method and concordance

Mutation Detection by Type of Test and Disease Stage

323 patients 73-gene commercial platform.

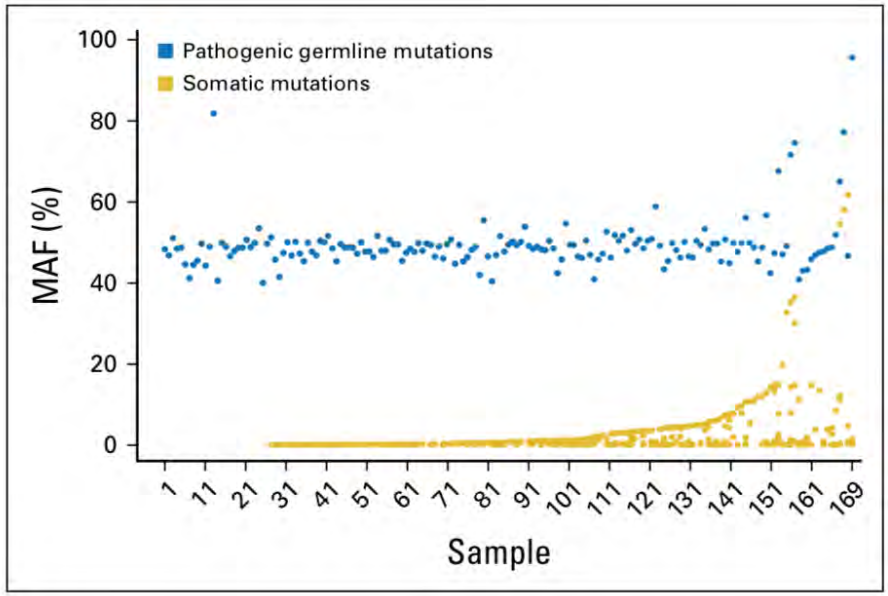


85.7% who received a targeted therapy based on the plasma result achieved a complete or a partial response or stable disease. The plasma-based targeted mutation AF had no correlation with depth of Response Evaluation Criteria in Solid Tumors response

Aggarwal (Langer) et al,JAMA OCT 2018

Germline Mutations detected by next generation sequencing and/or liquid biopsy

10,888 unselected patients with advanced cancer (stage III/IV)
lung (41%)
Guardant360 testing



156 pts (**1.4%**) had suspected hereditary cancer mutations **in 11 genes**.

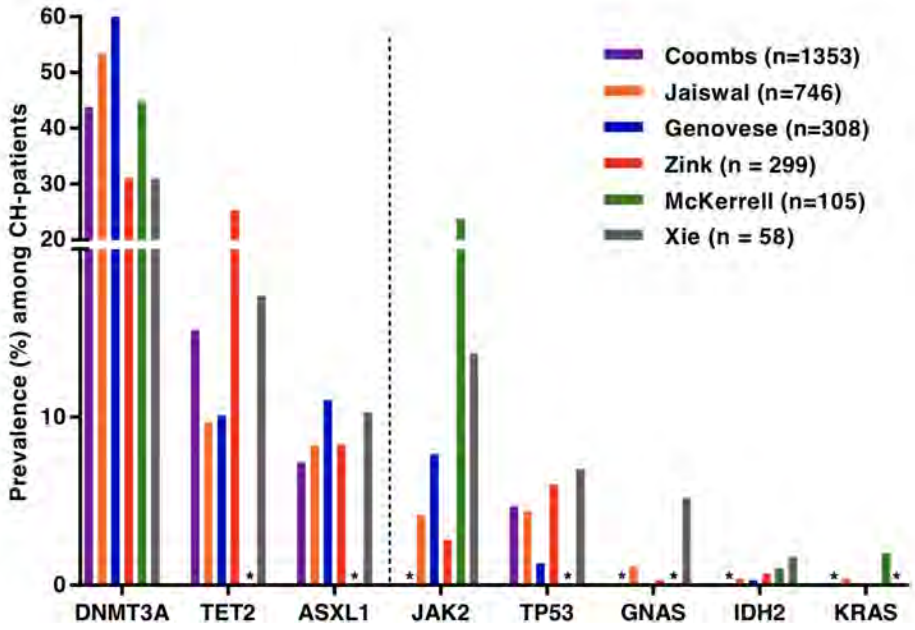
Putative germline mutations were **more frequent in individuals younger** than 50 years versus those 50 years and older (3.0% v 1.2%, respectively; P , .001).

Genetic counseling advise is madatory in these patients

Slavinetal, JCO, 19 October 2018

A new problem: Clonal Hematopoeisis

Genes commonly mutated



Clonal hematopoiesis (CH) is the somatic acquisition of genomic alterations in hematopoietic stem and/or progenitor cells, leading to clonal expansion.

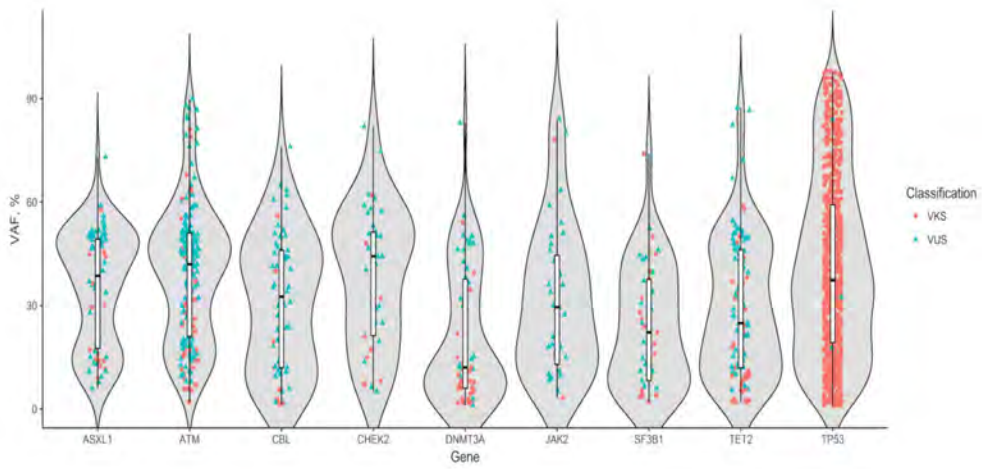
- A **large proportion of cfDNA is derived from peripheral blood cells** (PBC), therefore somatic mutations within non-malignant hematopoietic cells, known clonal hematopoiesis (CH).
- CH might be a recurring source of discordance between tumor genotyping and plasma cfDNA genotyping.

Hu (Oxnard) et al,Clin Cancer Res, March 2018

Ptashkin et al , *JAMA Oncol*. June 2018

CLONAL HEMATOPOEISIS

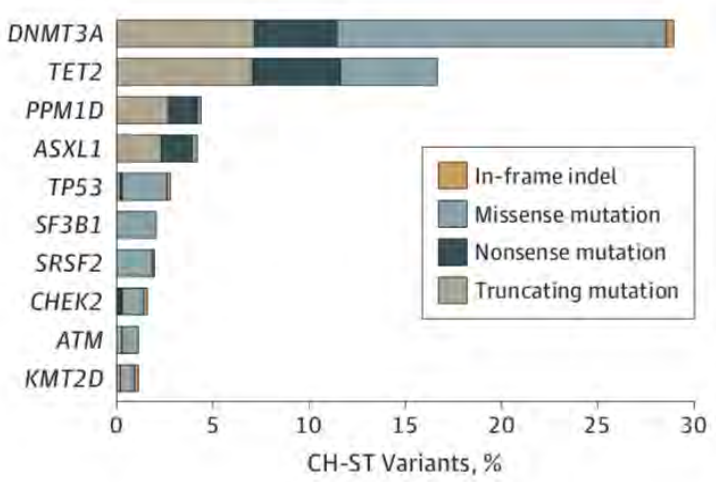
Genes commonly mutated



Mutations in genes that are frequently altered in **clonal hematopoiesis were identified in 65%** (**1139/1757**) of patients undergoing next-generation sequencing.

DNA isolated from tumor tissue and matched peripheral blood using the MSK-IMPACT assay

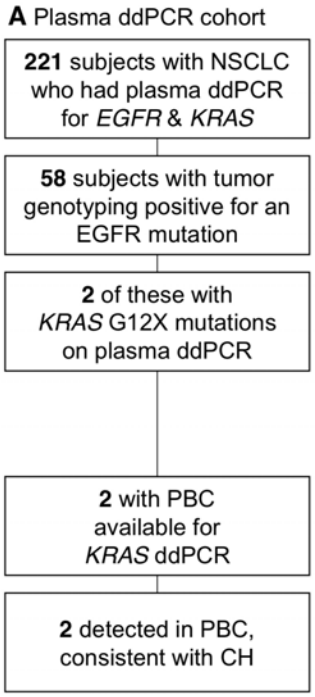
17469 patients with advanced cancer, paired next-generation sequencing results show that **5% of the patients would have at least 1 CH-associated mutation** misattributed as tumor derived in the absence of matched blood sequencing.

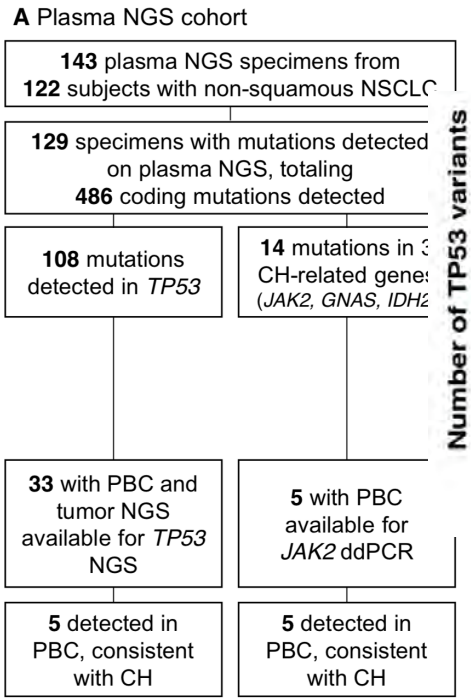


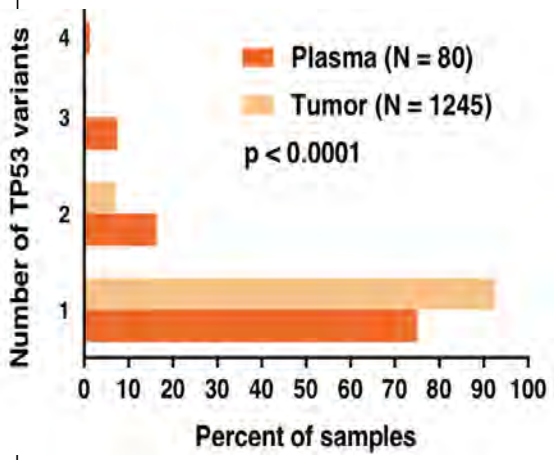
Coombs et al, Clin Cancer Res, Jun 2018

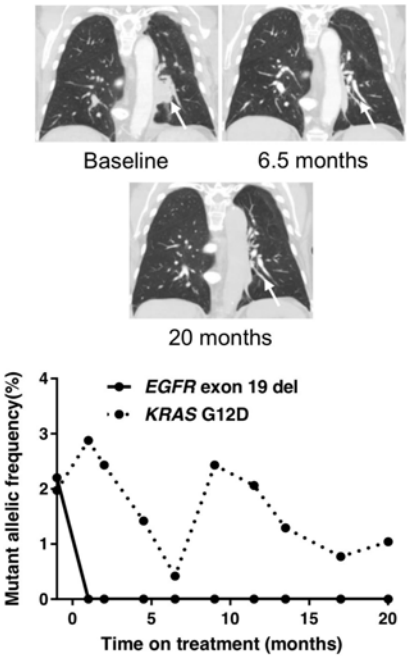
Ptashkin et al , *JAMA Oncol*. June 2018

False positive plasma genotyping due to clonal hematopoiesis (CH) peripheral blood cells (PBC)









- ***JAK2* mutations, some *TP53* mut, and rare *KRAS* mut detected in cfDNA are derived from CH not tumor**

Hu (Oxnard) et al,Clin Cancer Res, March 2018

Clinical Case

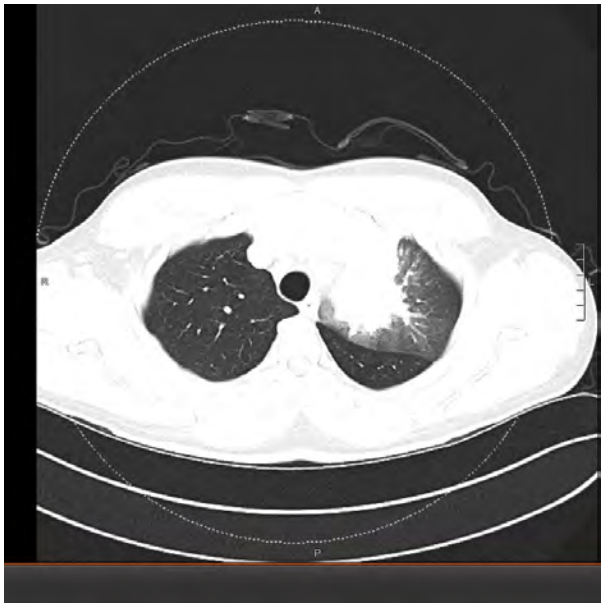
- A 43 yo M, never smoker,
- December 2017 and later was found to have LUL mass (7*5cm) with enlarged left hilar and mediastinal nodes, and numerous bone lesions(Chest CT, 1/22/18).
- A sternal bone biopsy (1/24/18) showed metastatic adenocarcinoma consistent w/ lung origin.
- PET/CT showed LUL mass with widely spread lesions in brain, chest, abdomen, pelvic, and bones. Brain MRI (3/7/18) revealed multiple small metastatic lesions.
- EGFR mutation… PD-L1 expression 50%.

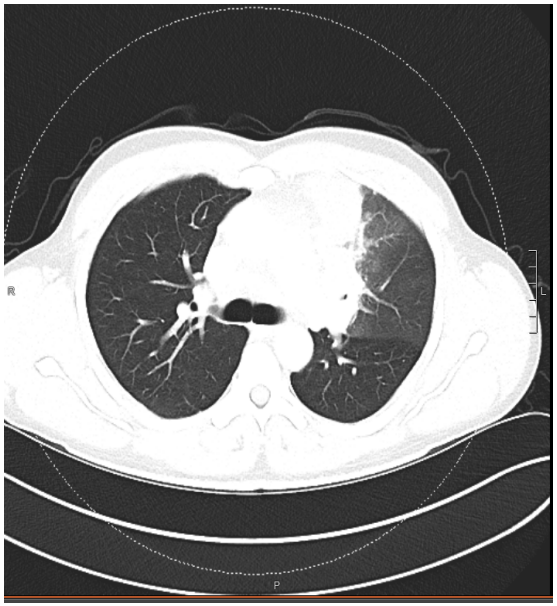
EGFR mutation G719X

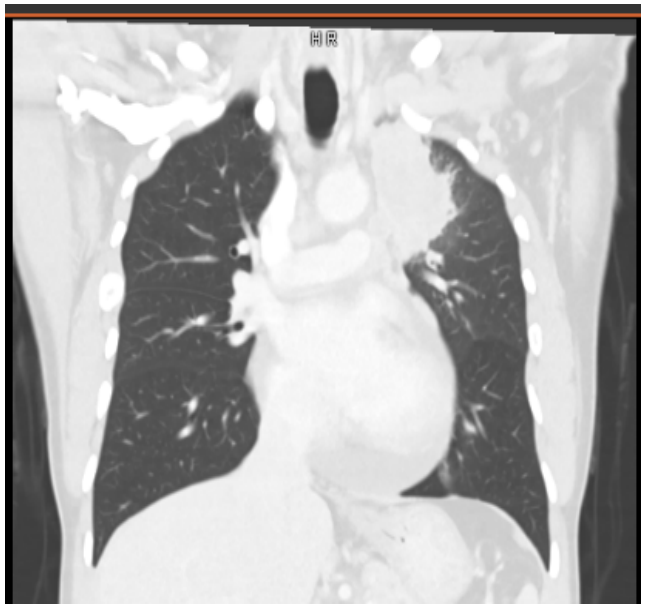
Mutation Detected G719X B1 Base change: 2156G>C, 2155G>A, 2155G>T
Missense mutation exon 18 of the EGFR gene

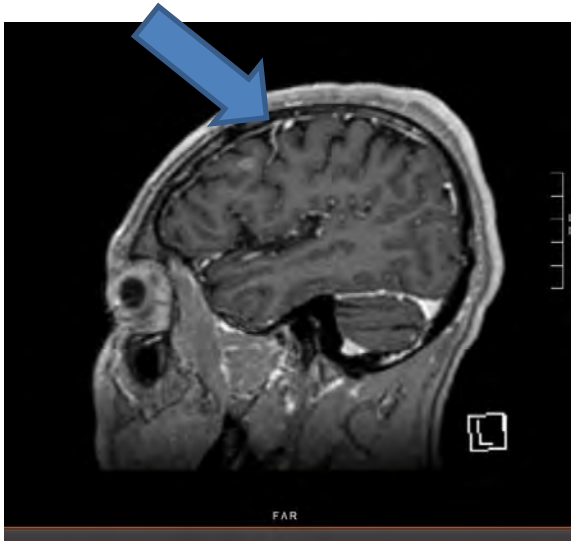
- Compared with other EGFR mutations, L861Q, G719X or S768I substitution mutations are associated with a poorer prognosis

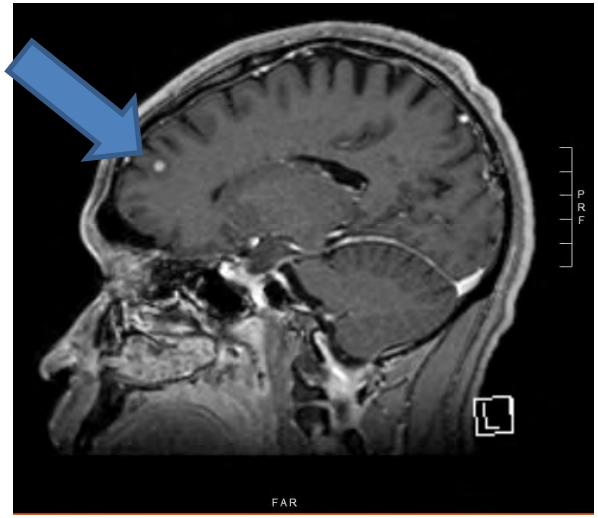
Clinical Case








FAR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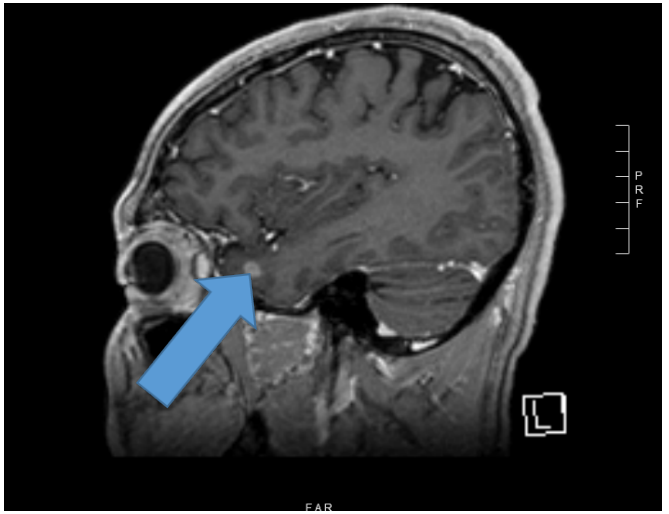
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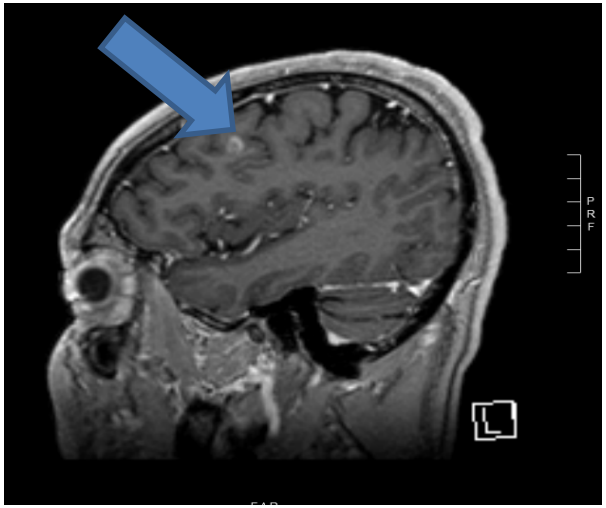

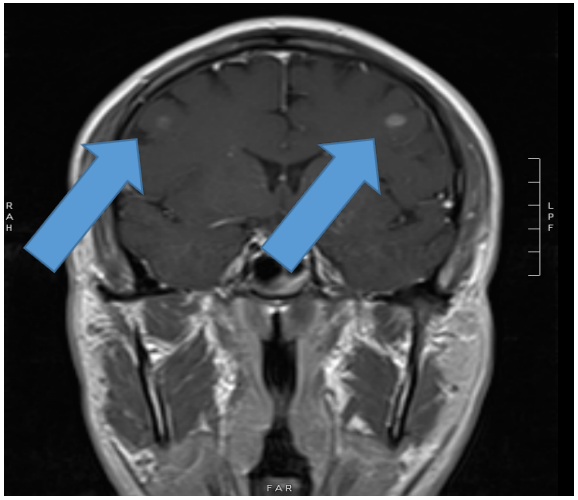
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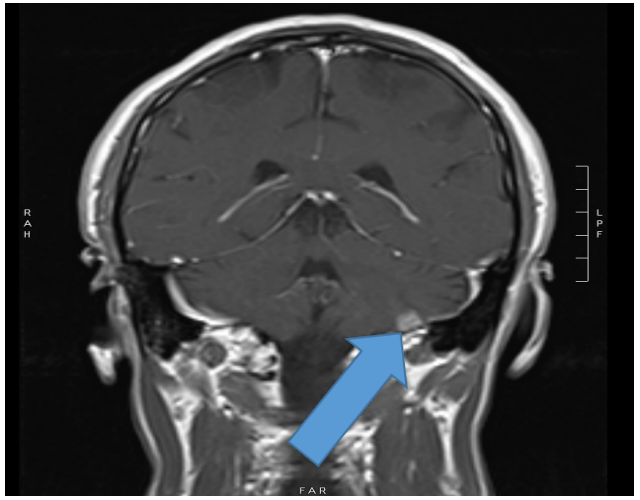

FAR

Clinical Case

PATIENT RESULTS[‖]

4 genomic findings

7 therapies associated with potential clinical benefit

0 therapies associated with lack of response

10 clinical trials

[‖] Reduced sensitivity due to sample quality – See Appendix: Performance Specifications for details.

TUMOR TYPE: LUNG ADENOCARCINOMA

Genomic Alterations Identified[†]

EGFR E709V, G719A

Additional Findings[†]

Microsatellite status Cannot Be Determined

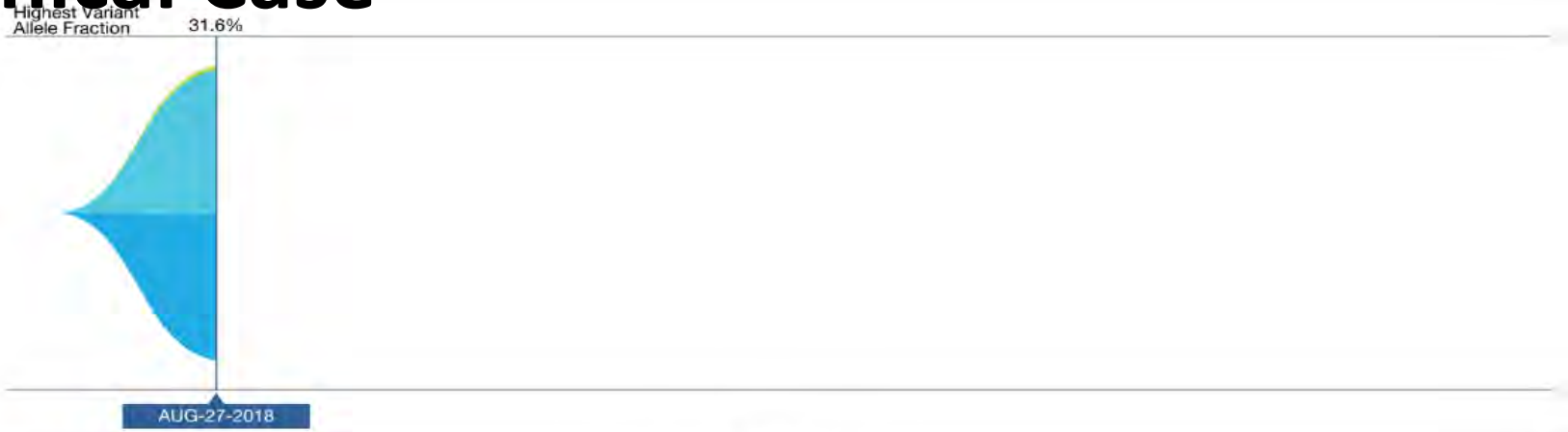
Tumor Mutation Burden Cannot Be Determined

Additional Disease-relevant Genes with No Reportable Alterations Identified[†]

RET
ROS1
ALK
BRAF
KRAS
ERBB2
MET

[†] For a complete list of the genes assayed and performance specifications, please refer to the Appendix

Clinical Case



Alteration	% cfDNA or Amp	
EGFR E709V	31.6%	
EGFR G719A	31.4%	
NRAS Q61R	0.2%	
ARID1A R2164W	0.2%	Variant of Uncertain Significance §
AR Amplification Amplifications not graphed above	Low (+) Plasma Copy Number: 1.6	

The table above annotates the variant allele fraction (% cfDNA) detected in this sample, listed in descending order.

§ See definitions section for more detail

Clinical Case

THERAPEUTIC IMPLICATIONS

Genomic Findings Detected	FDA-Approved Therapies (in patient's tumor type)	FDA-Approved Therapies (in another tumor type)	Potential Clinical Trials
EGFR E709V, G719A	Afatinib Erlotinib Gefitinib Osimertinib	Cetuximab Lapatinib Panitumumab	Yes, see clinical trials section
Microsatellite status Cannot Be Determined	None	None	None
Tumor Mutation Burden Cannot Be Determined	None	None	None

Clinical Case

Summary of Somatic Alterations & Associated Treatment Options

KEY ✓ Approved in indication ~ Approved in other indication ✗ Lack of response

Alteration	% cfDNA or Amplification	Associated FDA-approved therapies	Clinical trial availability (see page 3)
AR Amplification	Low (+)	✗ Abiraterone, Enzalutamide	Yes
EGFR G719A	31.4%	✓ Afatinib ~ Erlotinib, Gefitinib, Neratinib, Osimertinib	Yes
EGFR E709V	31.6%	~ Afatinib, Erlotinib, Gefitinib, Neratinib, Osimertinib	Yes
NRAS Q61R	0.2%	~ Binimetinib, Cobimetinib, Trametinib	Yes

Variants of Uncertain Significance

ARID1A R2164W (0.2%)

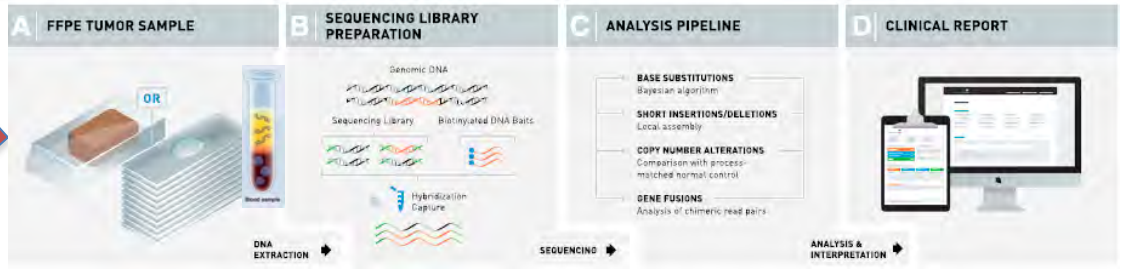
The functional consequences and clinical significance of alterations are unknown. Relevance of therapies targeting these alterations is uncertain.

Our New Way to Work . . .
Molecular Tumor Board

Patient case is derived from his doctor

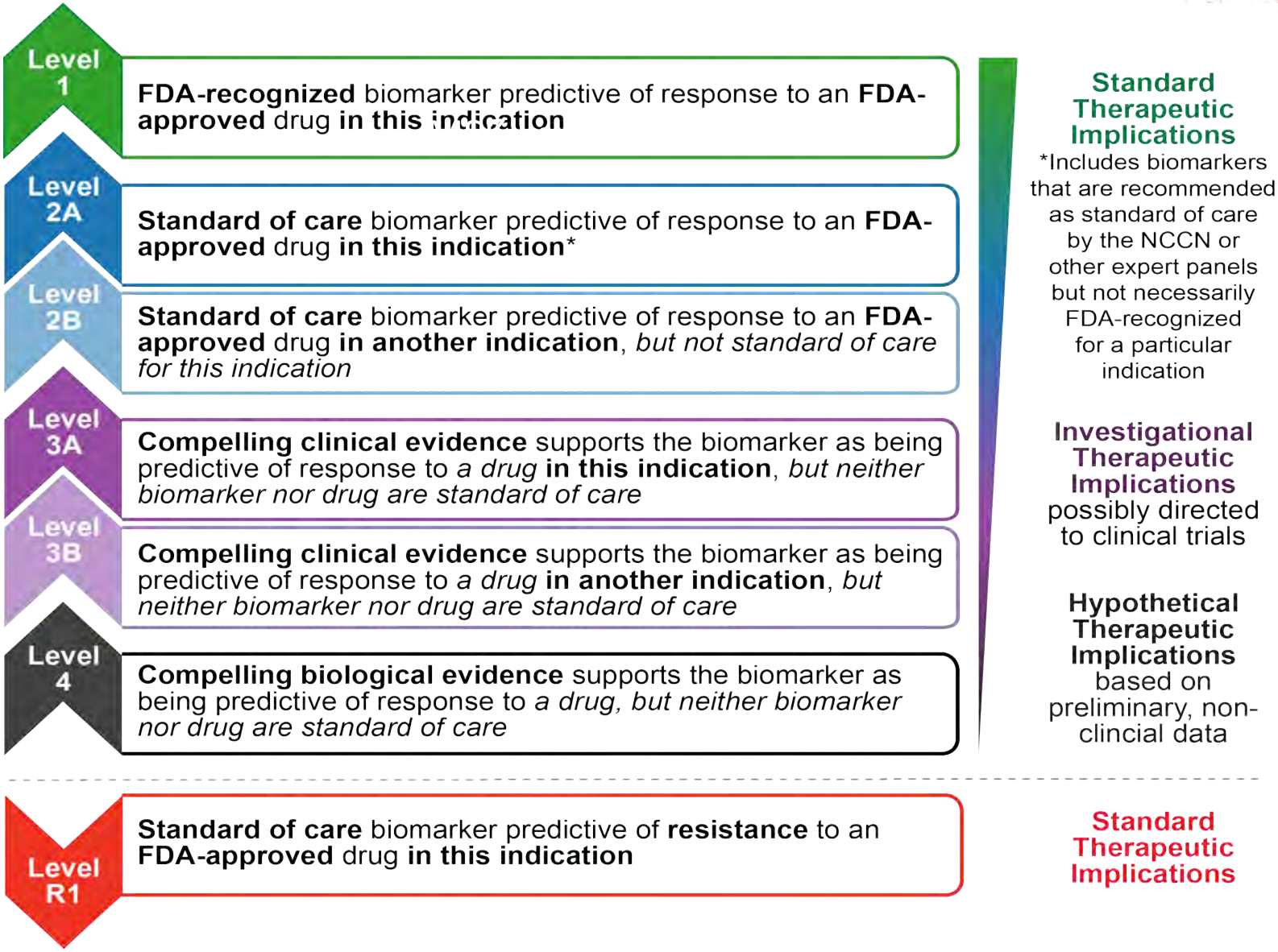
Molecular Tumor Board

Oncologist	Mol. Pathol	Bioinformat	Surgeon	Geneticist	Pediat	Nav. nurse



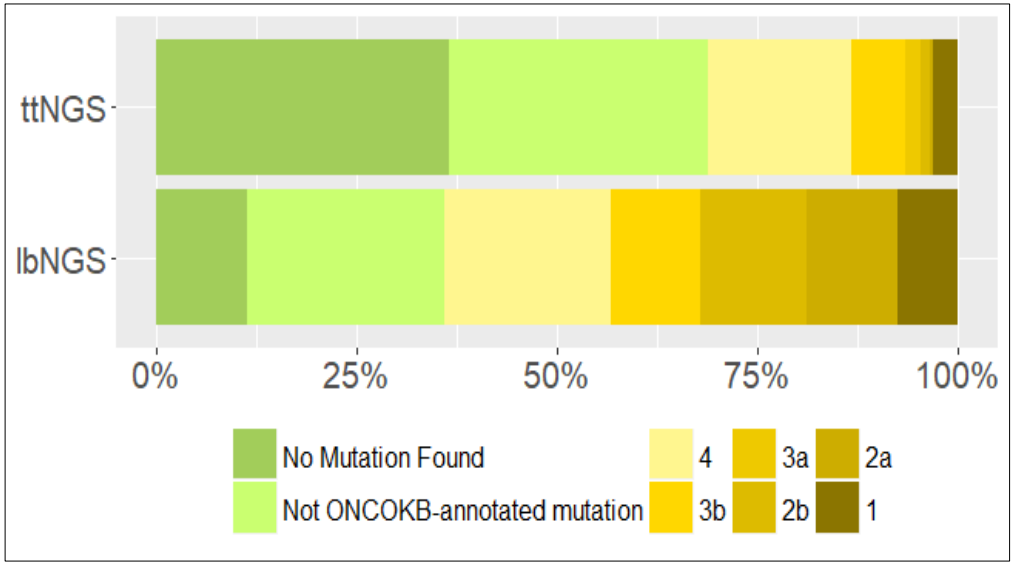
Molecular Tumor Board → **Report with therapeutic proposal** → Referral Doctor Discussion

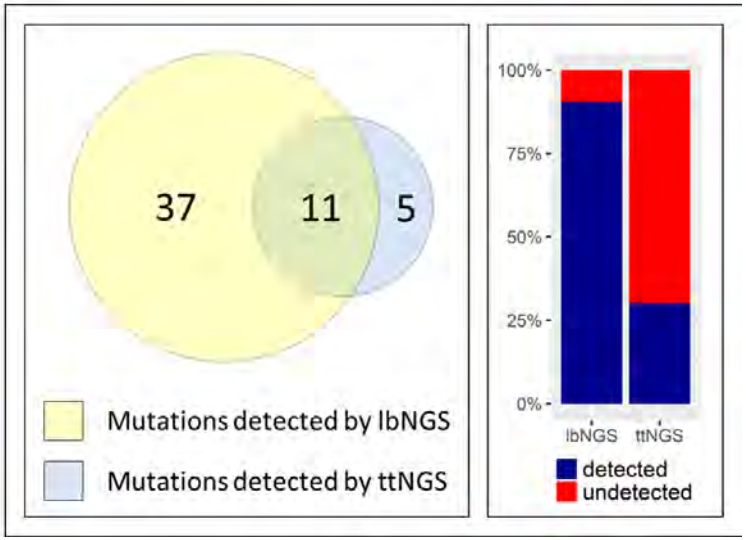
OncoKB



J Clin Oncol 34, 2016 (suppl; abstr 11583)

Effects of molecular tumor board and different NGS panels implementation for the treatment of patients with cancer.

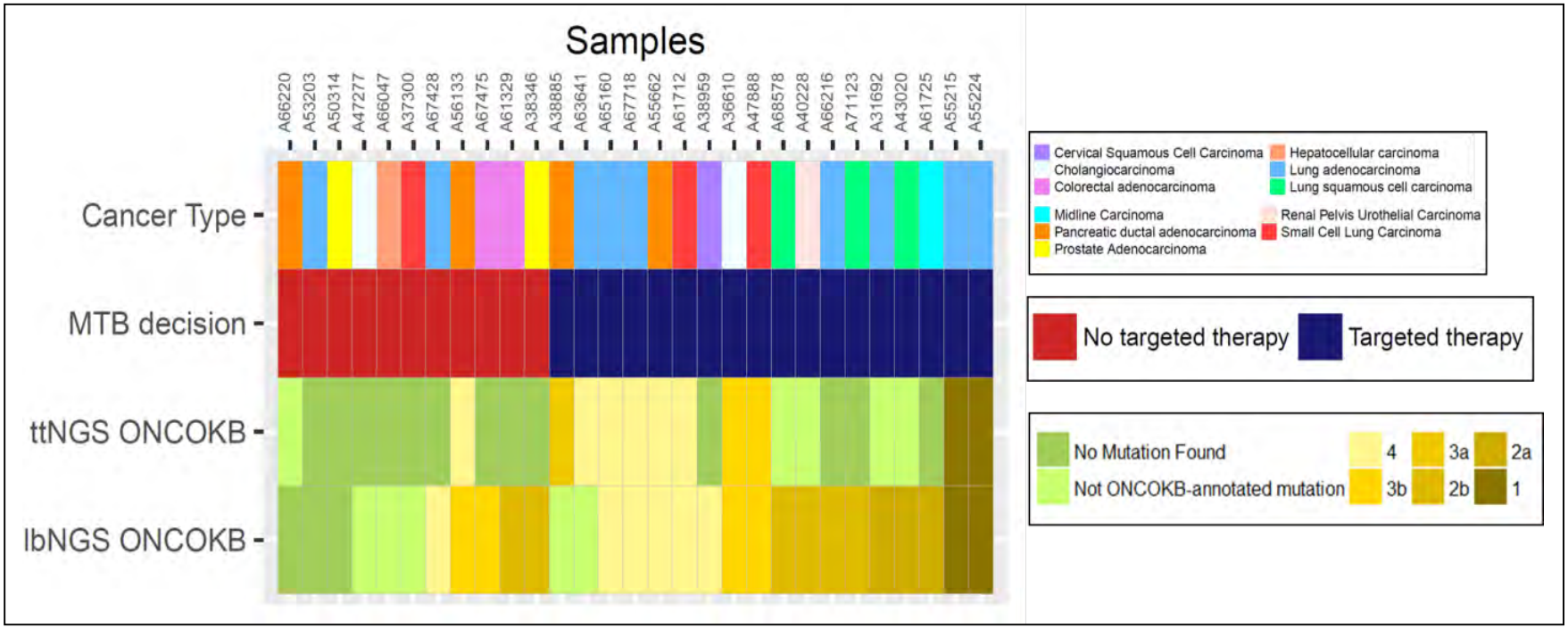




It looks like lbNGS can provide patients with alteration-driven treatment recommendations more effectively than ttNGS

Rolfo et al, ESMO Open August 2018

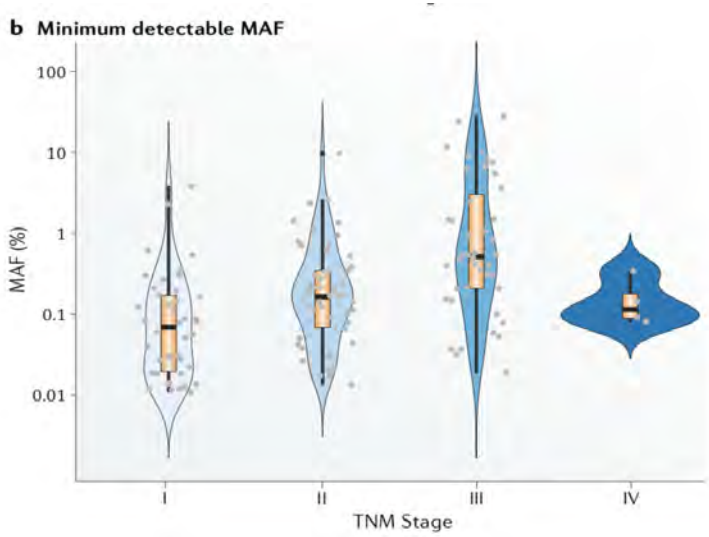
Effects of molecular tumor board and different NGS panels implementation for the treatment of patients with cancer.



Rolfo et al ESMO Open August 2018

Minimal Residual disease

The Role of Liquid Biopsy

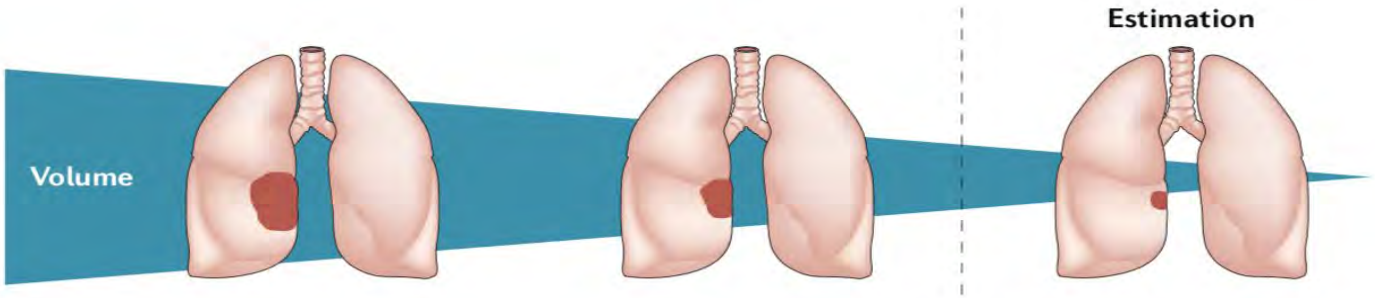


Minimum detectable mutant allele frequencies (MAFs) for 142 patients with detectable ctDNA, from a total of 301 patients analysed.

Technique (purpose)	Panel size (base pairs)	Enrichment technology	Stage I	Stage II	Stage III
CAPP-Seq (detection & MRD)	128 genes (188 kbp)	Hybridization	5/5 **(100 %)**	4/6 **(67%)**	20/21 **(95%)**
TEC-Seq (detection)	58 genes (80.9 kbp)	Hybridization	13/29 **(45%)**	23/31 **(74%)**	4/5 **(80%)**
CancerSEEK (detection)	16 genes (4.6 kbp)	Multiplex PCR	2/46 **(4%)**	10/26 **(38%)**	11/31 **(35%)**
TRACERx (MRD)	18 patient-specific SNV (1.5 kbp)	Multiplex PCR	22/37 **(59%)**	16/23 **(70%)**	8/14 **(57%)**

Abbosch, Birkbak & Swanton, Nat Rev Clin Oncol, Sep 2018
M. Tsao, WCLC 2018

Mutant allele frequency (MAF) in Early Stage NSCLC

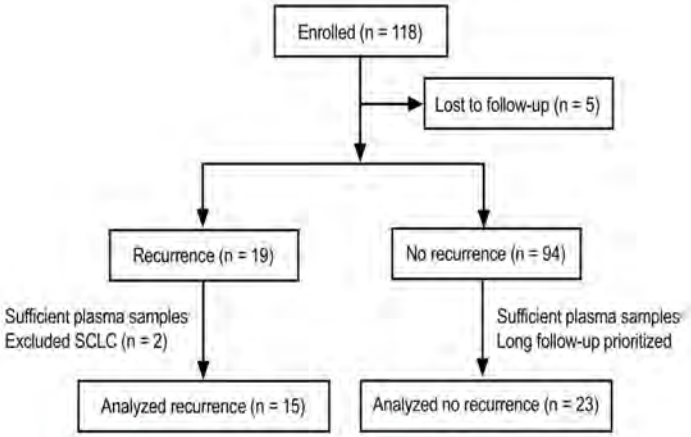


MAF	1.4% (0.62–3.1%)	0.1% (0.06–0.18%)	0.008% (0.002–0.03%)
Nodule diameter	5.8 cm	2.6 cm	1.2 cm
Nodule volume	100 cm^3	10 cm^3	1 cm^3
T stage	T3	T1c	T1b

Early detection of small NSCLC (<2 cm; T1a – T1b) using ctDNA will be limited by the technical and physical constraints of detecting mutations present at a low MAF (<0.1%).

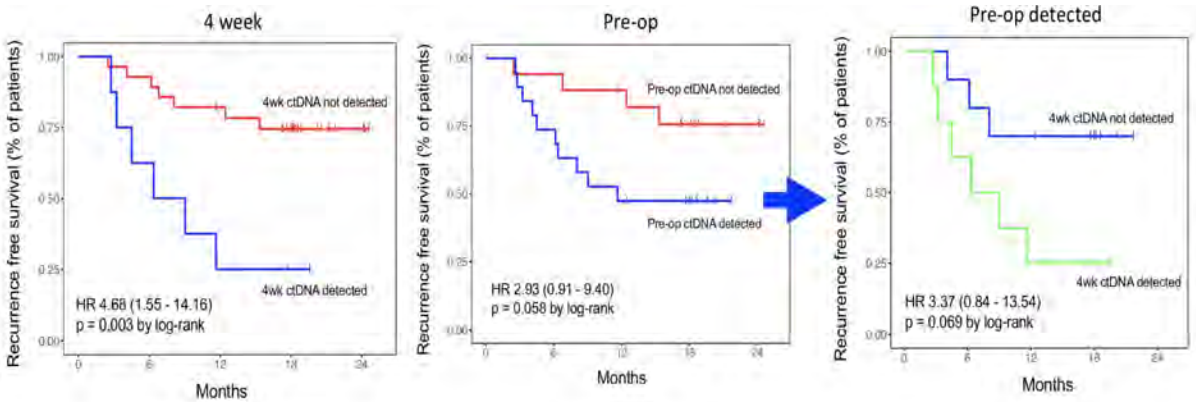
Abbosch, Birkbak & Swanton, Nat Rev Clin Oncol, Sep 2018

ctDNA detection at 4 weeks identifies high-risk pts



Patients with recurrence (39%)
Stage II/III (93%)
SCC (7%)
Neoadjuvant tx (40%)
PORT (33%)

Patients without recurrence (61%)
Stage I/II (96%)
SCC (22%)
Neoadjuvant tx (9%)
PORT (1%)



75% PPV / 75% NPV

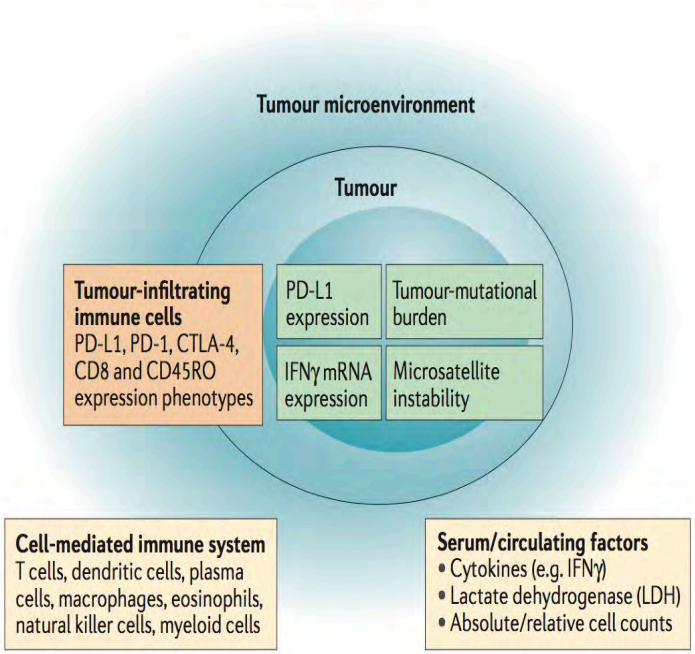
53% PPV / 77% NPV

- 4 week ctDNA detection has high accuracy for recurrence
- 4 week ctDNA detection associated with worse RFS in multivariate model accounting for stage, histology, neoadjuvant/adjuvant treatment (p = 0.01)

Lam V (Heymach) et al, WCLC 2018

Immunotherapy in Cancer

Liquid Biopsy and Immunotherapy in Cancer



Unmeet Medical Need:

Validated Biomarkers in Blood!

Potential Utility of Liquid Biopsy in Immunotherapy

- Diagnostic
- Prognostic
- Predictive of Response
- Monitoring
- Mechanisms if Resistance

Current tools:

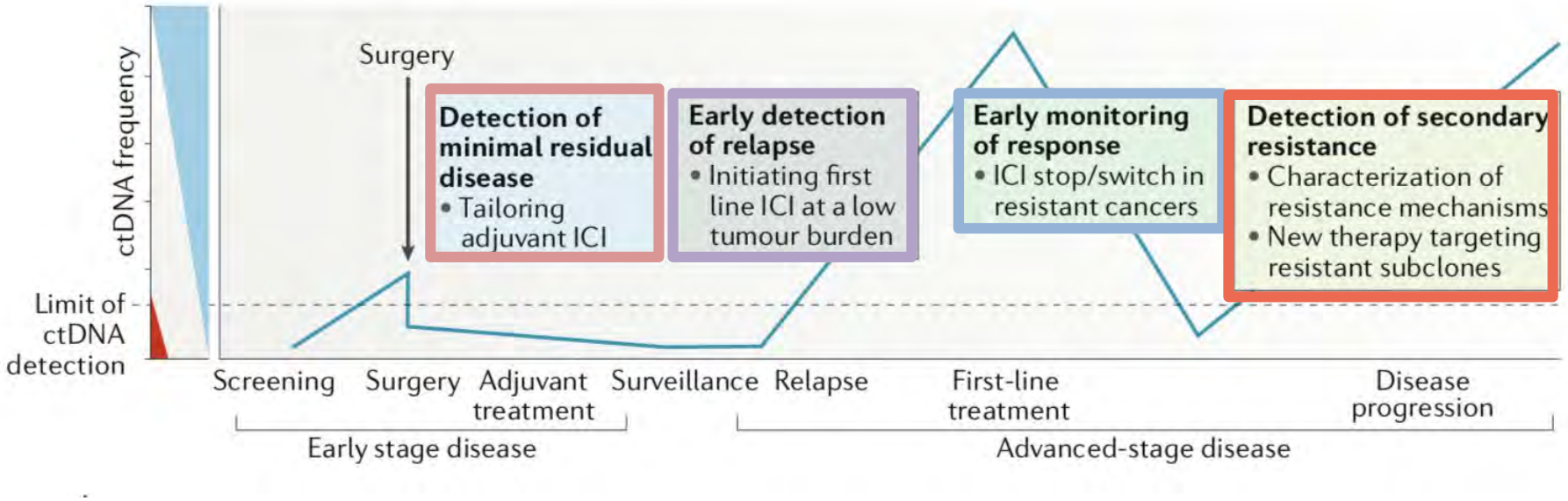
- Calculation of circulating TMB
- Detection of bPDL1
- Alellic Fraction Variation Dynamic

Liquid Biopsy in Immunotherapy is challenging!

A complex microenvironment

Image from Nishino et al, Nature Reviews Clinical Oncology, June 2017

Clinical Application of liquid biopsy in Immunotherapy

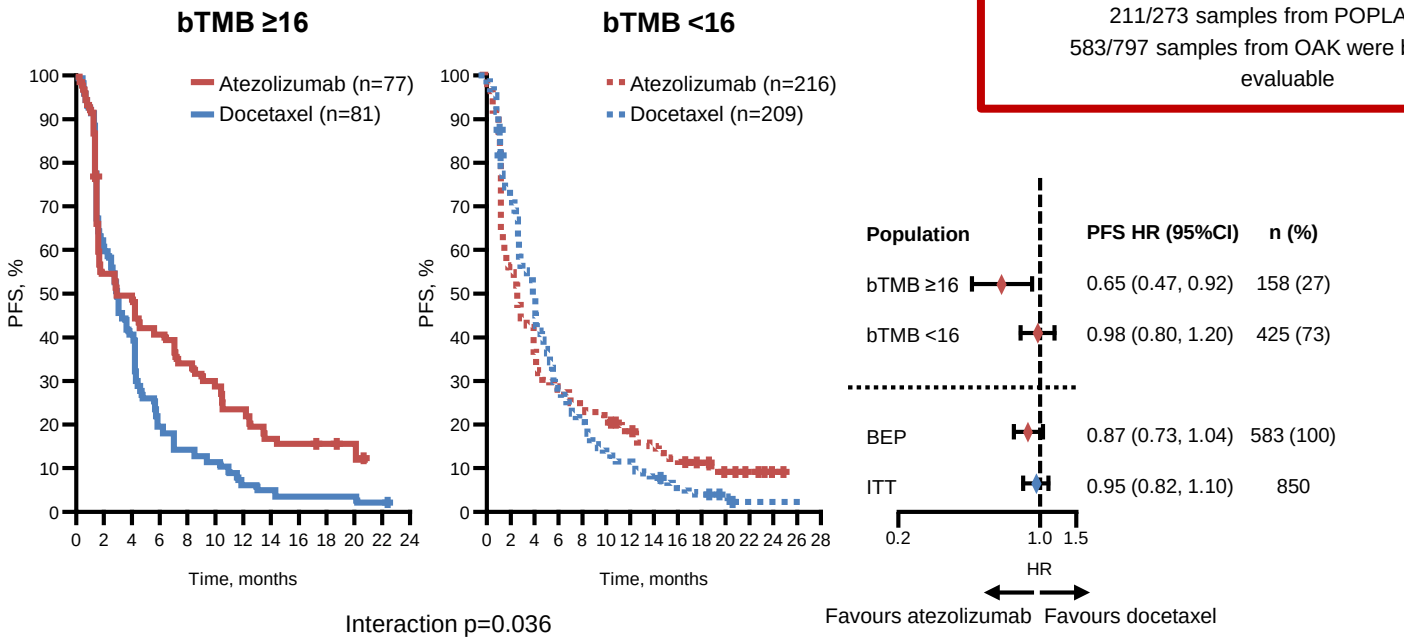


Not so easy!!

Cabel et al, Nature Rev Clin Onc, Oct 2018

Blood-based biomarkers for cancer immunotherapy: Tumor mutational burden in blood (bTMB) is associated with improved atezolizumab (atezo) efficacy in 2L+ NSCLC (POPLAR and OAK)

Atezolizumab PFS benefit in bTMB subgroups: OAK

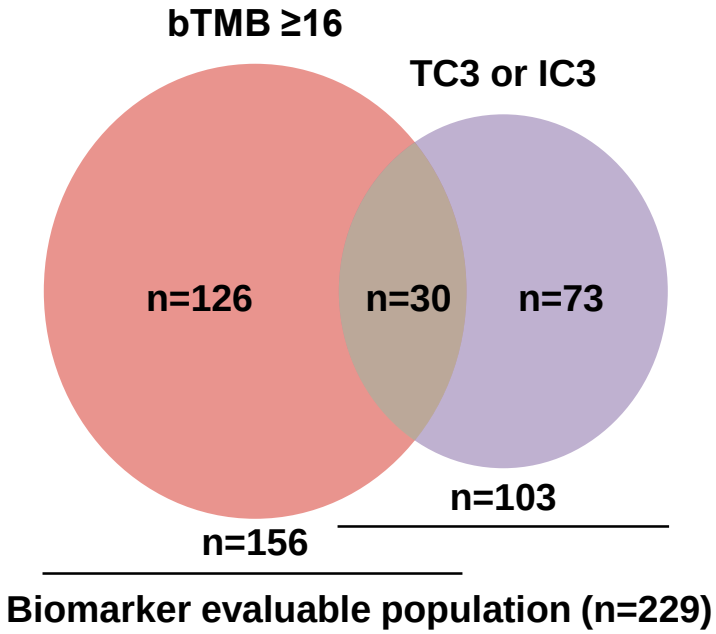


BEP, biomarker-evaluable population

Gandara DR et al. Ann Oncol 2017;28(suppl 5):Abstr 1295O

Blood-based biomarkers for cancer immunotherapy: Tumor mutational burden in blood (bTMB) is associated with improved atezolizumab (atezo) efficacy in 2L+ NSCLC (POPLAR and OAK)

Limited overlap between bTMB ≥16 and PD-L1 expression: OAK



	PFS HR (95%CI)	OS HR (95%CI)
bTMB ≥16	0.64 (0.46, 0.91)	0.64 (0.44, 0.93)
TC3 or IC3	0.62 (0.41, 0.93)	0.44 (0.27, 0.71)
bTMB ≥16 and TC3 or IC3	0.38 (0.17, 0.85)	0.23 (0.09, 0.58)

Gandara DR et al. Ann Oncol 2017;28(suppl 5):Abstr 1295O

Key Results

Conclusions

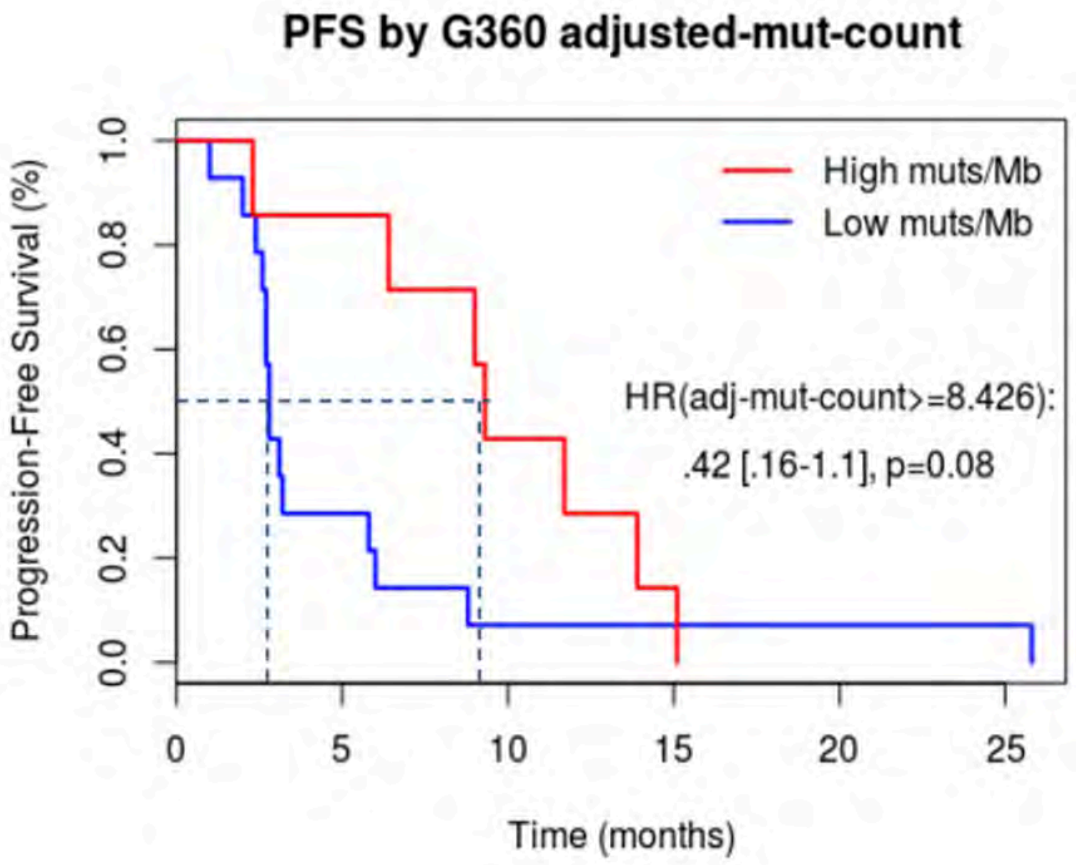
- This exploratory analysis demonstrated that TMB can be measured in blood
- The cut-point of bTMB ≥16 was identified in POPLAR, and independently validated to predict PFS benefit in OAK
- bTMB identified a unique patient population which was not significantly associated with PD-L1 status

Comments

- Great News

- The cut-point of bTMB ≥16 was is a real cut-off?
- Great News: to be validated
- No wildly applicable in clinical practice

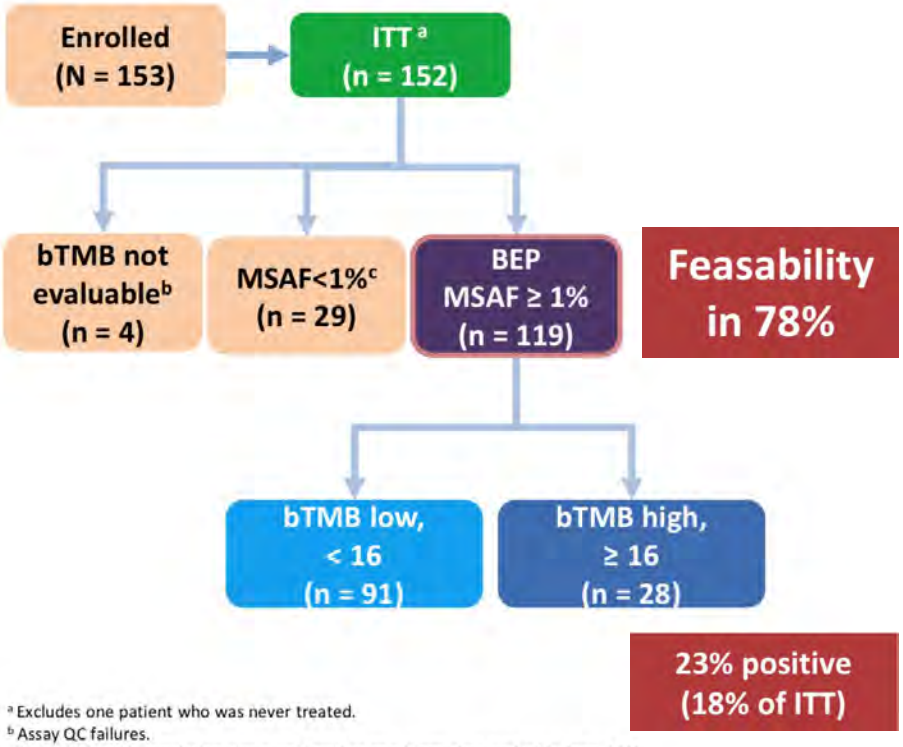
Digital Tumor Mutation Burden Predicts IO Response in NSCLC (top tertile vs. lower tertiles) 73 genes panel



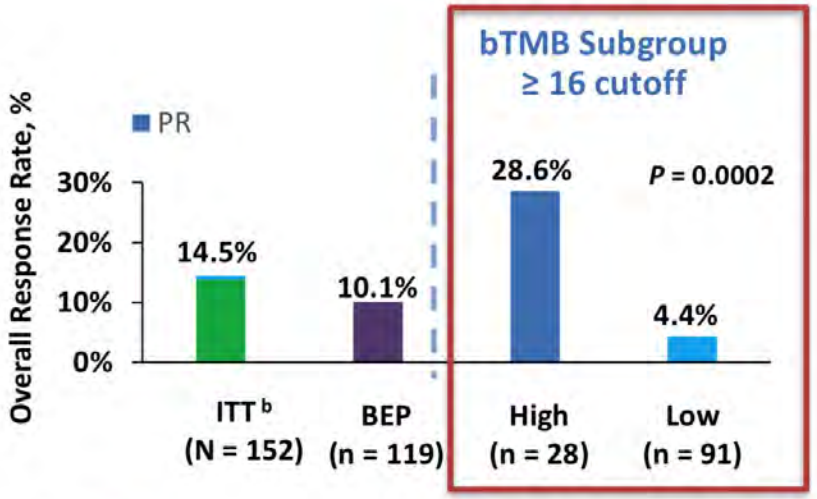
Grinberg (Peled) et al. 2018 Abstract ELCC, Geneva, Switzerland

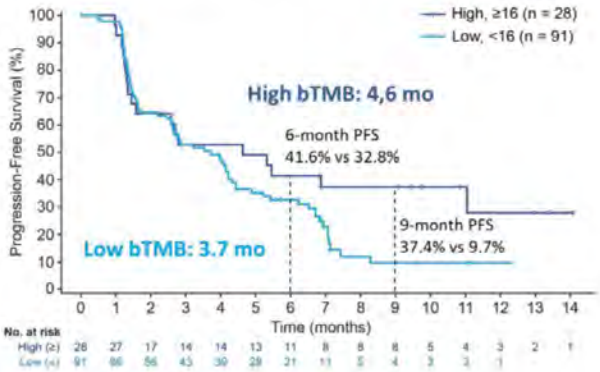
N = 27, 12 IO responders and 15 non-responders

B-F1RST :Blood-Based Tumour Mutational Burden as a Biomarker of Atezolizumab Activity in First-Line NSCLC Treatment



[a] Excludes one patient who was never treated.
[b] Assay QC failures.
[c] The MSAF < 1% population was considered as non-biomarker evaluable (non-BEP).

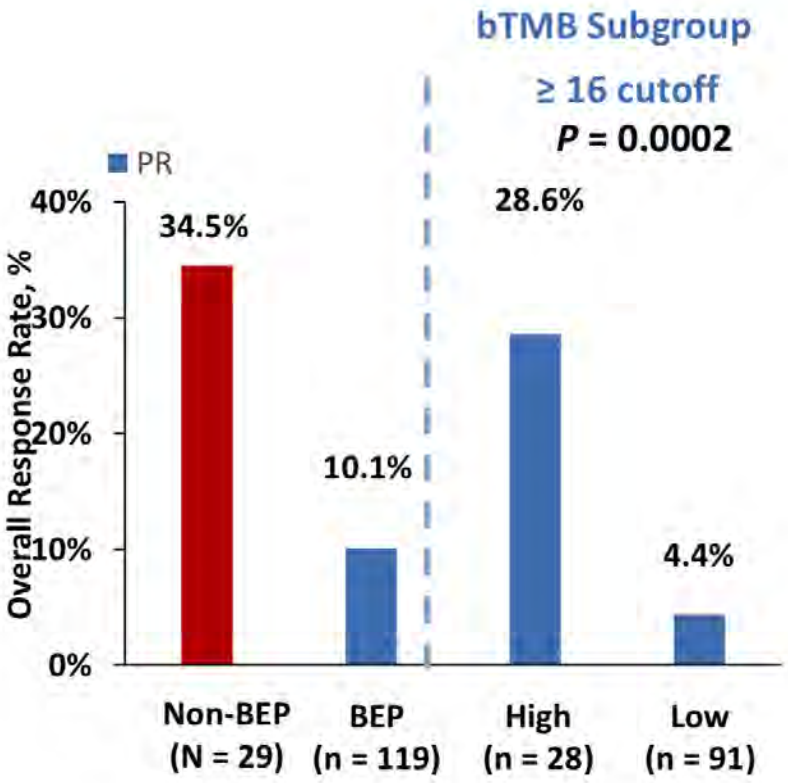


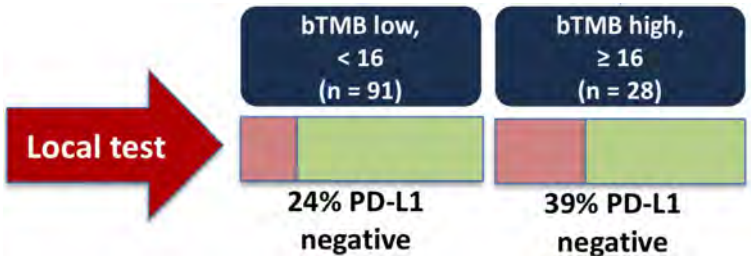


Kim ESMO 2018

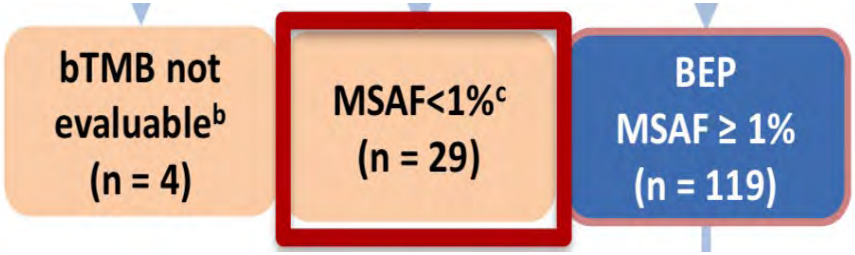
Adapted from B. Besse, ESMO 2018

B-F1RST: strengths and weaknesses





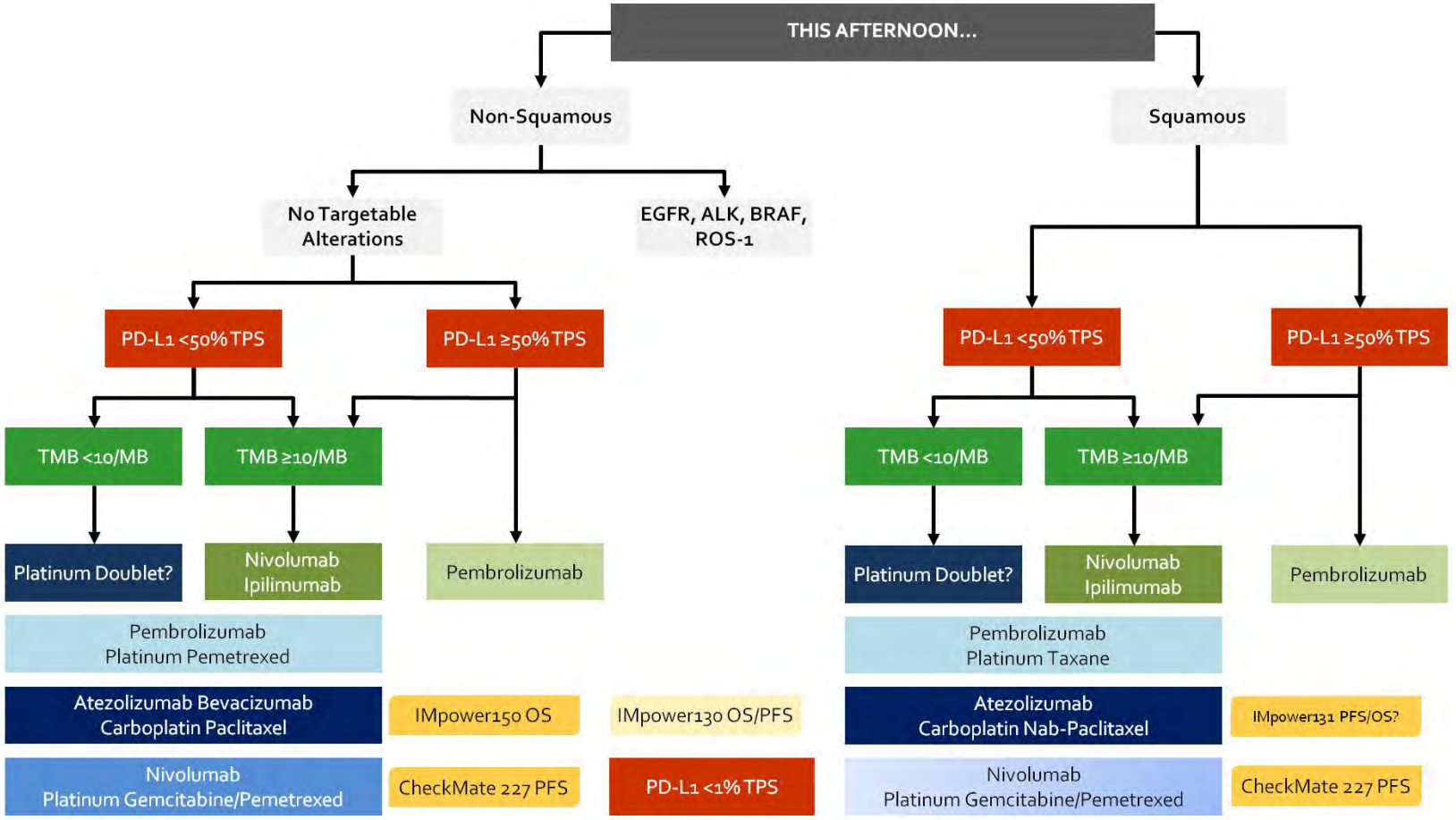
Major limitations No tissue collection No central PD-L1 testing No tissue TMB



Median overall survival (OS) was not estimable (NE) in patients with blood TMB high compared to 13.1 months in blood TMB low patients, HR 0.77; 90% CI, 0.41 – 1.43 (p = 0.48).

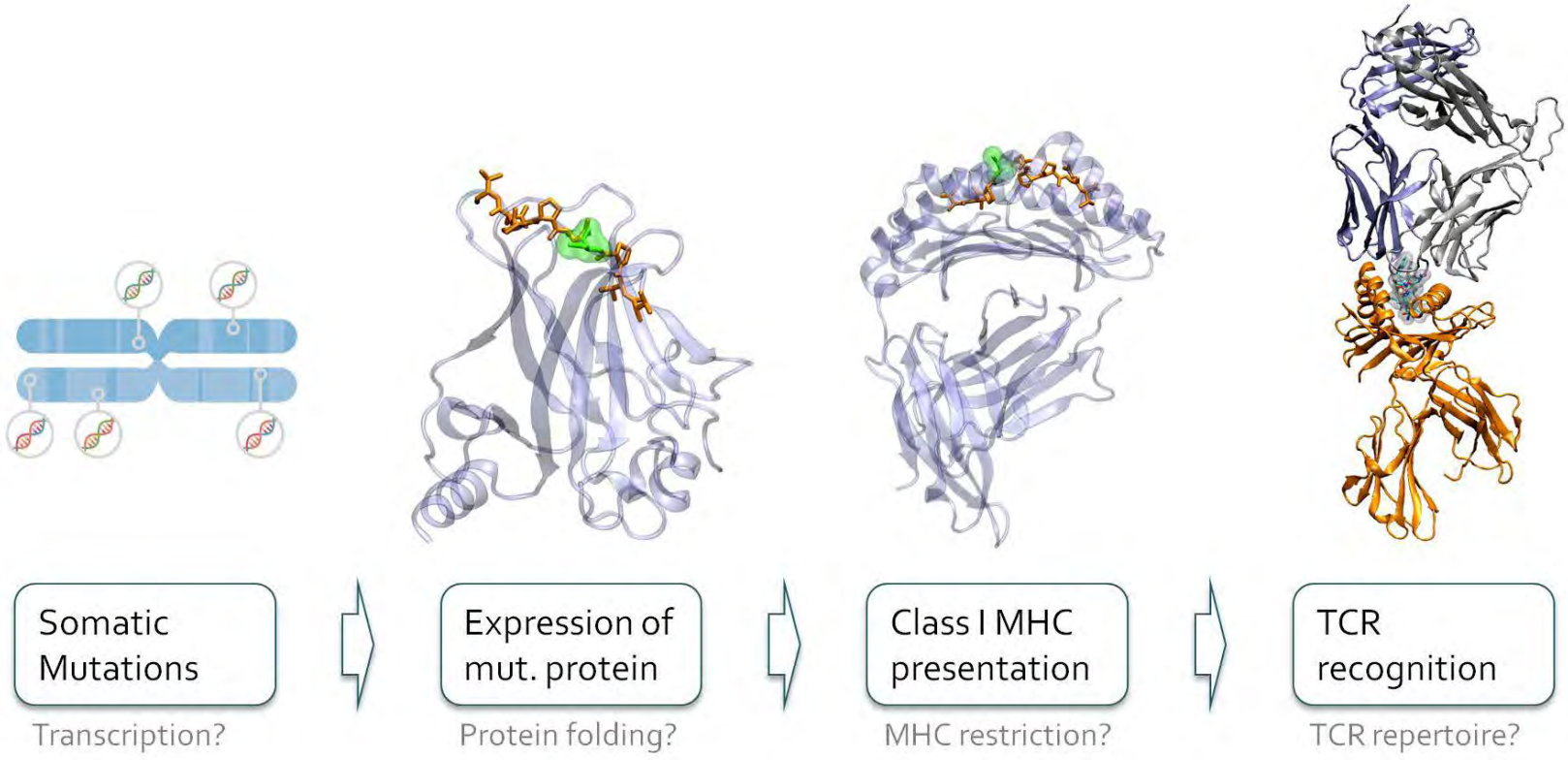
LOW TUMOR BURDEN! LESS REPLICATIVE? IS MASF<1% THE BEST PREDICTIVE MARKER?

Adapted from B. Besse, ESMO 2018


THIS AFTERNOON…
Non-Squamous
Squamous
No Targetable Alterations
EGFR, ALK, BRAF, ROS-1
PD-L1 <50% TPS
PD-L1 ≥50% TPS
PD-L1 <50% TPS
PD-L1 ≥50% TPS
TMB <10/MB
TMB ≥10/MB
TMB <10/MB
TMB ≥10/MB
Platinum Doublet?
Nivolumab Ipilimumab
Pembrolizumab
Platinum Doublet?
Nivolumab Ipilimumab
Pembrolizumab
Pembrolizumab Platinum Pemetrexed
Pembrolizumab Platinum Taxane
Atezolizumab Bevacizumab Carboplatin Paclitaxel
IMpower150 OS
IMpower130 OS/PFS
Atezolizumab Carboplatin Nab-Paclitaxel
IMpower131 PFS/OS?
Nivolumab Platinum Gemcitabine/Pemetrexed
CheckMate 227 PFS
PD-L1 <1% TPS
Nivolumab Platinum Gemcitabine/Pemetrexed
CheckMate 227 PFS

Quantity or quality of mutations?

Present antigents is the matter…



S. Peters at 2018 ASCO

Mutational Load

Mutation Load
Analysis Report

Mutation Load per MB: 79.73

Analysis

B810145_C_T_v7_88998a78-1907-4e0d-89d0-fe74354f29e7

Ion Reporter Version	Launched by	Launched on	Workflow
5.6	Ion User	December 13, 2017 11:26 PM	Oncomine Tumor Mutation Load - w1.0 - DNA - Single Sample r.0

Annotations	Reference
All r.0	Oncomine Tumor Mutation Load Hotspots v1.0,Oncomine Tumor Mutation Load Regions v1.0,hg19

Samples

B810145_C_T_v7

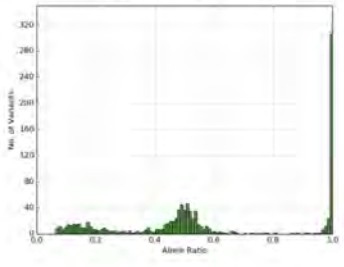
Gender	Relationship	Chip Type	Sample Type
Unknown	Proband	540	DNA

QC Metrics

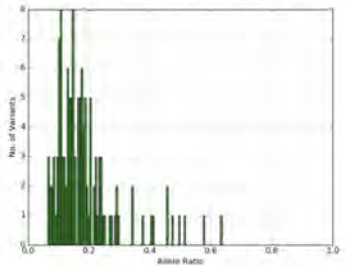
Average Coverage	Total Variants Called	Estimated SNP proportion consistent with Deamination (mainly FFPE)
583.0	1,064	20

Analysis Results

Germ-line and Somatic Variants: 1,064

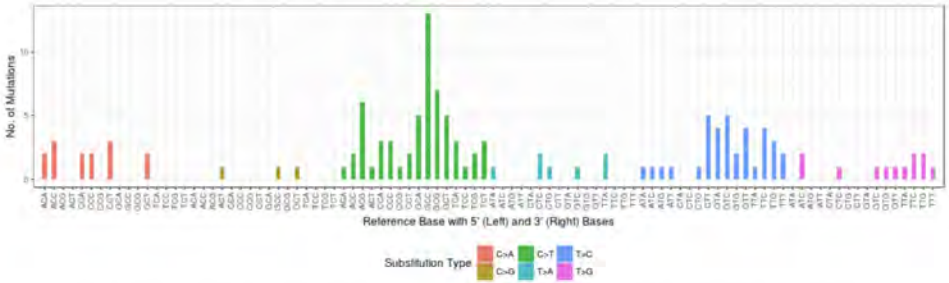


Only Somatic Variants: 129
Nonsynonymous: 65: Synonymous: 64

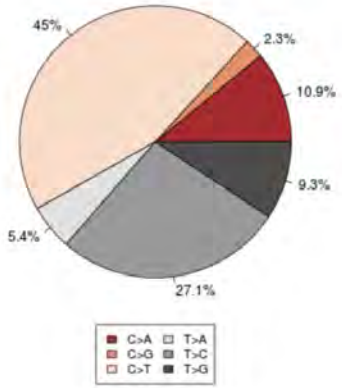


Number of Somatic Variants Present in COSMIC: 14

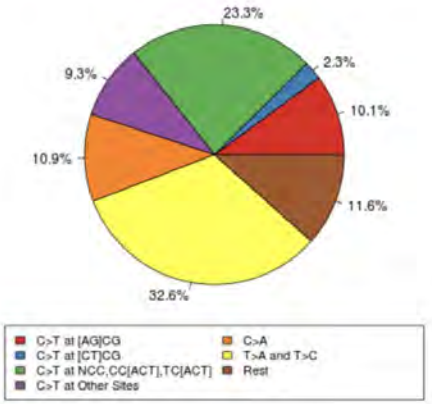
Substitution Type and Context of Somatic Mutations



Substitution Type of Somatic Mutations



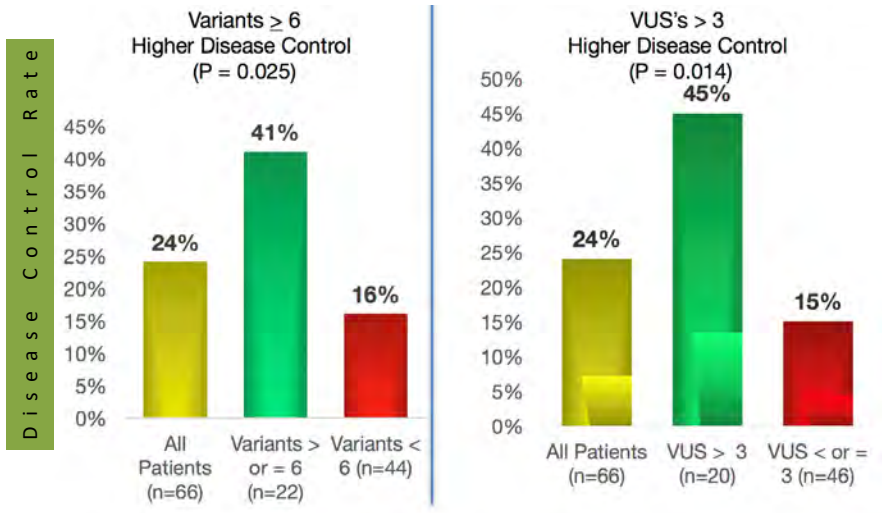
Signature Pattern of Somatic Mutations



Oncomine sample report

Hypermutated Circulating Tumor DNA

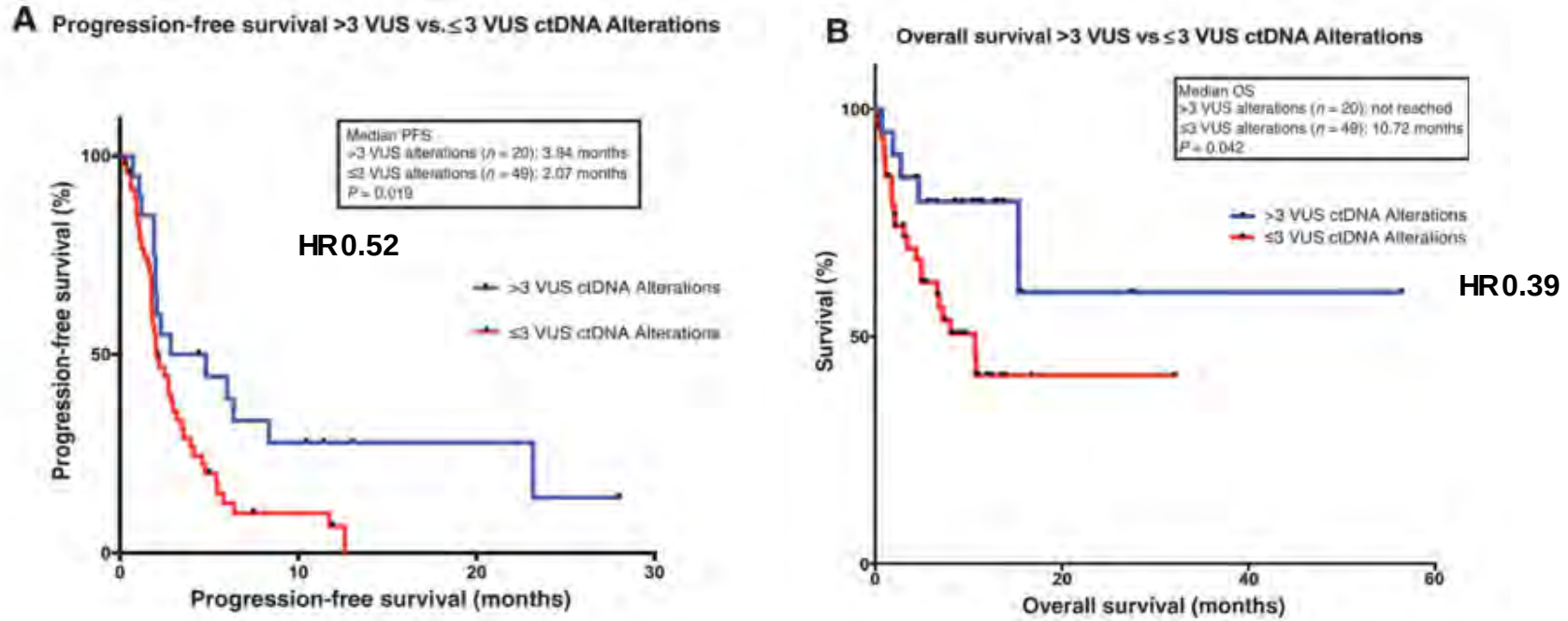
Hypermutated Circulating Tumor DNA: Correlation with Response to Checkpoint Inhibitor–Based Immunotherapy



Khagi (Kurzrock) et al. Oct 2017 Clinical Cancer Research

Disease Control Rate: CR+ PR + SD

HYPERMUTATED CIRCULATING TUMOR DNA



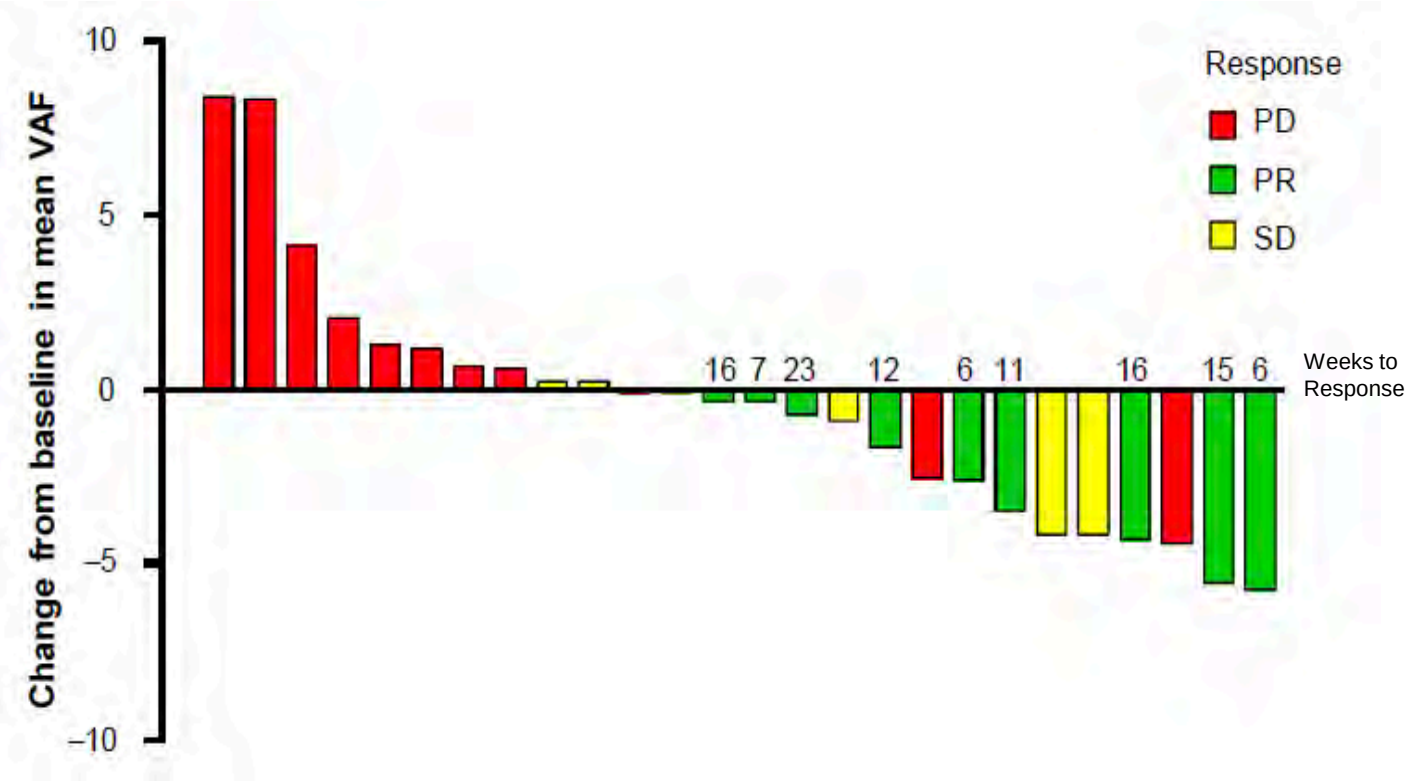
In patients undergoing therapy with IO a higher amount of mutations was associated with a better PFS and OS

Khagi (Kurzrock) et al. 2017 Clinical Cancer Research

Monitoring ctDNA during immune-checkpoint inhibition in patients with metastatic cancer

n	Detection method	Patients with detectable ctDNA at baseline (%)	Timing of second blood sample	Criteria used for ctDNA response during therapy	Correlation with PFS	Correlation with OS
Non-small-cell lung cancer						
23	Targeted NGS	65	8 weeks	ctDNA <0.006 ng/μl vs >0.006 ng/μl	Median not reached vs 1.8 months; $P=0.003$	Median not reached vs 2.2 months; $P=0.044$
28	Targeted NGS	Focus on patients with detectable ctDNA	Serial (every other week)	>50% decrease in ctDNA level vs <50% decrease (in 2 consecutives samples)	HR 0.2; $P=0.03$	HR 0.13; $P=0.0034$
28	Targeted NGS	NA	8 weeks	Increased VAF vs decreased VAF	NA	NA
14	Targeted NGS	50	2 weeks	Increased VAF vs decreased VAF (2 weeks)	NA	NA

“ctDNA Velocity”: Change in ctDNA Allele Fractions at 6 weeks Predicts IO Response in NSCLC

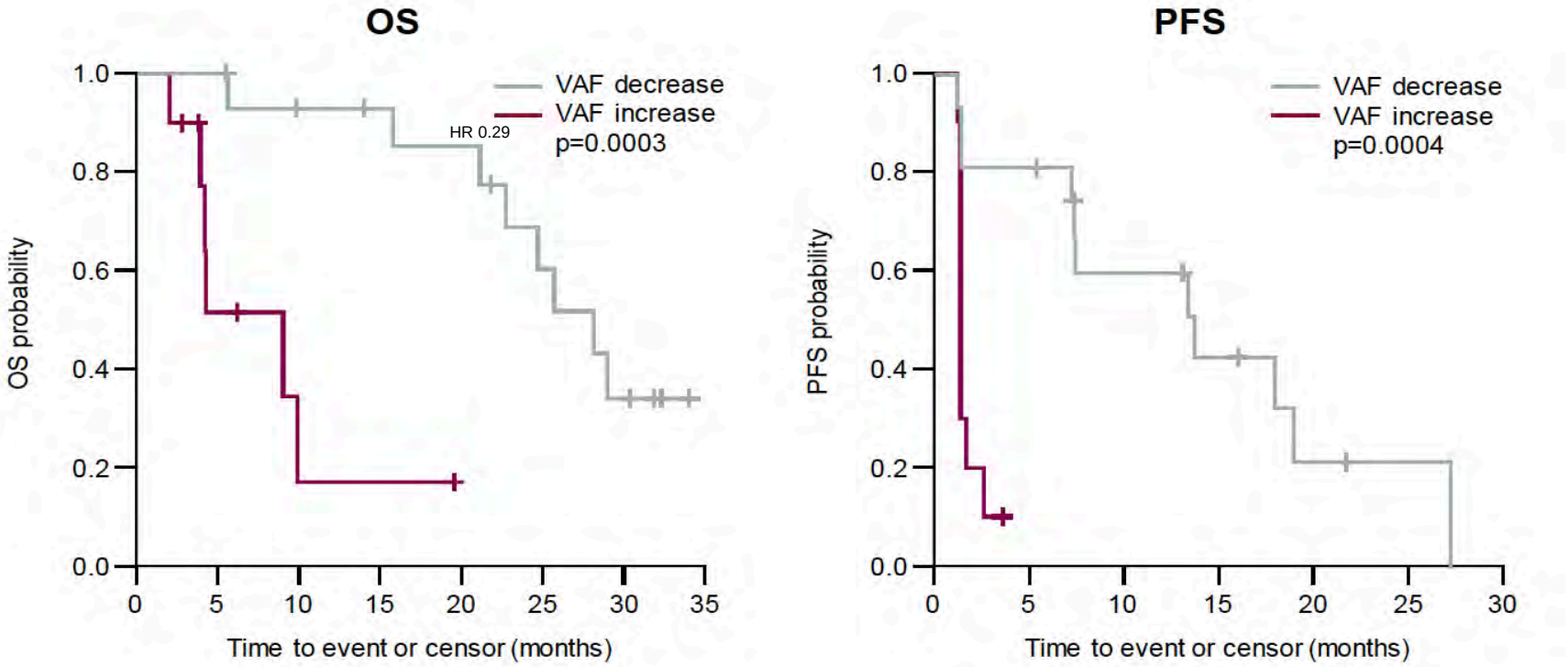


The delta in variant allele fractions (VAF) was calculated by subtracting the mean VAF pre-dose from the mean VAF post-dose. VAF decreased in 9/9 PR patients and 4/6 SD subjects. The time (in weeks) to investigator determination of PR response is shown.

Kuziora (Ranadne) et al. 2017 Abstract AACR

A Decrease in Mean VAF After 6 Weeks of Durvalumab Treatment was Associated with Improved OS and PFS

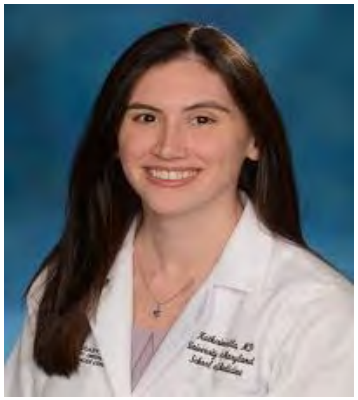
"ctDNA Dynamics": Change in ctDNA Allele Fractions at 6 weeks Predicts IO Response in NSCLC



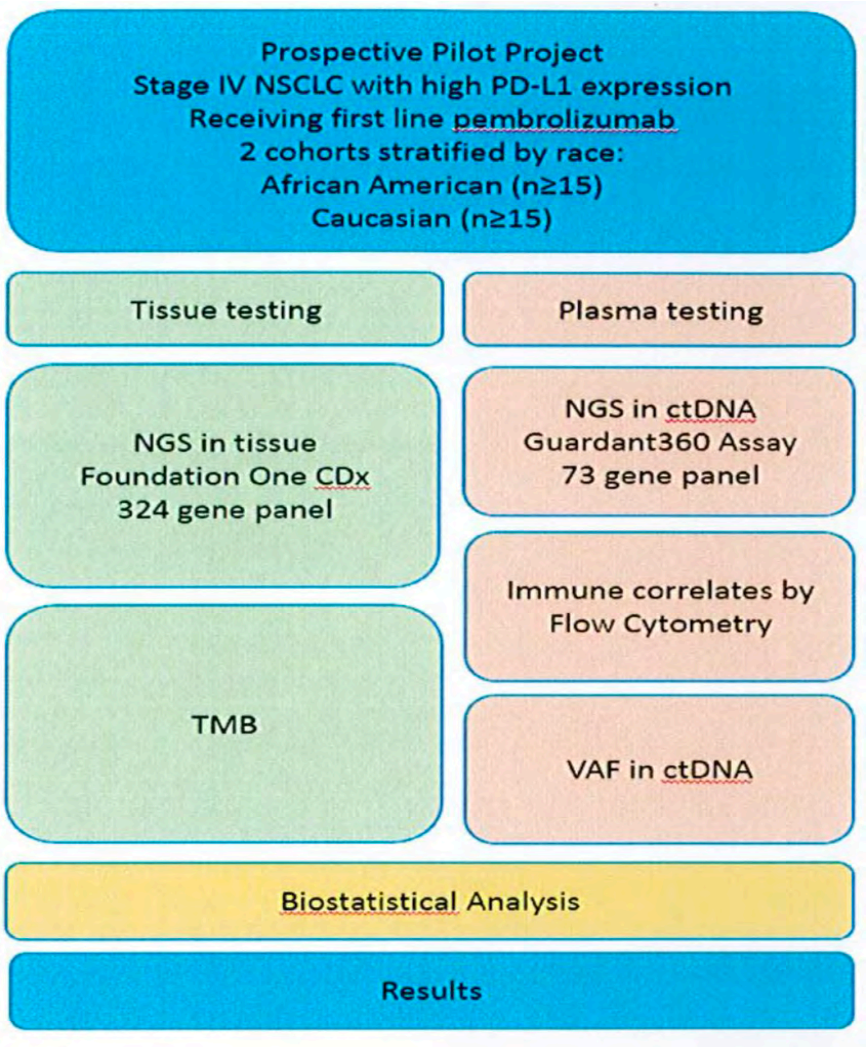
Kuziora (Ranade) et al. 2017
Abstract 582 AACR

UNIVERSITY of MARYLAND
MARLENE AND STEWART GREENEBAUM
COMPREHENSIVE CANCER CENTER

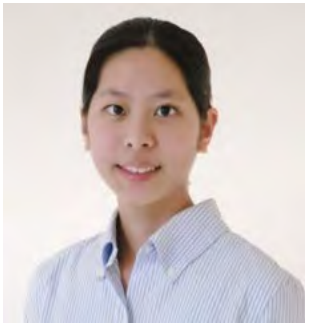
Immunologic Differences by Race among Stage IV Non-small Cell Lung Cancer Patients treated with First Line Immunotherapy



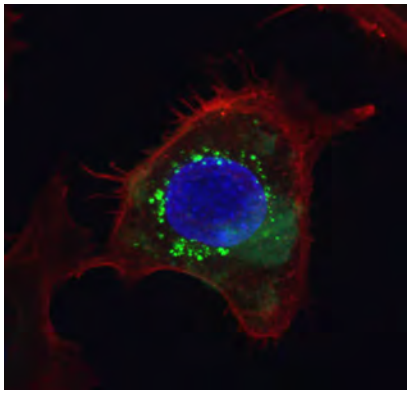
Dr. Katherine Scilla



Exosomes in lung cancer

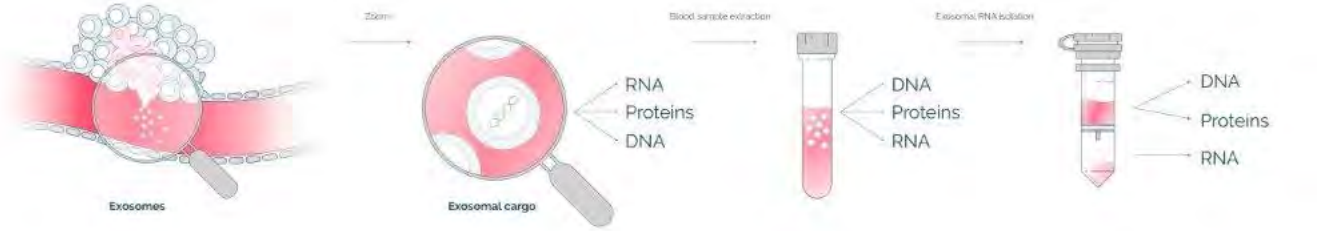


Elizabeth Chang, PhD

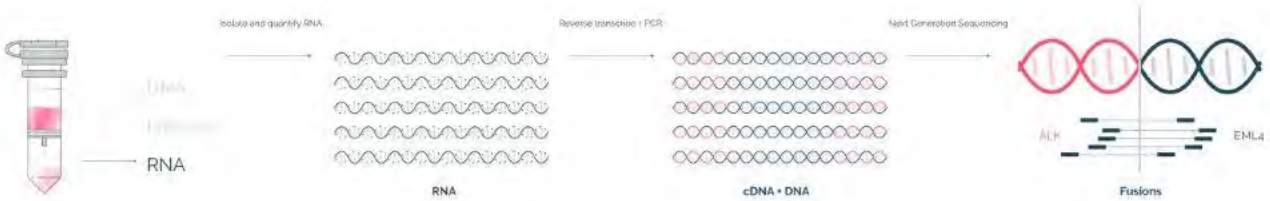


S. Taverna, M. Giallombardo, (C. Rolfo) . Oncotarget., 2016 May 10;7(19):28748-60
Fig. unpublished P.Reclusa (Rolfo Lab)

EML4-ALK translocation identification in RNA exosomal cargo (*ExoALK*) in NSCLC Patients: a novel role for liquid biopsy



Pablo Reclusa



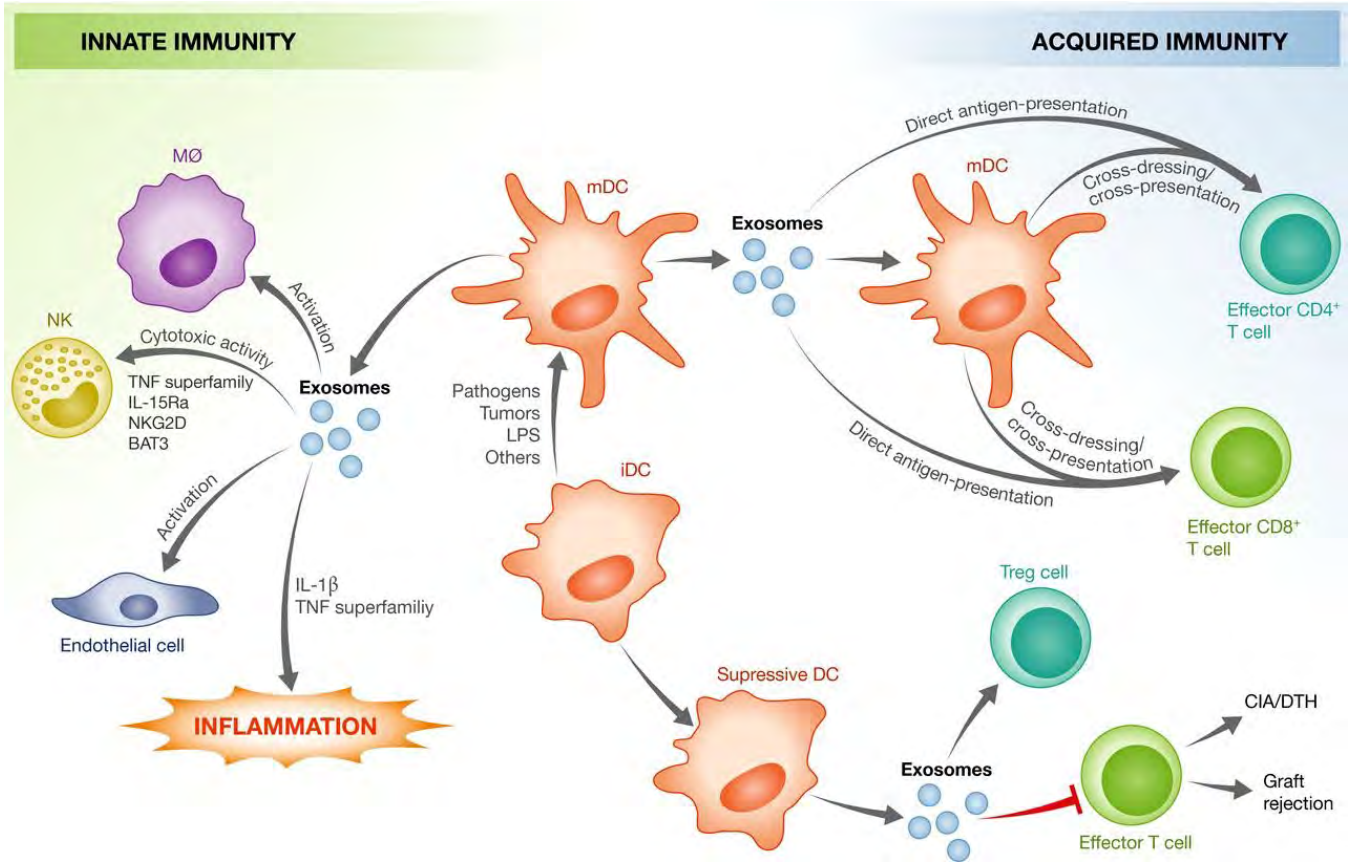
	ALK-EML4 FISH detection in Tissue (n=19)	
	Positive (16)	**Negative (3)**
Positive (9)	9	0
Negative (8)	5	3
	Sensitivity 64%	**Specificity 100%**

The concordance between tissue and exosomes was 63% (9 / 16 patients). All three patients being negative for the fusion gene in tissue resulted also negative in the *ExoALK* analysis, representing a specificity of 100%.

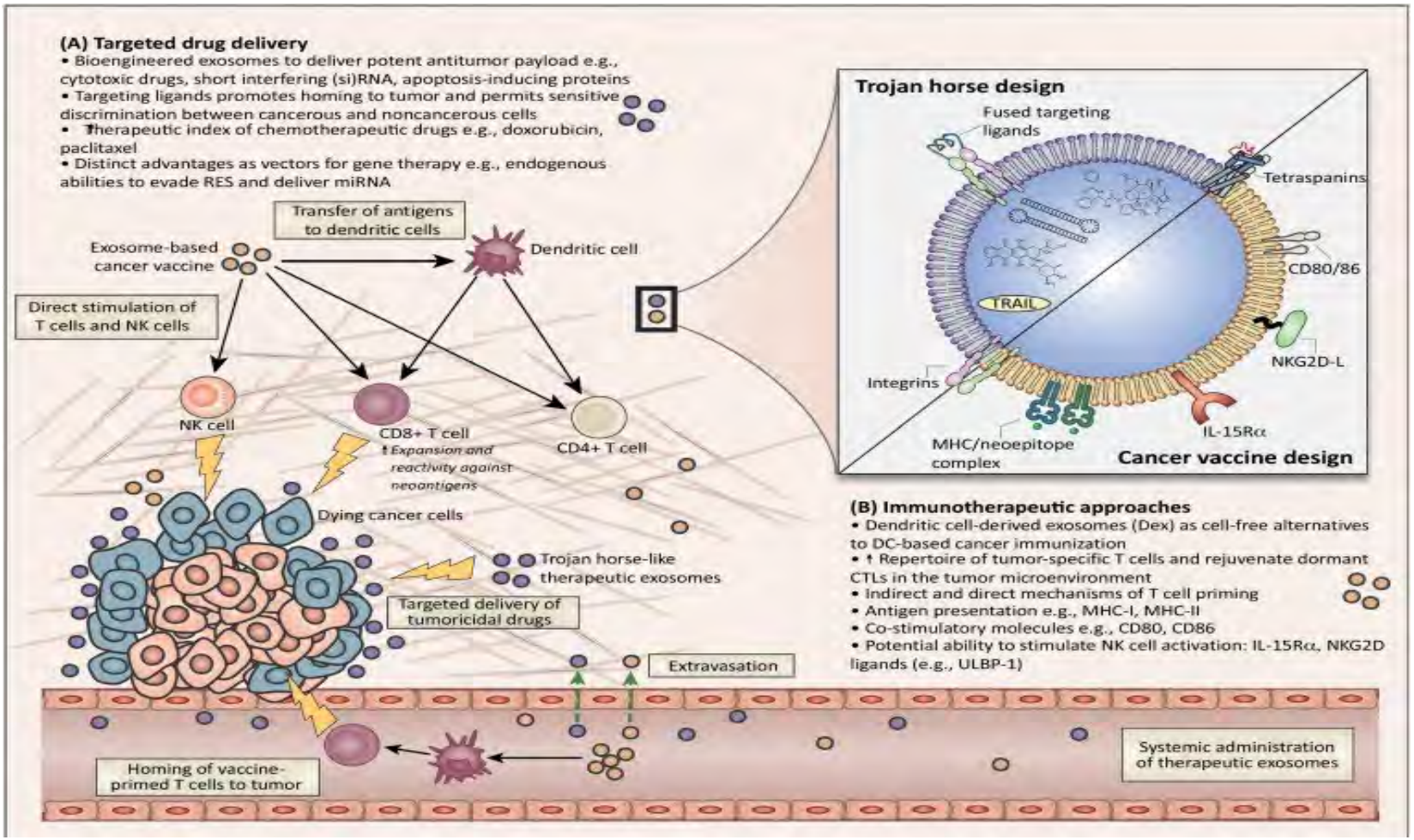
Reclusa (Rolfo) et al, Translational Cancer Research,In Press Nov 2018

Immune-response

Muthukumar Gunasekaran, PhD



Exosomes in IO: potential therapeutic implication



Trends in Biotechnology 2017 Jul;35(7):665-676

Take home message

- Liquid biopsy are entering in our clinicla practice in oncology
  Important tool in NSCLC, as a non invasive method.
- Free tDNA nowdays have a high concordance with tissue and more easy.
- LB Immunoterapy: several questions to be answered: correlation with tumor, standarize isolation, mutations.
- Exosomes represents a step forward with multiple possibilities for clinical application
- More trials grants, academia, cooperative groups and pharma efforts are needed.

Liquid biopsy Program
University Antwerp & University Maryland


Dr. Simona Taverna


Pablo Reclusa


Dr Karen Zwaenepoel

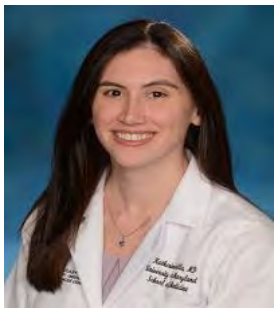

Laure Sorber


Prof. Patrick Pauwels


Prof. Rena Lapidus

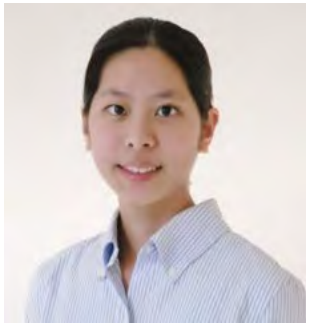

Dr. Colleen Damcot


Prof. Nick Ambulos


Dr. Katherine Scilla


Allison Gittens, RN,Onc


Muthukumar Gunasekaran, PhD


Elizabeth Chang, PhD


Brandon Carter, Cooper, BsC

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Thanks

christian.rolfo@umm.edu