UNIVERSITY of MARYLAND
SCHOOL OF MEDICINE

Liquid Biopsy
From Target to Immunotherapy

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MARLENE AND STEWART GREENEBAUM
COMPREHENSIVE CANCER CENTER

ISLB
INTERNATIONAL SOCIETY
OF LIQUID BIOPSY

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

DISCLOSURE INFORMATION

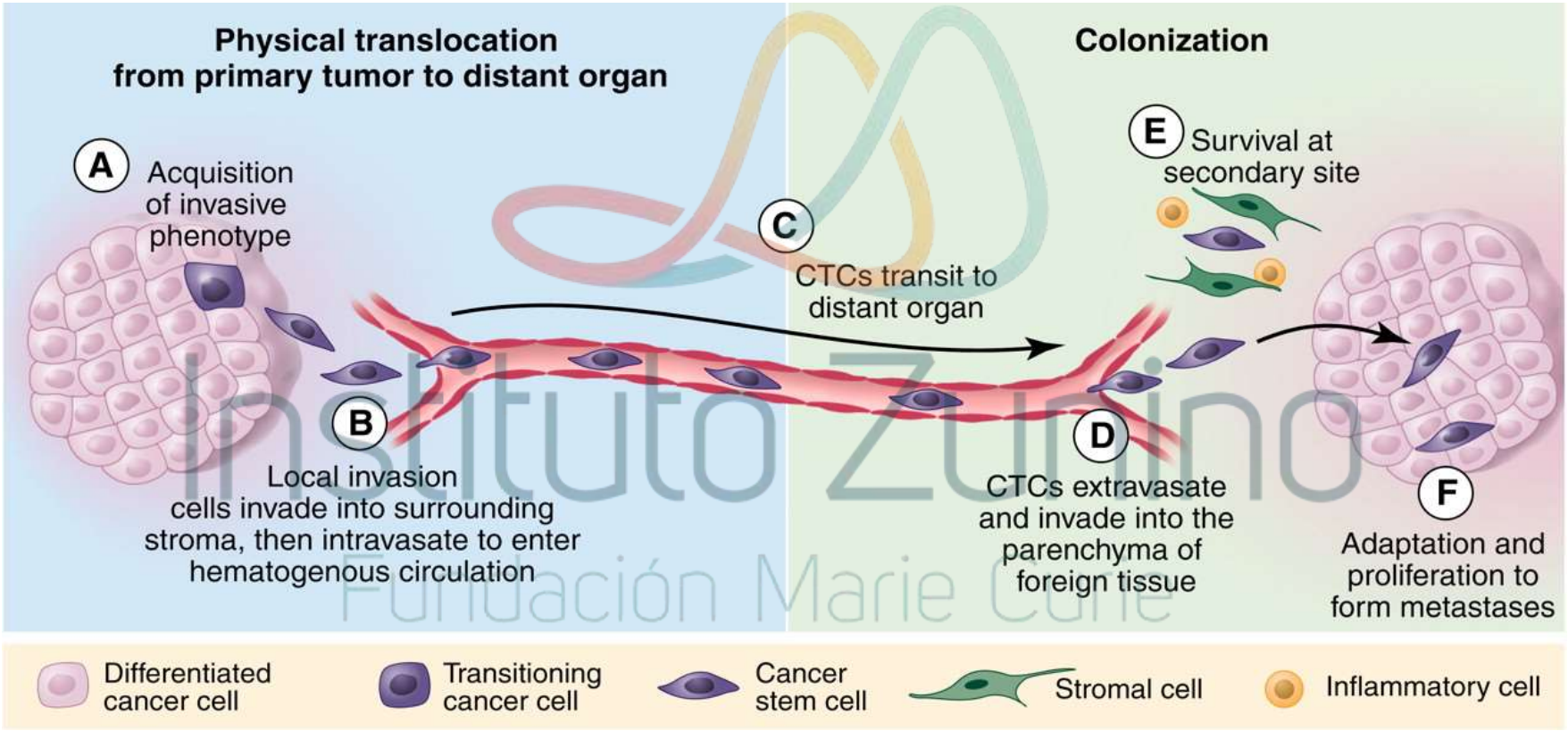
- **Personal financial interests**
  Speaker bureau: MSD, Novartis, GuardantHealth; Scientific advisor: Mylan

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

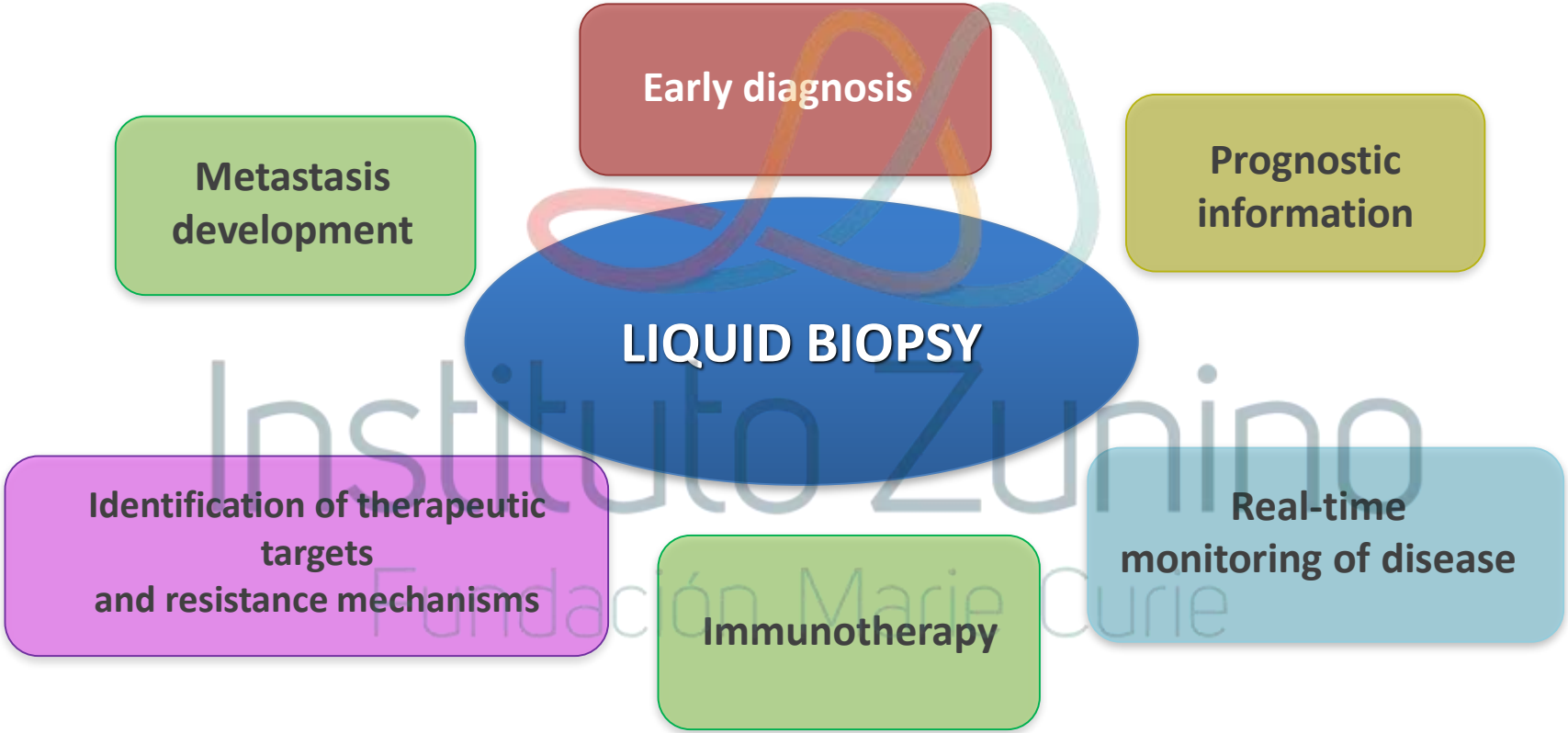
- **Non-financial interests**: Oncompass Steering scientific committee;
  OncoDNA: Research collaboration no remunerated for Exosomes (2017)

- **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

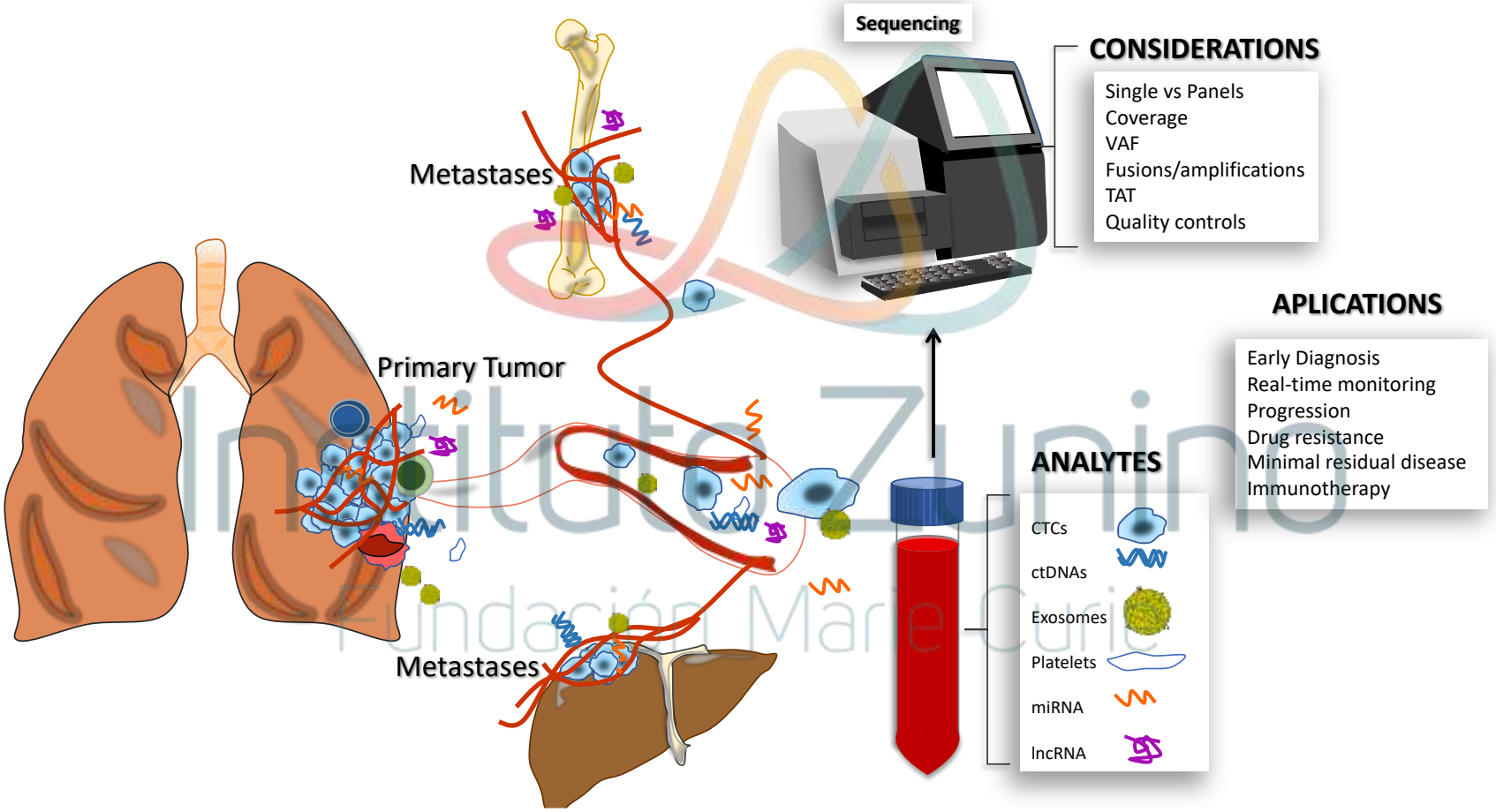


Liquid Biopsy: clinical application



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

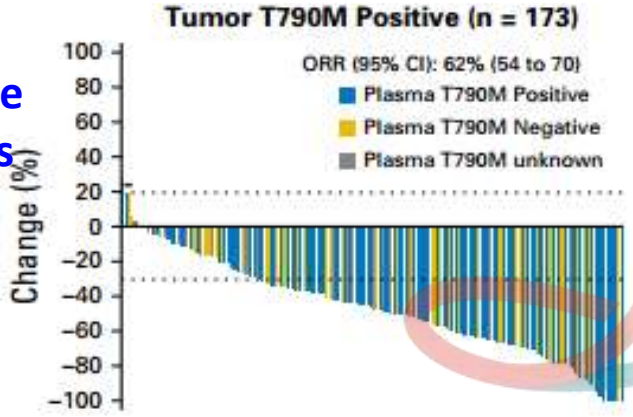
Some liquid Biopsy components



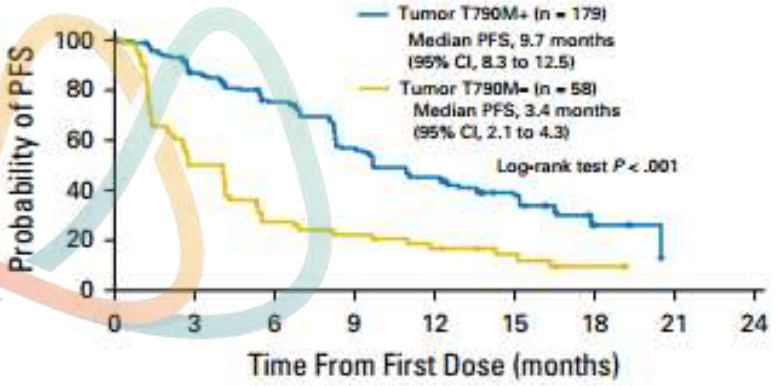
Scilla K & Rolfo C. Current Treatment Options in Oncology in press 2019

RR to Osimerinib according to T790M in plasma or tumor tissue

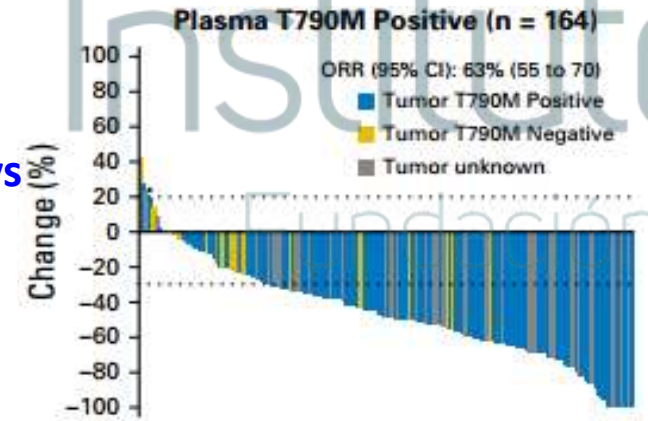
Tumor tissue
ORR: 62% vs 26%



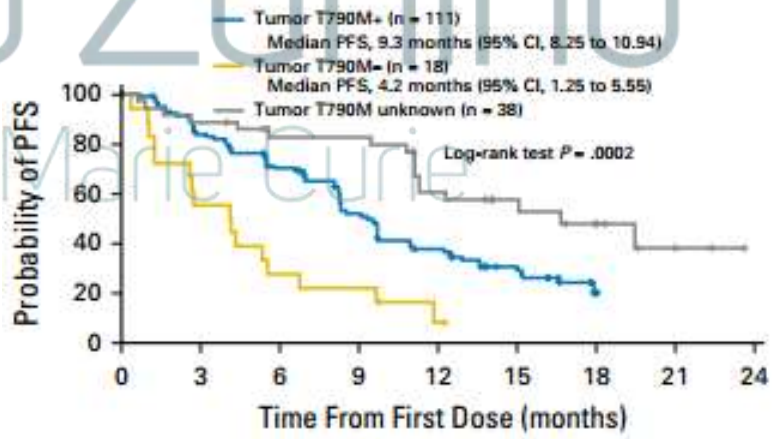
Tumor T790M+ vs T790M-



Plasma
ORR: 63% vs 46%



PlasmaT790M+ by tissue status



Oxnard, JCO 2016

Oxnard, JCO 2016

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

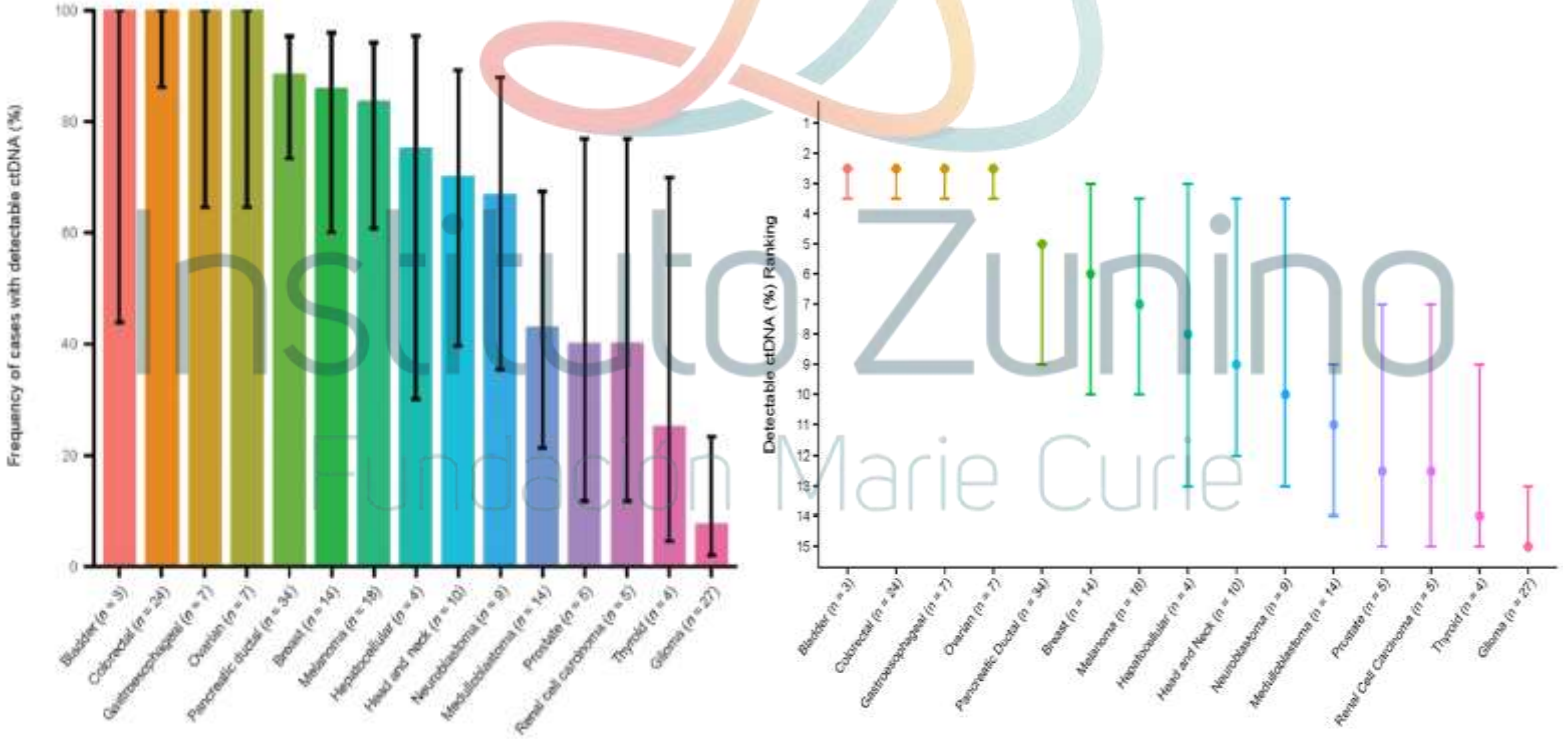
Christian Rolfo, MD, PhD, MBA,[a] Philip C. Mack, PhD,[b] Giorgio V. Scagliotti, MD, PhD,[c] Paul Baas, MD, PhD,[d] Fabrice Barlesi, MD, PhD,[e] Trever G. Bivona, MD, PhD,[f] Roy S. Herbst, MD, PhD,[g] Tony S. Mok, MD,[h] Nir Peled, MD, PhD,[i] Robert Pirker, MD,[j] Luis E. Raez, MD,[k] Martin Reck, 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] Ignacio I. Wistuba, MD,[r] Murry W. Wynes, PhD,[s] David P. Carbone, MD, PhD,[t] Fred R. Hirsch, MD, PhD,[u,*] David R. Gandara, MD[b]

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

SPECIAL CONSIDERATIONS...

Liquid biopsy: ctDNA

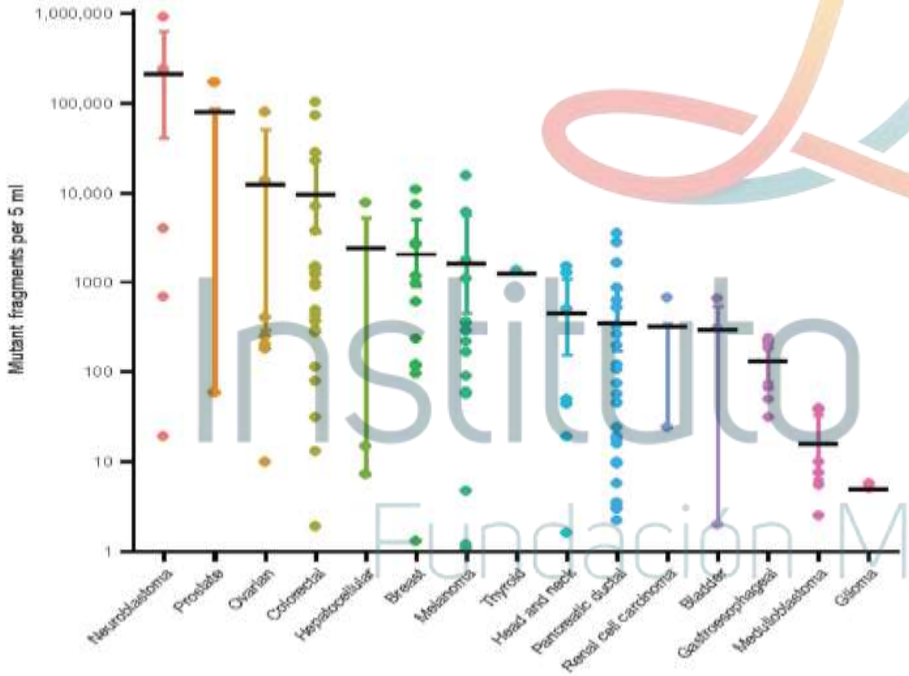
Does different tumor types release the same amount of DNA in the blood?

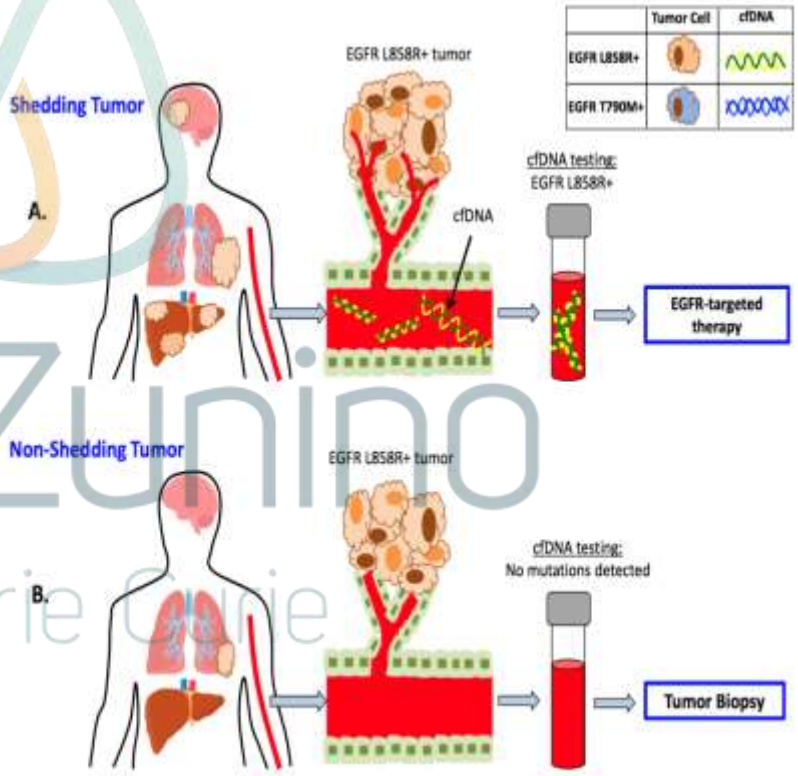


Bettegowda et al., Sci Trans Med, 2014

Liquid biopsy: ctDNA

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

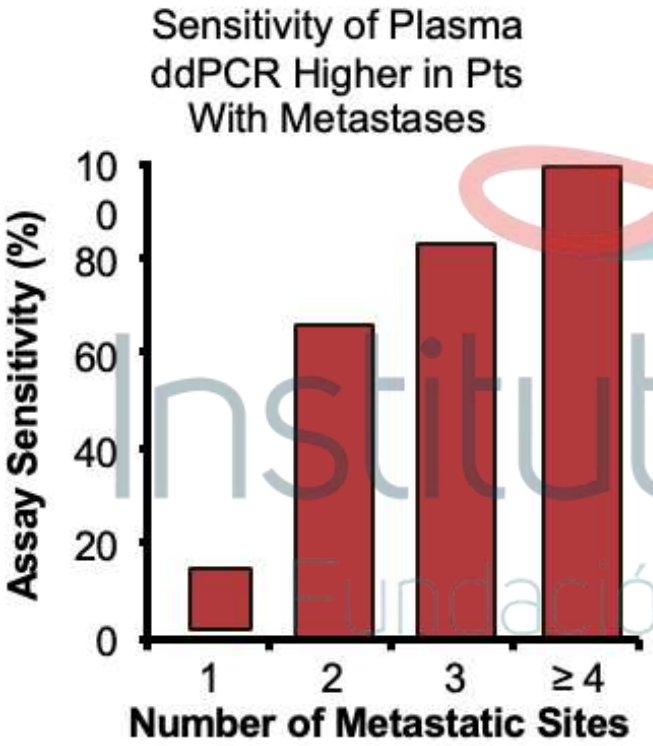




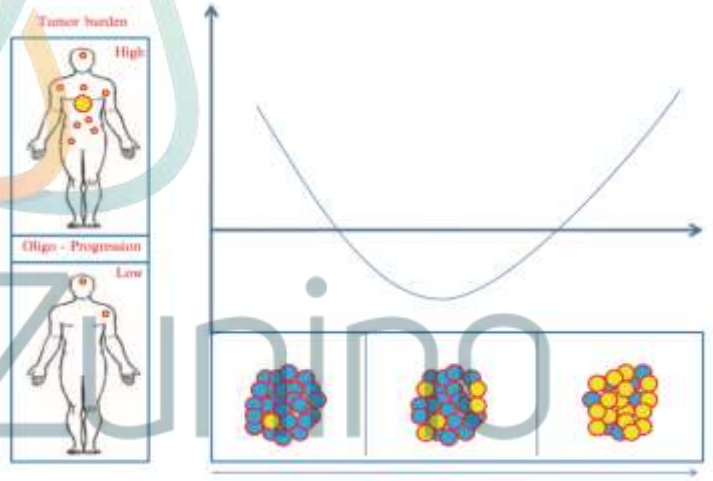
Bettegowda et al., Sci Trans Med, 2014

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

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

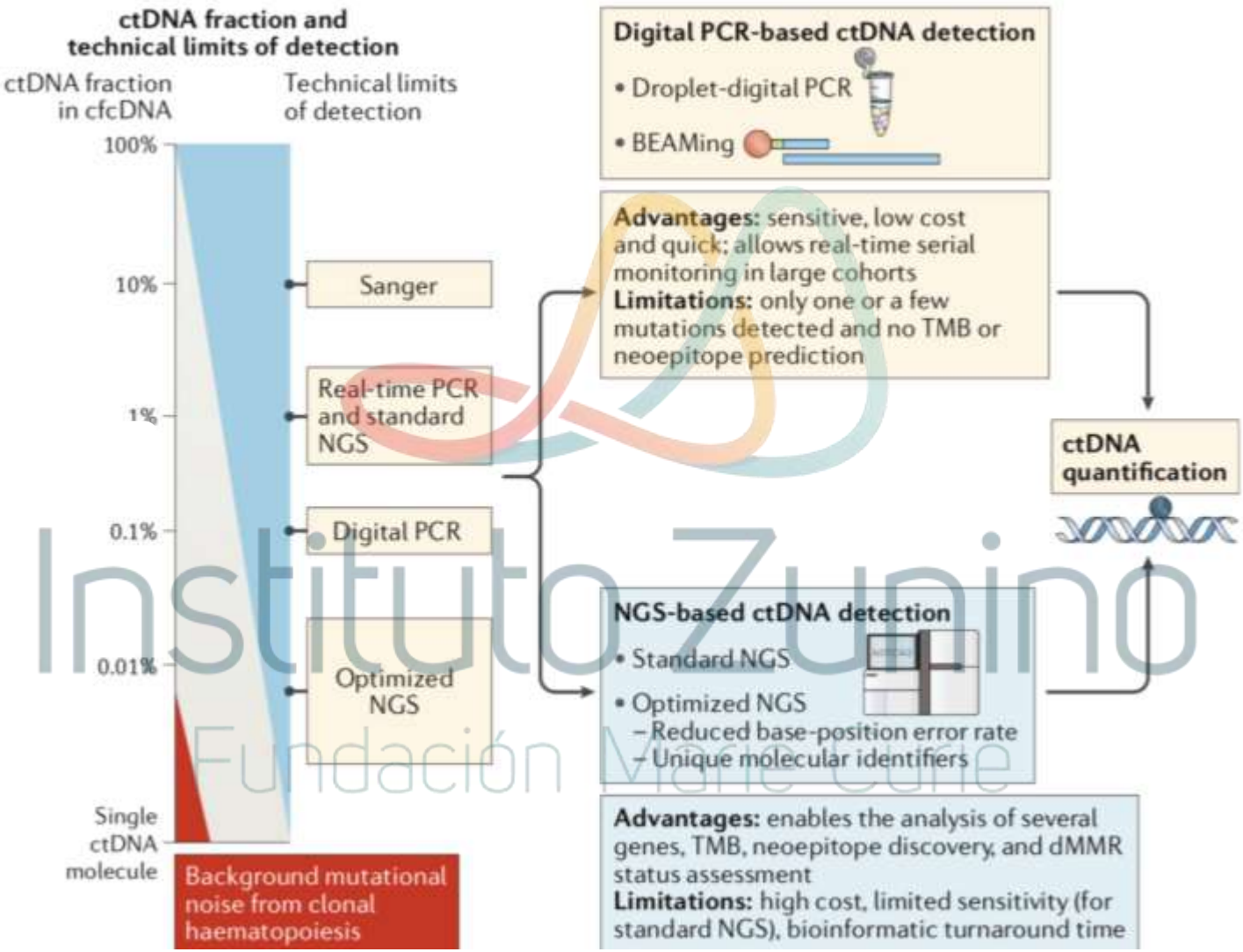
Whole target SNV/Indel
- 1.0% AF

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

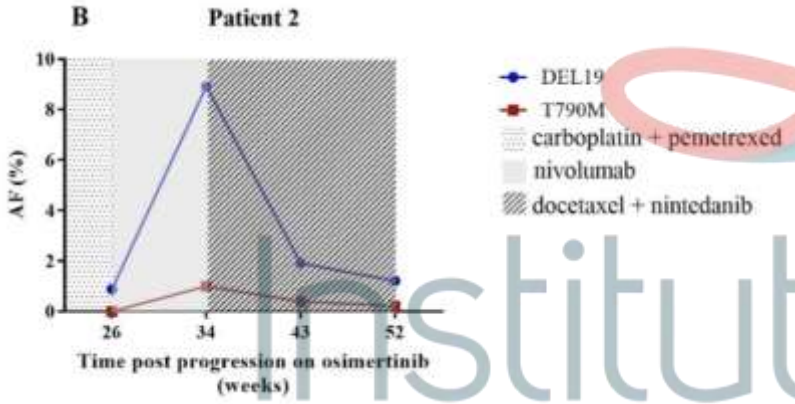
Oncomine data.



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

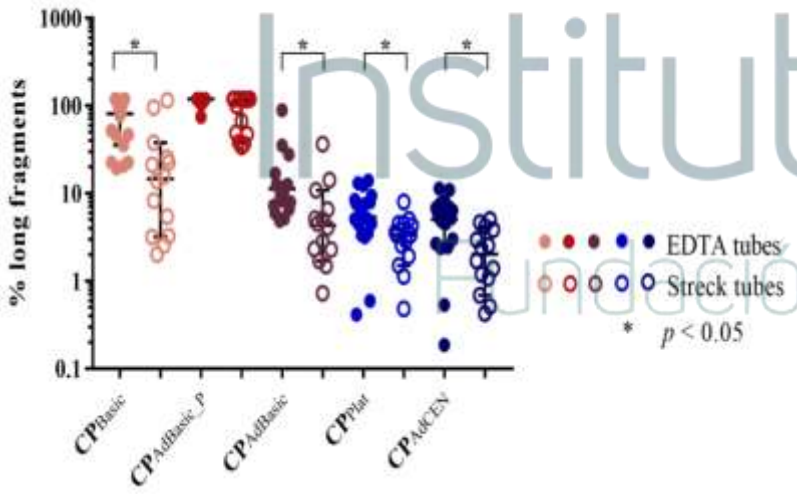
cancers

MDPI

Article

Circulating Cell-Free DNA and RNA Analysis as Liquid Biopsy: Optimal Centrifugation Protocol

Laure Sorber [1,2,*], Karen Zwaenepoel [1,2], Julie Jacobs [1,2], Koen De Winne [2], Sofie Goethals [3], Pablo Reclusa [1], Kaat Van Casteren [1,2,4], Elien Augustus [1,2], Filip Lardon [1], Geert Roeyen [5], Marc Peeters [1,6], Jan Van Meerbeeck [1,7], Christian Rolfo [1,8] and Patrick Pauwels [1,2,3]

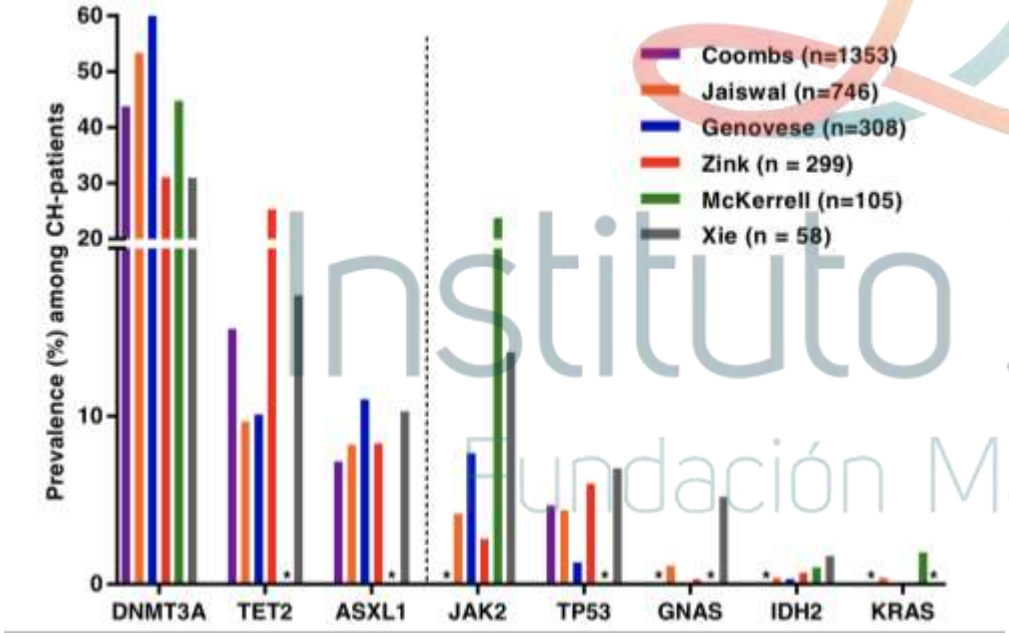


- **Two-step**, high-speed centrifugation protocols were associated with high cfDNA but low cfRNA concentrations. High cfRNA concentrations were generated by a one-step, low-speed protocol.
- In **Streck tubes**, two-step, high-speed centrifugation protocols also generated good quality, high cfDNA concentration. However, these tubes are not compatible with cfRNA analysis.

April 2019

A new problem: Clonal Hematopoeisis

Genes commonly mutated



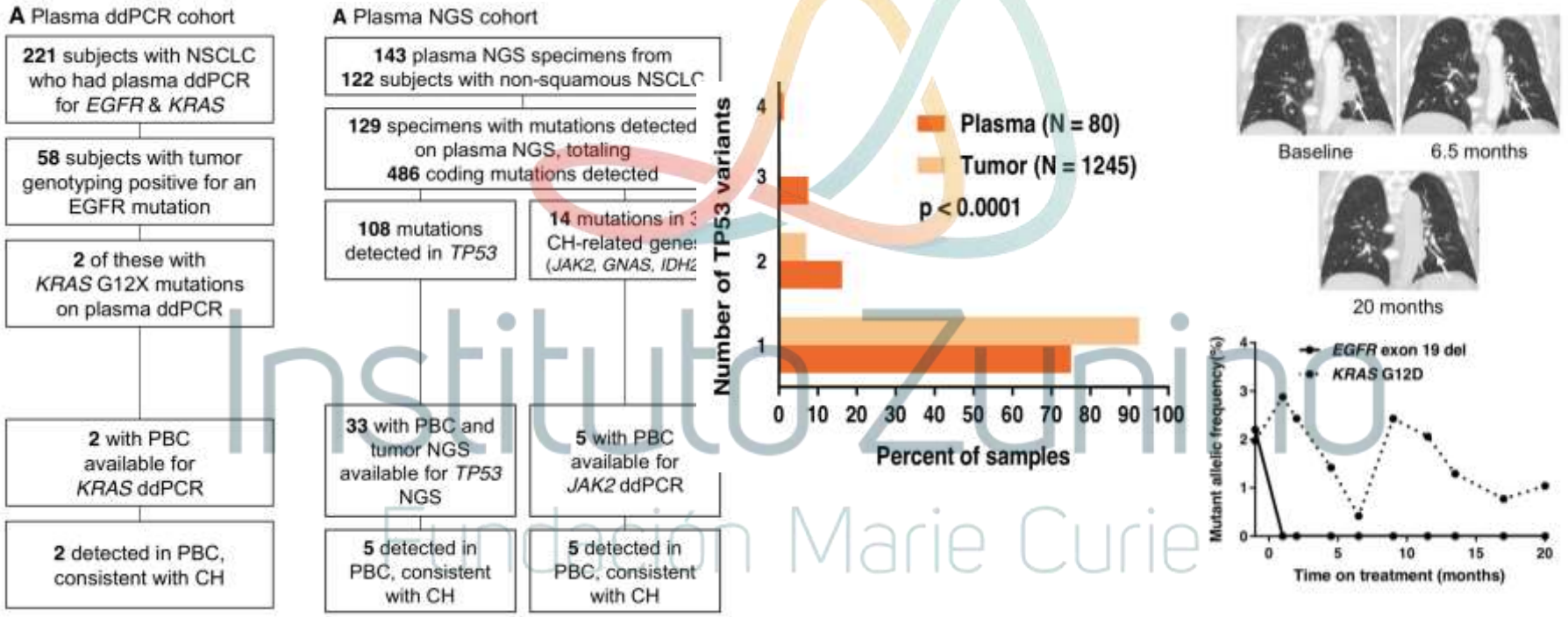
Clonal hematopoiesis (CH) is the somatic acquisition of genomic alterations in hematopoietic stem and/or progenitor cells, leading to clonal expansion.

- 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

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

Our New Way to Work . . .
Molecular Tumor Board

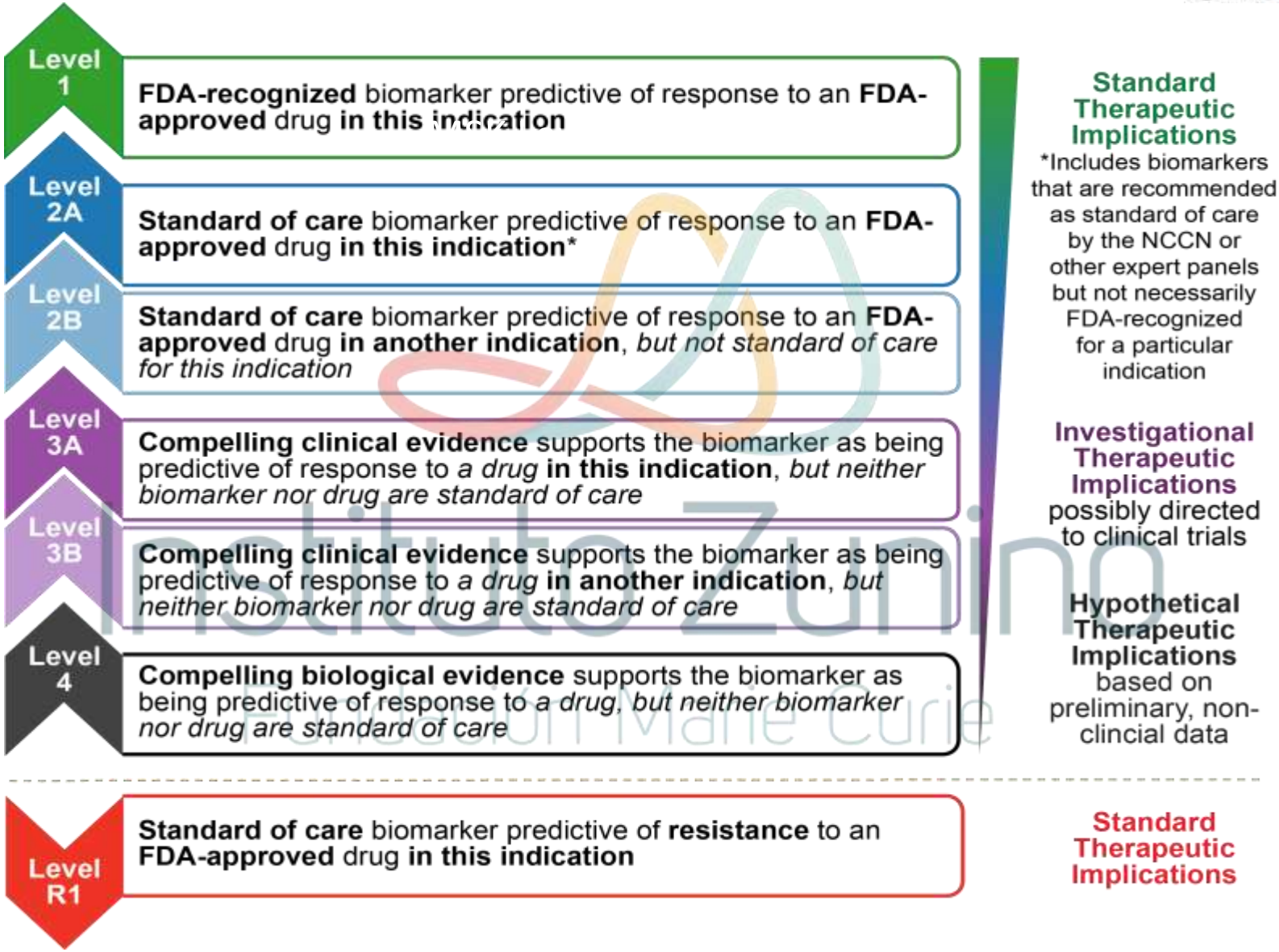
Patient case is derived from his doctor

Molecular Tumor Board

Oncologist | Mol. Pathol | Bioinformat | Surgeon | Geneticist | Pediat | Nav. nurse

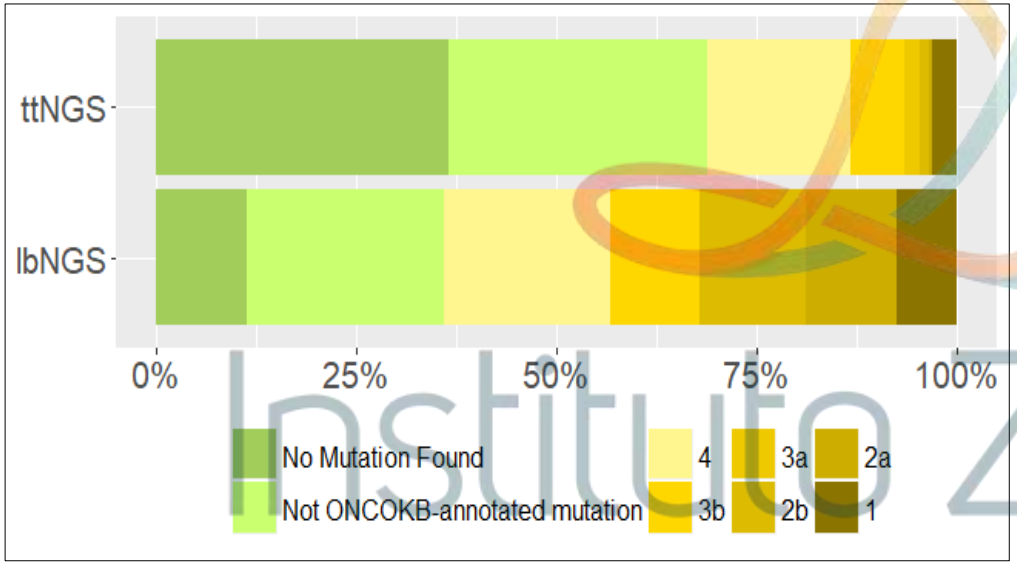
Molecular Tumor Board → **Report with therapeutic proposal** → Referral Doctor Discussion

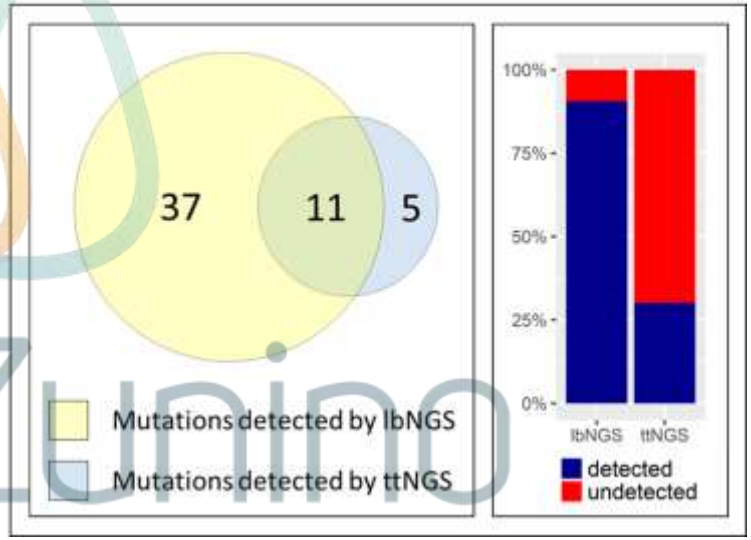
OncoKB



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.

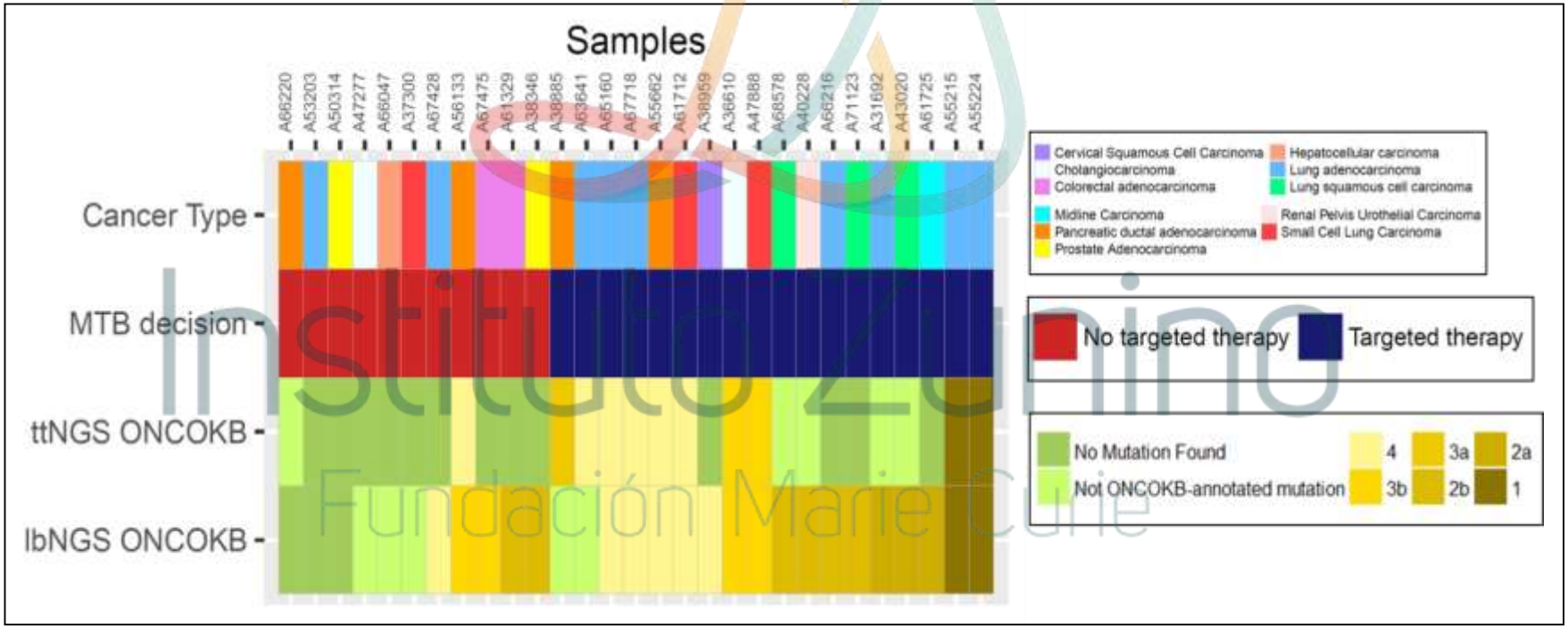




It looks like lbNGS can provide patients with alteration-driven treatment recommendations more effectively than ttNGS

Rolfo et al, ESMO Open August 2018

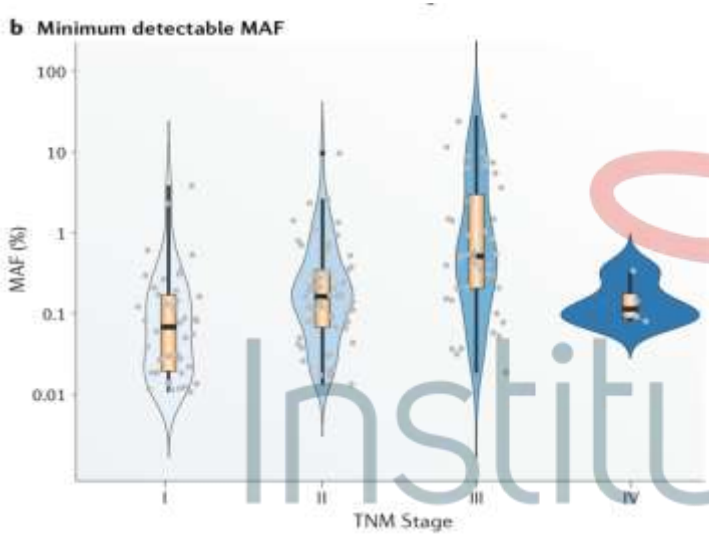
Effects of molecular tumor board and different NGS panels implementation for the treatment of patients with cancer.



Rolfo et al ESMO Open August 2018

Minimal Residual disease

The Role of Liquid Biopsy

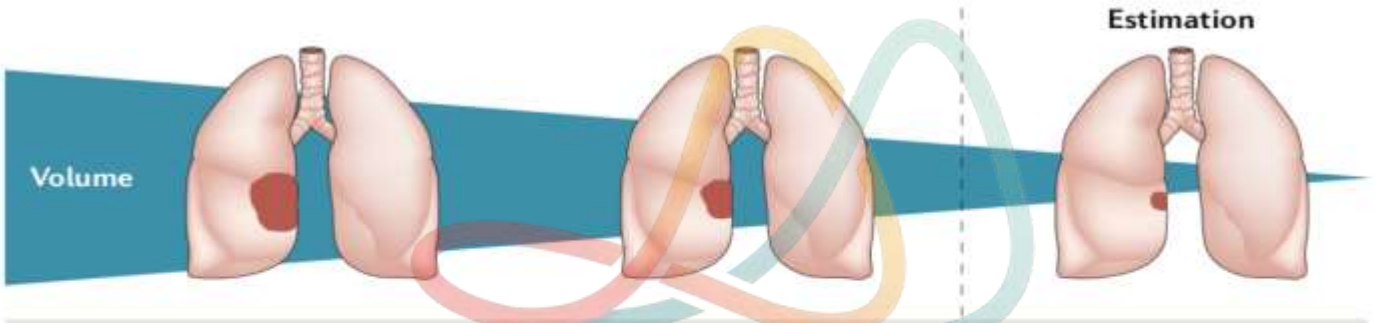


Minimum detectable mutant allele frequencies (MAFs) for 142 patients with detectable ctDNA, from a total of 301 patients analysed.

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



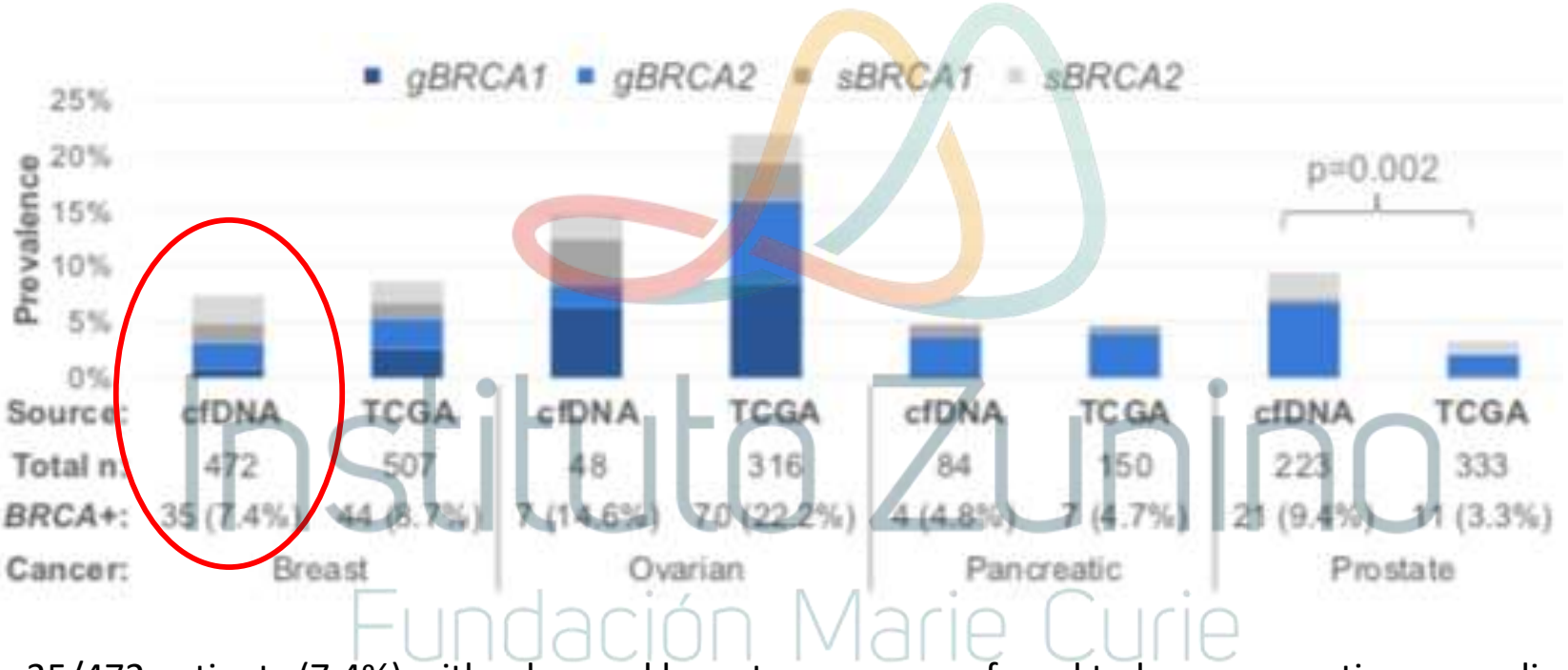
MAF	1.4% (0.62–3.1%)	0.1% (0.06–0.18%)	0.008% (0.002–0.03%)
Nodule diameter	5.8 cm	2.6 cm	1.2 cm
Nodule volume	100 cm^3	10 cm^3	1 cm^3
T stage	T3	T1c	T1b

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

LIQUID BIOPSY IN BREAST CANCER

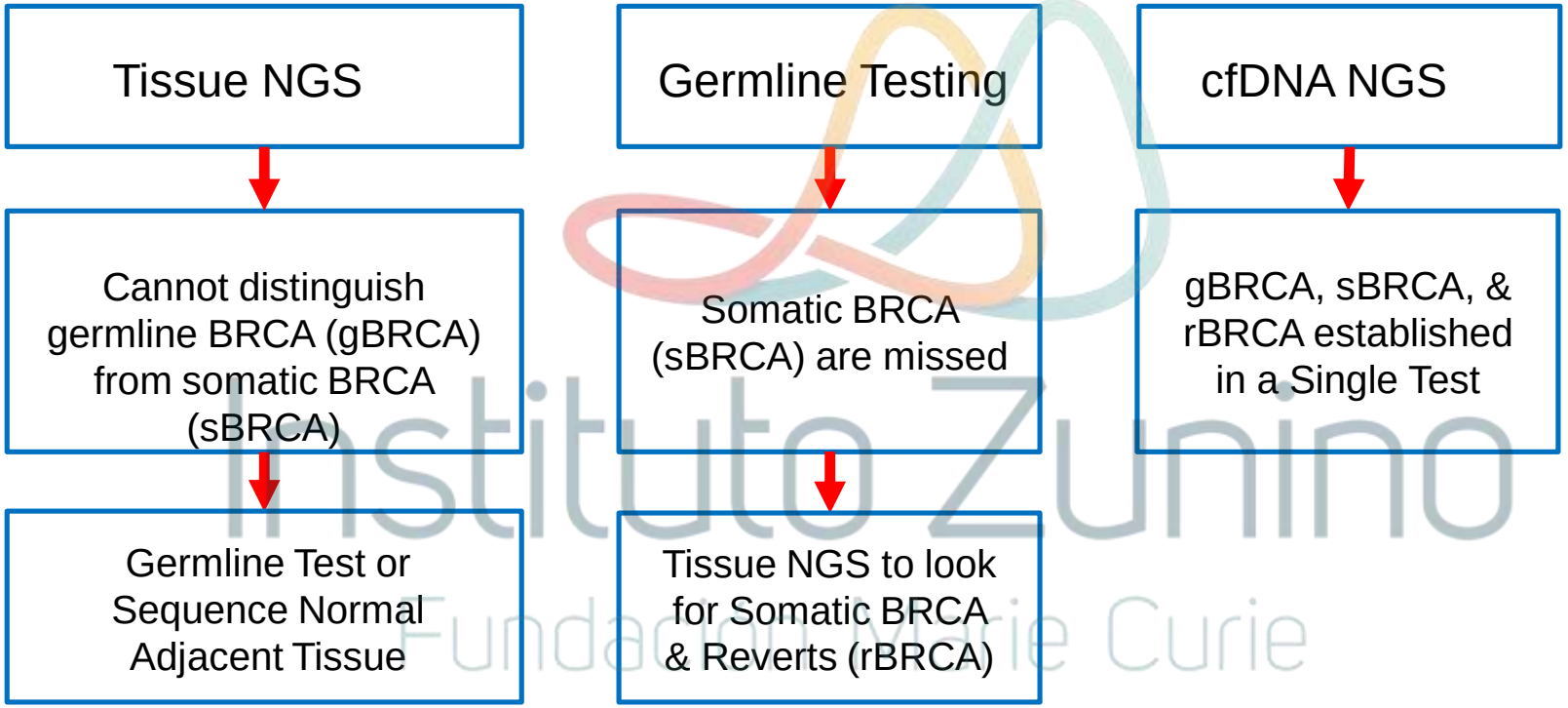
Incidence of *BRCA* alterations in advanced breast cancer found by ctDNA analysis



- 35/472 patients (7.4%) with advanced breast cancer were found to have a somatic or germline BRCA mutation with ctDNA analysis
  - Approximately half of the BRCA+ alterations were somatic only
- Reversion BRCA were identified in a significant percentage (13%) of BRCA+ pts without foreknowledge of germline- or tissue-based testing, and may identify pts unlikely to respond to PARPi.

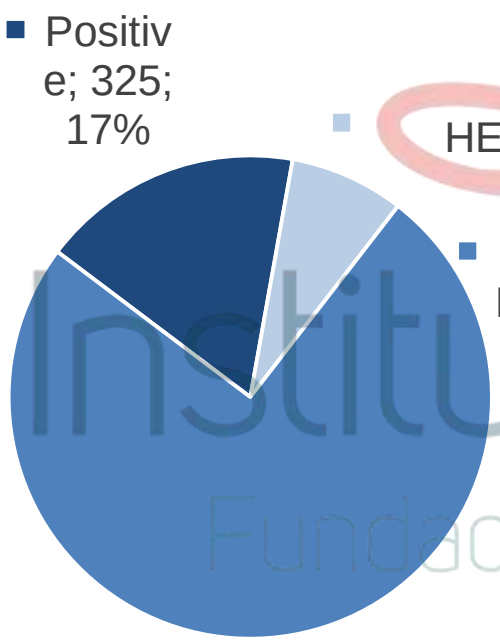
Bardia et al. 2018 ASCO

cfDNA NGS – A Simpler Path to PARP Inhibitor Decision-Making



- Germline BRCA = gBRCA, Somatic BRCA = sBRCA, BRCA revert = rBRCA
- *gBRCA vs. sBRCA essential for familial risk assessment

Plasma-detected *ERBB2* (HER2) "flips" predict response to targeted HER2 therapy



Plasma *ERBB2* (HER2) Results

8.5% (12 of 142 HER2 Equivocal on tissue) were *ERBB2* amplification on cfDNA

+

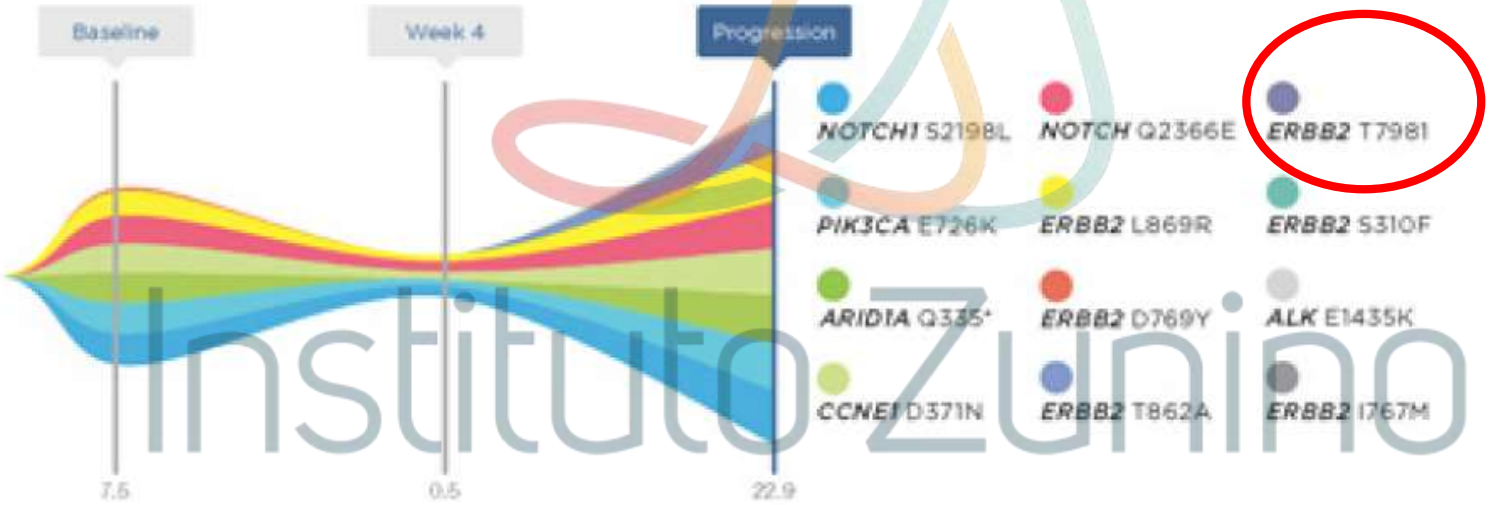
2.1% (29 of HER2 Negative on tissue) were *ERBB2* amplification on cfDNA

Of the 29 HER2 "Flips": 6 were treated with anti-HER2 Rx
5/6 (83%) of these patients had good clinical response

Tissue HER2 status, N=1,853

Raymond (Lanman) Cell-free DNA Analysis Identifies Actionable *ERBB2* Amplifications in Patients with HER2 Negative Breast Cancer (SABCS, 2017)

Molecular Response and Resistance: *ERBB2* L869R Targeted with Neratinib, Followed by Emergence of *ERBB2* T798I Mutation

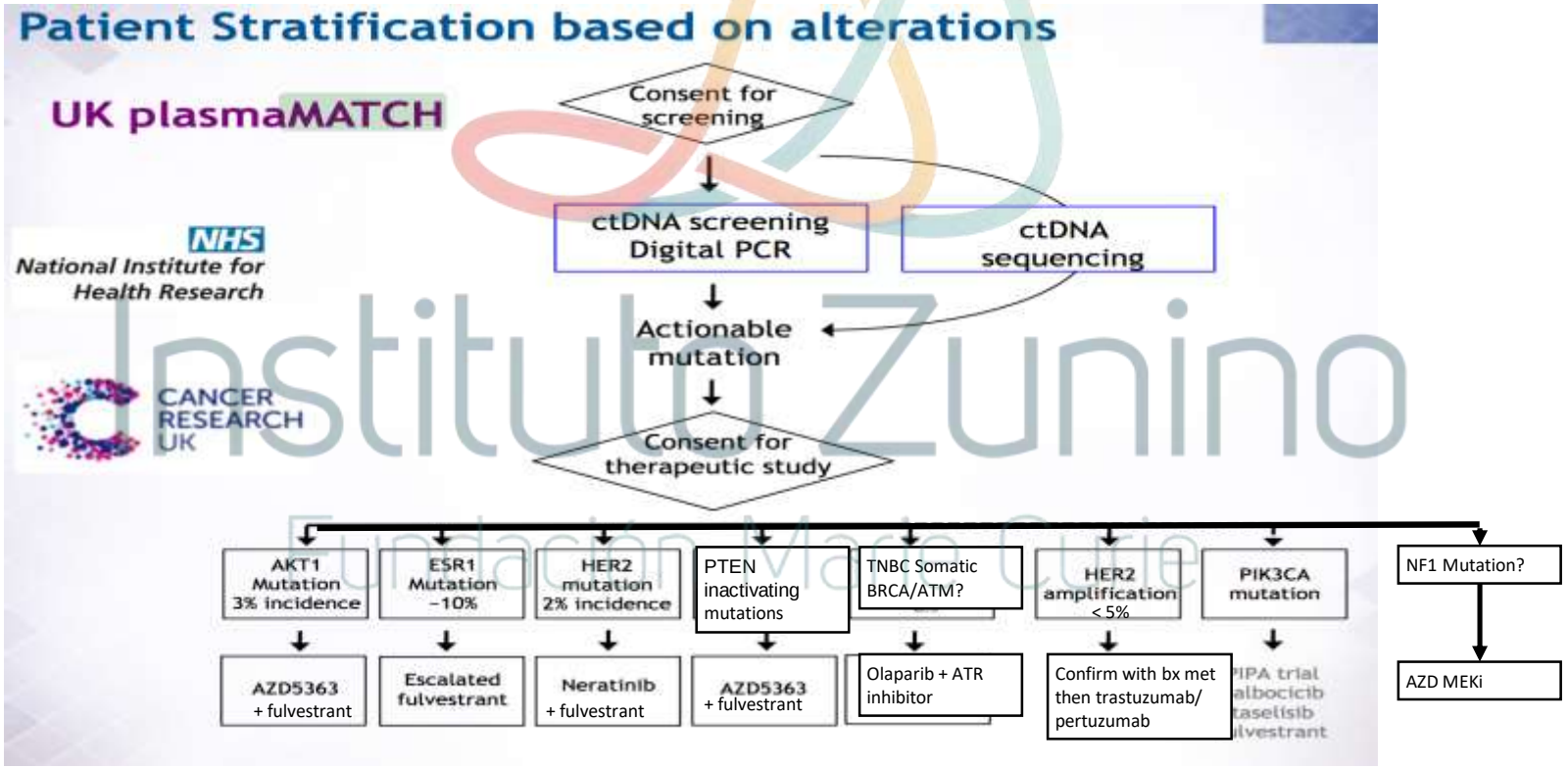


ERBB2 L869R is homologous to *EGFR* L861, and *ERBB2* T798I is homologous to *EGFR* T790M

plasmaMATCH – Prospective Umbrella Trial for Targeted Therapy in Metastatic Breast Cancer

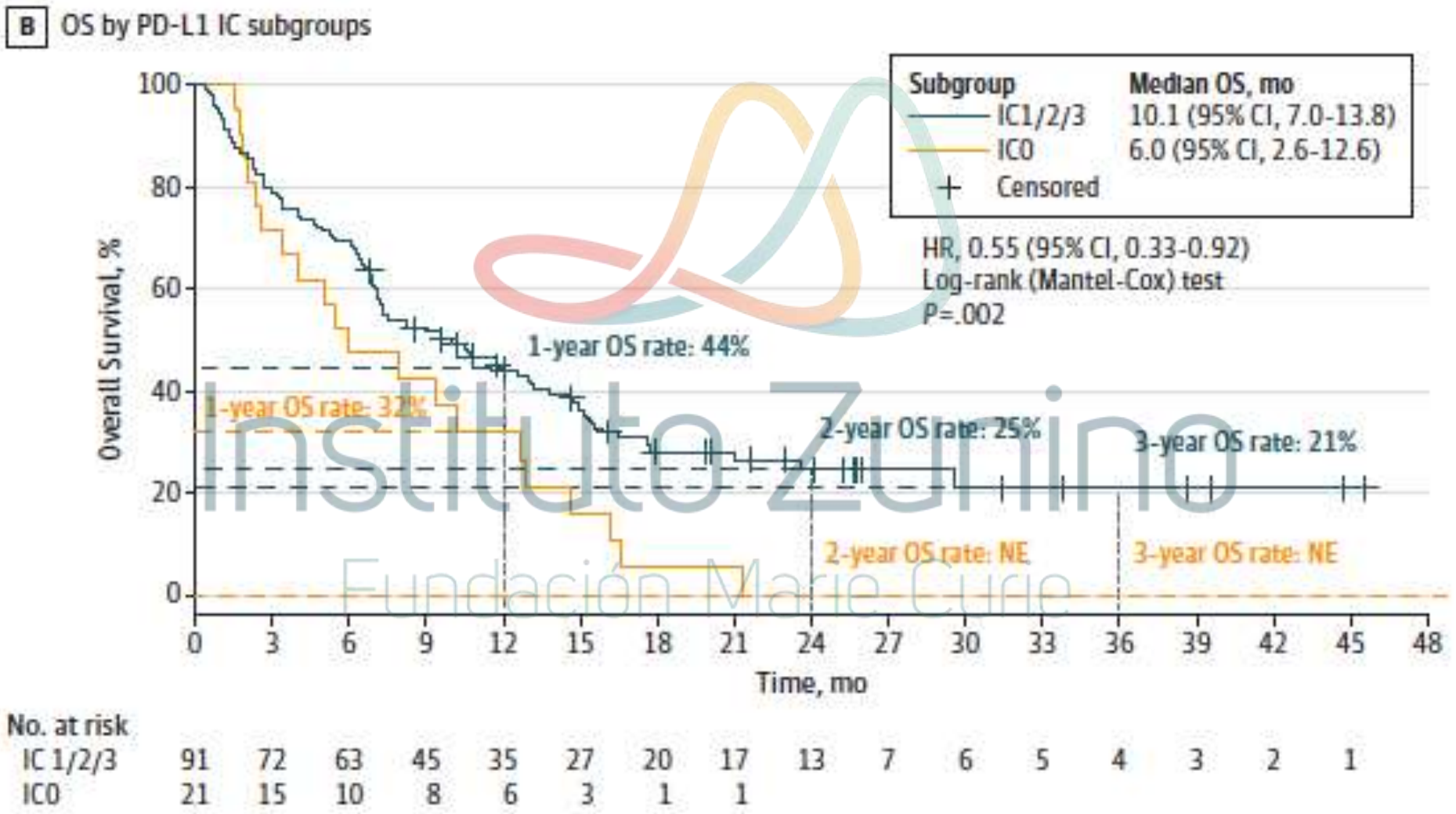
Enrolling on Plasma (ddPCR and/or cfDNA NGS (Guardant360))

- Courtesy of Nicholas Turner MD, PhD, The Royal Marsden NHS Trust



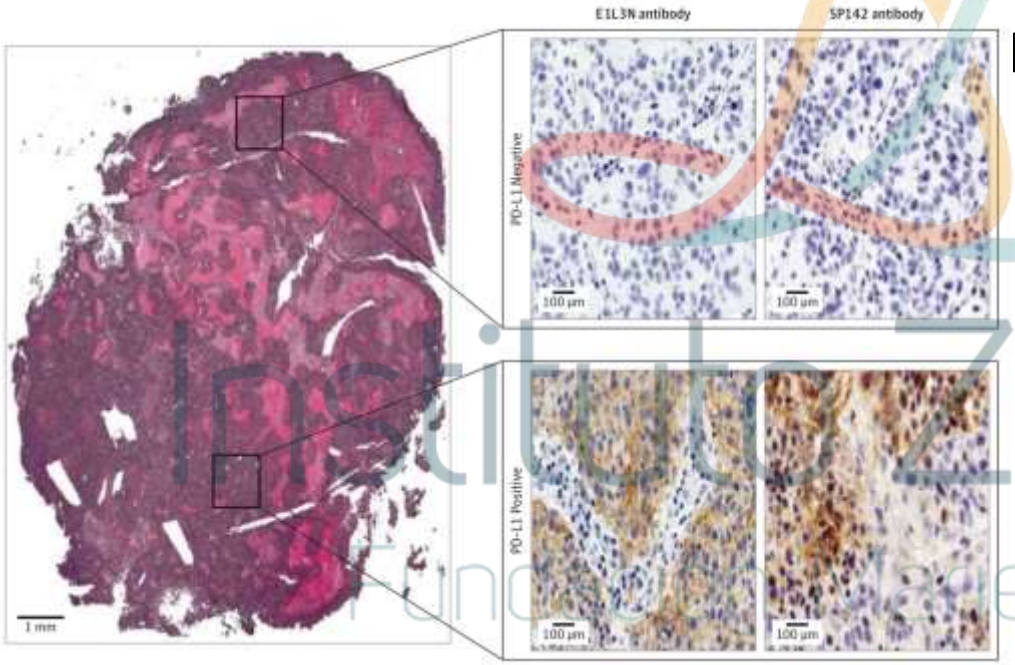
Immunotherapy in Cancer

Atezolizumab phase I study in metastatic TNBC



Emens L et al. JAMA Oncol 2018.

Heterogeneity of PD-L1 Expression



marker

- **Intratumor** heterogeneity
- **Intrapatient** Heterogeneity

McLaughlin et al, JAMA Oncol. 2016;2(1):46-54

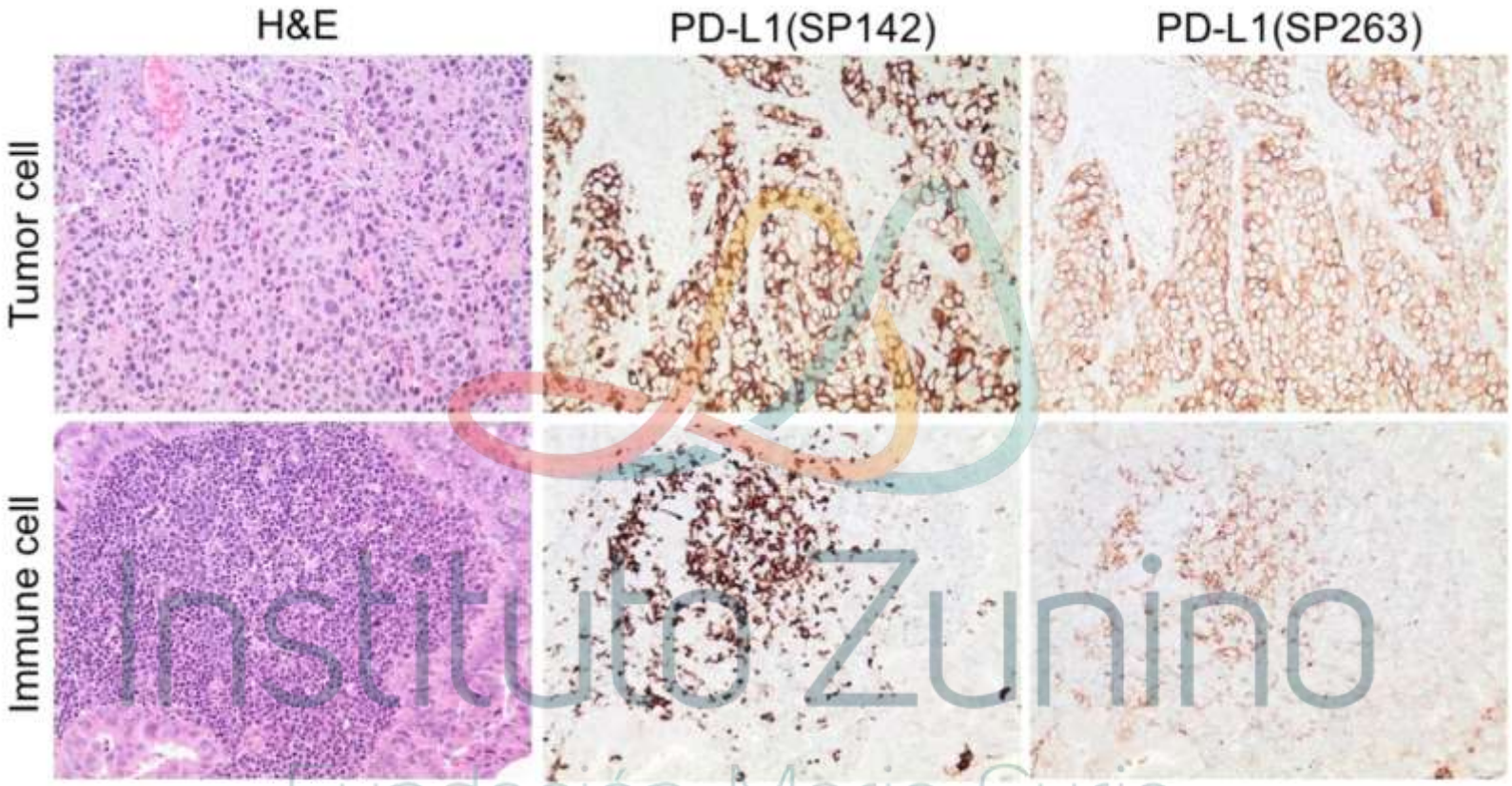
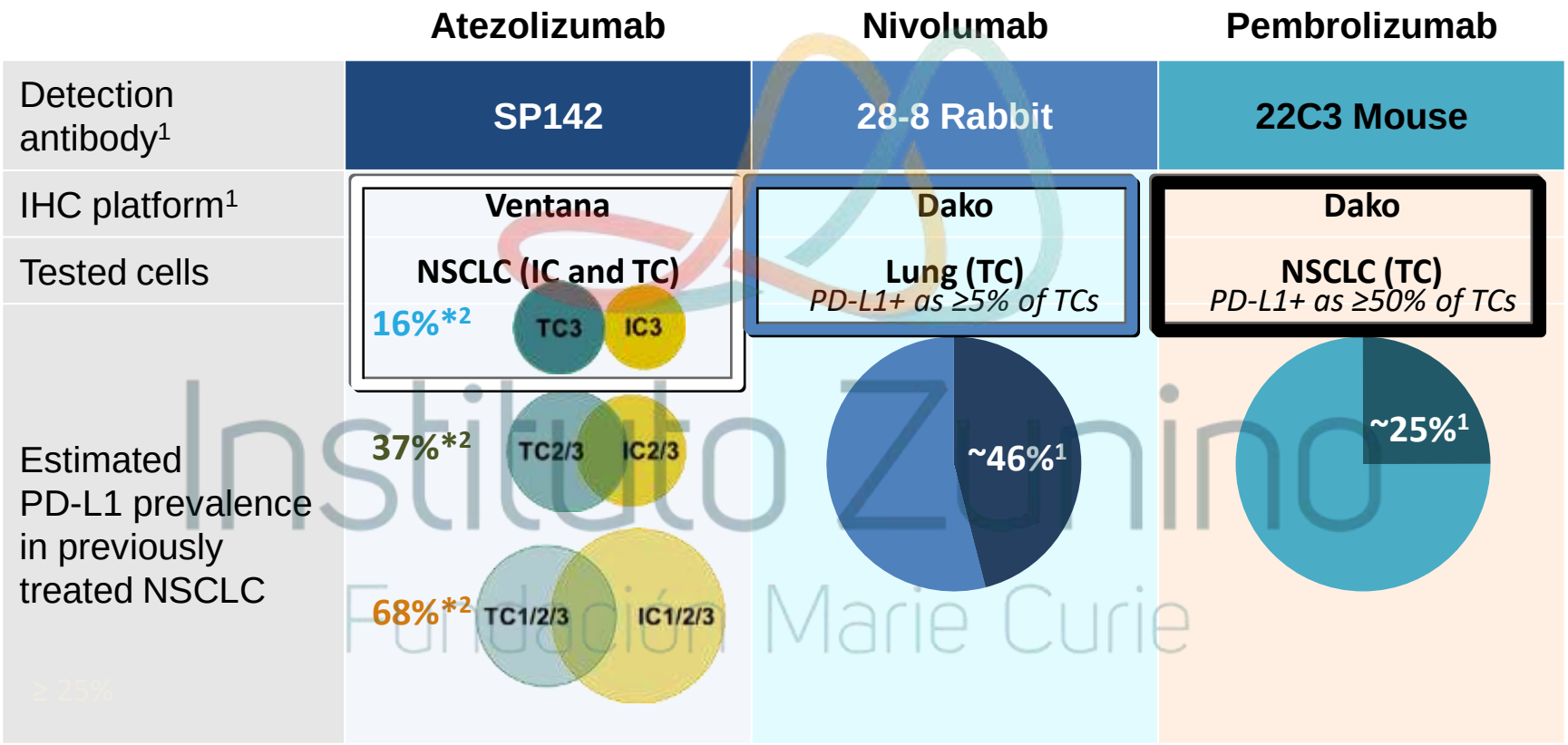


Figure 1: Staining with PD-L1 monoclonal antibodies in tumor and immune cells. Histology of urothelial carcinoma (upper panels) and metastatic lung adenocarcinoma (lower panels). Tissues were stained with hematoxylin-eosin and PD-L1 monoclonal antibodies (SP142 and SP263, respectively).

S.Patel ACCC 2017

Many PD-L1 Biomarker assays are there and they are not the same …At All !!



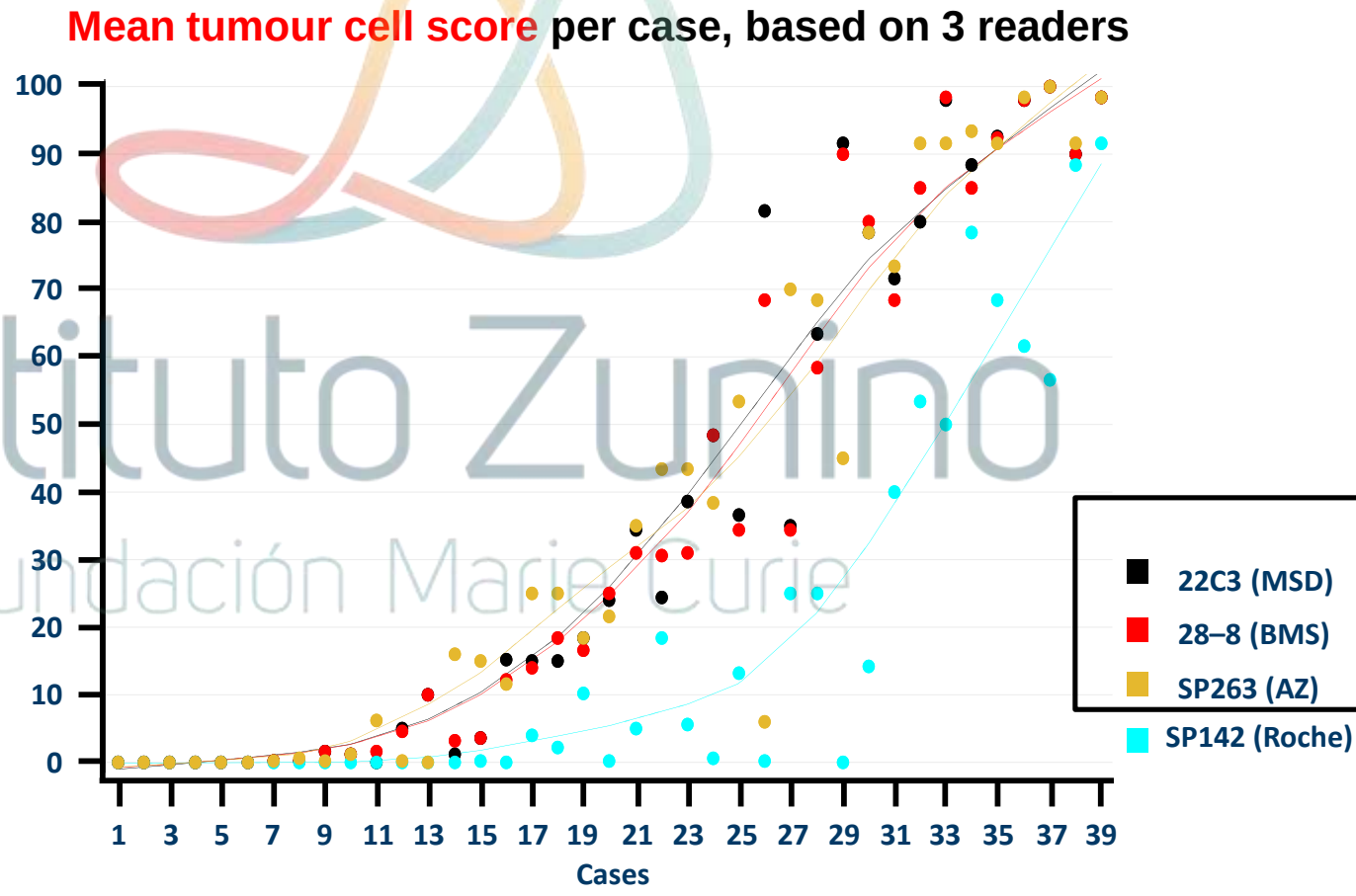
*TC3 or IC3 = TC ≥ 50% or IC ≥ 10% PD-L1+; TC2/3 or IC2/3 = TC or IC ≥ 5% PD-L1+; TC1/2/3 or IC1/2/3 = TC or IC ≥ 1% PD-L1+; TC0 and IC0 = TC and IC < 1% PD-L1+, respectively.
IC = tumor infiltrating immune cell; IHC = immunohistochemistry; NSCLC = non-small cell lung cancer; PD-L1 = programmed death ligand 1; TC = tumor cell; UBC = urothelial bladder cancer.

1. Kerr KM et al. *J Thorac Oncol.* 2015;10(7):985-989. 2. Spira AI et al. Oral presentation at ASCO 2015. 8010.
3. Petrylak DP et al. Oral presentation at ASCO 2015. 4501.

Blueprint Initiative

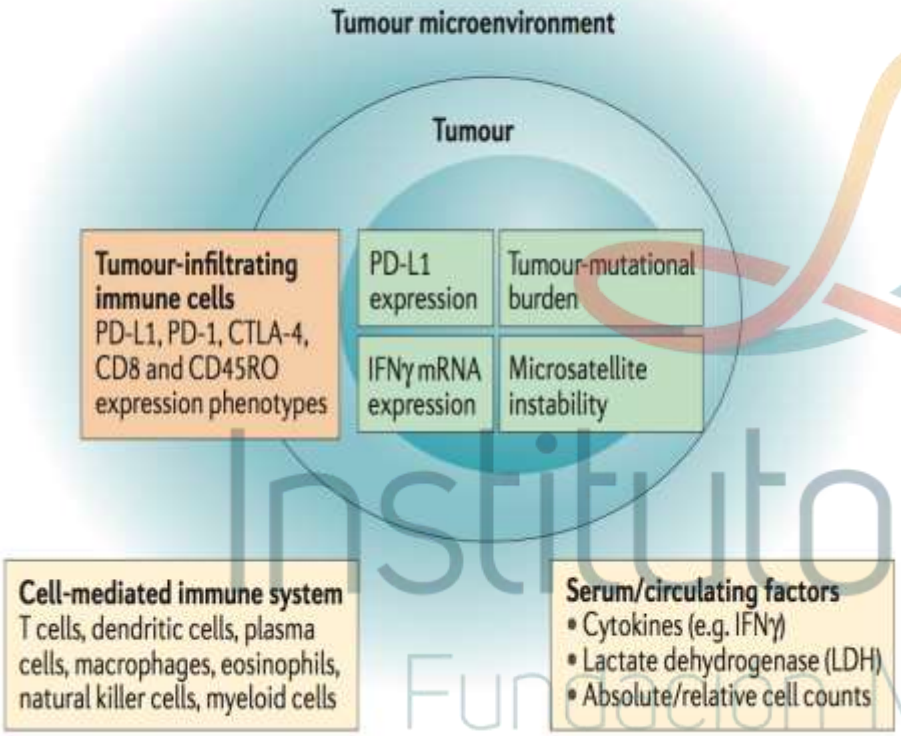
- Three assays (22C3, 28–8, SP263) demonstrate similar performance
- SP142 (Roche/Genentech) consistently labels fewer TC

PD-L1 IHC 22C3 pharmDx (Dako) is the only FDA-approved companion diagnostic for selecting NSCLC patients for treatment with pembrolizumab



- Hirsch FR, et al. Oral presentation at AACR 2016b.

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:

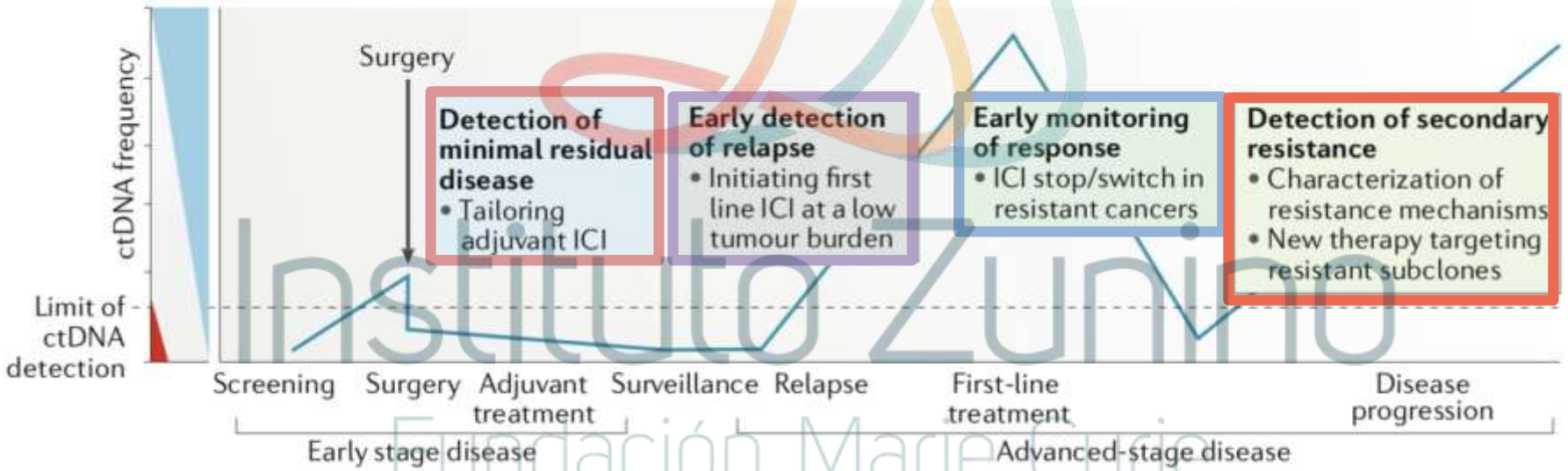
- Calculation of circulating TMB
- Detection of bPDL1
- Alellic Fraction Variation Dynamic

Liquid Biopsy in Immunotherapy is challenging!

A complex microenvironment

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

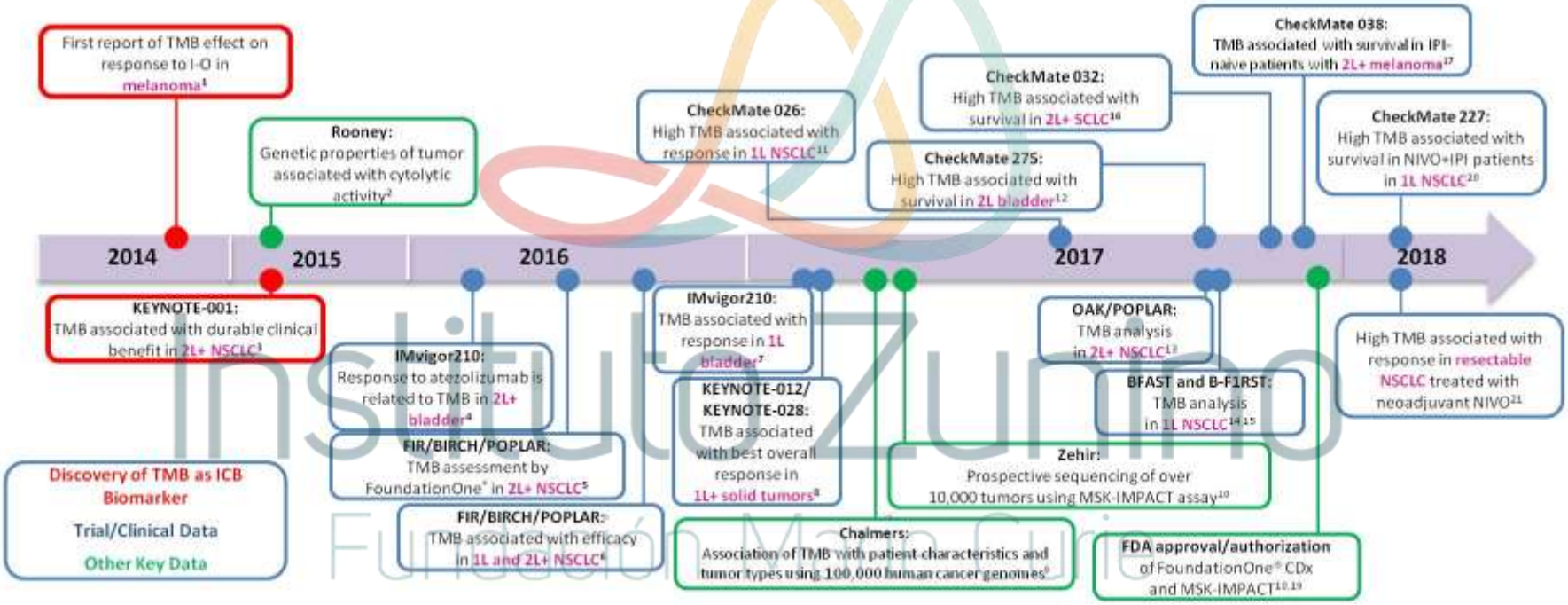
Clinical Application of liquid biopsy in Immunotherapy



Not so easy!!

Cabel et al, Nature Rev Clin Onc, Oct 2018

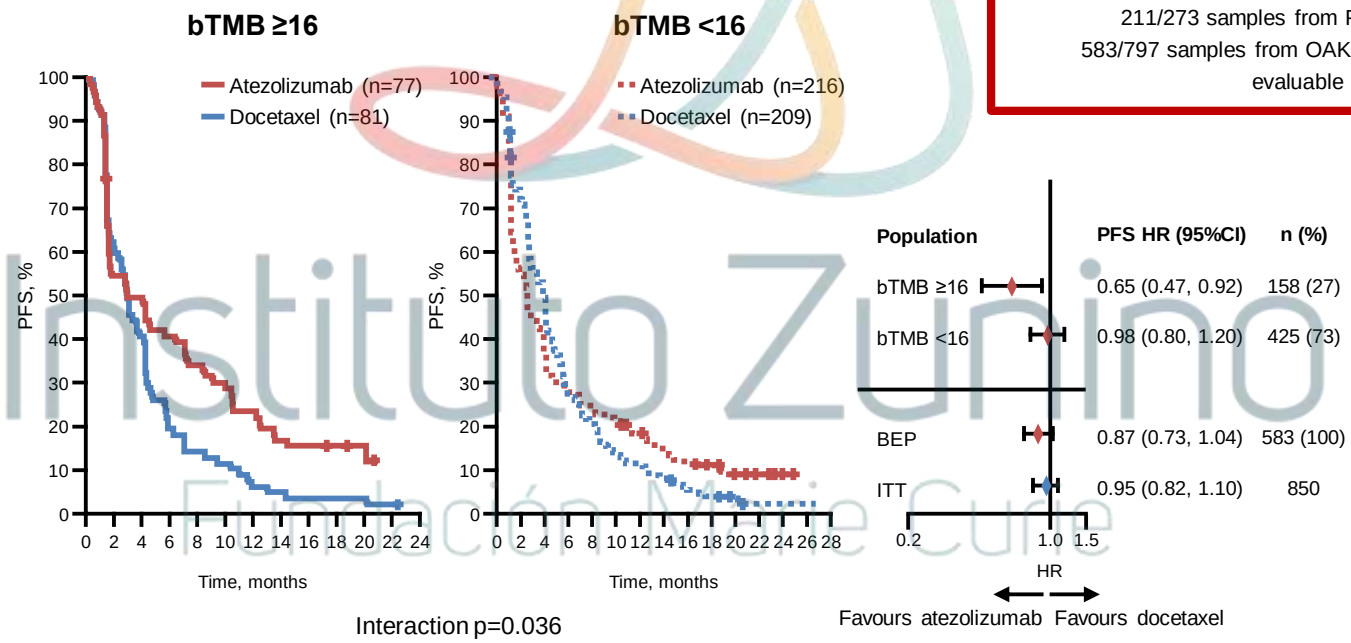
Tumor Mutational Burden Timeline



T. Chan at 2018 ASCO

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



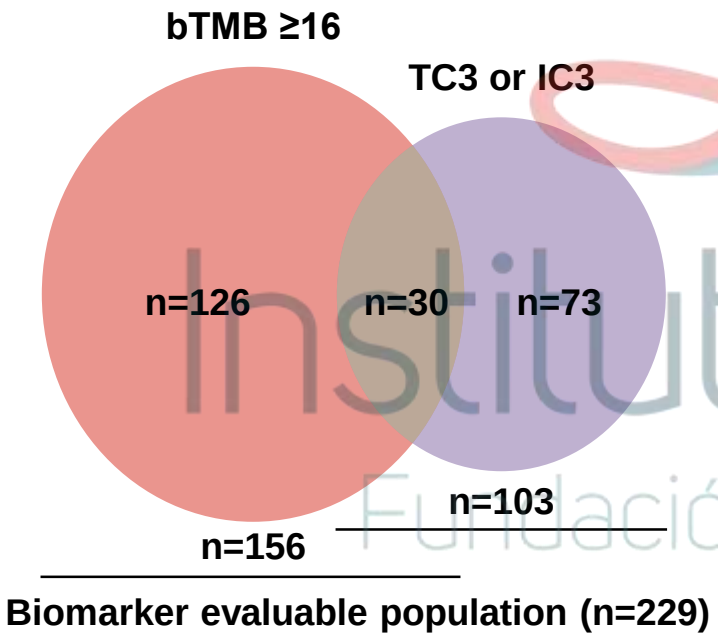
211/273 samples from POPLAR and 583/797 samples from OAK were biomarker-evaluable

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

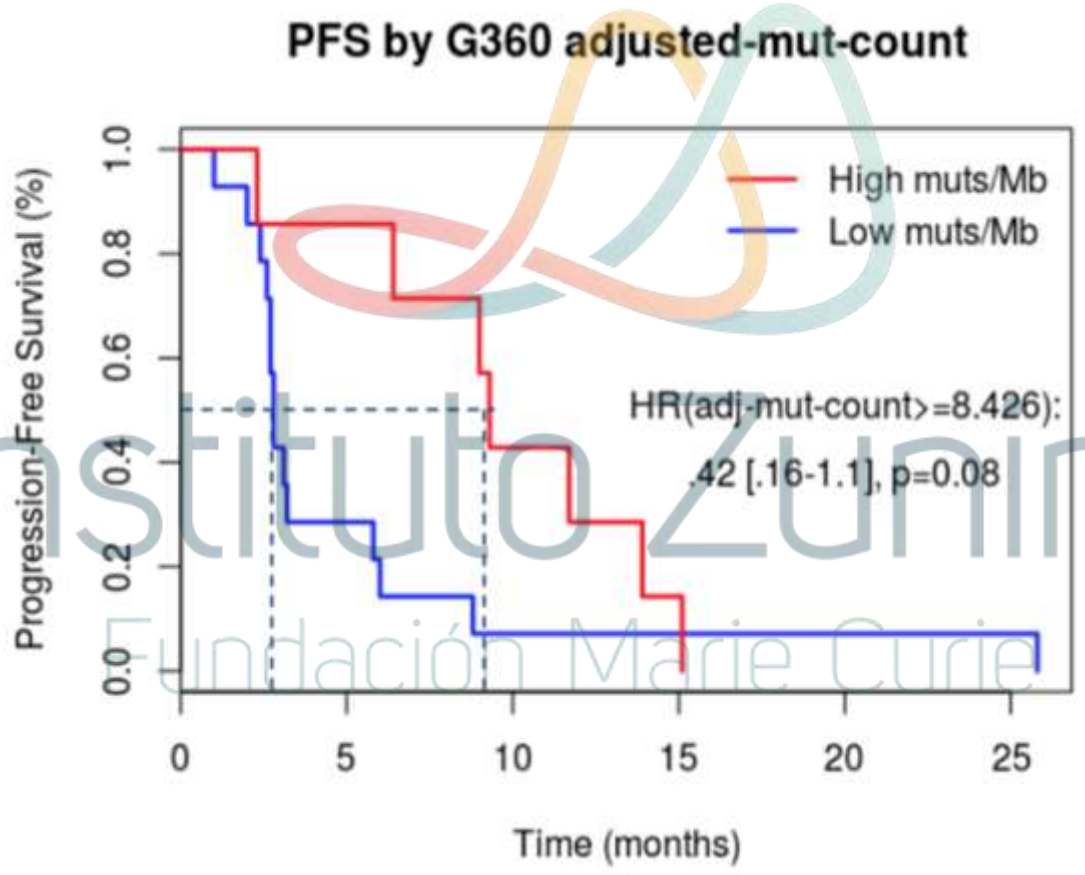
Conclusions

- 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

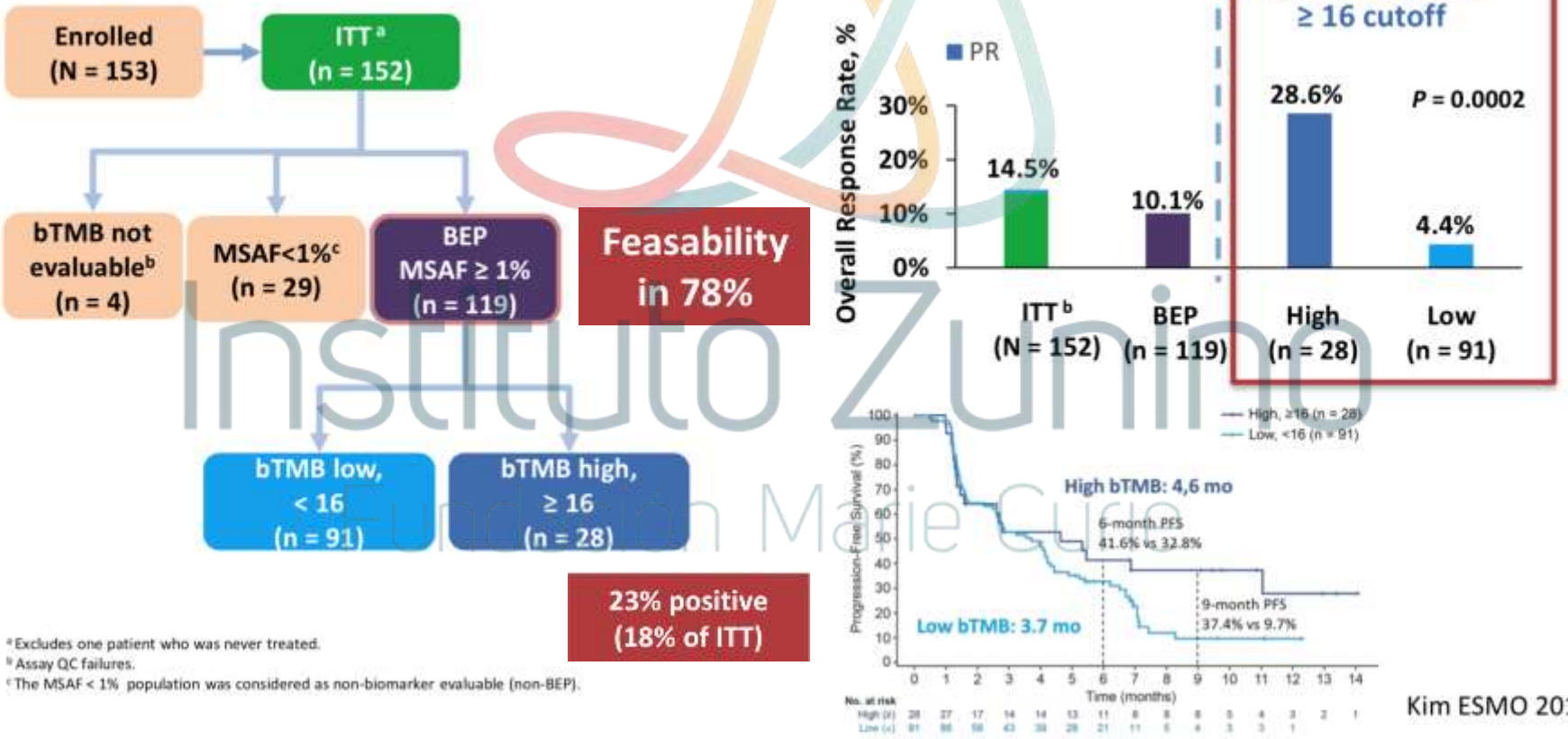
Digital Tumor Mutation Burden Predicts IO Response in NSCLC (top tertile vs. lower tertiles) 73 genes panel



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

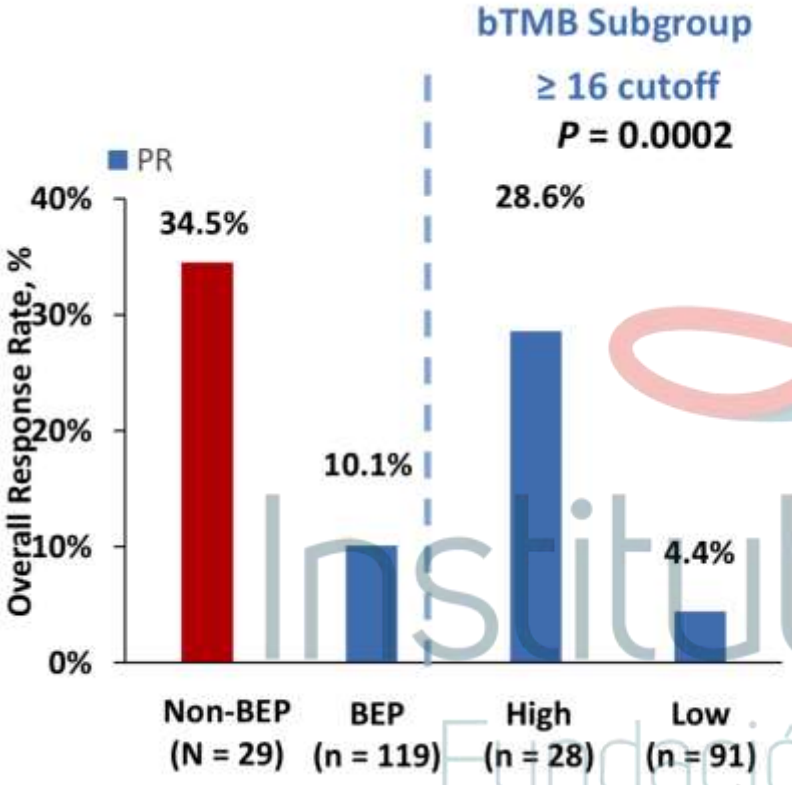
N = 27, 12 IO responders and 15 non-responders

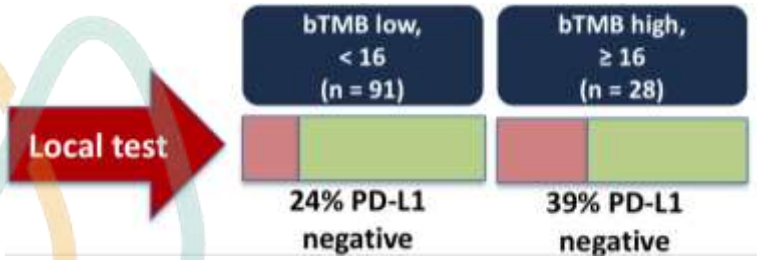
B-F1RST :Blood-Based Tumour Mutational Burden as a Biomarker of Atezolizumab Activity in First-Line NSCLC Treatment



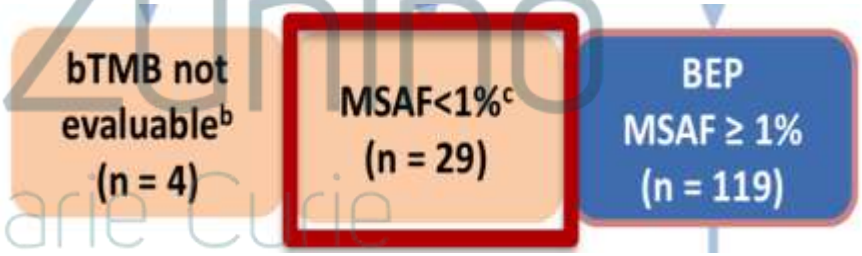
Adapted from B. Besse, ESMO 2018

B-F1RST: strengths and weaknesses





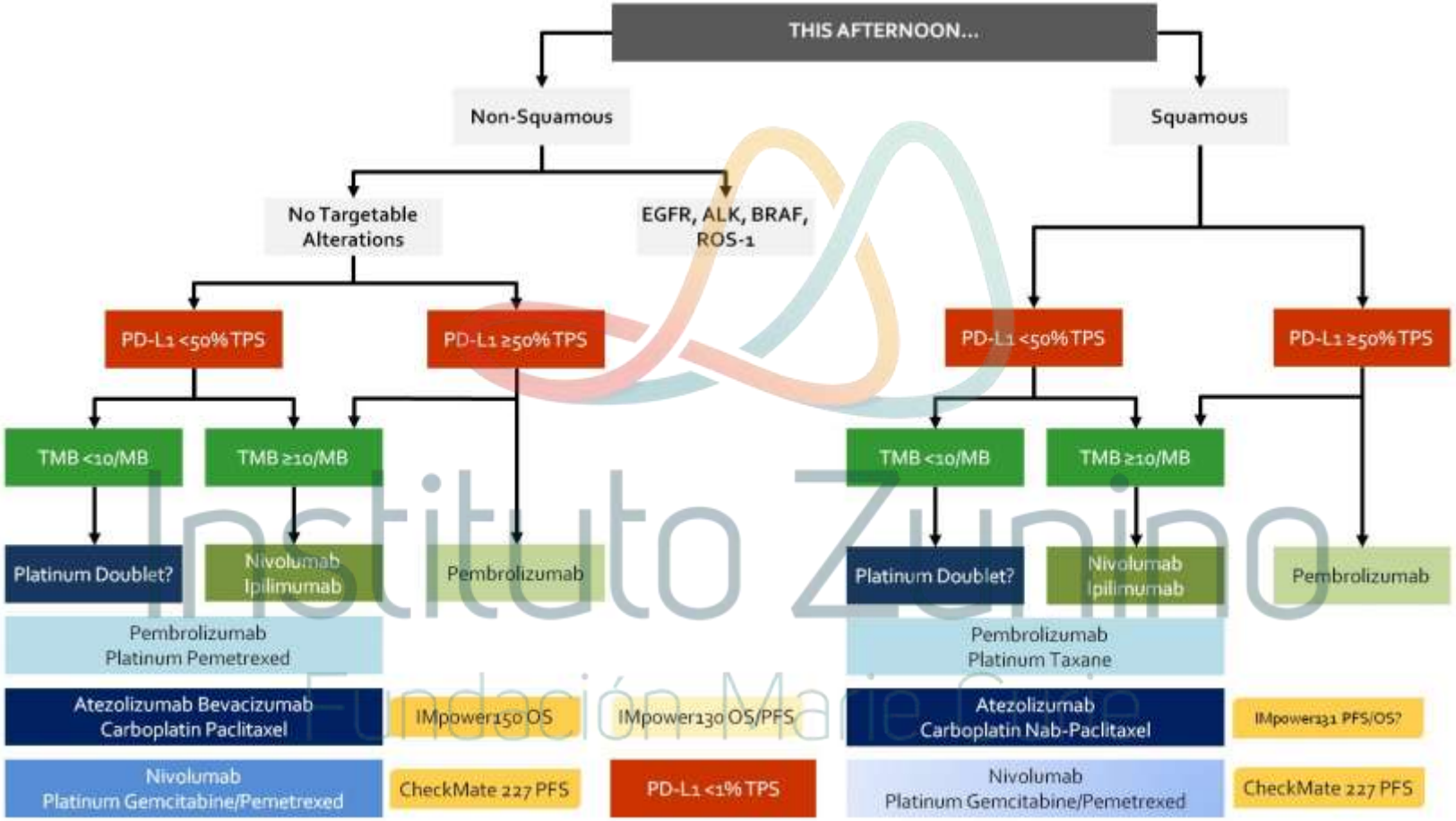
Major limitations No tissue collection No central PD-L1 testing No tissue TMB



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?

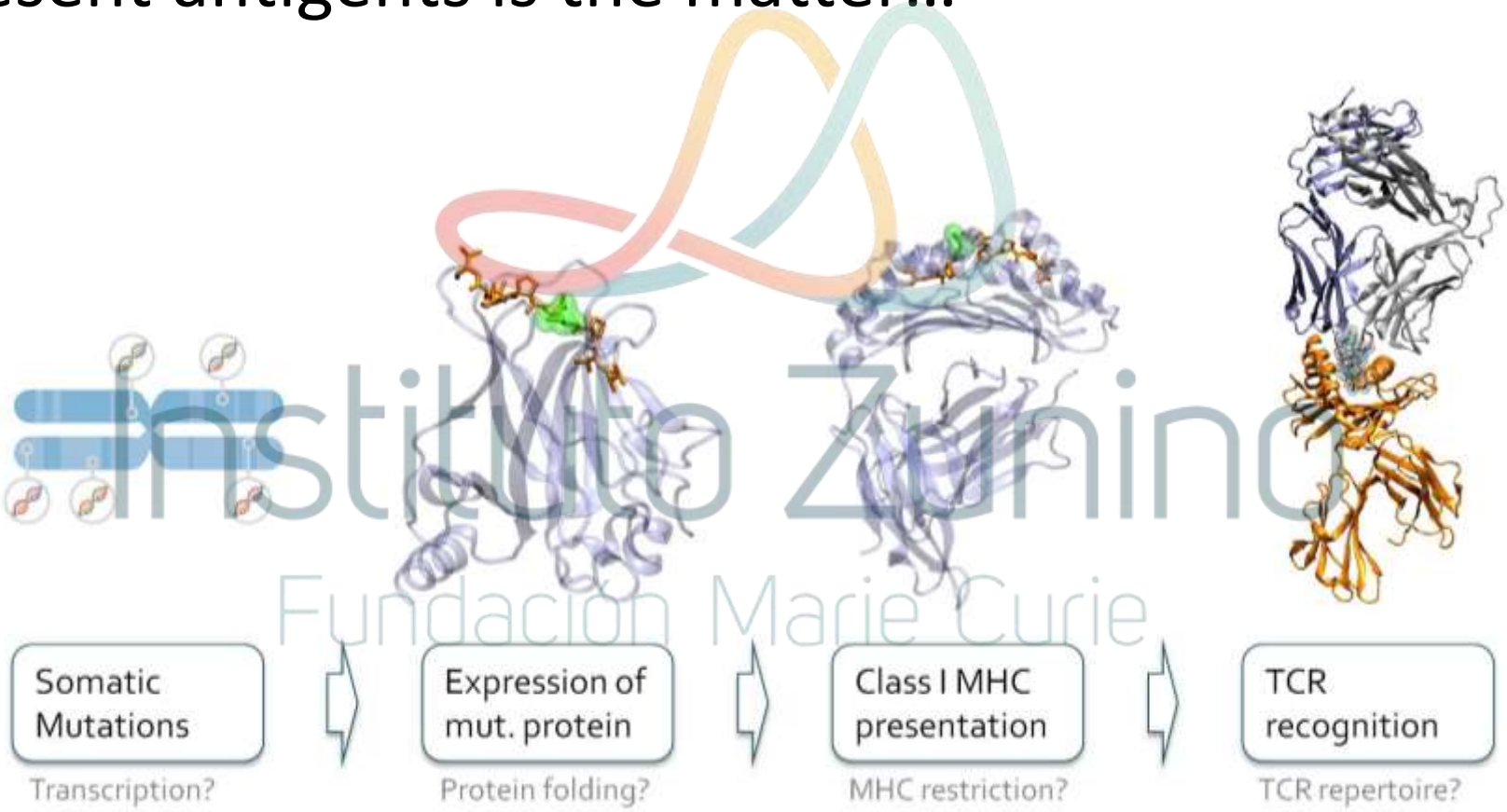
Adapted from B. Besse, ESMO 2018



Solange Peters at 2018 ASCO Annual Meeting

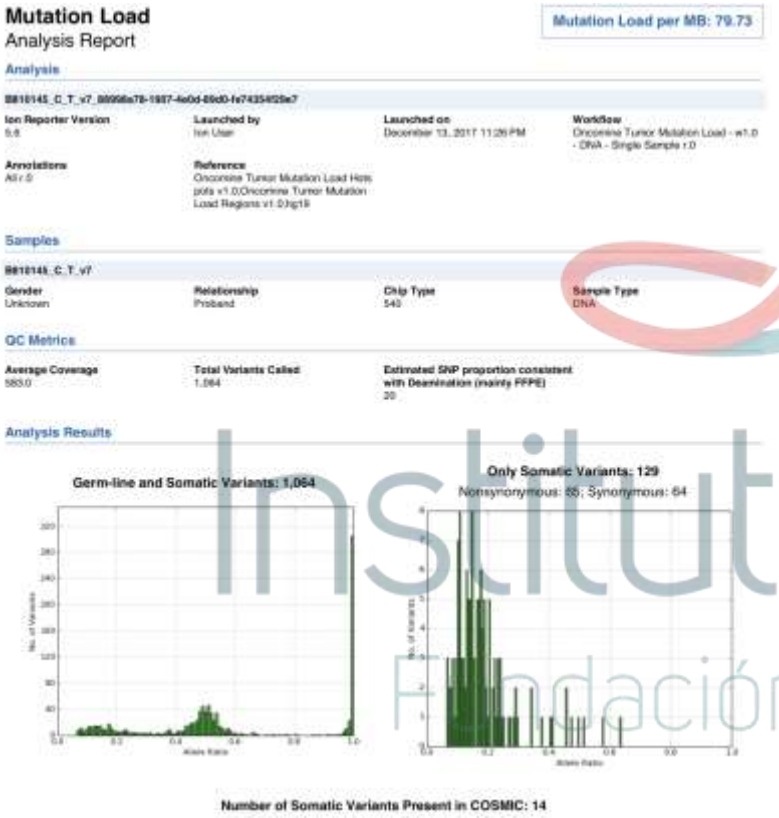
Quantity or quality of mutations?

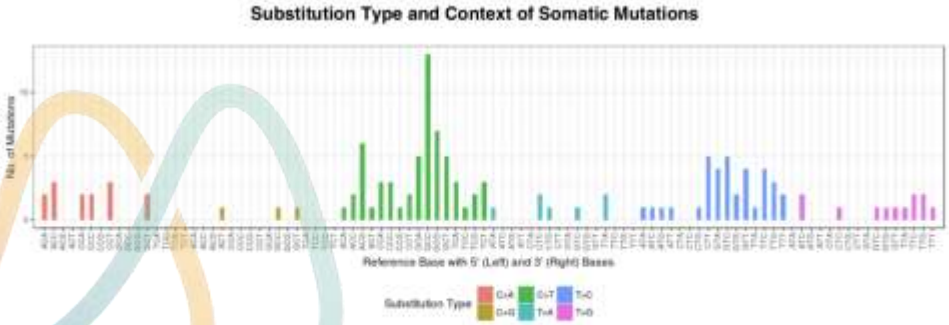
Present antigents is the matter…

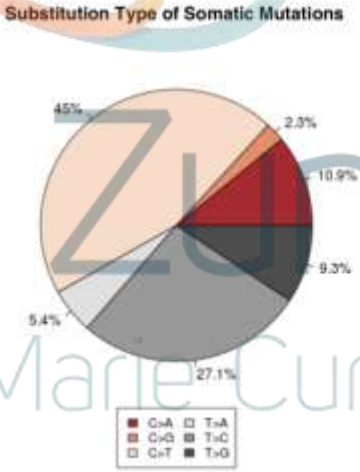


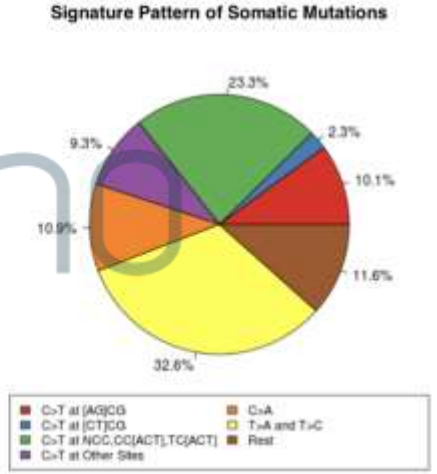
S. Peters at 2018 ASCO

Mutational Load





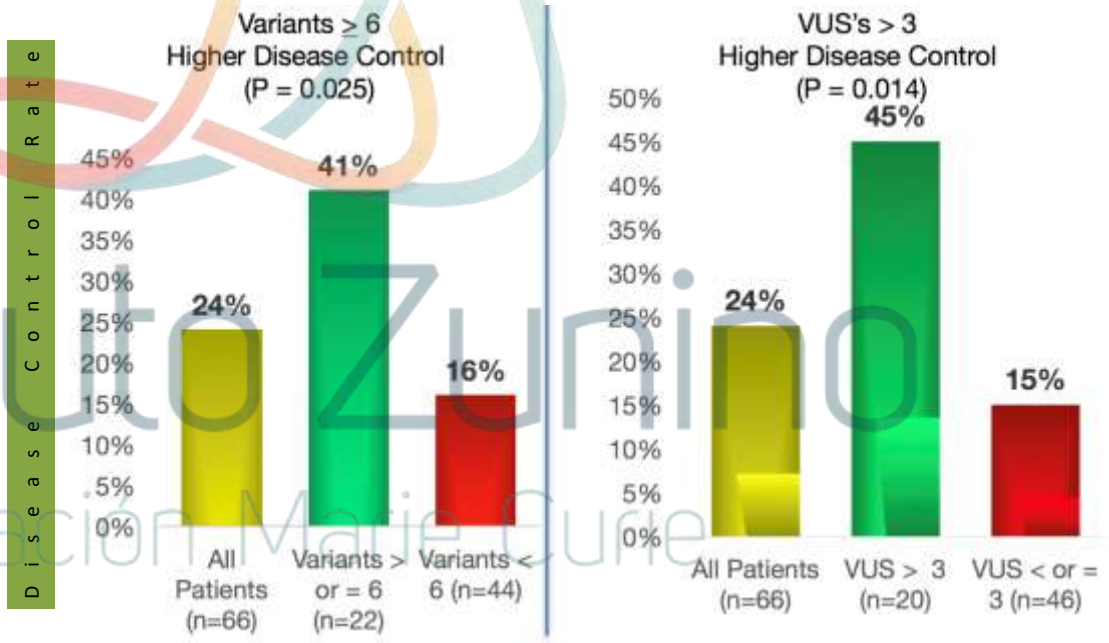




Oncomine sample report

Hypermutated Circulating Tumor DNA

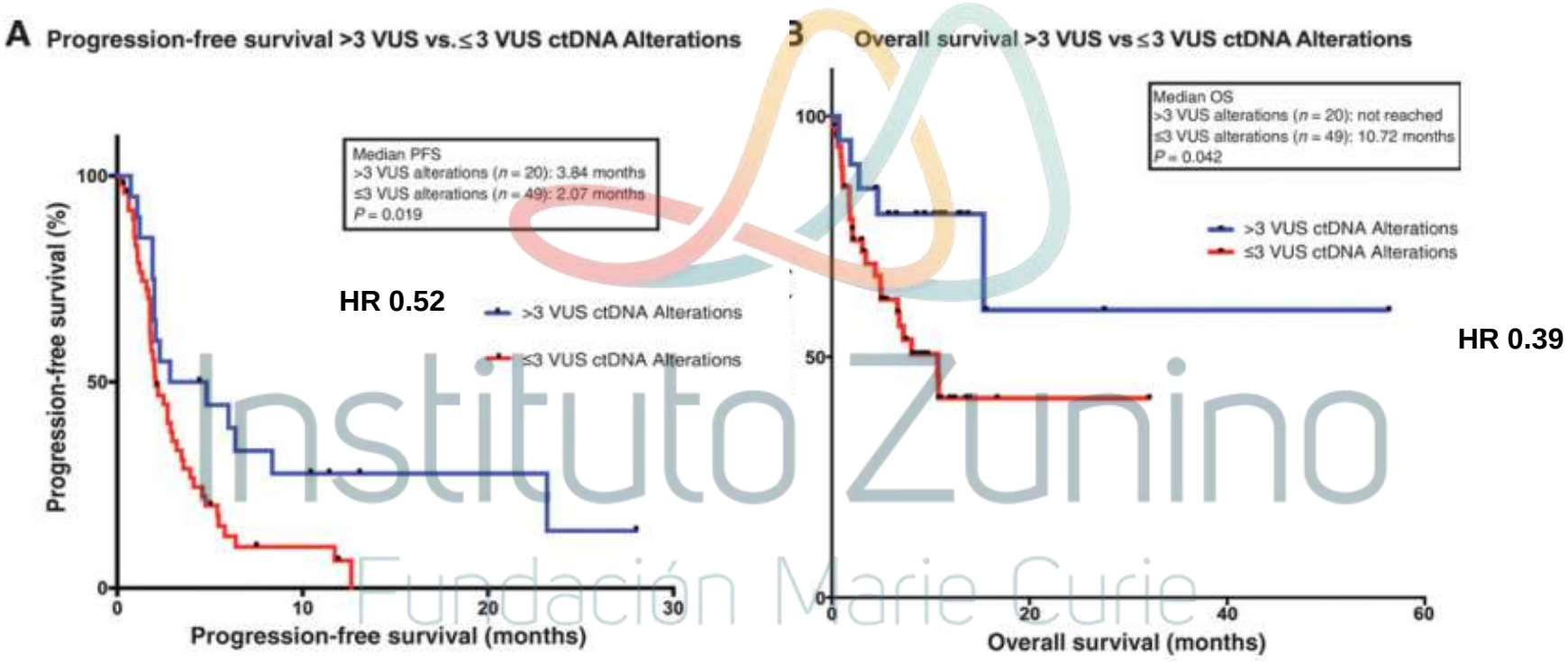
Hypermutated Circulating Tumor DNA: Correlation with Response to Checkpoint Inhibitor–Based Immunotherapy



Disease Control Rate: CR+ PR + SD

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

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

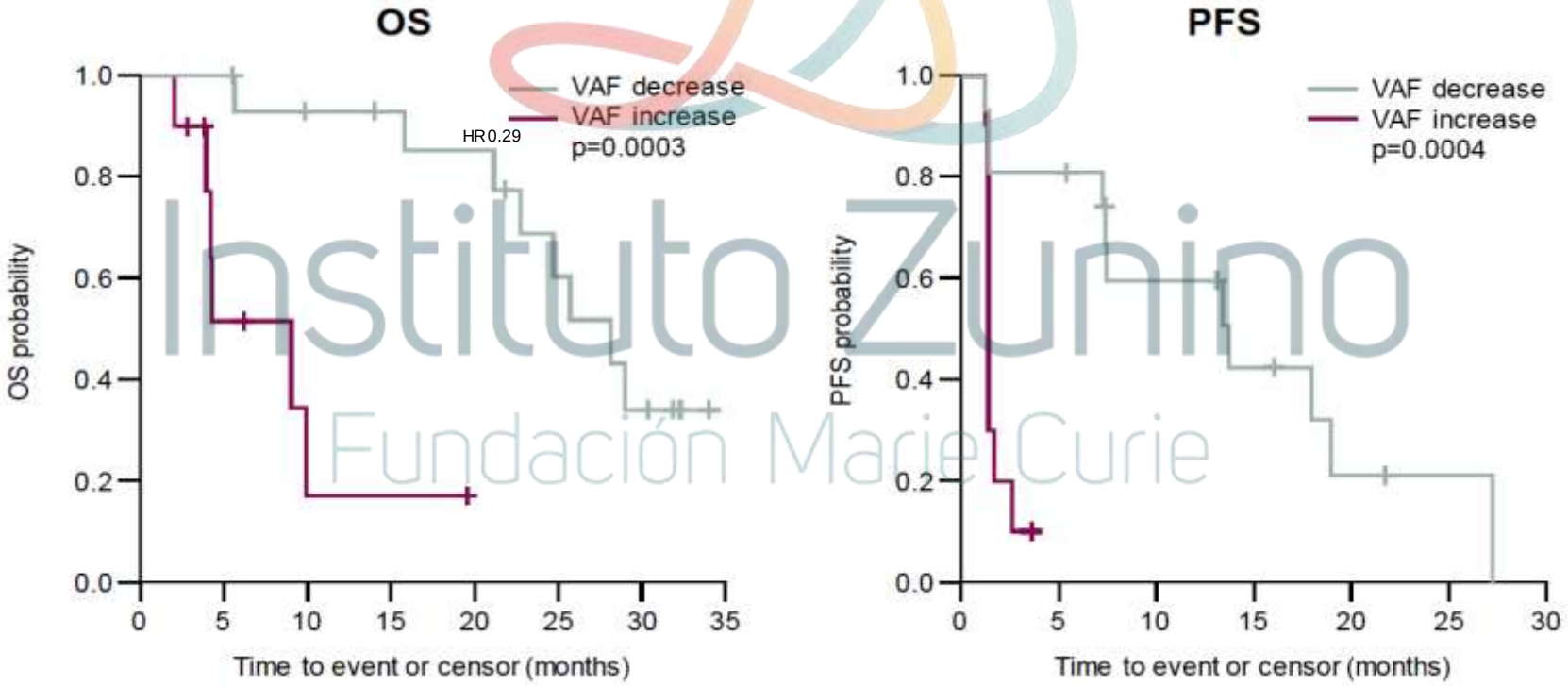
“ctDNA Velocity”: Change in ctDNA Allele Fractions at 6 weeks Predicts IO Response in NSCLC

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

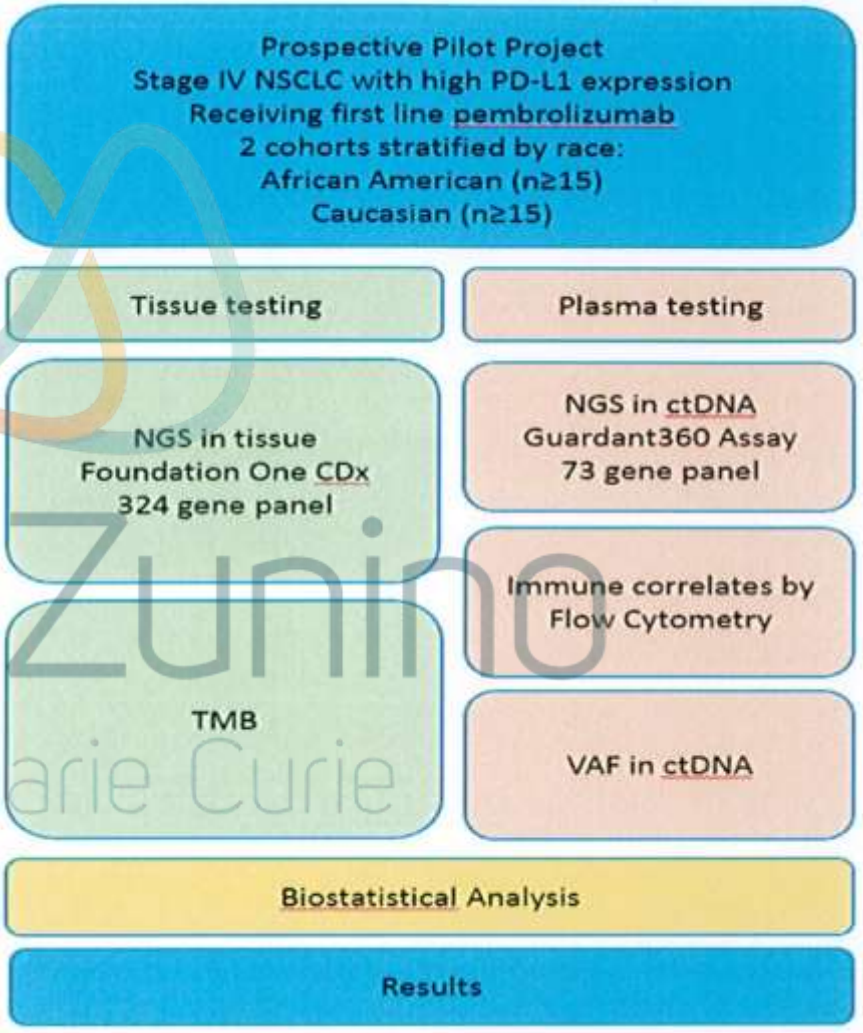


Kuziora (Ranade) et al. 2017
Abstract 582 AACR

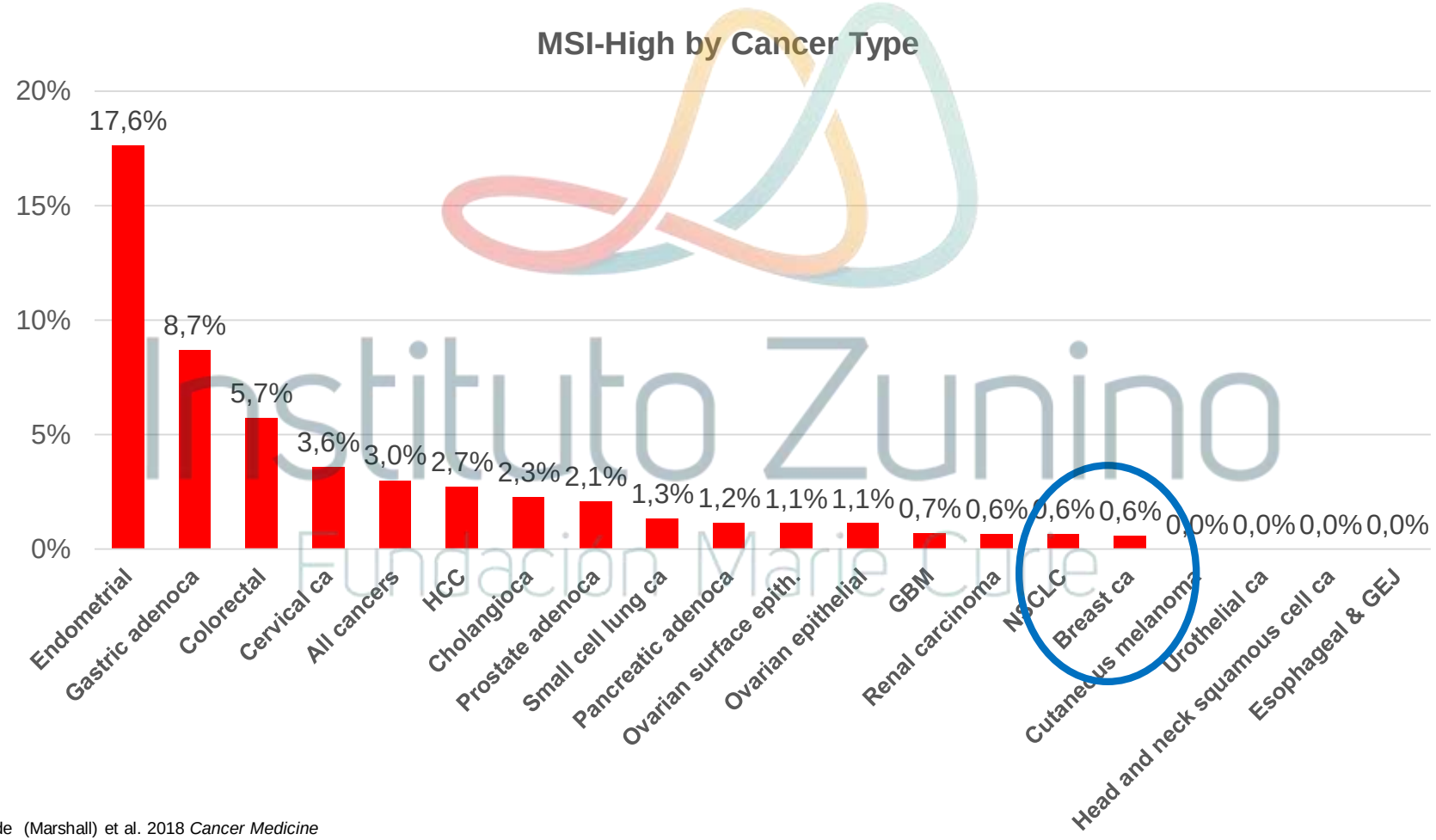
UNIVERSITY of MARYLAND
MARLENE AND STEWART GREENEBAUM
COMPREHENSIVE CANCER CENTER

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

Dr. Katherine Scilla

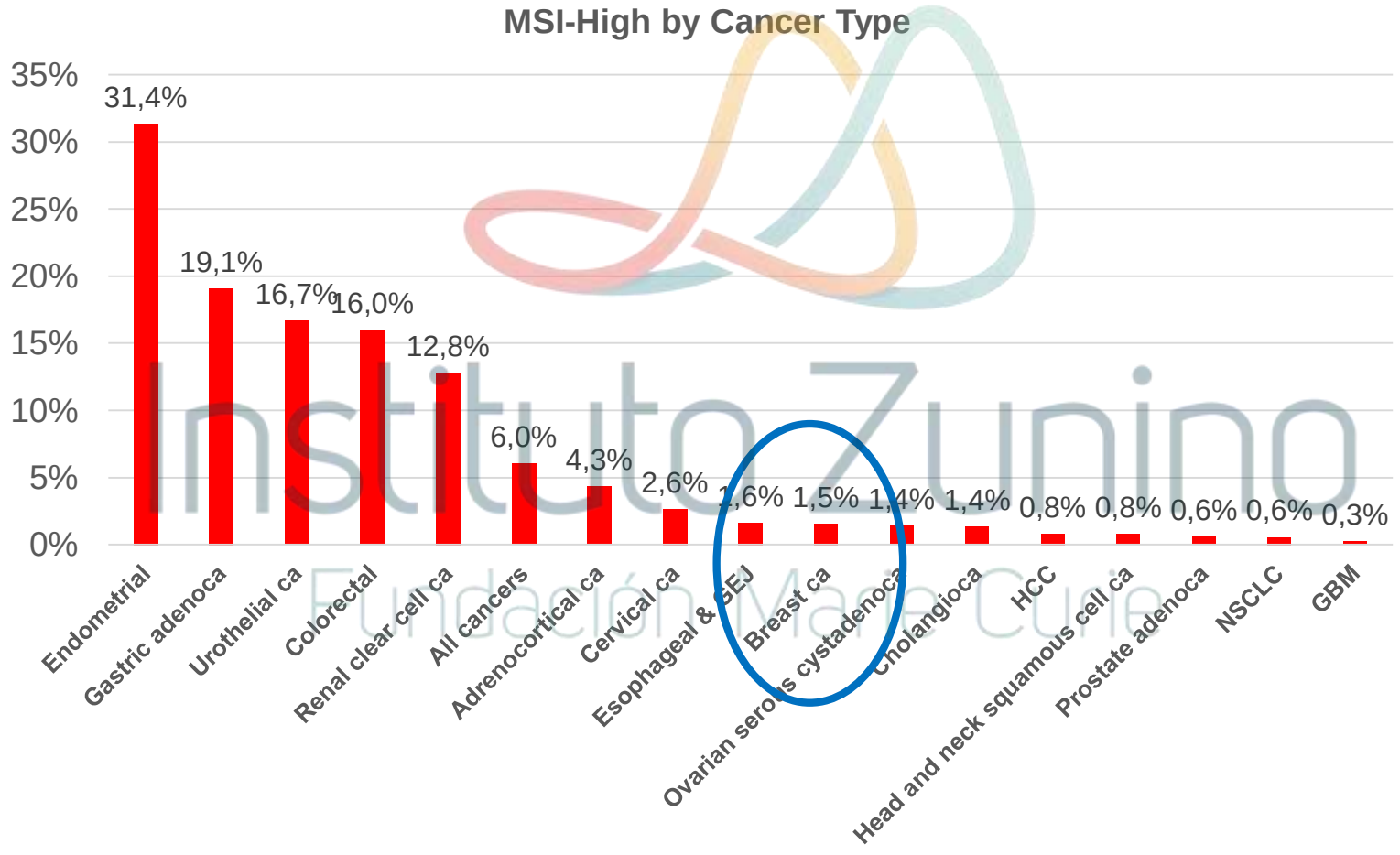


MSI-High in 2,189 Patients by Cancer Type with NGS



Vanderwalde (Marshall) et al. 2018 *Cancer Medicine*
N=2,189 with PCR and NGS tested by Caris Life Sciences

MSI-High Prevalence in TCGA by Whole Exome Seq

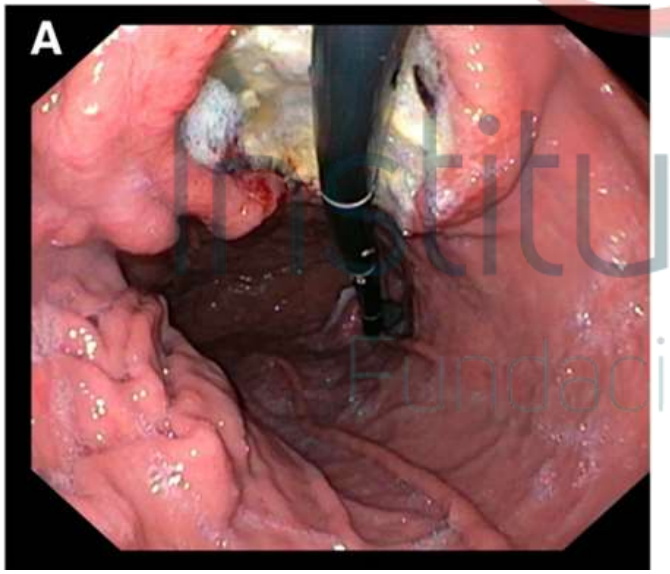


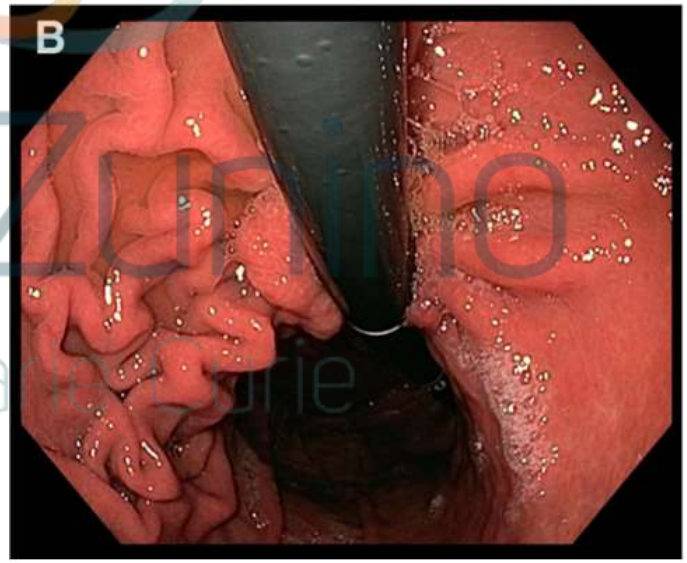
Bonneville (Chowdhury) et al. 2017 *JCO Precision Oncology*
11,139 paired tumor tissue - normal tissue samples; 10,415 from TCGA, 280 from TARGET, and 444 from other studies

Dramatic Response to Nivolumab in MSI-High Triple Negative Breast Cancer – A Case Report

69 yoF with TNBC 3 years earlier, now with mets in lung, stomach, & abdominal lymphadenopathy, multiple biopsies confirm TNBC in all affected organs, PD-L1 tissue expression not observed

IHC showed loss of MLH1 and PMS2 expression, and somatic hypermethylation of the MLH1 promotor was found. MSI was confirmed using PCR - after 3 cycles of nivolumab dramatic response to ulcerated 5 cm metastatic lesion in stomach





Kok (van Dieren) et al. 2017 *JCO Precision Oncology*

GuardantOMNI

- GuardantOMNI (OMNI), a highly sensitive **500-gene cfDNA** sequencing test requiring as little **as 2 mL** of plasma and designed for broad genomic detection of **somatic single-nucleotide variants (SNVs)** and **small indels in 497 genes, copy number amplifications** (CNAs) **in 106 genes, and fusions in 21 genes.**

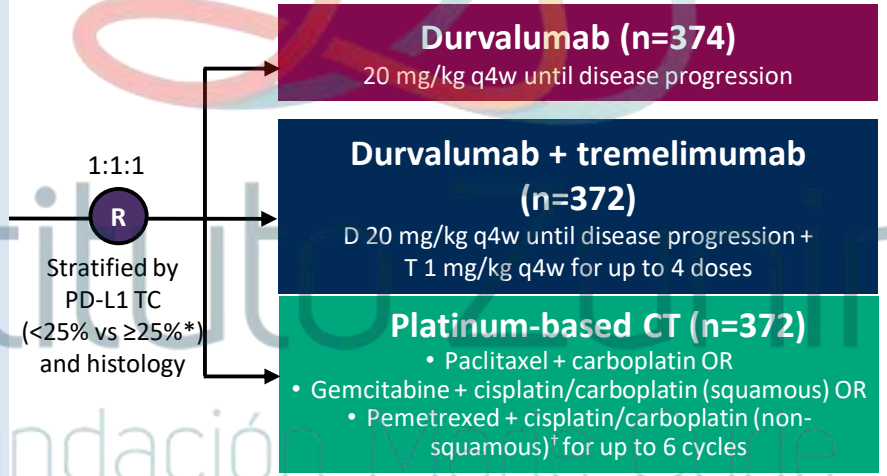
- Additionally, tumor mutational burden (**TMB**), and **DNA damage and mismatch repair**, with coverage of over **30 genes** associated with the DDR pathway.

Helman et al, AACR Annual Meeting 2018

MYSTIC study design: Phase 3, open-label, multicenter study

- Stage IV NSCLC
- All-comers population (i.e. irrespective of PD-L1 status)
- *EGFR–/ALK–*
- ECOG PS 0/1
- Immunotherapy- and CT-naïve

N=1118 randomized



Primary endpoints (PD-L1 TC ≥25%*)
- OS (D vs CT)
- OS (D+T vs CT)
- PFS (D+T vs CT)

Key exploratory endpoints
- OS by bTMB and tTMB

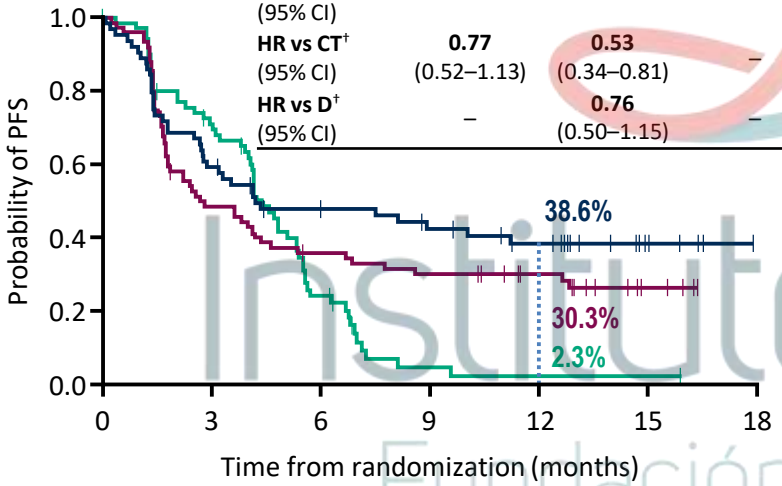
- *Ventana PD-L1 (SP263) assay using newly acquired or archival (<3 months) tumor biopsy; †Followed by pemetrexed maintenance therapy if eligible; bTMB, blood tumor mutational burden; CT, chemotherapy;
- D, durvalumab; ECOG, Eastern Cooperative Oncology Group; mNSCLC, metastatic non-small cell lung cancer; OS, overall survival; PD-L1, programmed cell death ligand-1; PFS, progression-free survival; PS, performance status; T, tremelimumab; TC ≥25%, ≥25% of tumor cells with membrane staining for PD-L1; tTMB, tissue tumor mutational burden
- 1. Garassino MC, et al. Lancet Oncol 2018;19:521–536; 2. Kowalski D, et al. Presented at ESMO 2018, #1378O; 3. Rizvi N, et al. Presented at ESMO I-O 2018, #LBA6

S. Peeters et al, AACR 2019

PFS in Patients With Blood TMB ≥20 and <20 mut/Mb

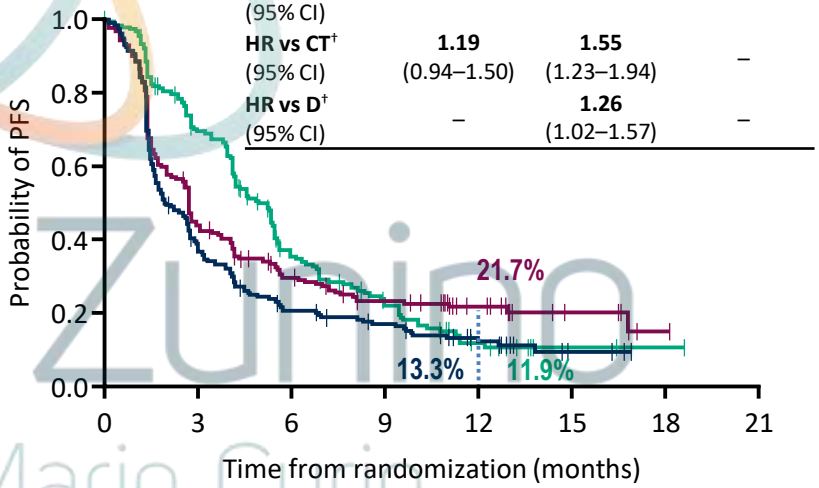
bTMB ≥20 mut/Mb

	D (n=77)	D+T (n=64)	CT (n=70)
mPFS,* months (95% CI)	**2.7** (1.8–4.4)	**4.2** (2.8–NR)	**4.4** (4.1–5.4)
HR vs CT† (95% CI)	**0.77** (0.52–1.13)	**0.53** (0.34–0.81)	–
HR vs D† (95% CI)	–	**0.76** (0.50–1.15)	–



bTMB <20 mut/Mb

	D (n=209)	D+T (n=204)	CT (n=185)
mPFS,* months (95% CI)	**2.8** (2.2–3.1)	**2.0** (1.7–2.8)	**5.0** (4.2–5.5)
HR vs CT† (95% CI)	**1.19** (0.94–1.50)	**1.55** (1.23–1.94)	–
HR vs D† (95% CI)	–	**1.26** (1.02–1.57)	–



No. at risk

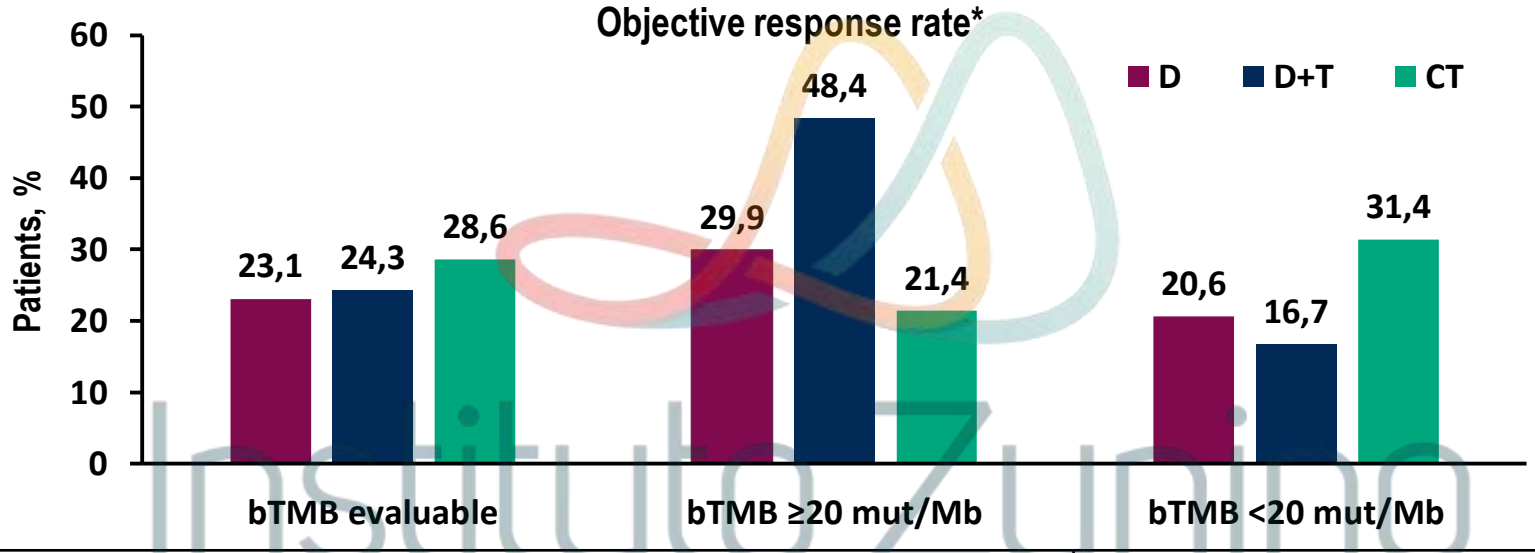
D	77	35	25	21	16	4	0
D+T	64	38	28	23	18	5	0
CT	70	47	14	2	1	1	0

D	209	86	53	38	20	6	1	0
D+T	204	77	37	28	14	3	0	0
CT	185	116	52	30	10	2	1	0

- *Blinded independent central review per RECIST v1.1; †Unadjusted; data cut-off June 1, 2017
- mPFS, median progression-free survival; NR, not reported; RECIST, Response Evaluation Criteria for Solid Tumors.

S. Peeters et al, AACR 2019

Tumor Response in Patients With Blood TMB ≥20 and <20 mut/Mb

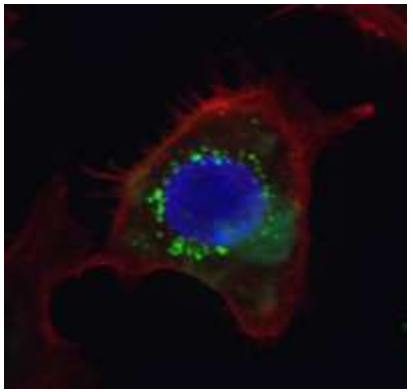


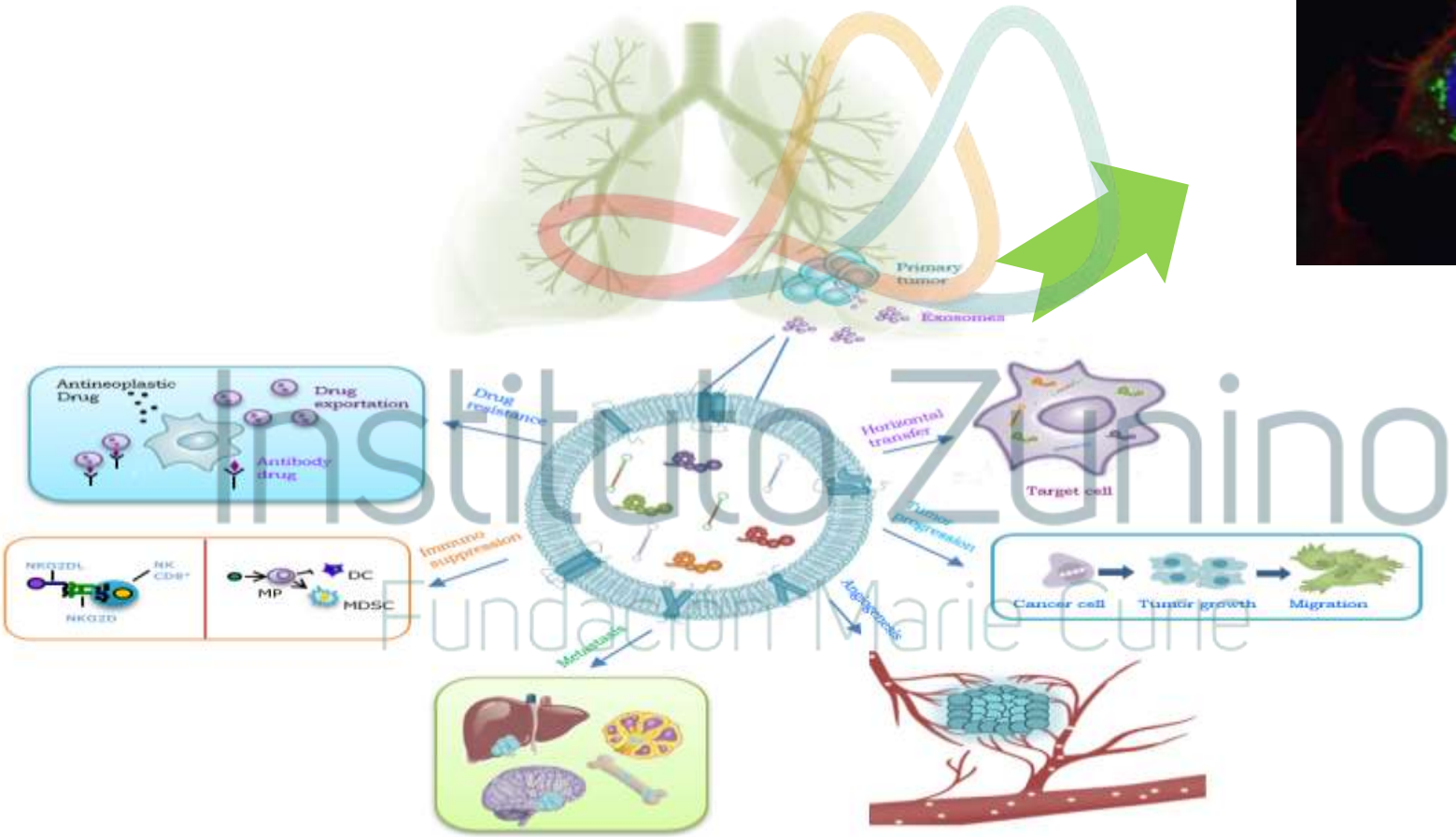
	bTMB ≥20 mut/Mb			bTMB <20 mut/Mb		
	D (n=77)	D+T (n=64)	CT (n=70)	D (n=209)	D+T (n=204)	CT (n=185)
Patients with response, n	23	31	15	43	34	58
Remaining in response at 6 mo, %	86.5	85.6	14.4	64.0	66.6	33.3
Remaining in response at 12 mo, %	80.3	81.7	7.2	59.1	48.2	14.3

*Blinded independent central review per RECIST v1.1; responses include unconfirmed responses; data cut-off June 1, 2017

S. Peeters et al, AACR 2019

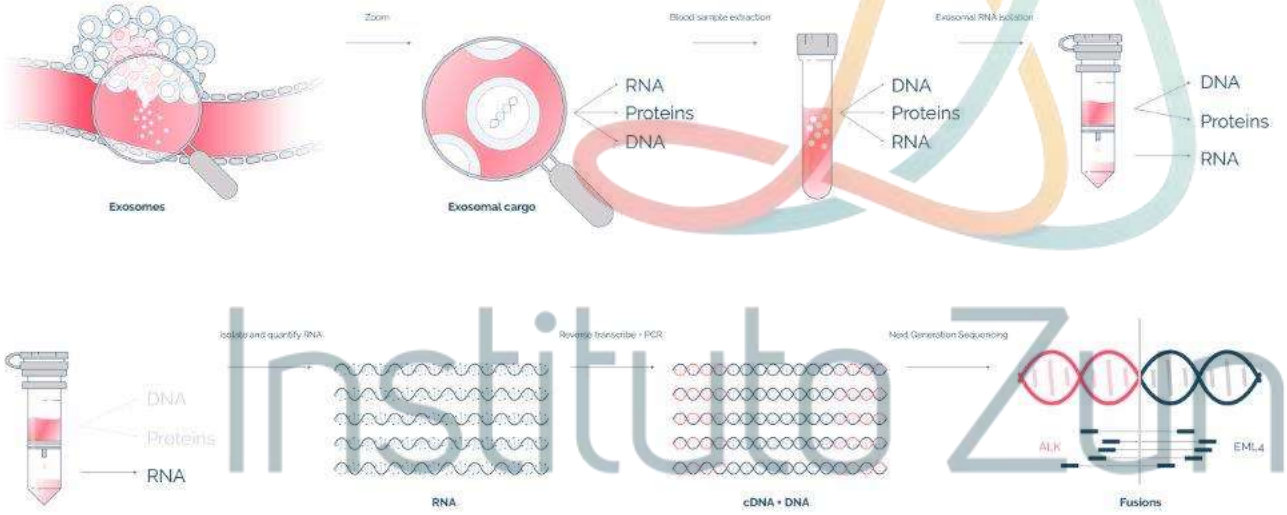
Exosomes in lung cancer





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

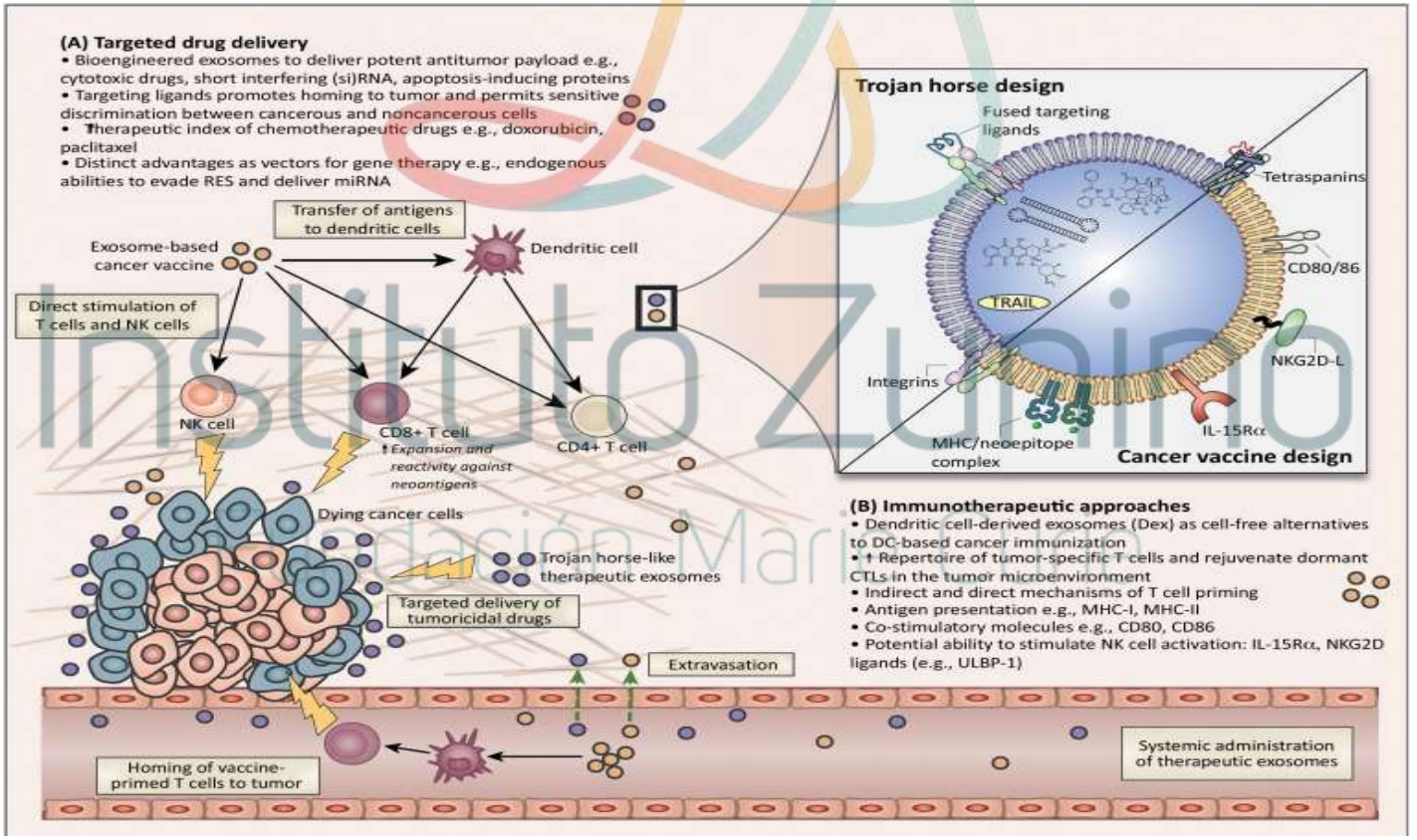
ALK-EML4 FISH detection in Tissue (n=19)		
	Positive (16)	Negative (3)
Positive (9)	9	0
Negative (8)	5	3
	Sensitivity 64%	Specificity 100%

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

Exosomes in IO: potential therapeutic implication

Muthukumar Gunasekaran, PhD



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.

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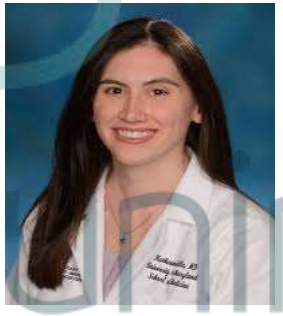
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Thanks

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