Liquid Biopsy in Cancer:

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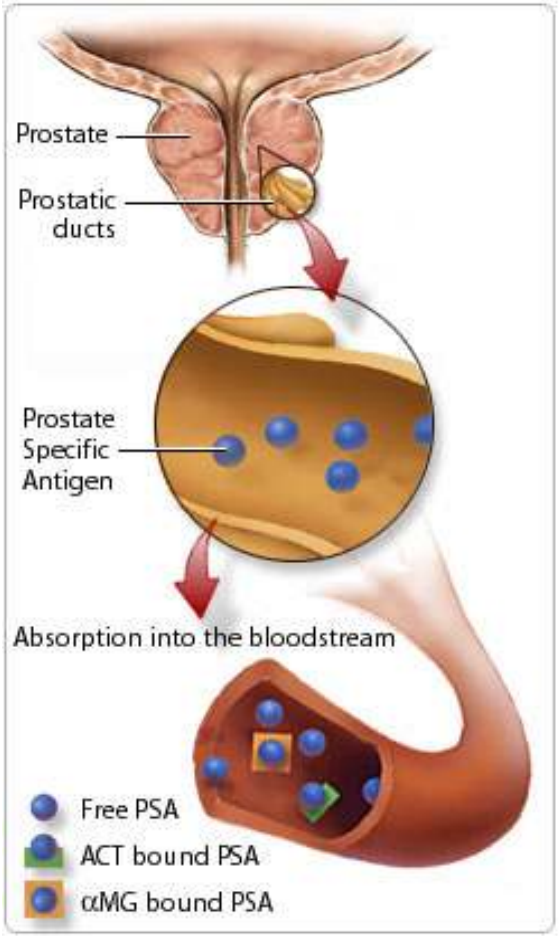
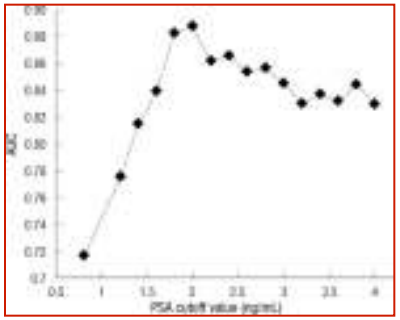
Disclosures

- Novartis International Speaker bureau
- Boeringher Speaker Bureau
- MSD – Merck Speaker Bureau
- Oncompass Molecular Profile Steering Committee board Member
- Mylan Biosimilars Advisor for NSCLC
- Guardant Health speaker bureau
- OncoDNA research grant for exosomes

The concept of Non invasive test....





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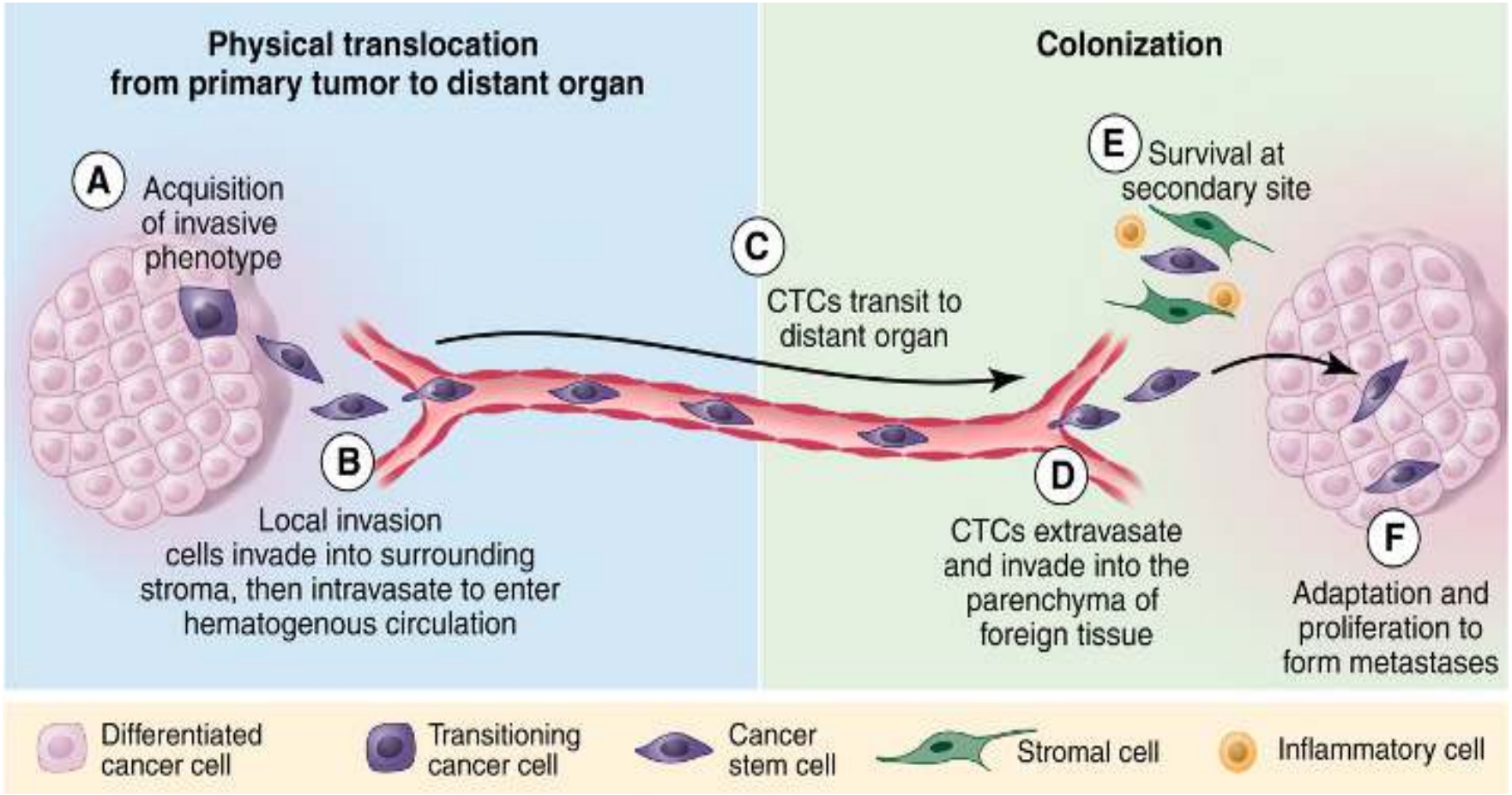
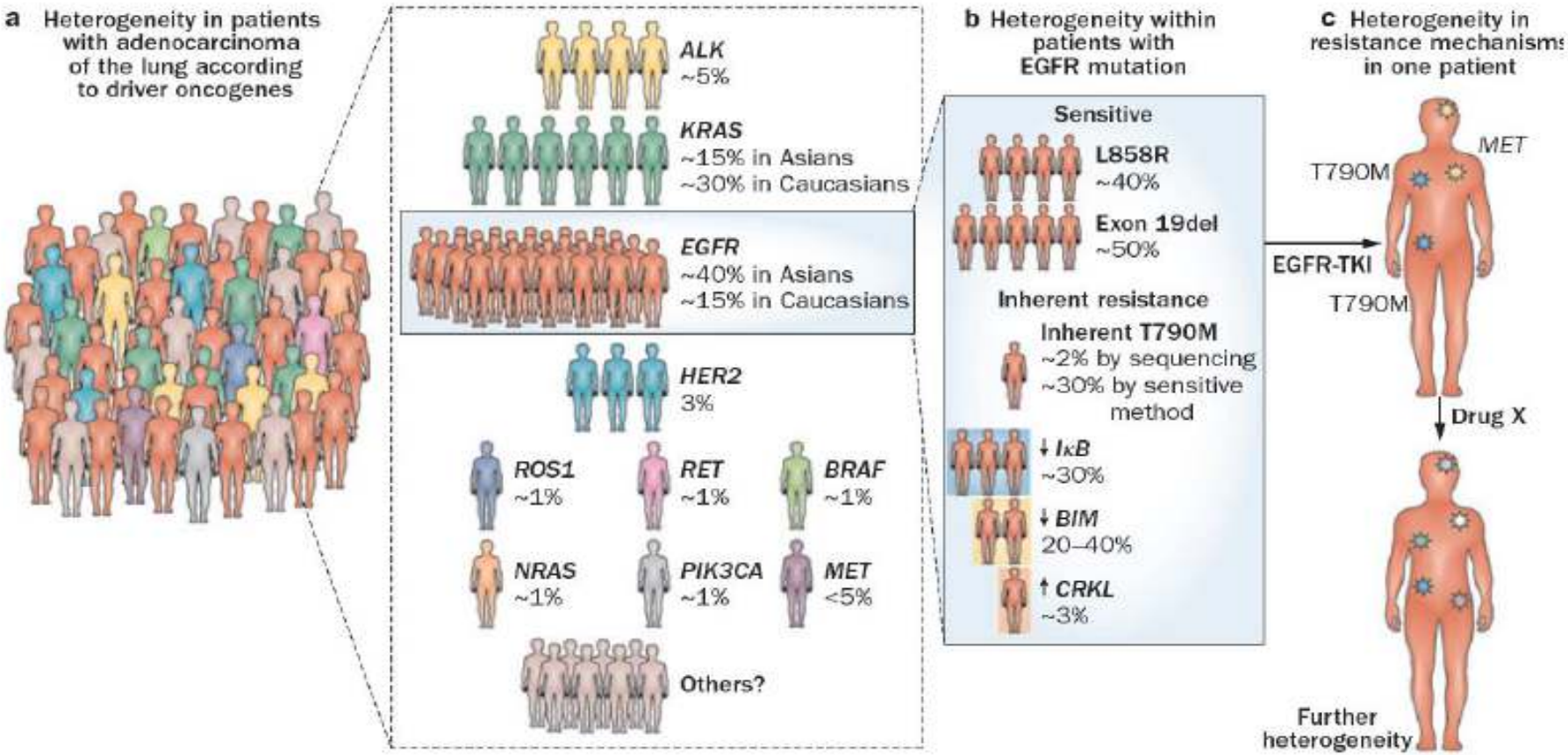
Beginning of Concept of Liquid Biopsy



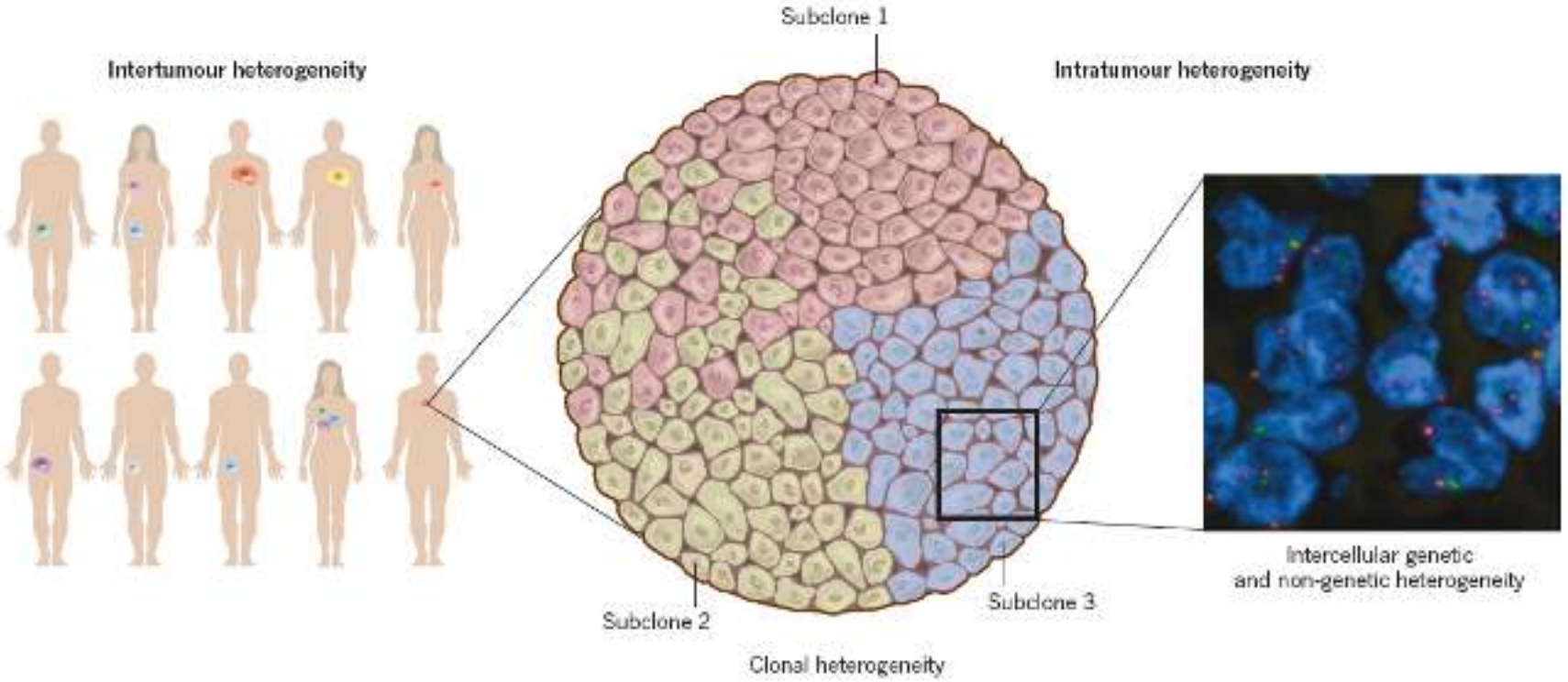
The efficacy of target therapy is affected by…

TUMOR HETEROGENEITY



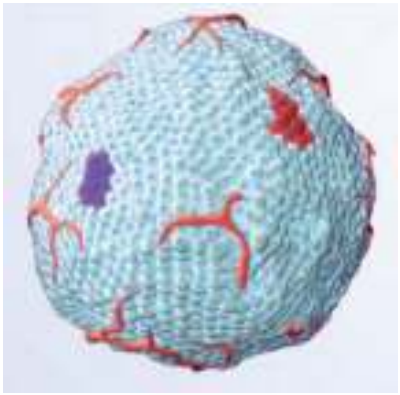
Mitsudomi Nat Rev Clin Oncol 2013

Intertumor and intratumor heterogeneity



Identify biomarkers to define phenotypic similarity, yet genetically diverse, to guide treatment – Still A Challenge

Temporal Heterogeneity: Tumors Evolve Over Time to Develop Treatment Resistance

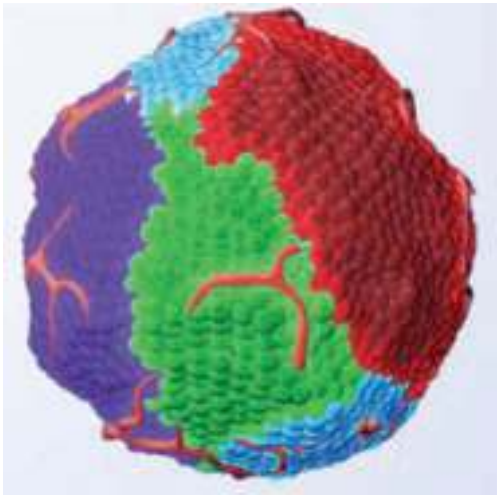


Sensitive Clone

Response

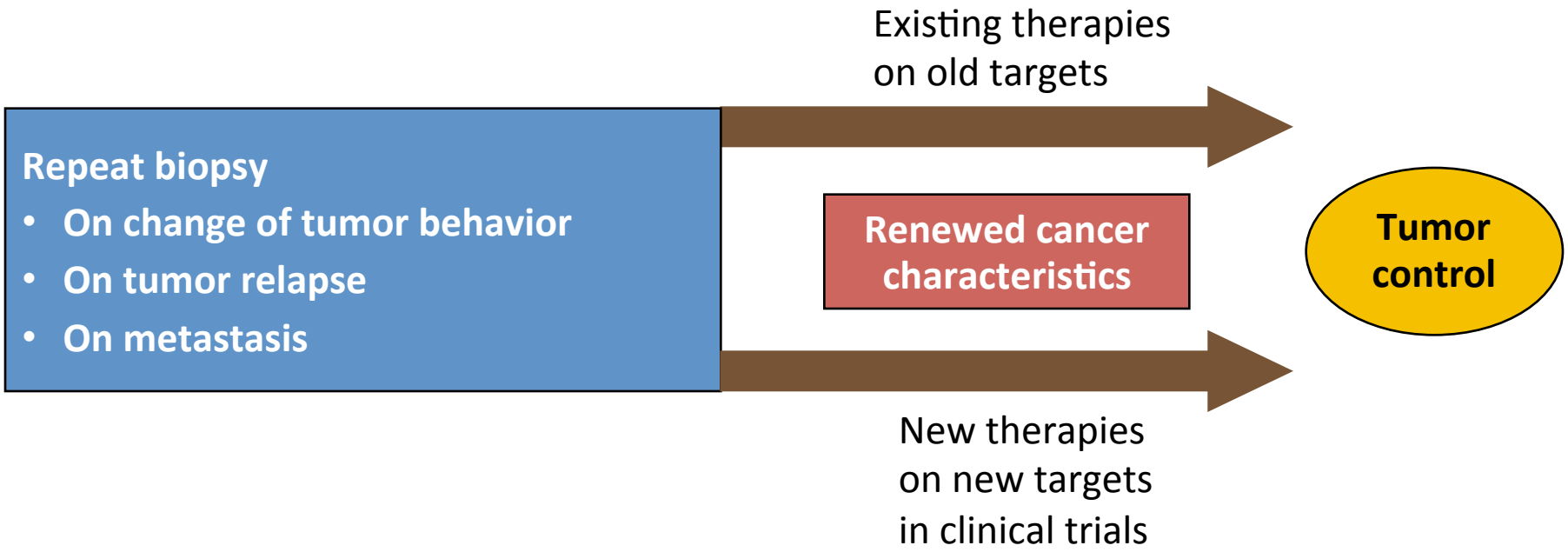
Resistant Clones

Progression



Rebiopsies May Give Insight into Resistance Mechanisms

- Repeat biopsies can drive our understanding and could lead to future treatment
- They have the potential to predict future therapy response



4.Jekunen AP. *J Oncol.* 2015;2015:809835.

Multiple Tests Require Large Tissue Volume

Tumor Biopsy

Finite tissue

Histology

↓

Cancer

≥2 slides

Anatomy

↓

Adeno-carcinoma

≥1 slide

cMET

EGFR

5 µm tissue

BRAF

PI3K

FGFR

≥5 slides

IHC (ALK+)

≥2 slides but no tissue

Confirmatory FISH

≥2 slides

RELAPSE

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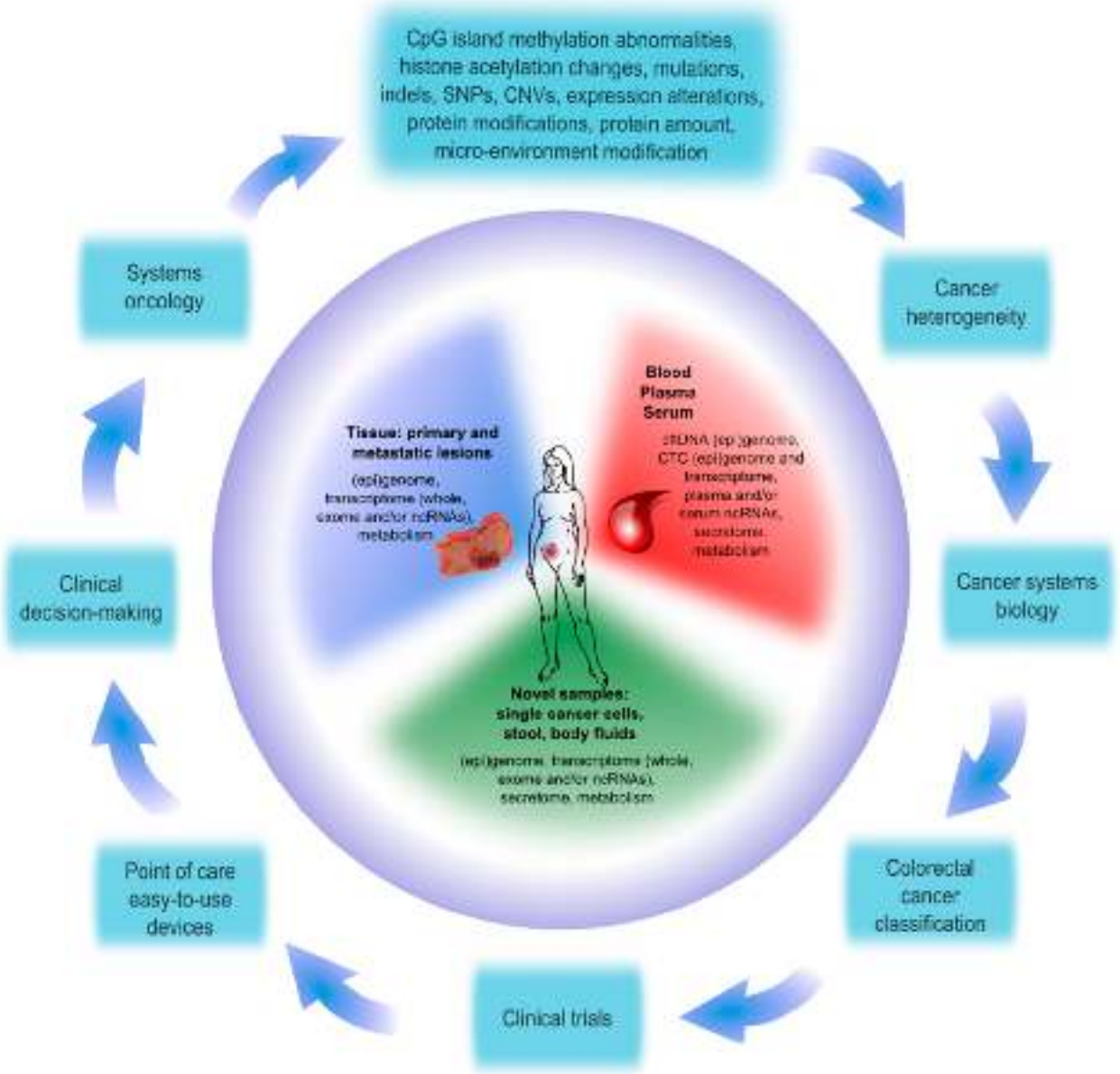
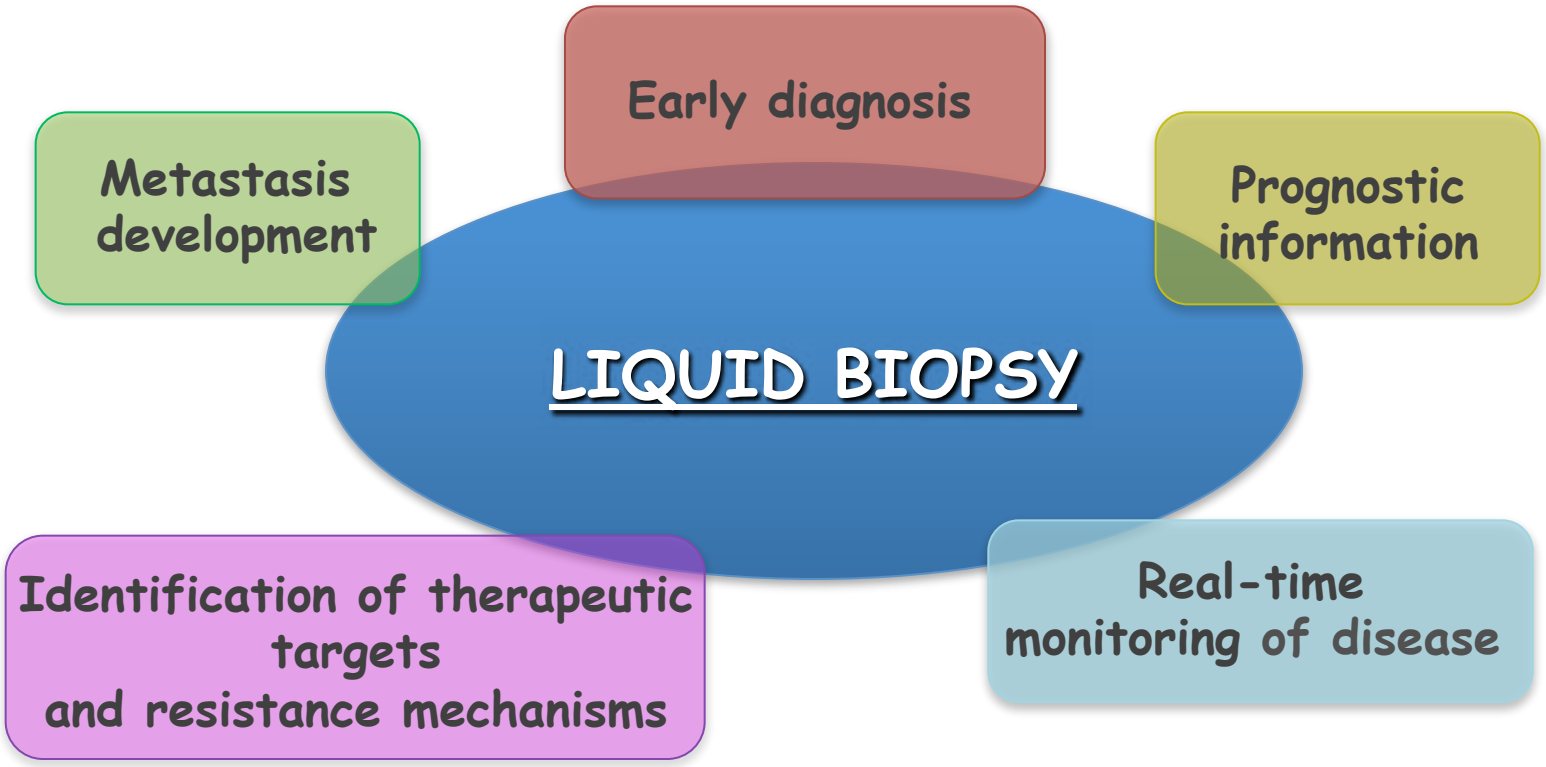
Why the need of Liquid Biopsy?

1. **Tumor biopsies** of both primary and metastases is often **difficult for practical reason**
2. **Tissue** biopsy is **not always representative** for the all tumor (especially for fine-needle biopsy) → Tumor Heterogeneity
3. **Lack of sensitive and specific biomarker** for tumor early detection and monitoring (treatment response, relapse, etc…)
4. The concept of **Tempo-spatial heterogeneity**

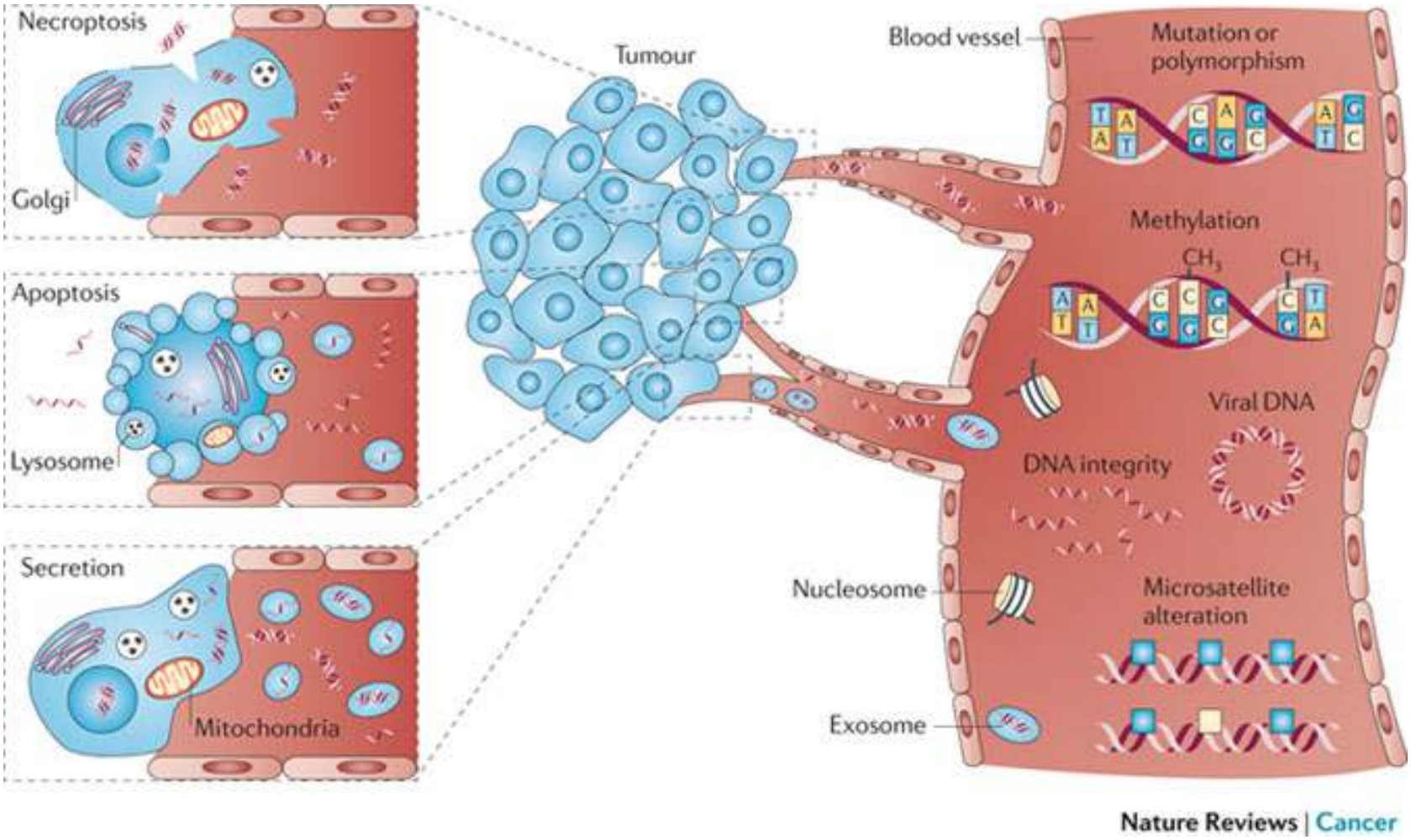
The complete picture…



Liquid Biopsy: clinical application

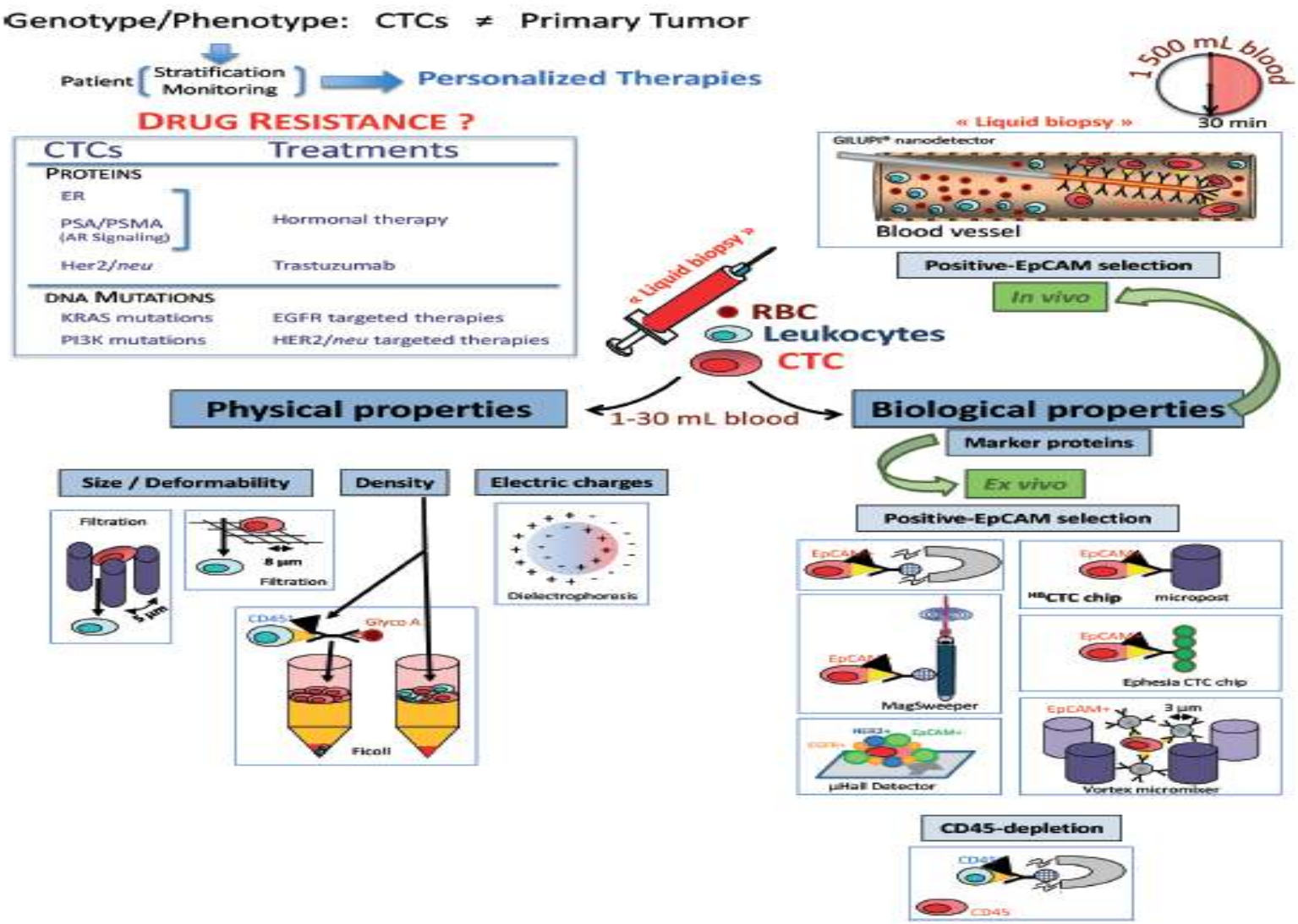


Some liquid Biopsy components



Wei-Lun Huang et al. BioMed Research International Volume 2015, Article ID 340732

CTCs enrichment techniques

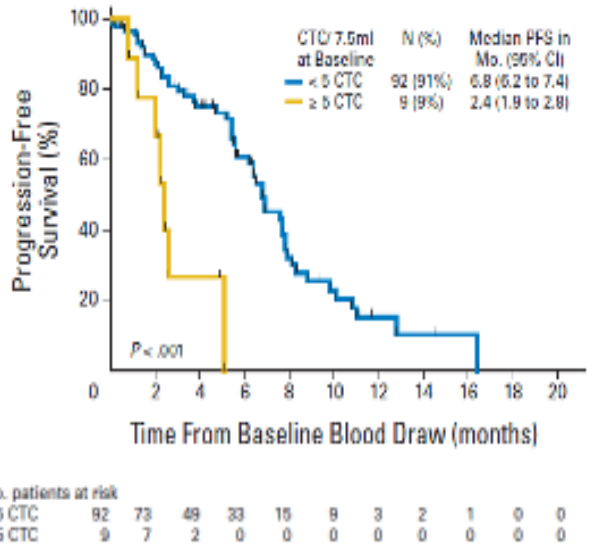


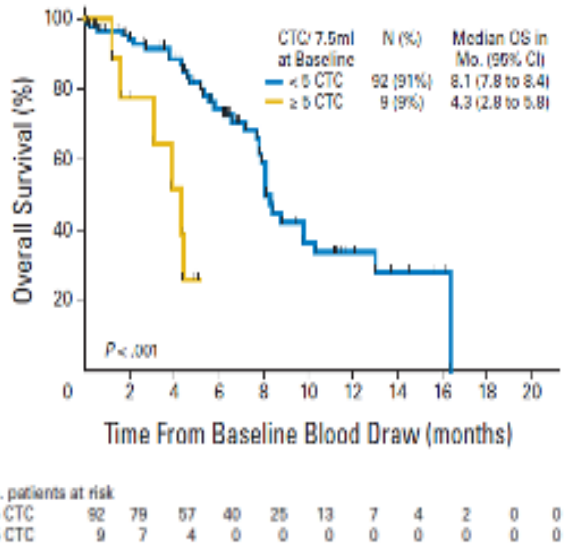
Alix-Panabières C, Pantel K. Lab Chip. 2014

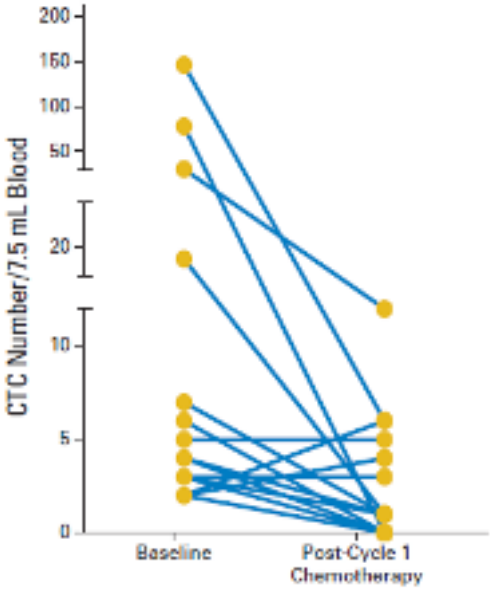
CTC clinical application

Evaluation and Prognostic Significance of Circulating Tumor Cells in Patients With Non-Small-Cell Lung Cancer

- Single-center prospective study
- Blood samples for CTCs analysis from 101 NSCLC patients (untreated, stage III or IV) collected before and after one cycle of standard chemotherapy







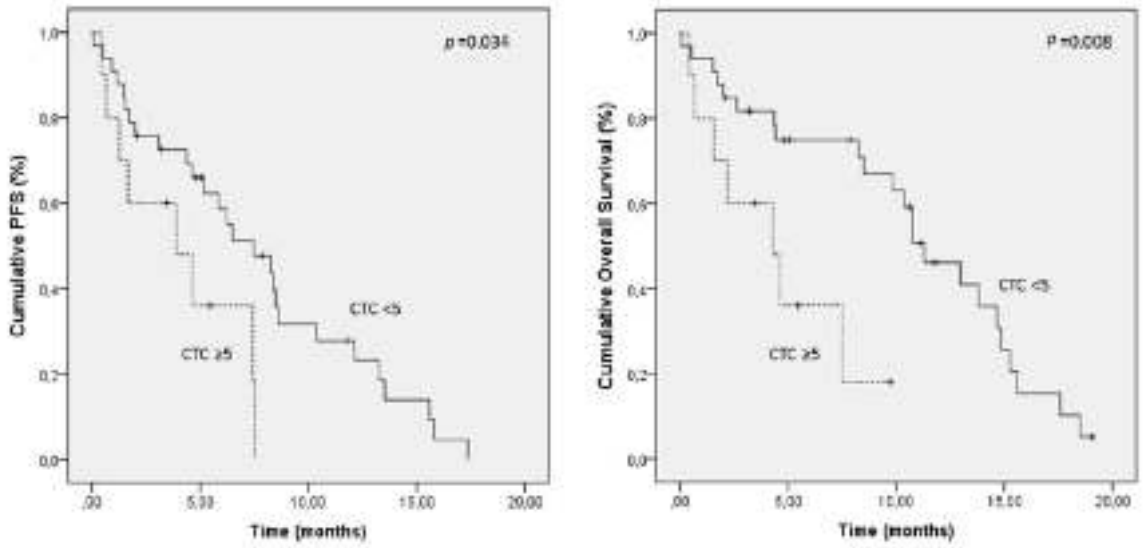
Kaplan-Meier curves for PFS and OS of patients with < 5 and >5 CTC in 7.5ml at baseline

Krebs et al. JCO 2011

CTC clinical application

Evaluation of Circulating Tumor Cells and Related Events as Prognostic Factors and Surrogate Biomarkers in Advanced NSCLC Patients Receiving First-Line Systemic Treatment

"The clinical value of CTC as a surrogate biomarker relies on how consistently and accurately CTC can reflect tumor burden, prognosis and response to therapy. The possibility that CTC enumeration could stratify patients into prognostic subgroups with differential outcomes, and modify treatment plans to alter the course of NSCLC, would have an impact on patient management."



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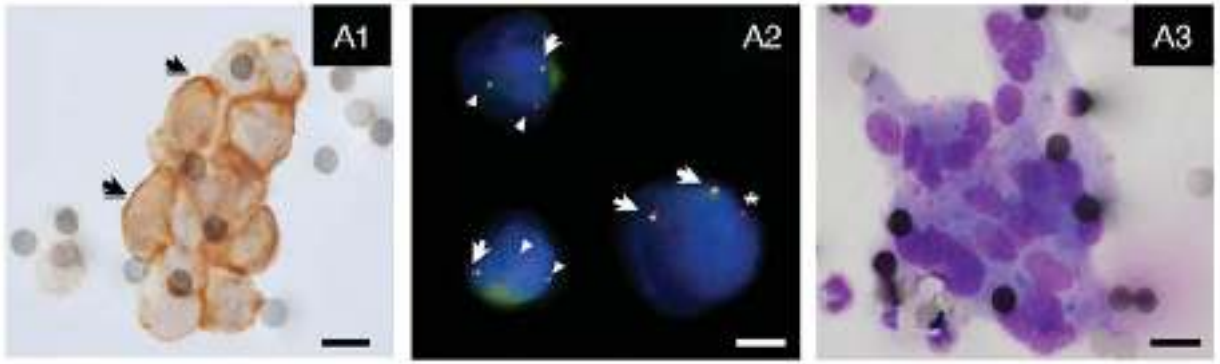
Muinelo-Romay L. et al., Cancers 2014

CTCs: FISH and IHC testing

ALK-gene rearrangement: a comparative analysis on circulating tumors cells and tumors tissue from patients with lung adenocarcinoma.



➢Circulating tumor cells showing an intense and cytoplasmic staining with some membrane reinforcements for ALK

➢Circulating cell nuclei hybridized with a dual-color 2p23 LSI ALK locus-specific split probe. The two probes show a distinct separation of the red and green signals indicating a rearrangement in the 2p23 ALK-gene locus.

Ilie M; at al, Ann Oncol 2012

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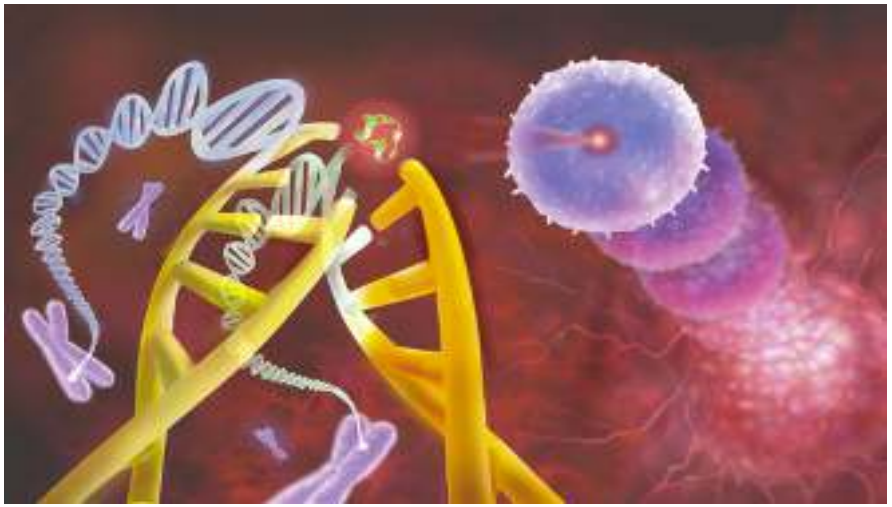
Cell Free DNA and Circulating Tumor DNA



Pantel K, Diaz LA Jr, Polyak K. Tracking tumor resistance using 'liquid biopsies'. Nat Med. 2013

- Cell-free DNA (cfDNA) → DNA released in the bloodstream from apoptotic and necrotic cell
- Circulating-tumor DNA (ctDNA)→ proportion of the cfDNA released from tumor cells
- ctDNA can be used and as a tool to evaluate in real time the "molecular condition" of the disease

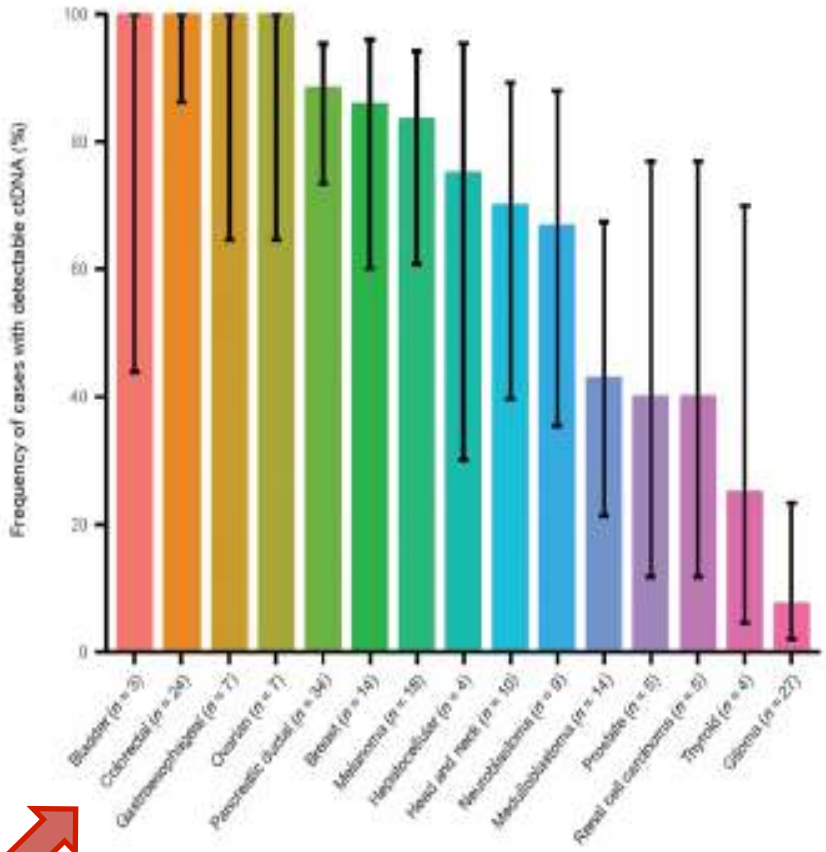
PRO & CON

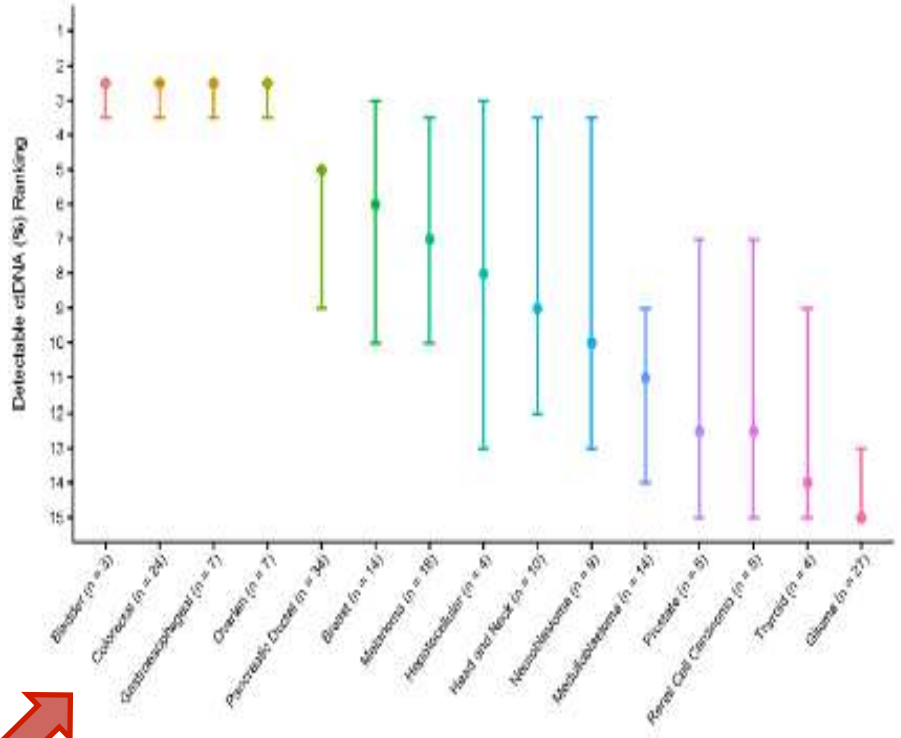
1. Minimal invasive marker
2. Early detection of drug resistance development
3. Driver mutation detection from blood samples
4. Solving the issue regarding "insufficient material for analysis"

1. Lack of standardized and widely approved methods for analysis
2. Contamination with cfDNA from healthy cells

Liquid biopsy: ctDNA

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





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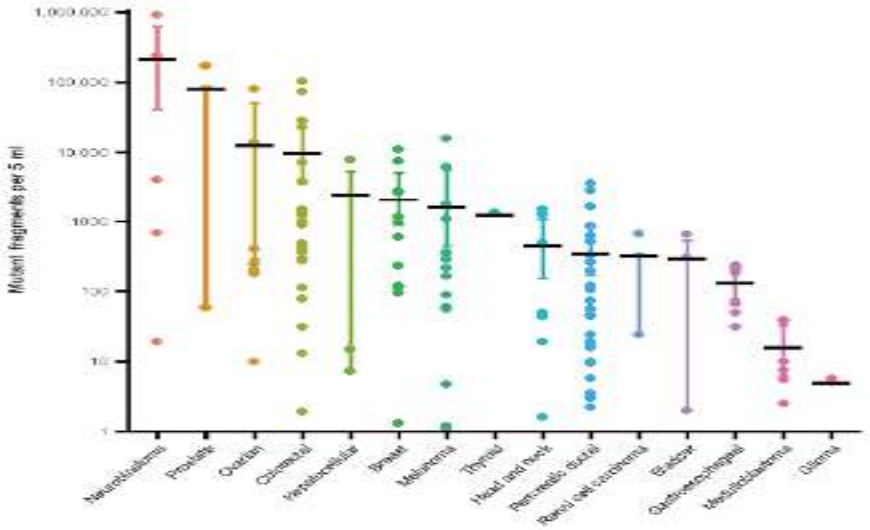
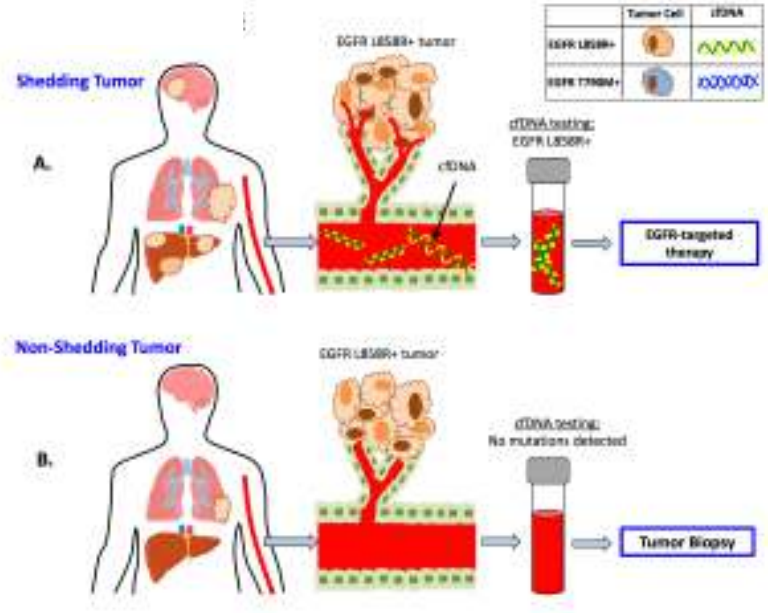
Bettegowda et al., Sci Trans Med, 2014

Liquid biopsy: ctDNA

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

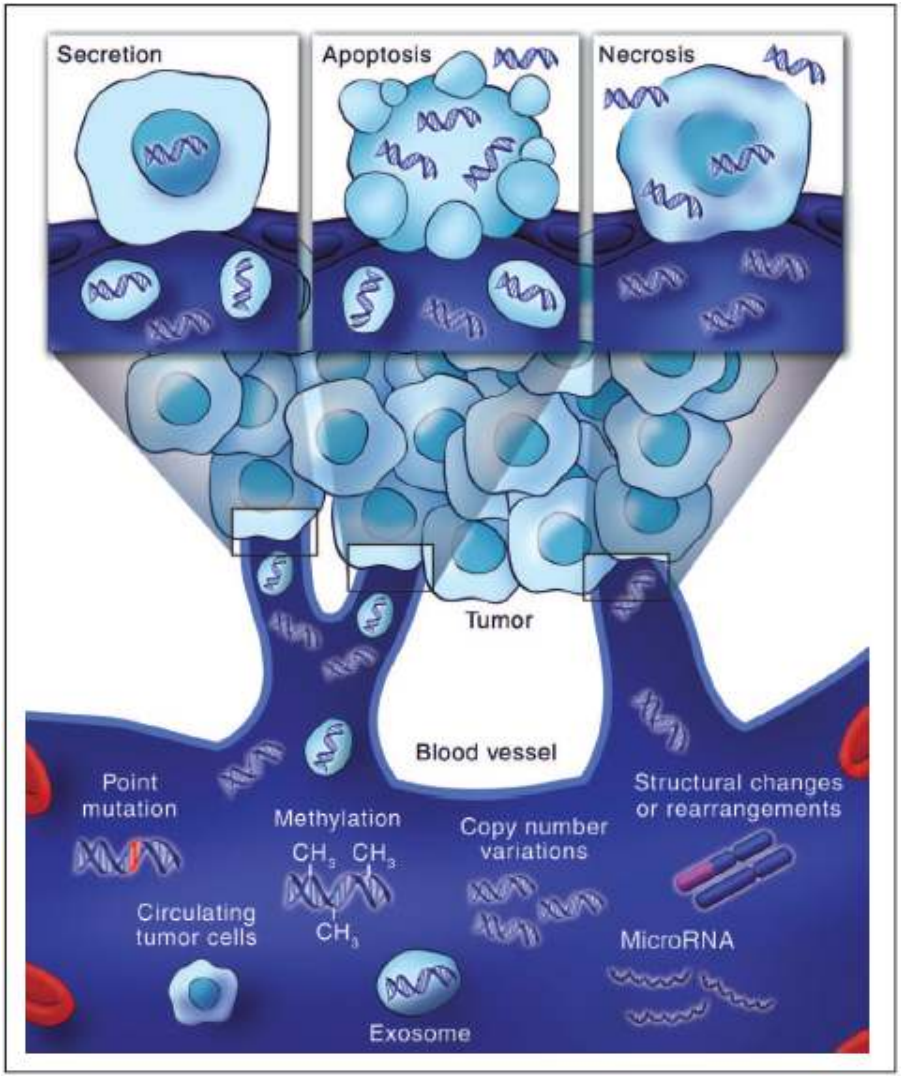


Bettegowda et al., Sci Trans Med, 2014



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

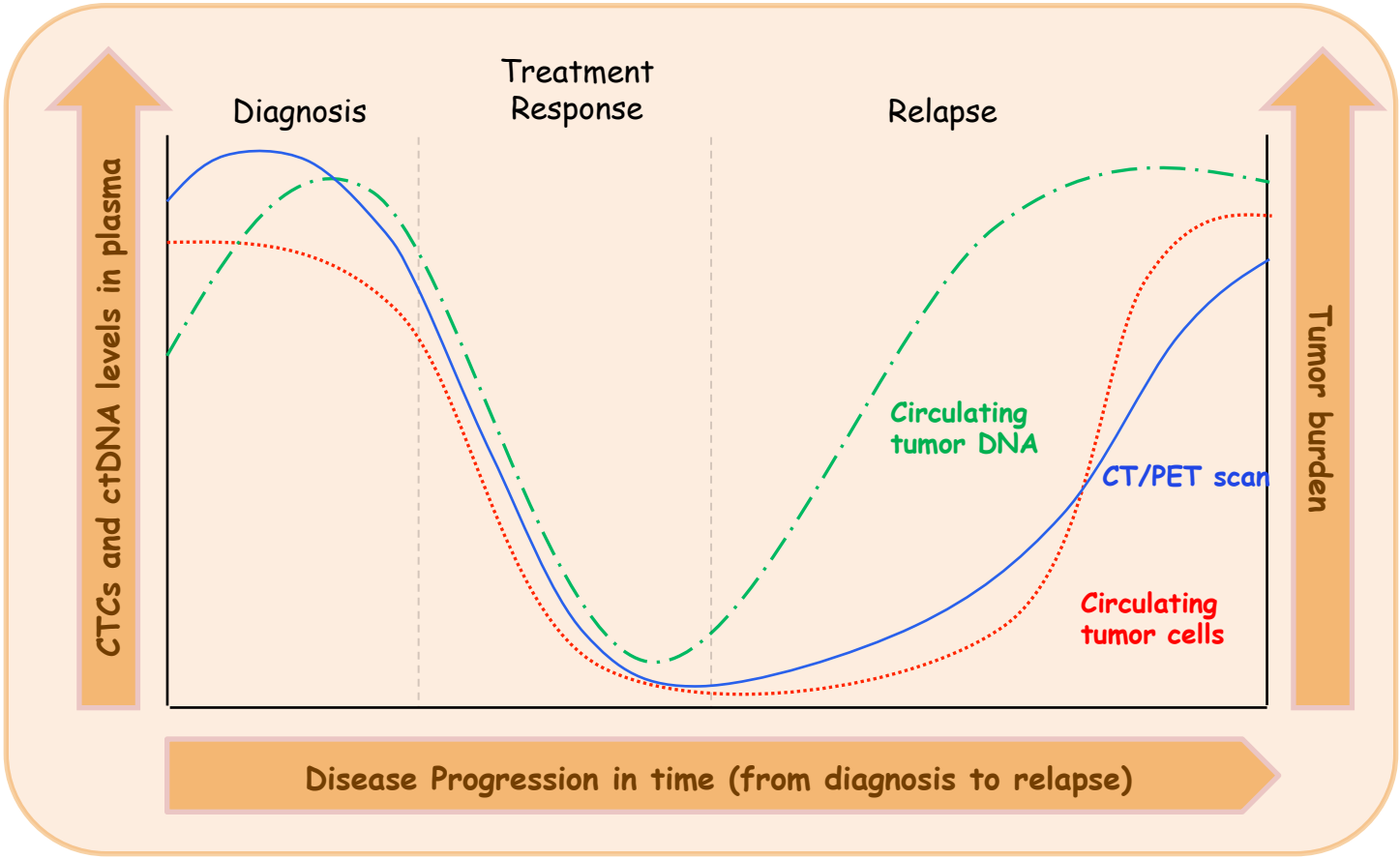
Liquid Biopsies: Circulating Tumor DNA



Technique	Sensitivity	Optimal Application
Sanger sequencing	> 10%	Tumor tissue
Pyrosequencing	10%	Tumor tissue
Next-generation seqeuncing	2%	Tumor tissue
Quantative PCR	1%	Tumor tissue
ARMS	0.10%	Tumor tissue
BEAMing, PAP, Digital PCR, TAM-Seq	0.01% or lower	ctDNA, rare variants in tumor tissue

Diaz L. and Bardelli A. JCO , 2014

Modifications of CTCs and ctDNA during three phases of cancer disease



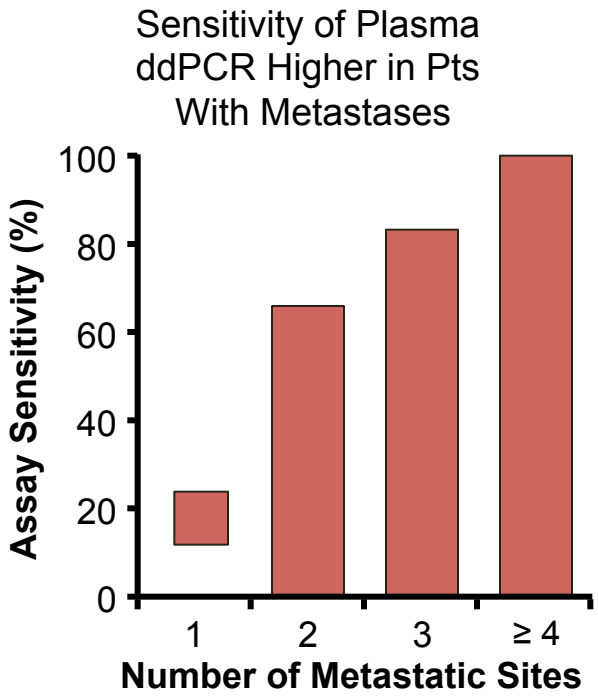
Rolfo, Castiglia, Russo et al. Biochim Biophys Acta. 2014 Dec;1846(2):539-46

Druggable targets : tissue and liquid biopsies

Genetic Source	Heterogeneity	Drug	Clinical Significance	Sample Source	Analysis
RAS (KRAS, NRAS)	Mutations	Anti-EGFR antibodies	Predictive	Primary and metastatic tissue, CTC, cfDNA	Next-generation and Sanger sequencing, BEAMing®, high-performance liquid chromatography, dropled dPCR, qPCR
BRAF	Mutations	Chemotherapy and targeted agents	Prognostic, possible predictive (anti-EGFR antibodies)	Primary and metastatic tissue, cfDNA	Next-generation and Sanger sequencing, high-performance liquid chromatography, BEAMing®, qPCR
MMR system (e.g., MLH1 gene)	Mutations (hereditary CRC) or CpG island methylation (sporadic CRC)	Chemotherapy in adjuvant setting	Prognostic, possible predictive to adjuvant 5-FU-based regimens	Primary tissue	IHC, (q)PCR
PI3K	Mutations	Anti-EGFR antibodies	Possible predictive	Primary and metastatic tissue, cfDNA	Next-generation and Sanger sequencing, BEAMing®, qPCR
cMET	Expression	Anti-EGFR antibodies	Possible prognostic and predictive	Primary and metastatic tissue	Expression microarrays, IHC
EGFR	Mutations, amplifications	Anti-EGFR antibodies	Possible predictive	Primary and metastatic tissue, cfDNA	Next-generation and Sanger sequencing, BEAMing®, qPCR, FISH

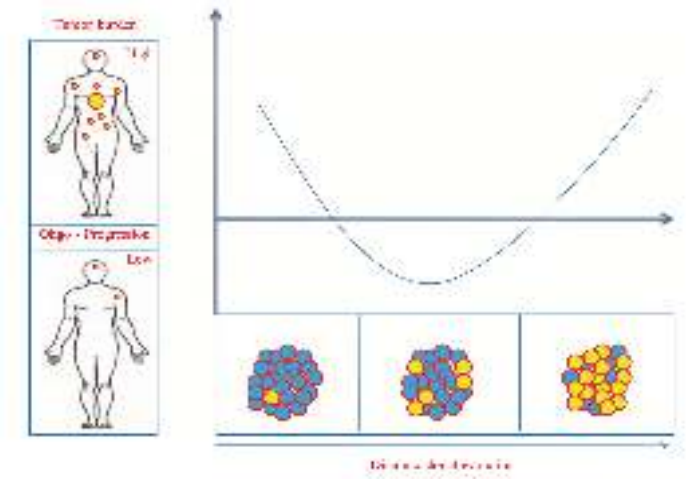
Int. J. Mol. Sci. **2015**, *16*

Some considerations



Sacher AG, et al. JAMA Oncol. 2016

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

Pisapia, Malapelle, Troncone, Springer Book 2017

Turnaround Time Shorter for Plasma ddPCR vs Tissue Genotyping

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

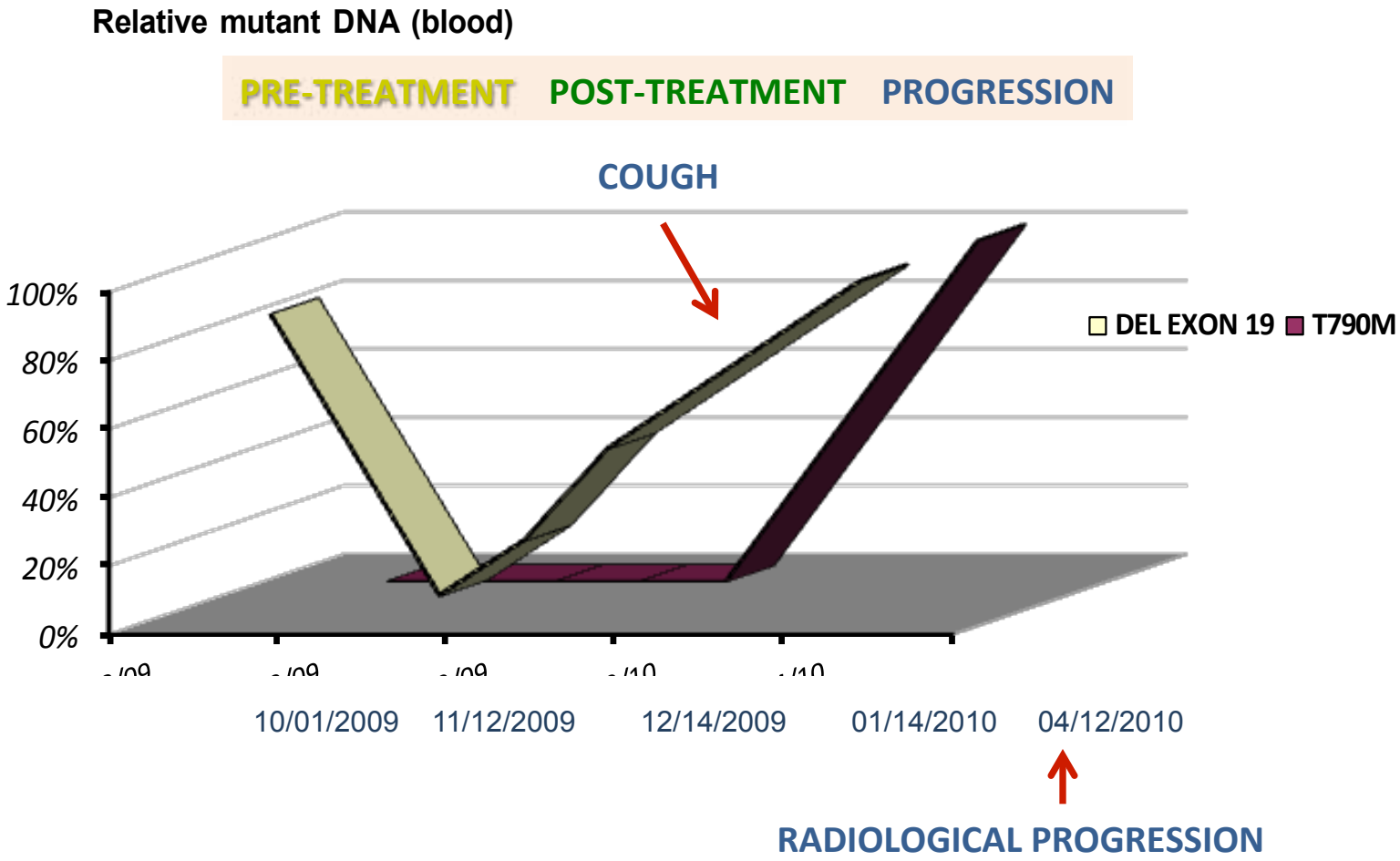
Turnaround Time, Median Days (Range)	Cohort 1, Newly Diagnosed (n = 115)	Cohort 2, Acquired Resistance (n = 59)
Plasma genotyping*	3 (1-7)	2 (1-4)
Tissue genotyping†	12 (1-54)	27 (1-146)

*Plasma 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;[Epub ahead of print].

Patient Follow up :One case of our experience



Courtesy Rafael Rosell

EGFR-T790M genotyping of matched urine, plasma and tumor tissue

High sensitivity in plasma (82%), urine (75%), and combined (93%)

A Urine vs Tissue

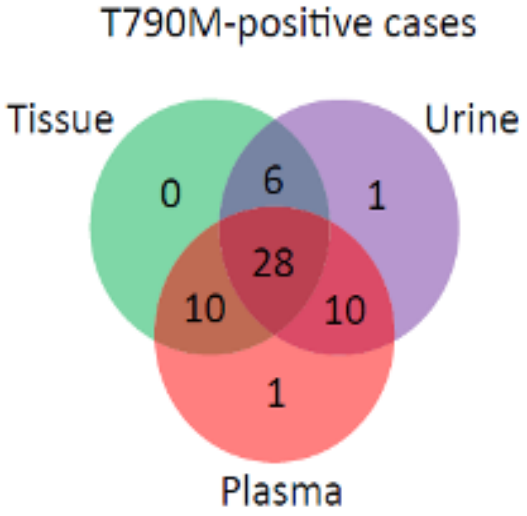
10-100 mL urine

T790M		FFPE Tumor, n			*Total*
		Positive	Negative	Inadequate	
Urine, n	Positive	34	9	2	45
	Negative	13	4	1	18
Total		47	13	3	63

B Plasma vs Tissue

T790M		FFPE Tumor, n			*Total*
		Positive	Negative	Inadequate	
Plasma, n	Positive	38	9	2	49
	Negative	3	4	1	8
	Failed	3	0	0	3
Total		44	13	3	60

D



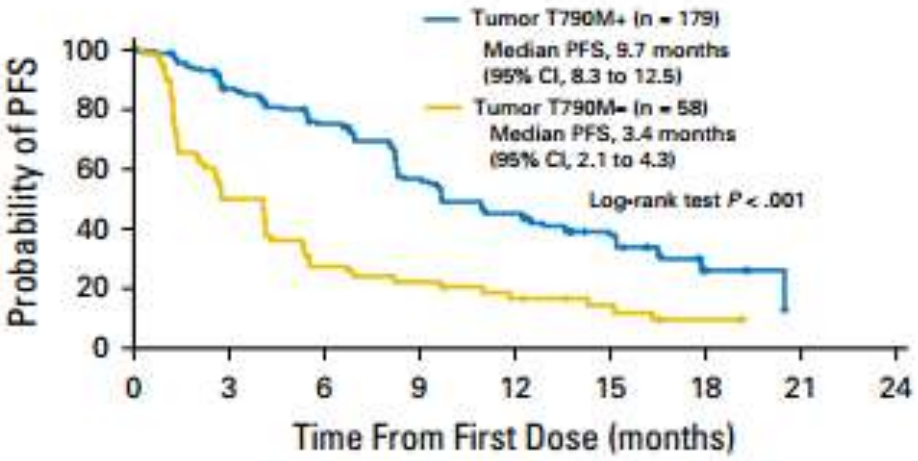
Positive by any one specimen type: 56 of 60 (93%)
Positive by tissue: 44 of 60 (73%)
Positive by plasma: 49 of 60 (82%)
Positive by urine: 45 of 60 (75%)
Positive by urine and plasma combined: 56 of 60 (93%)

Combined plasma and urine testing identified 12 additional T790M+ pts
7/9 (78%) had PR/SD with Rociletinib

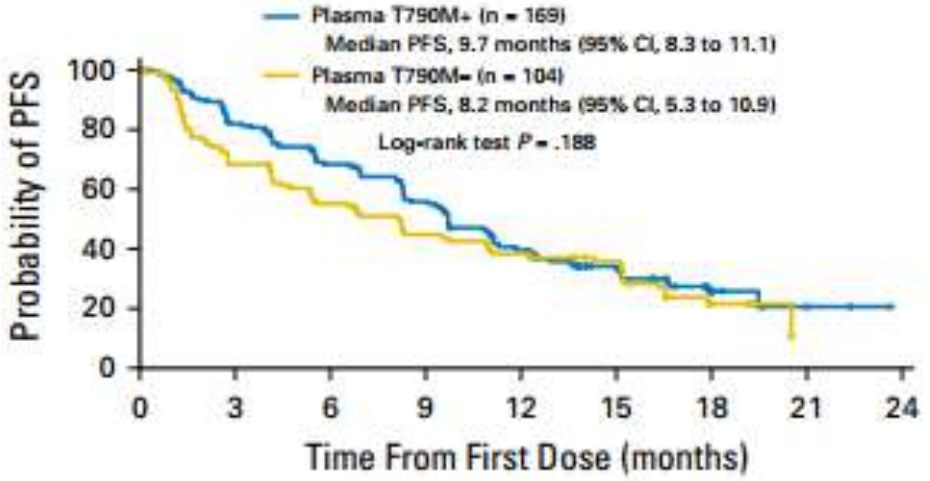
Reckamp et al., JTO 2016

PFS to Osimerinib according to T790M in plasma or tumor tissue

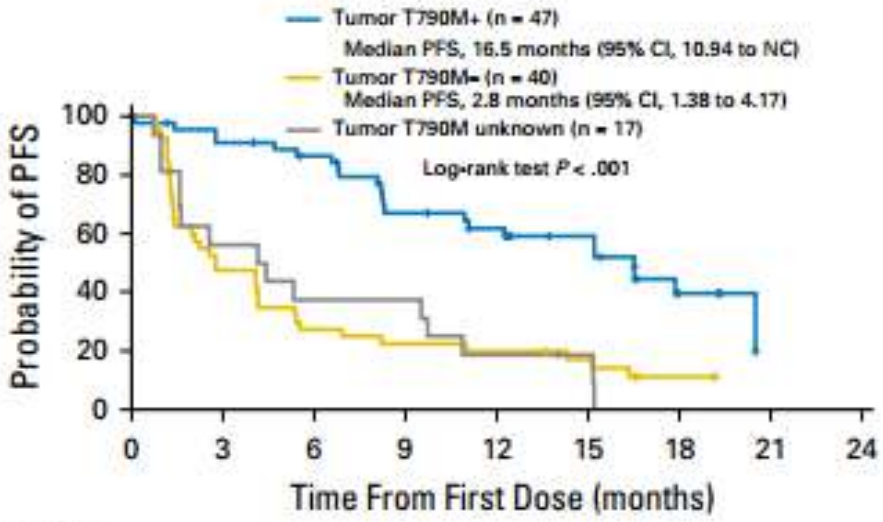
Tumor T790M+ vs T790M-



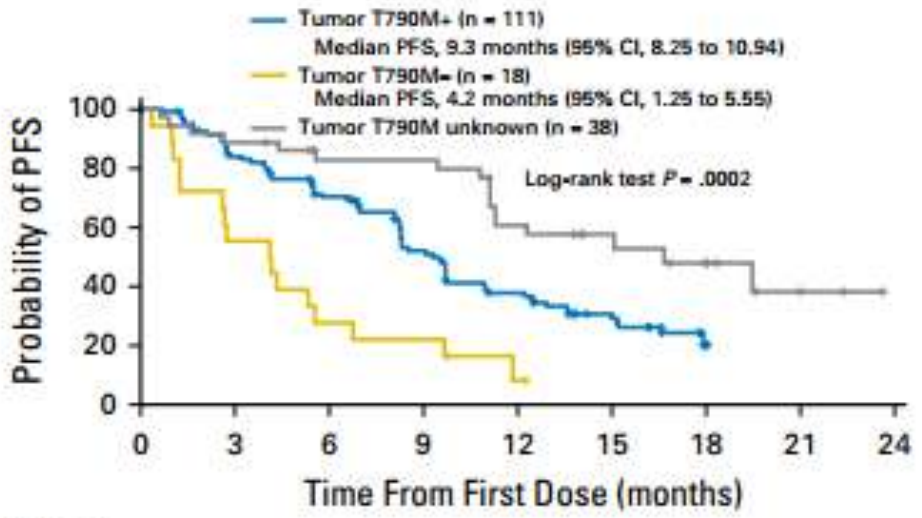
Plasma T790M+ vs T790M-



PlasmaT790M- by tissue status

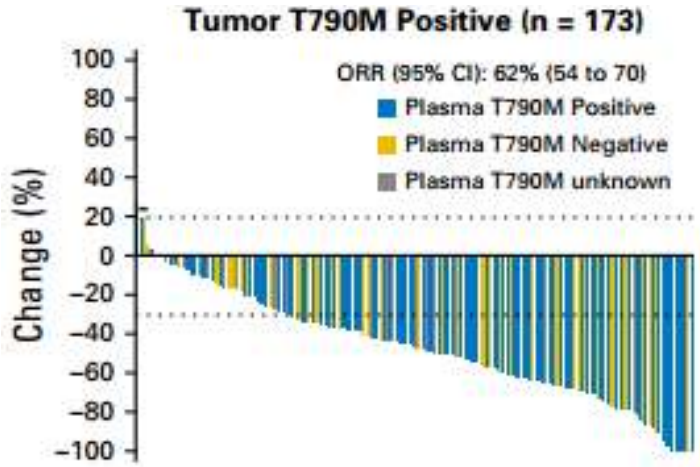


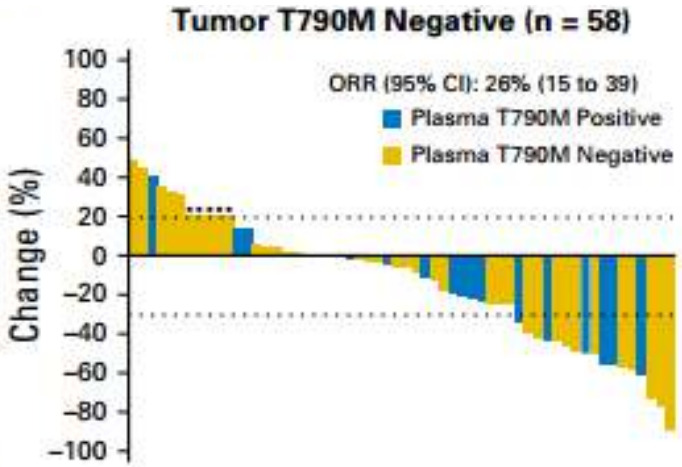
PlasmaT790M+ by tissue status



Oxnard, JCO 2016

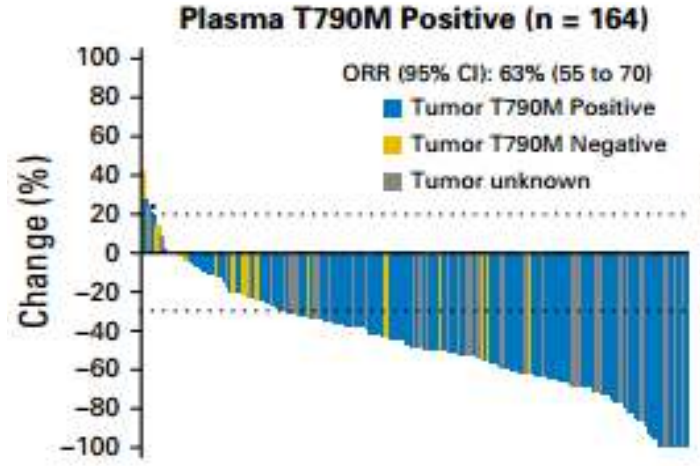
RR to Osimerinib according to T790M in plasma or tumor tissue

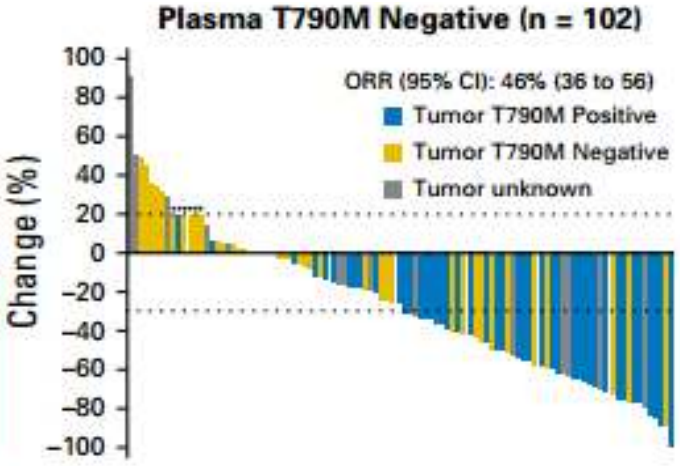




Tumor tissue

ORR: 62% vs 26%





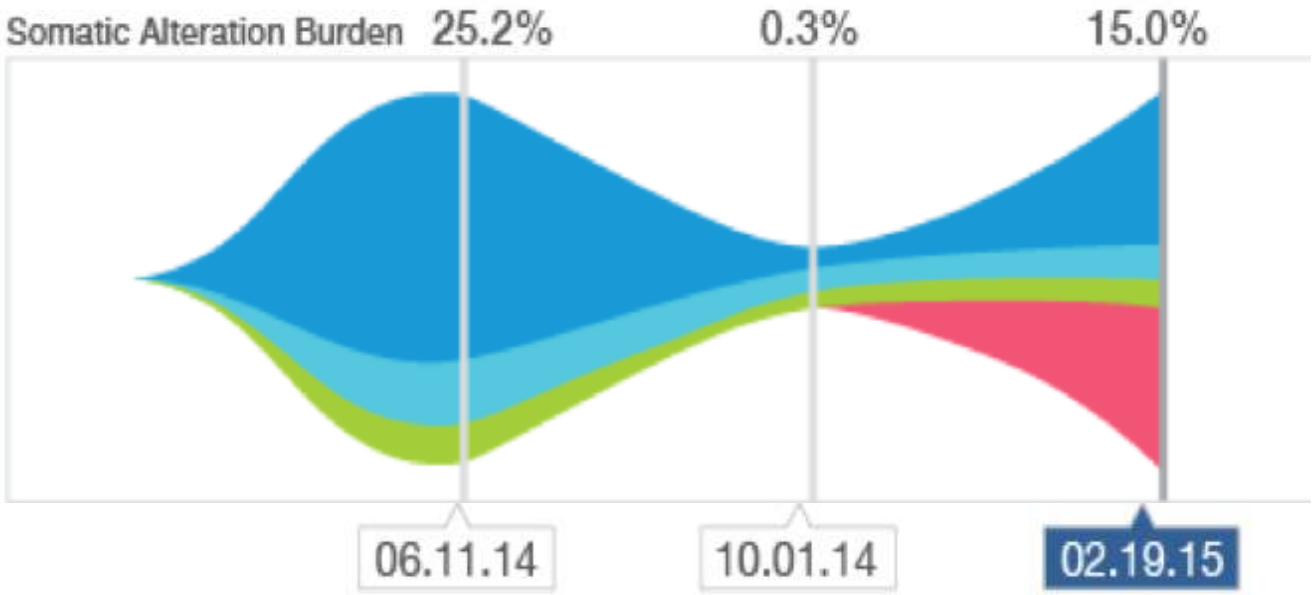
Plasma

ORR: 63% vs 46%

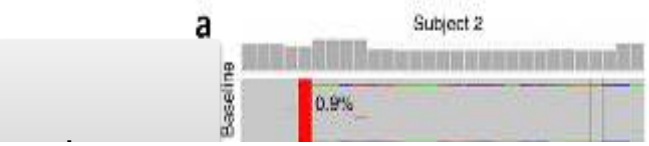
Tissue vs Plasma

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

Liquid Biopsy in clinical practice



Explorer new mechanism of resistance
Story of c797s



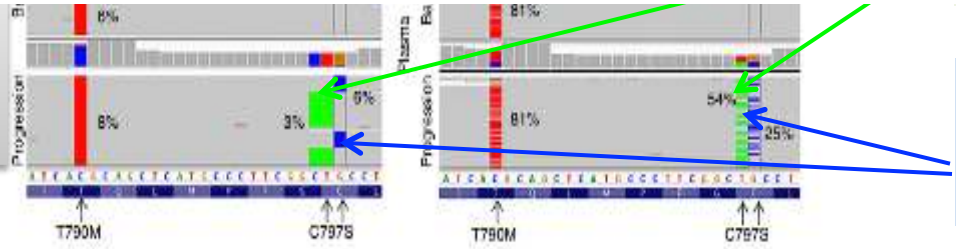
Acquired C797S G→C mutation on a

THE AMERICAN JOURNAL OF HEMATOLOGY/ONCOLOGY®

Case Report: Detection of c797s as a Mechanism of Resistance in a Patient With Lung Cancer With *EGFR* Mutations

Luis E. Raez, MD, and Christian Rolfo, MD, PhD, MBA

plasma



Plasma NGS additionally detects a second G→C mutation encoding for C797S

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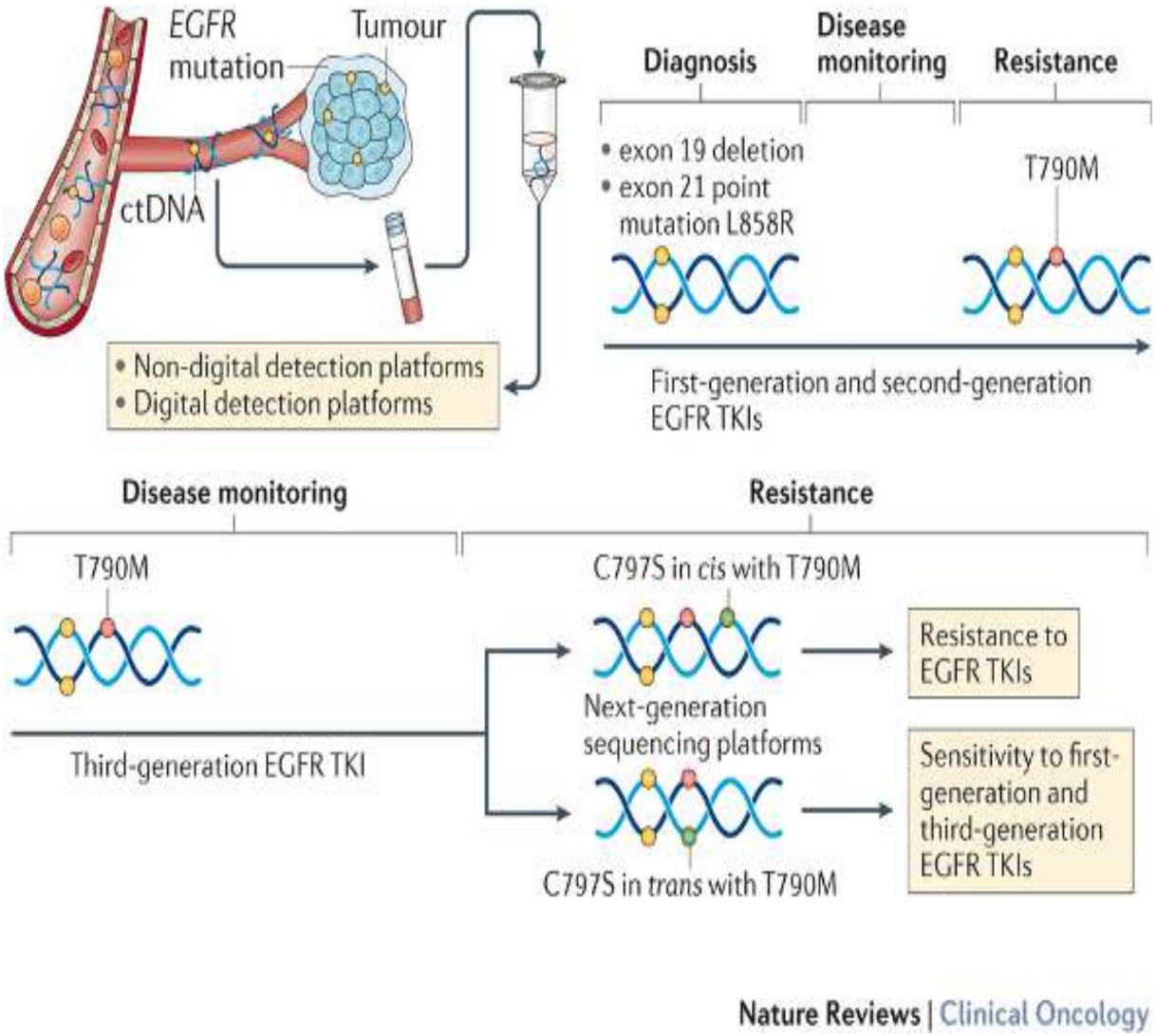
Thress KS, et al, Nat Med.2015 Jun;21(6):560-2.

Liquid Biopsies in Lung Cancer



ctDNA monitoring TKI resistance mechanisms in NSCLC

Other resistant mechanism:
T790M loss
Cmet amplification
Her2 amplification
BRAF mutation
And more…

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Nat Rev Clin Oncol 2016, 13(7):401-402.

Special considerations...

Guardant360 Panel 2015

All NCCN Somatic Genomic Targets in a Single Test

Point Mutations - ***Complete**** *or Critical Exon Coverage in 73 Genes*

AKT1	*ALK*	***APC***	***AR***	*ARAF*	***ARID1A***	*ATM*	***BRAF***	***BRCA1***	***BRCA2***
CCND1	***CCND2***	***CCNE1***	*CDH1*	***CDK4***	***CDK6***	***CDKN2A***	***CDKN2B***	*CTNNB1*	***EGFR***
ERBB2	*ESR1*	*EZH2*	*FBXW7*	***FGFR1***	***FGFR2***	*FGFR3*	*GATA3*	*GNA11*	*GNAQ*
GNAS	*HNF1A*	***HRAS***	*IDH1*	*IDH2*	*JAK2*	*JAK3*	***KIT***	***KRAS***	*MAP2K1*
MAP2K2	***MET***	*MLH1*	*MPL*	***MYC***	***NF1***	*NFE2L2*	*NOTCH1*	*NPM1*	***NRAS***
NTRK1	***PDGFRA***	***PIK3CA***	***PTEN***	*PTPN11*	***RAF1***	***RB1***	*RET*	*RHEB*	*RHOA*
RIT1	*ROS1*	*SMAD4*	*SMO*	*SRC*	*STK11*	*TERT*	***TP53***	*TSC1*	*VHL*

AMPLIFICATIONS

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

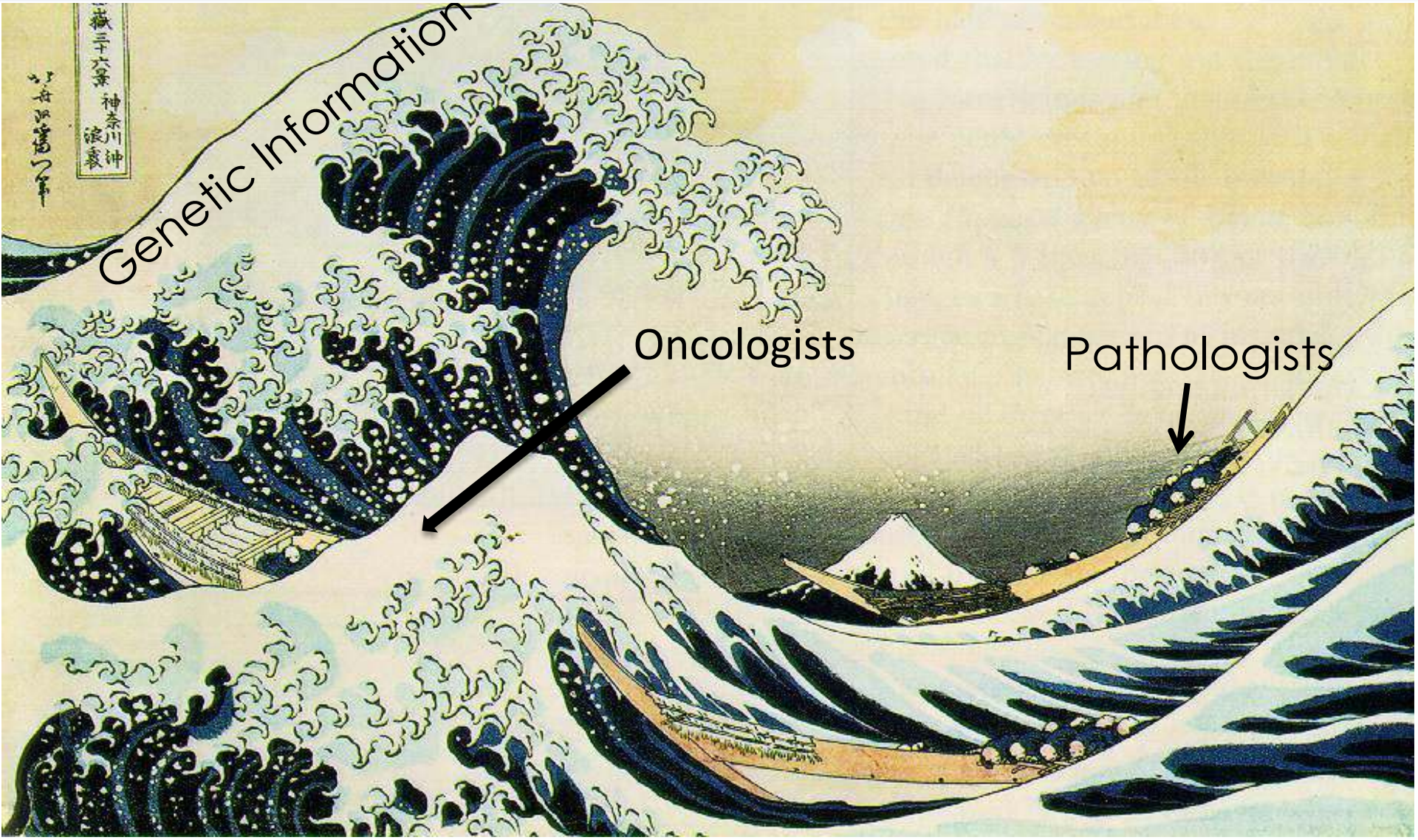
FUSIONS

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

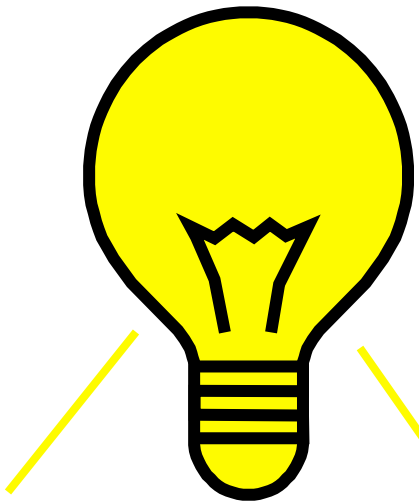
INDELS

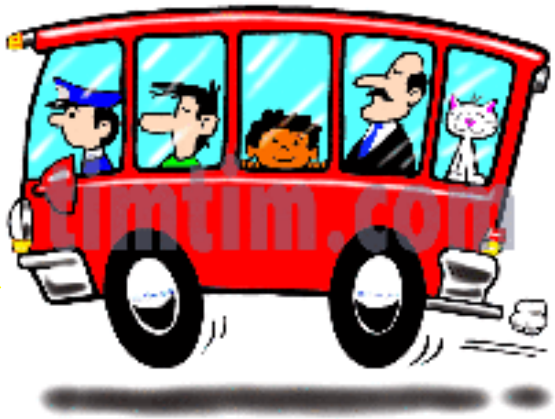
EGFR exons 19/20	*ERBB2* exons 19/20	*MET* exon 14 skipping

Data Tsunami



Discriminating a Driver and a Passenger Mutation in Early Phases Can Be Difficult





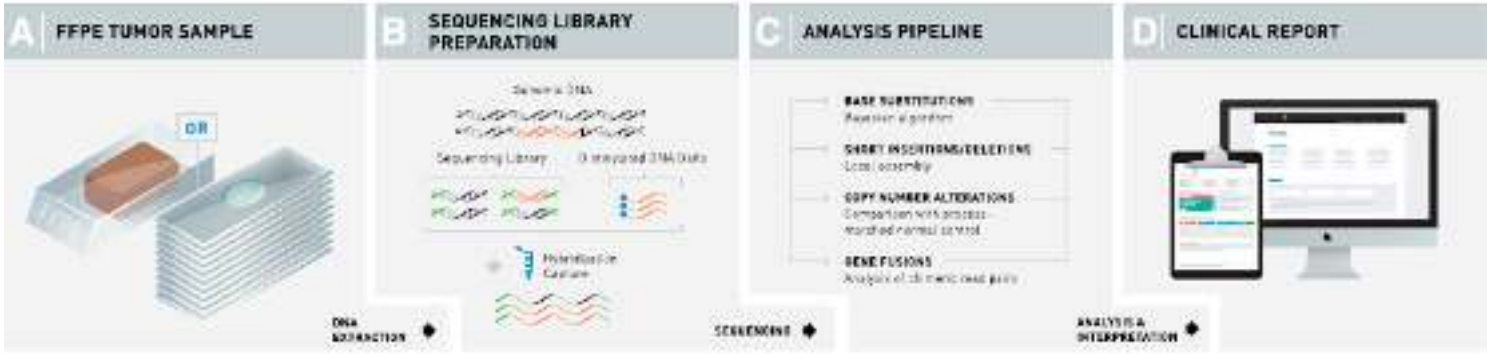
Stratified medicine creates multiple rare cancers

Our New Way to Work . . . Molecular Tumor Board

Patient case is derived from his doctor

Molecular Tumor Board

Oncologist	Mol. Pathol	Gyneco	Thorax	Geneticist	Pediat	Nav. nurse



Molecular Tumor Board

Kennis / Ervaring / Zorg

Report with therapeutic proposal

Referral Doctor Discussion

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MSK Levels of Evidence

OncoKB

Level 1 — **FDA-recognized** biomarker predictive of response to an **FDA-approved** drug **in this indication**

Level 2A — **Standard of care** biomarker predictive of response to an **FDA-approved** drug **in this indication***

Level 2B — **Standard of care** biomarker predictive of response to an **FDA-approved** drug **in another indication**, *but not standard of care for this indication*

Level 3A — **Compelling clinical evidence** supports the biomarker as being predictive of response to *a drug* **in this indication**, *but neither biomarker nor drug are standard of care*

Level 3B — **Compelling clinical evidence** supports the biomarker as being predictive of response to *a drug* **in another indication**, *but neither biomarker nor drug are standard of care*

Level 4 — **Compelling biological evidence** supports the biomarker as being predictive of response to *a drug*, *but neither biomarker nor drug are standard of care*

Level R1 — **Standard of care** biomarker predictive of **resistance** to an **FDA-approved** drug **in this indication**

Standard Therapeutic Implications
*Includes biomarkers that are recommended as standard of care by the NCCN or other expert panels but not necessarily FDA-recognized for a particular indication

Investigational Therapeutic Implications
possibly directed to clinical trials

Hypothetical Therapeutic Implications
based on preliminary, non-clincial data

Standard Therapeutic Implications

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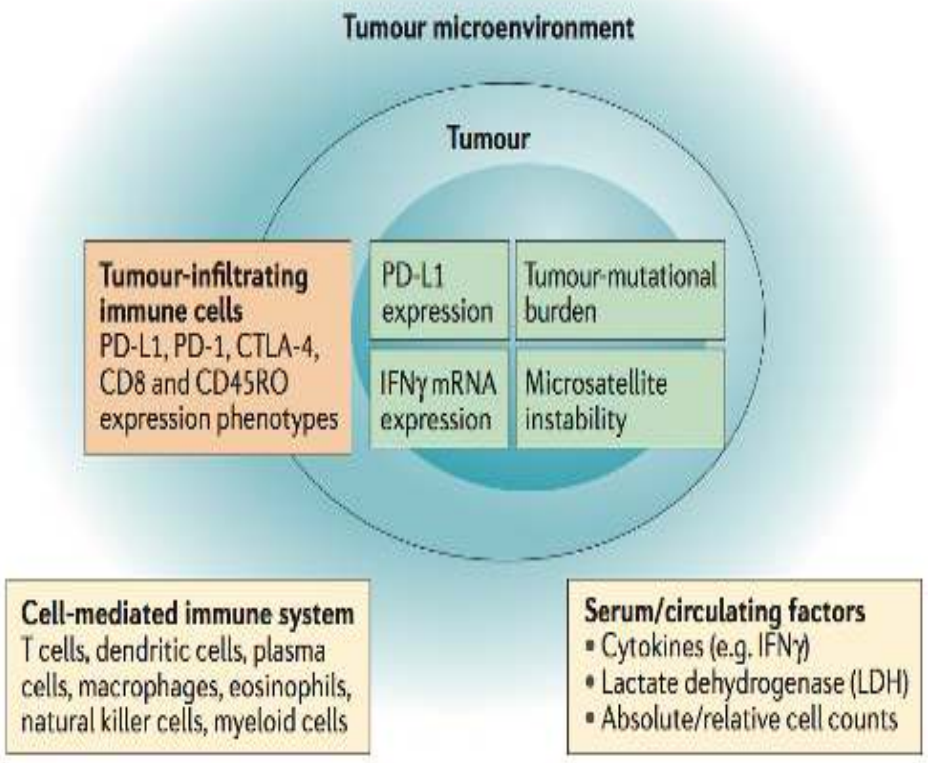
varing / Zorg

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

Immunotherapy in Cancer

Liquid Biopsies in Immunotherapy



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:

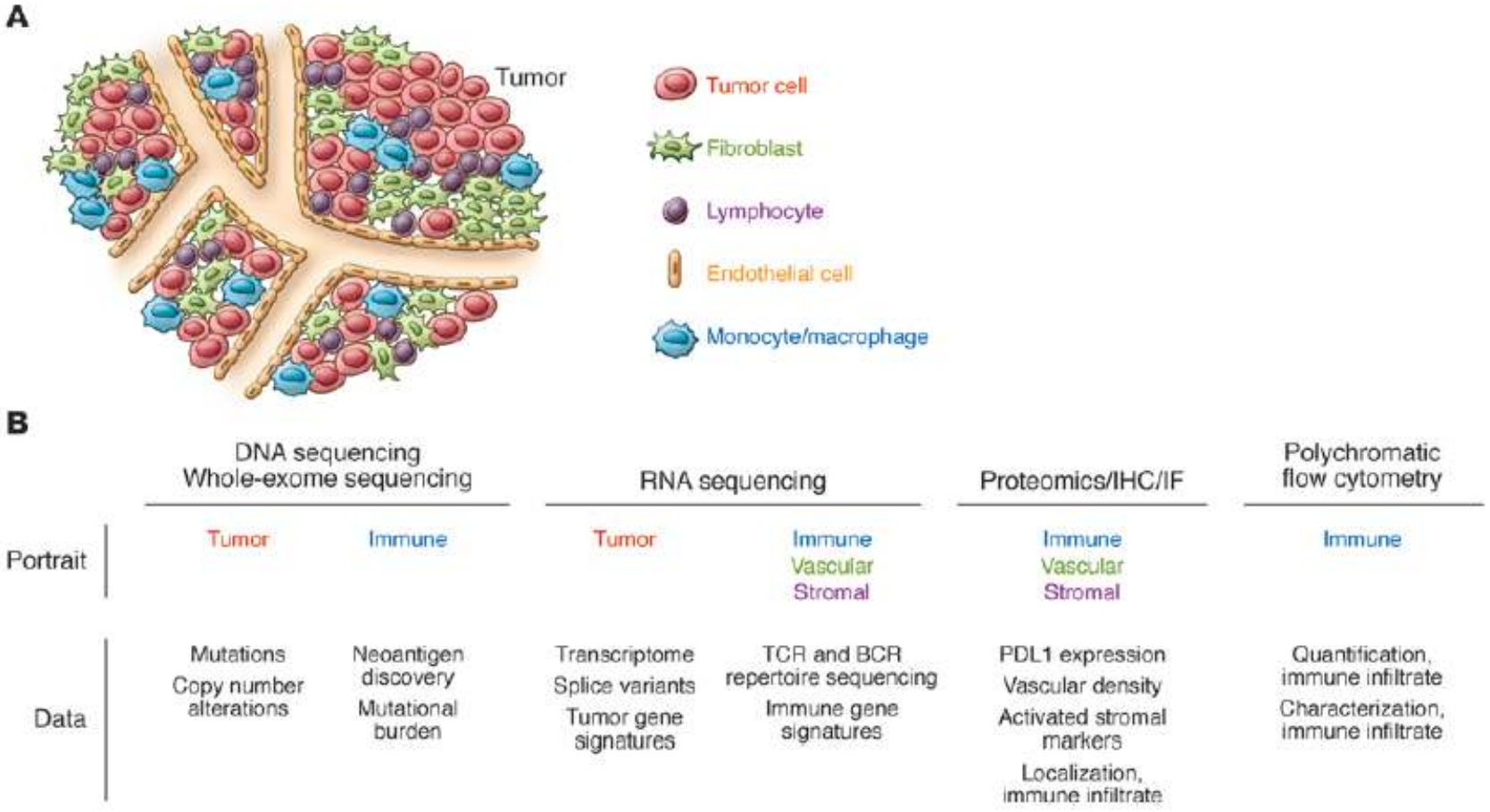
- Calculation of circulating TMB
- Detection of bPDL1
- Alellic Fraction Variation Dynamic

Liquid Biopsy in Immunotherapy is challenging!

A complex microenvironment

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

Next-gen sequencing and the complexity of human tumors.



Tumor mutational burden in blood (bTMB) and improved atezolizumab (atezo) efficacy in NSCLC

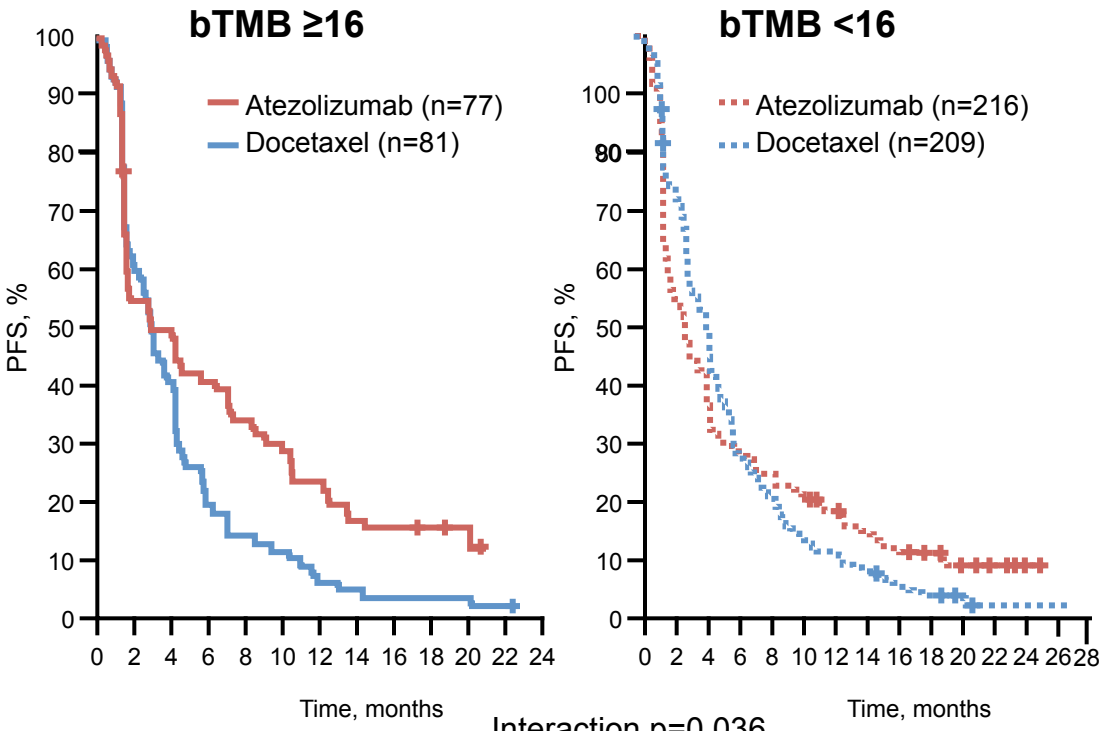
Blood-efficacy in 2L+ NSCLC (POPLAR and OAK)

Aim: to evaluate a method for the investigation of tumor mutational burden from peripheral blood and its predictive value on Atezolizumab therapy outcome

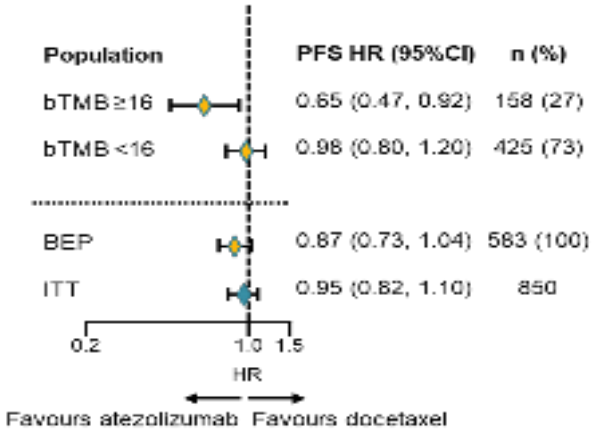
Methods: an NGS panel of 394 genes was used to measure the mutational burden from circulating tumoral DNA in peripheral blood

D. R. Gandara et al., ESMO 2017 abstract 1295O

MTB

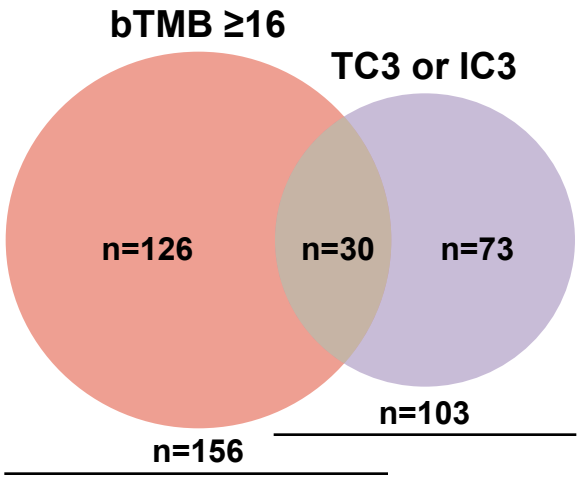


Interaction p=0.036



D. R. Gandara et al., ESMO 2017 abstract 1295O

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)

Biomarker evaluable population (n=229)

D. R. Gandara et al., ESMO 2017 abstract 1295O

MTB

Conclusions

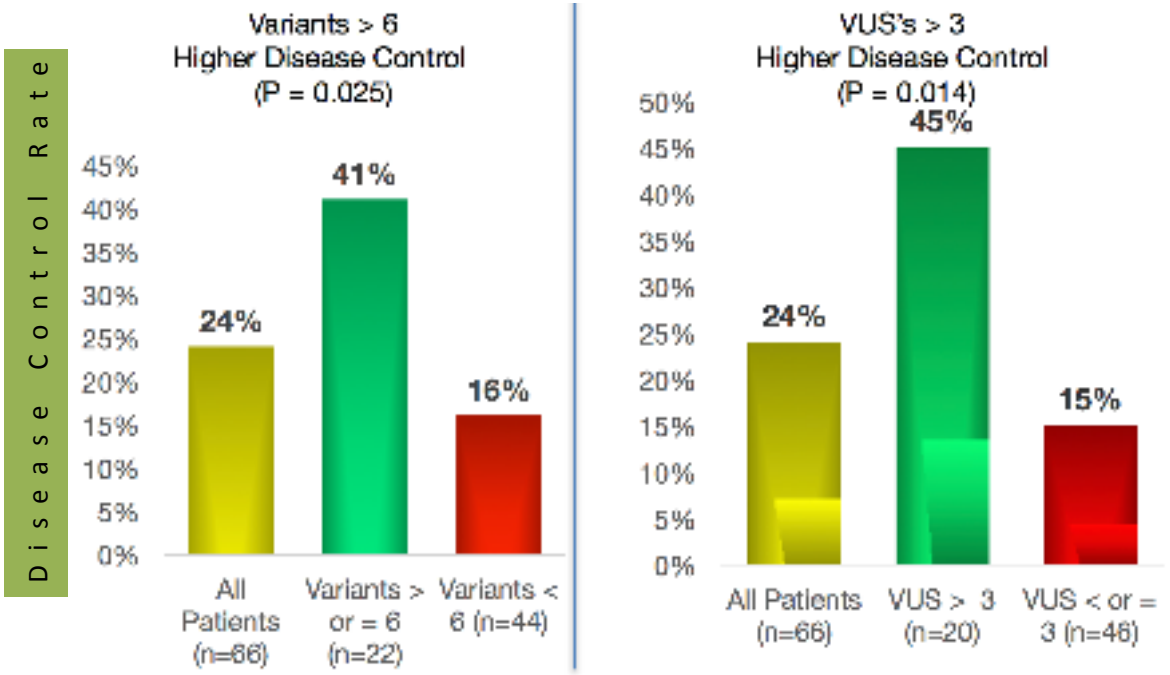
- This exploratory analysis demonstrated that TMB can be measured in blood
- The cut-point of bTMB ⩾16 was identified in POPLAR, and independently validated to predict PFS benefit in OAK
- bTMB identified a unique patient population which was not significantly associated with PD-L1 status

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

Hypermutated Circulating Tumor DNA

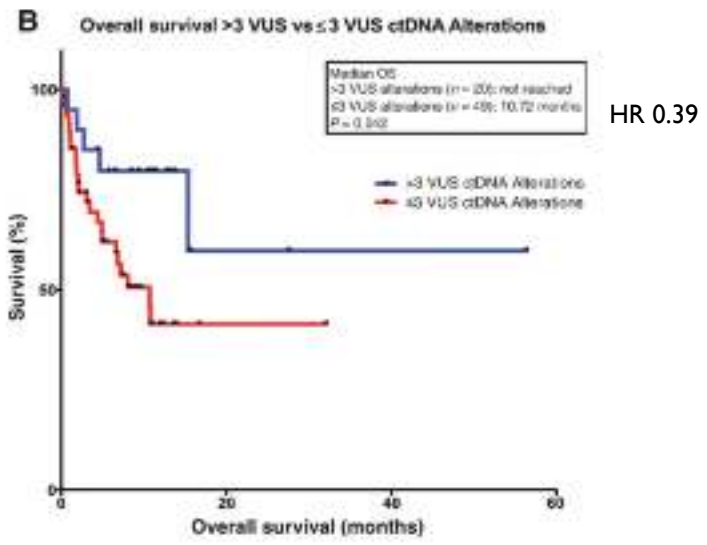
Hypermutated Circulating Tumor DNA: Correlation with Response to Checkpoint Inhibitor–Based Immunotherapy



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

Mutation Burden Predicts Disease Control Rates

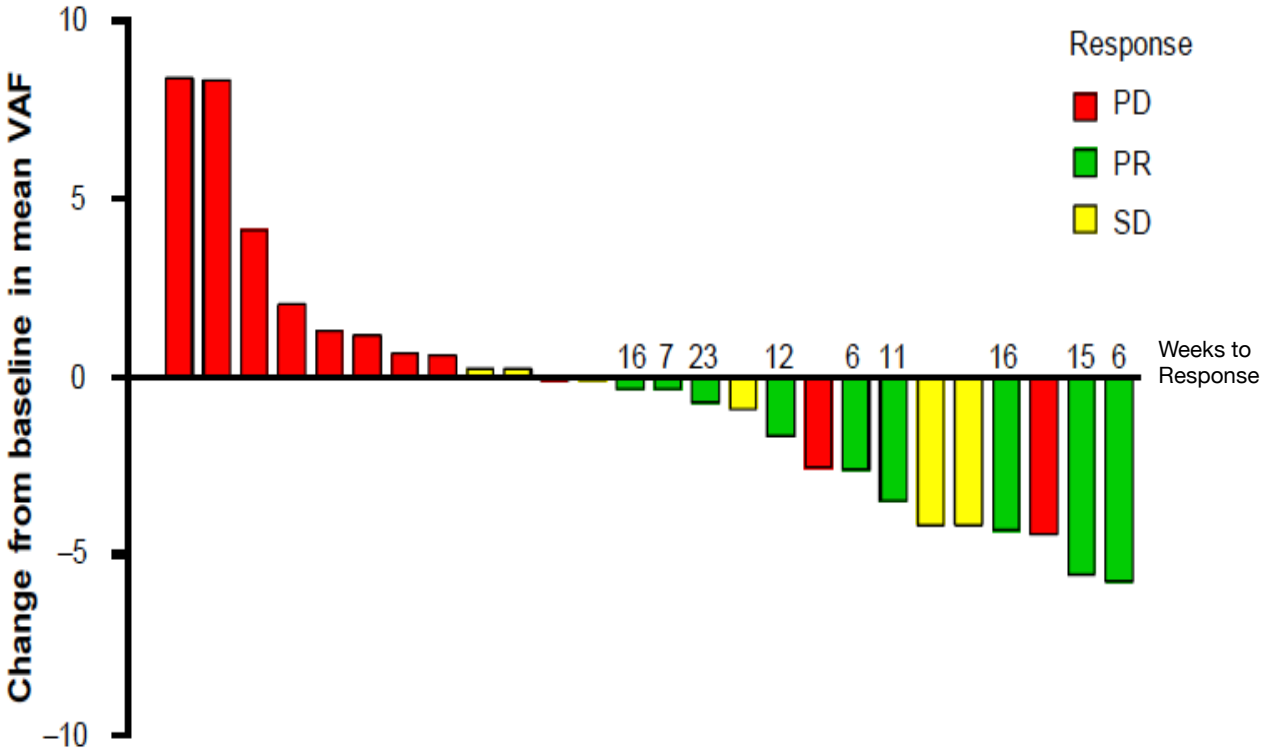
Variable	All Patients % (N, if applies)	VUS > 3 % (N, if applies)	VUS ≤ 3 % (N, if applies)	P value[c]
Disease Control Rate [SD/CR/PR] (% (N))[b]	24% (16/66)	45% (9/20)	15% (7/46)	P = 0.014
Median PFS, months[b]	2.3 (95%CI: 0.7-5.0)	3.84	2.07	P = 0.019 (HR 0.52; 95% CI 0.31-0.87)
Median OS, months[b]	15.3 (95%CI: 6.80-15.68)	Not Reached	10.72	P = 0.042 (HR 0.39; 95% CI 0.18-0.83)

Variable	All Patients % (N, if applies)	Variants ≥ 6 % (N, if applies)	Variants < 6 % (N, if applies)	P value[c]
Disease Control Rate [SD/CR/PR] (% (N))[b]	24% (16/66)	41% (9/22)	16% (7/44)	P = 0.025
Median PFS, months[b]	2.3 (95%CI: 0.7-5.0)	2.85	2.19	P = 0.046 (HR 0.59; 95% CI 0.35-0.99)
Median OS, months[b]	15.3 (95%CI: 10.6-23.9)	Not Reached	10.79	P = 0.042 (HR 0.39; 95% CI 0.2-0.8)

[b]N = 66 patients evaluable for SD ≥6 months/PR/CR; N=69 patients evaluable for PFS and OS
[c]p-values calculated only when at least 10 patients were assessable in a category

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

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

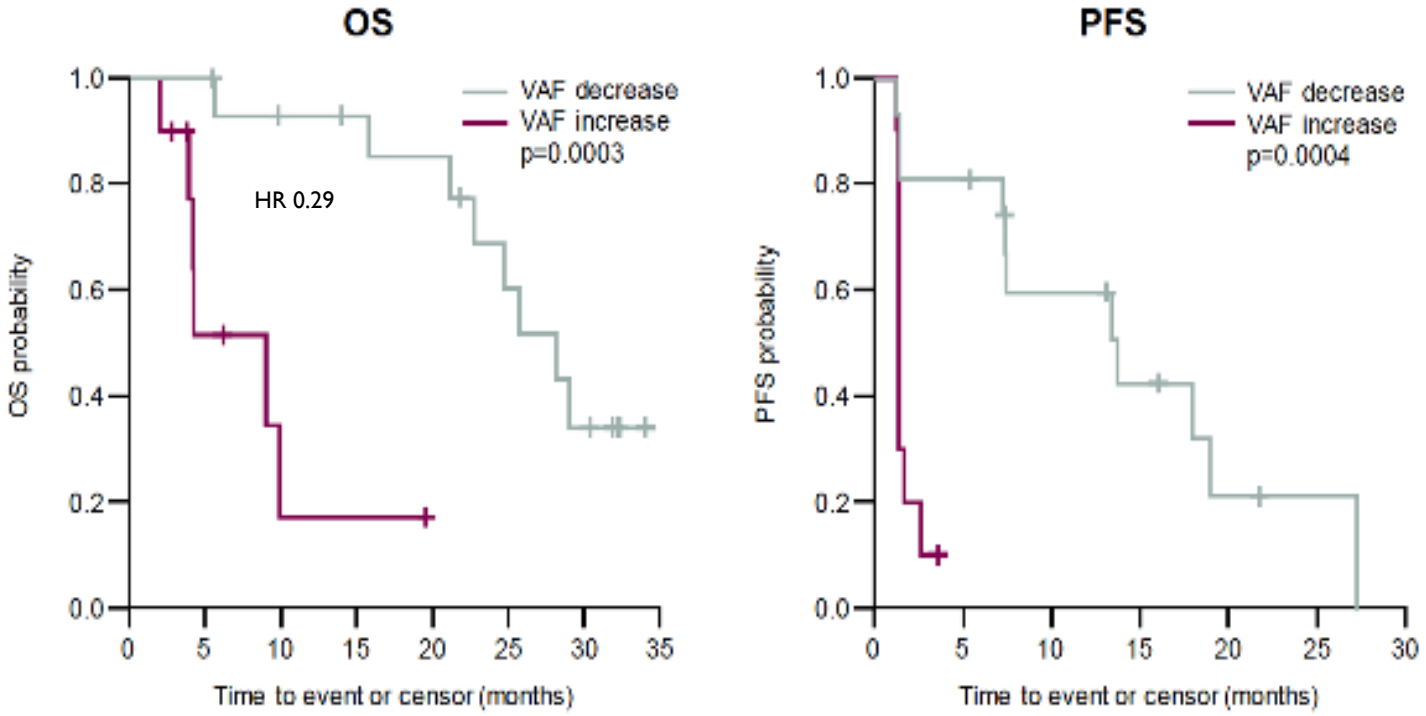


The delta in variant allele fractions (VAF) was calculated by subtracting the mean VAF pre-dose from the mean VAF post-dose. VAF decreased in 9/9 PR patients and 4/6 SD subjects. The time (in weeks) to investigator determination of PR response is shown.

Kuziora (Ranade) et al. 2017 Abstract 582 AACR

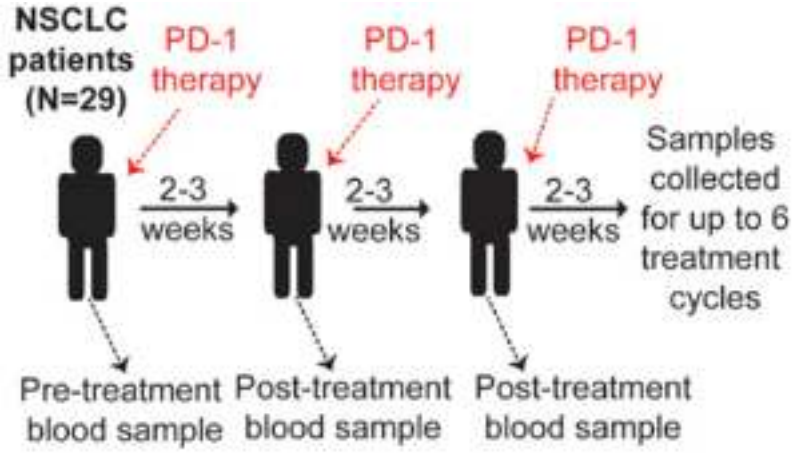
A Decrease in Mean VAF After 6 Weeks of Durvalumab Treatment was Associated with Improved OS and PFS

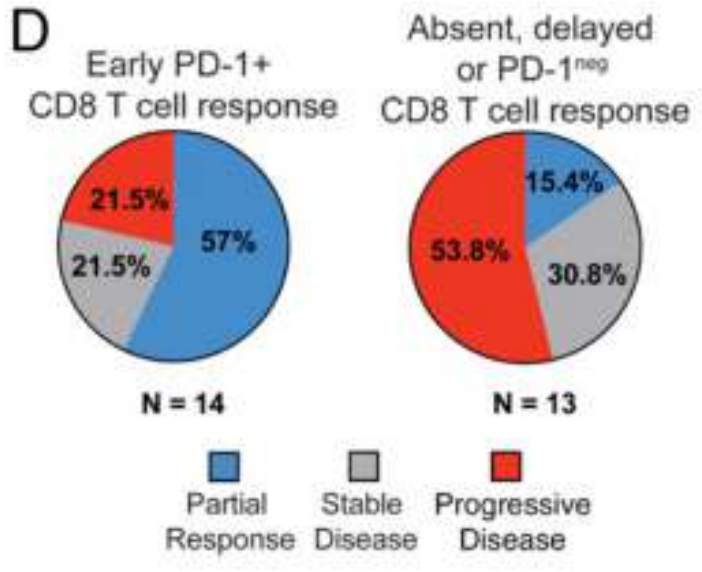
"ctDNA Dynamics": Change in ctDNA Allele Fractions at 6 weeks Predicts IO Response in NSCLC



Kuziora (Ranade) et al. 2017 Abstract 582 AACR

Proliferation of PD-1+ CD8 T cells in peripheral blood after PD-1–targeted therapy in lung cancer patients





70% of patients with disease progression had either a delayed or absent PD-1+ CD8 T-cell response, whereas **80% of patients with clinical benefit exhibited PD-1+ CD8 T-cell responses within 4 wk of treatment initiation**.

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Kamphorst. (Ramalingam), PNAS Spr 2017

Pleiotropic role of Tumors Derived Exosomes (TDEs)

Are involved in the mechanisms of signaling-molecules secretion

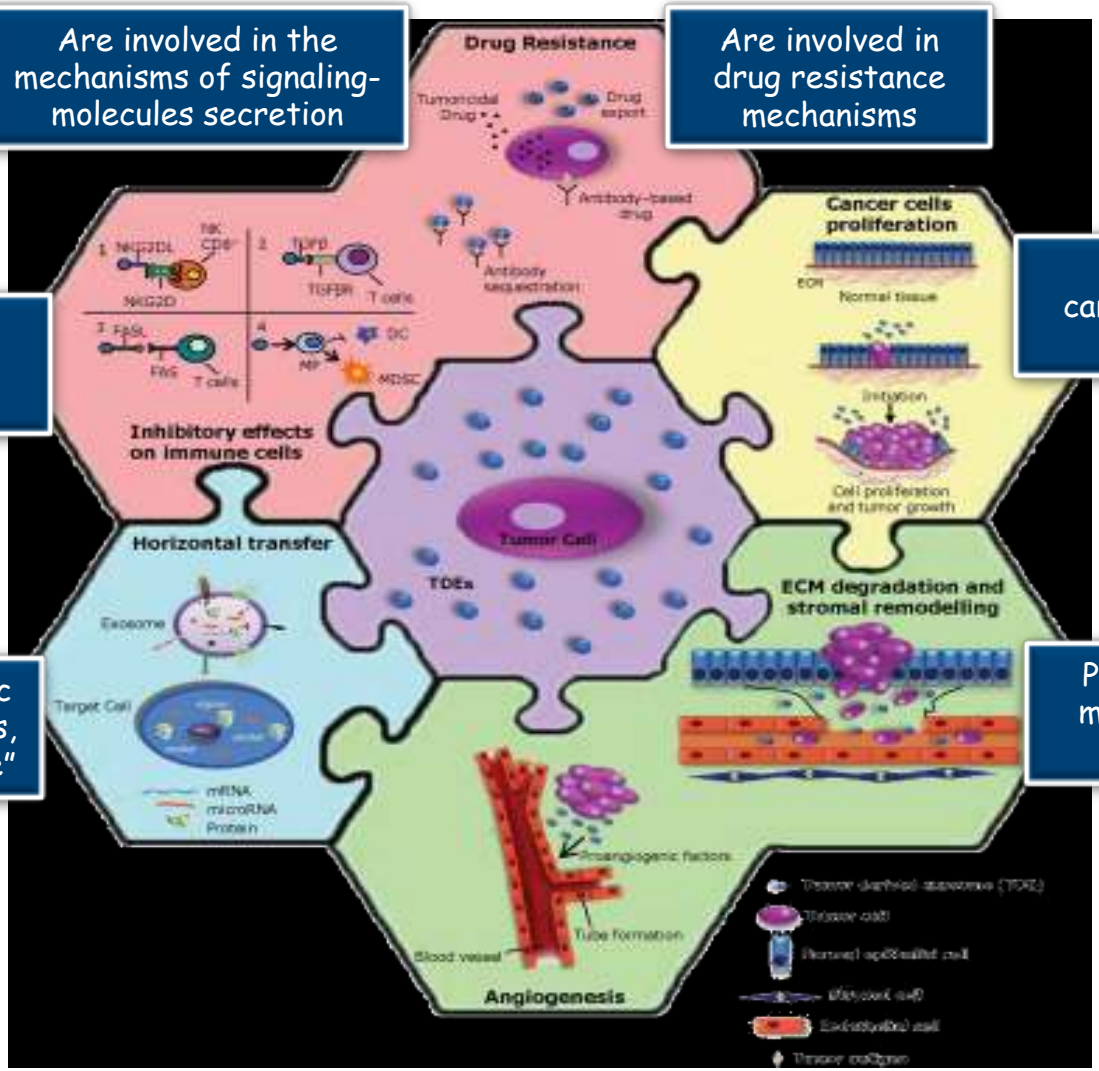
Are involved in drug resistance mechanisms

Have effects on different immune-system components

Promote carcinogenesis and tumor growth

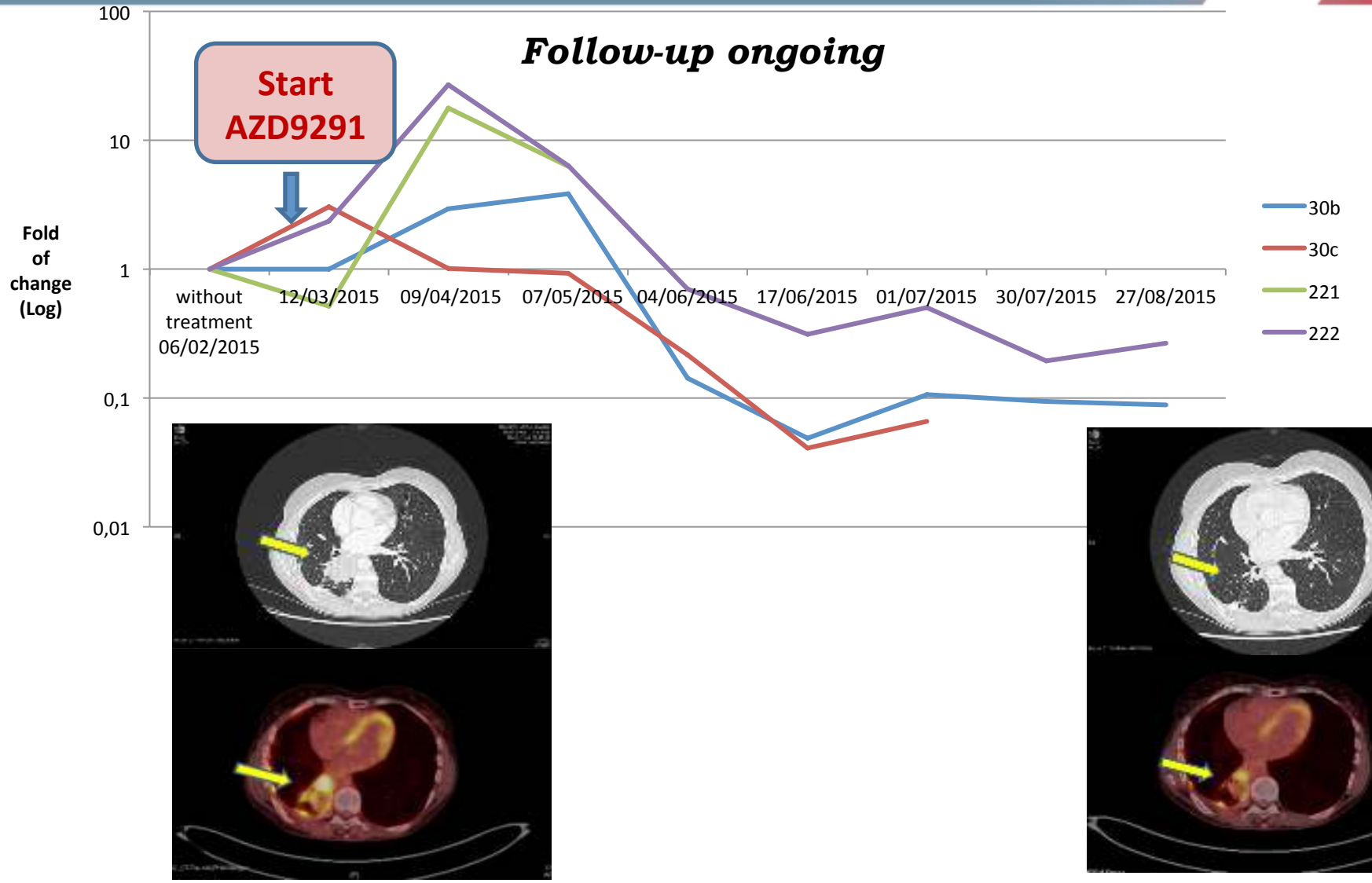
Have a role in genetic exchange mechanisms, and as " RNA Shuttle"

Promote extracellular matrix remodeling and angiogenesis



Fontana et al Proteomics 2012

Follow-up analysis of exosomal microRNAs of one EGFR (T790M exon 20) NSCLC patient (EGFR10)



Rolfo Lab, unpublished data , November 2015

Highly sensitive detection of low abundant somatic mutations in circulating exosomal RNA and cfDNA with next-generation sequencing

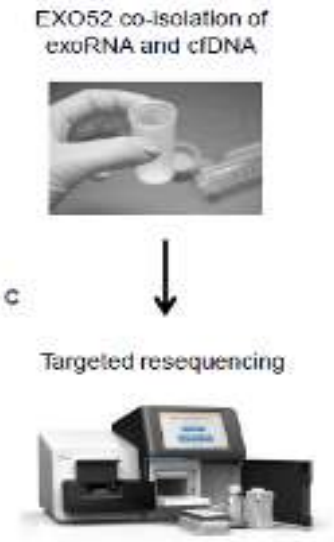
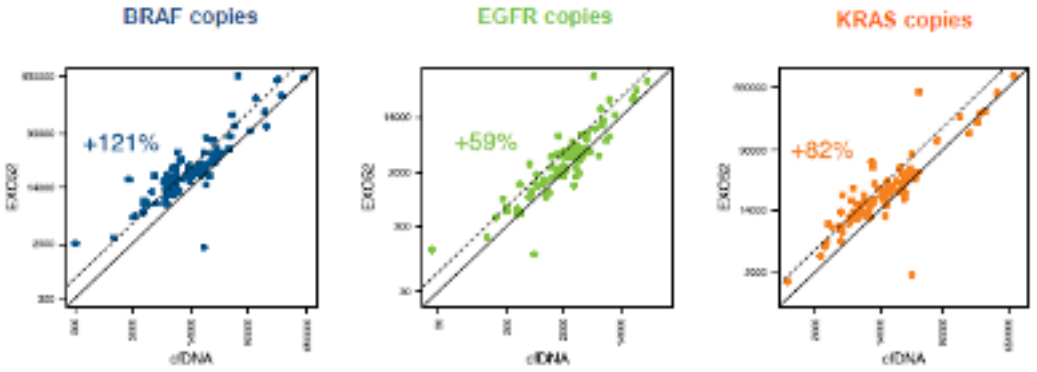


Fig. 2 - Single-step co-isolation of exoRNA + cfDNA yields consistently greater gene copies



Absolute quantification of extracted gene copies. Comparison of two fractions isolated from 86 different patient samples: cell-free DNA (cfDNA) and exoRNA+cfDNA (EXO52). In these three genes, the added molecules from RNA are around 100% (dotted line)

High positive concordance with tissue in late-stage cancers

COSMIC Mutations

Cohort	Tissue	EXO1000	Positive Concordance
All Samples (*n*=94)	**116**	**98**	**84%**
CRC (*n*=38)	43	40	93%
NSCLC (*n*=28)	45	31	69%
Melanoma (*n*=20)	21	21	100%
Others (*n*=8)	7	6	86%

Good sensitivity for challenging samples

Positive Concordance with Tissue

	EGFR L858R & del19	EGFR T790M
All stages	**81% (17/21)**	**75% (12/16)**
M0/M1a	67% (4/6)	40% (2/5)
M1b	87% (13/15)	82% (9/11)

Enderle D et al, ASCO 2015, Abstract 11061

Exo-ALK proof of concept: Exosomal analysis of ALK alterations in advanced NSCLC patients

Christian Rolfo[1], Jean François Laes[2], Pablo Reclusa[1], Anna Valentino[1], Maxime Lienard[2], Ignacio Gil Bazo[3], Umberto Malapelle[4], Rafael Sirera[1], Danilo Rocco[4], Jan Van Meerbeeck[1], Patrick Pauwels[1], Marc Peeters[1]

[1]Antwerp University Hospital, Belgium; [2]OncoDNA, Gosselies, Belgium; [3]University of Navarra, Pamplona, Spain; [4]University of Naples Federico II, Naples, Italy

Background

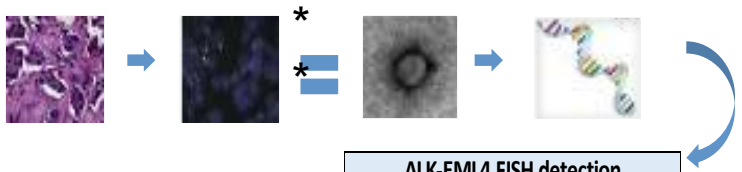
A subset of NSCLCs (approx. 5%), present alterations in the ALK gene. This produces abnormal ALK proteins that induce cells to grow and spread. Different generation of ALK inhibitors are available for targeted therapy and their indication depends on the detection of ALK alterations in the tissue. Thus, it is mandatory to develop new techniques that allow us to demonstrate ALK alterations in peripheral blood. The purpose of this study is to analyze the feasibility to determine ALK alterations in exosomes (Exo-ALK) in NSCLC patients and determine the sensitivity and specificity of the technique.

Methods

This study is performed in blind in a cohort of 19 NSCLC with and without known alterations of ALK in tumoral tissue. ALK-positive tissue samples were identified by FISH and patients were included independently of stage and time of disease. Exosomal RNA is isolated by exoRNeasy Serum/Plasma (Qiagen) and retrotranscripted by ProtoScript II First Strand cDNA Synthesis kit. The ALK gene present in the exosomes was determined by NGS and bio-informatic analysis by OncoDNA. Samples were provided by the Biobank of the University of Navarra, the UZA Biobank and by the University of Naples Federico II. Samples and data were processed following standard operating procedures approved by the local Ethical and Scientific Committees.

Results

The analyzed samples have been 16 ALK-EML4 tissue positive patients and 3 ALK-EML4 tissue negative, defined in this case by FISH. After analysis, we have been able to detect 9 positive ALK-EML4 patients, 8 negative samples and 2 samples where the RNA was degraded. Looking at the clinical data, the 9 positive samples detected in the exosomal RNA were positive also for ALK-EML4 translocation in the tissue, and comparing the 8 negative samples, 3 were tissue negative and 5 tissue positive. These data show a sensitivity of 64% and a specificity of 100%. No correlation has been found comparing treatment-naïve and pretreated patients.



		ALK-EML4 FISH detection in Tissue (n=19)	
		Positive (16)	Negative (3)
ALK-EML4 Exosomal RNA detection. OncoDNA/UZA (n=17)*	Positive (9)	9	0
	Negative (8)	5	3
		Sensitivity 64%	Specificity 100%

*2 RNA (positive) samples get degraded during the delivery

Conclusion

Exosomes are raising as one of the most promising tools to understand the tumor due to their stability in the blood and their similarity to the cells of origin. Our preliminary results show a high specificity for a proof of concept analysis. Further studies with a larger number of patients and a cross-validation analysis are required, but as we present in this abstract, exosomes can represent an important tool for the clinical management of this specific NSCLC population.

Correspondence:
Prof. Dr. Christian Rolfo
Head of Phase I – Early Clinical Trials Unit
Antwerp University Hospital – Belgium
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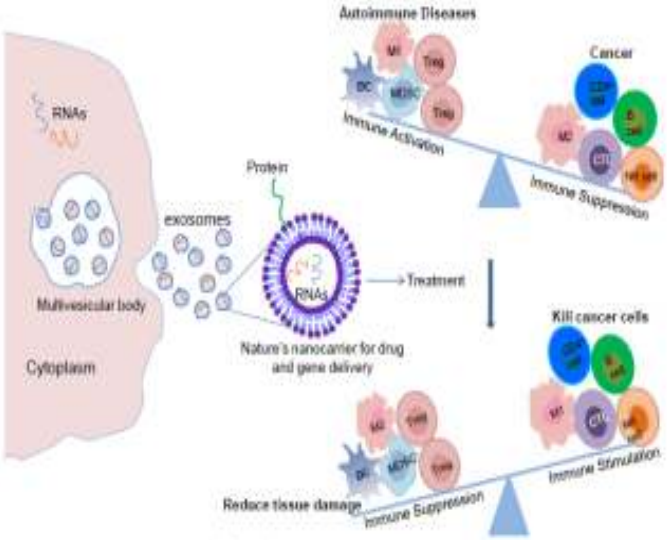
Abstract ID 5471

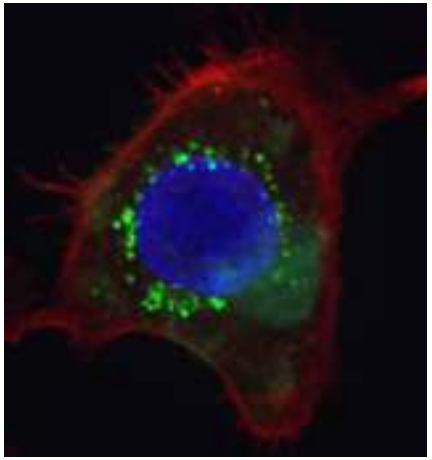
Exosomes in Immunotherapy

Exosomes as platform for liquid biopsy in immune-oncology

- Tumour-derived exosomes carry **multiple immunoinhibitory signals**, disable anti-tumour immune effector cells and **promote tumour escape** from immune control.
- Exosomes delivering negative signals to immune cells in cancer may interfere with therapy and influence outcome.



Tran et al, Clin Immun 2015



Rolfo Lab, Unpublished data

Analysis of PD-L1 in Lung Cancer Tissue and Plasma Exosome Before and After Radiotherapy (NCT02869685)

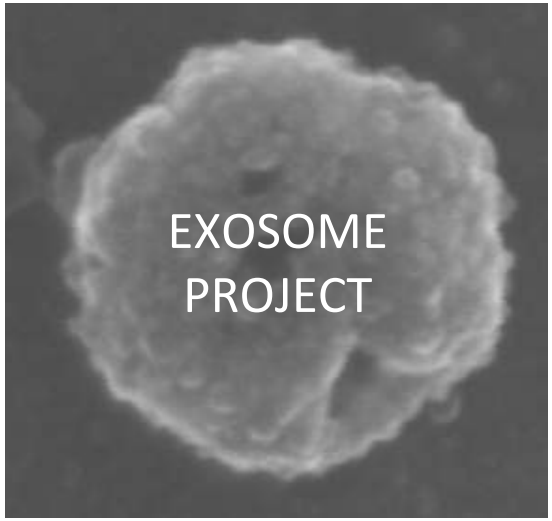
International Multidisciplinary Collaboration

Universidad de Navarra

Oncosalud

ONCODNA

vito



THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

UNIVERSITÀ DEGLI STUDI DI PALERMO

Memorial Cancer Institute

Biocept
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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.
- Exosomes represents a step forward with multiple possibilities for clinical application
- Exosomes have important biological implications
- More grants, cooperative groups and pharma efforts are needed.

Project Team members

Oncology – Phase I Early Clinical Trials Unit
Prof. Dr. Christian Rolfo -
Prof. Dr. Marc Peeters – head oncology and MOCA
Dr. Marika Rasschaert – Dr. Katrine De Block
Fellows: Dr. Helena Oliveres. Dr. Mariana Rocha
Rolfo Lab:
Exosomes: Senior Dr. SimonaTaverna
PhD students: Dr. Pablo Reclusa Asiain
Dr. Marzia Pucci
Dr. Mahafarin Maralani
tFree DNA: Dr. Laura Sober – Karen Zwaenepoel
Cell Lines & cMET: Dr. Nele Van Der Steen
Logistics: Sam Van Gerwen, BsC
Clinical Study –co: Amelie Lyessens, BsC
Molecular Pathology Unit
Prof. Dr. Patrick Pauwels
Dr. Amelie Dendooven
Dr. Karen Zwaenepoel
Tumor - Serum Bank
Dr. Annemieke De Wilde
Dr. Sofie Goethals

Next Generation Sequencing
Dr. Christine Weyn – UZA
Dr. Suzanne Lambin
Dr. Ken Op De Beeck - UA
Database: Dr. R. Mauceri
Dr. Andreia Coelho

Proteomics
Prof. Inge Mertens
Prof. Geert Baggerman
Dr. Evelien Maes

MOCA **2014 Research Grant**

vito vision on technology

2015 Stichting tegen Kanker

Universiteit Antwerpen / UZA

Dank u voor uw aandacht

Thank you for your attention

Gracias por vuestra atención

Kennis / Ervaring / Zorg

christian.rolfo@uza.be

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