CURA.R.T.E.
ALIMENTAZIONE, RICERCA, TERAPIA, EMOZIONE

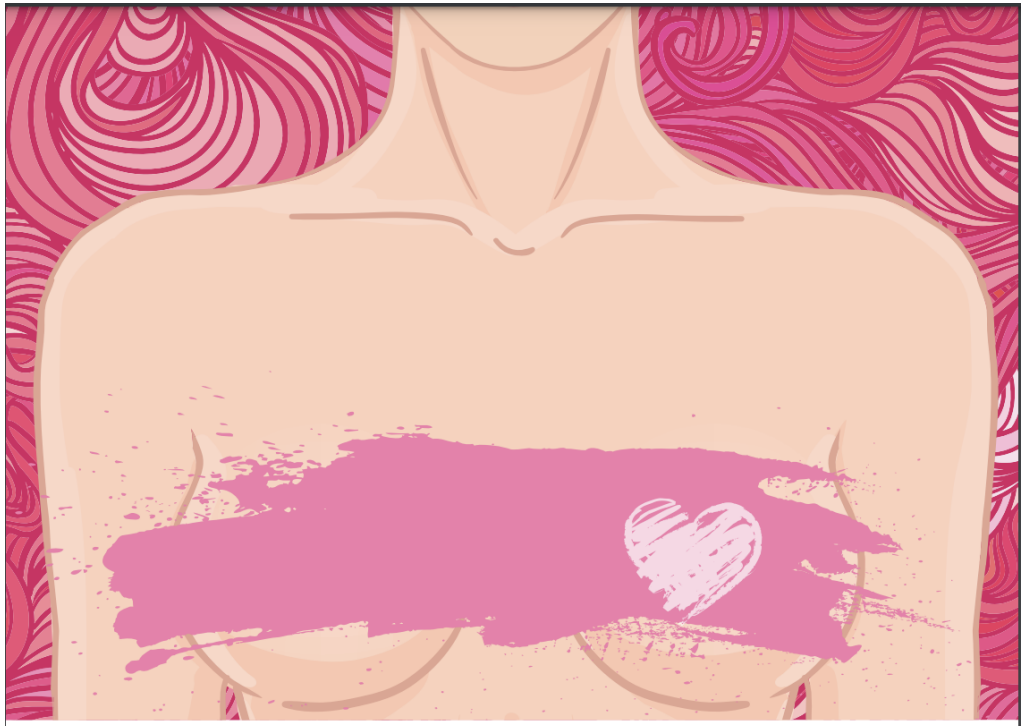
Convegno di **Fondazione IncontraDonna** | PRIMA EDIZIONE

ROMA, 14|06|2023
BOSCOLO CIRCO MASSIMO

Fondazione Incontra donna
OCCUPIAMOCI DI SALUTE

BIOPSIA LIQUIDA E GENETICA

Antonio Russo



CURA.R.T.E.

ALIMENTAZIONE, RICERCA, TERAPIA, EMOZIONE

Convegno di **Fondazione IncontraDonna** | PRIMA EDIZIONE

ROMA, 14|06|2023

BOSCOLO CIRCO MASSIMO

FONDAZIONE Incontra donna OCCUPIAMOCI DI SALUTE

UNIVERSITÀ DEGLI STUDI DI PALERMO

"P. GIACCONE" - UOC DI ONCOLOGIA MEDICA - AOUP

RETE TUMORI RARI Sicilia

A.O.U.P. "P. Giaccone"

Dipartimento di Discipline Chirurgiche, Oncologiche e Stomatologiche

UOC di Oncologia Medica

(Dir.: Prof. Antonio Russo)

La biopsia liquida nell'era dell'oncologia di precisione

Antonio Russo

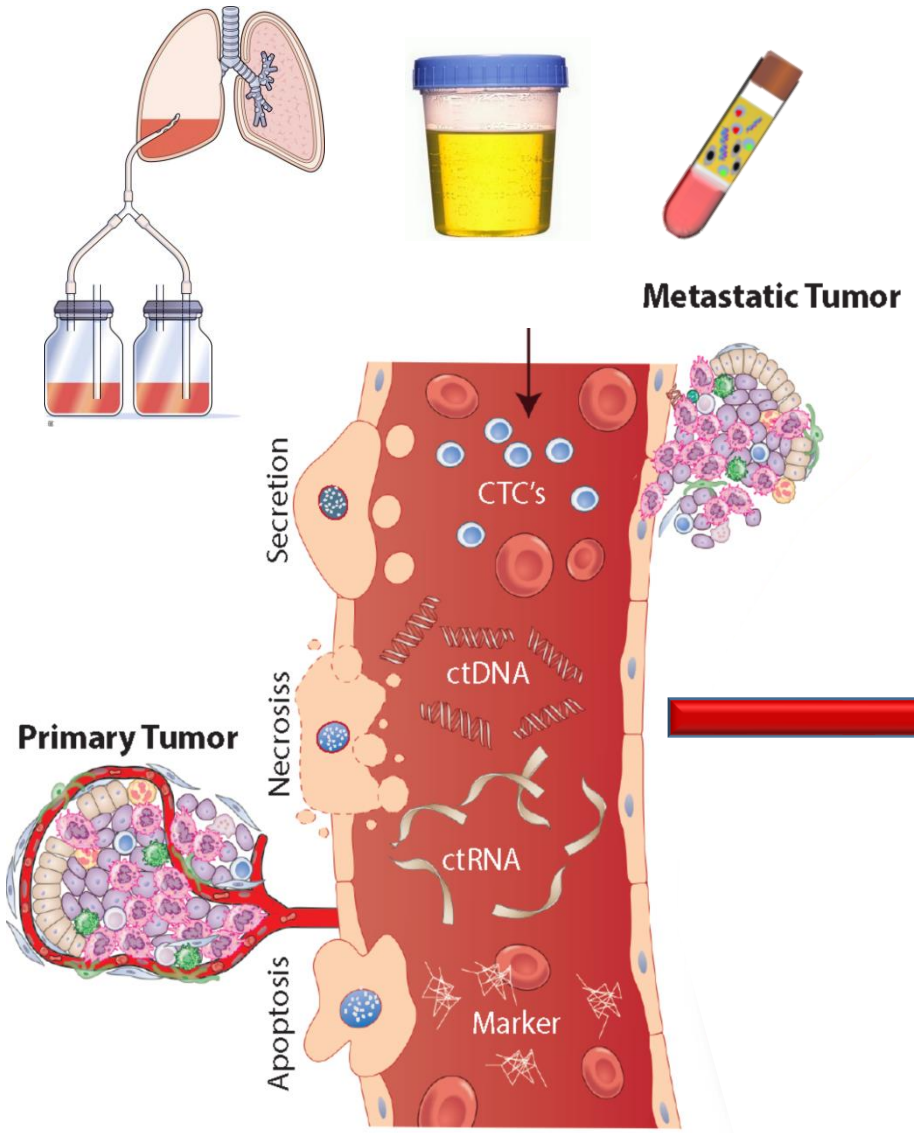
DISCLOSURE INFORMATIONS

All financial reports made in the last two years

- **Honorarium for advisory boards**: Bristol, Pfizer, Bayer, Kyowa Kirin, Ambrosetti

- **Speaker honorarium:** Roche Diagnostic

Liquid Biopsy
Definition



Circulating Tumor Cells (CTCs)

- Protein expression
- Gene expression
- DNA abnormalities
- miRNAs
- Epigenetic alteration
- Functional studies
- Single cell analysis
- Tumor heterogeneity

Circulating Tumor DNA (ctDNA)

- Tumor mutational burden
- Amplifications/deletions
- Translocations
- Point mutations
- Chromosomal abnormalities
- Tumor heterogeneity

ctDNA/methylation

- Epigenetic alterations
- DNA methylation
- Tumor heterogeneity

Circulating miRNAs

Extracellular vesicles

Liquid Biopsy

Where does the need for liquid biopsy come from?

Liquid Biopsy		Tissue Biopsy
- Patient-friendly, Minimally invasive - ↓ costs and risks of complications		- Often invasive and expensive - Risks of complications
Easily repeatable procedure		Not always feasible (multiple sampling?)
Rapid turnaround time	VS	Low turnaround time
Representative of the tumor TUMOR HETEROGENEITY → GLOBAL INTEGRATED		Not always representative for the all tumor TUMOR HETEROGENEITY → LOCALIZED
SERIAL: Real-Time monitoring/Dynamic		SINGLE (snapshot of the tumor)
Treatment selection and assignment to clinical trials. (larger sample sizes)		Difficulties in tissue sampling (smaller sample sizes)

De Mattos-Arruda et al. Nat Rev Clin Oncol 2014;
Siravegna et al. Annals Oncol 2019;
Wan et al Nature Reviews Cancer 2017

Precision Oncology

The efficacy of target therapy is affected by… Tumor Heterogeneity

Heterogeneity in pts with NSCLC according to driver oncogenes

ALK
≃5%

KRAS
≃15% in Caucasians
≃30% in Asians

EGFR
≃15% in Caucasians
≃40% in Asians

HER2
≃3%

ROS1
≃2%

RET
≃1%

BRAF
≃1%

NRAS
≃1%

PIK3CA
≃1%

MET
<5%

Others?

Heterogeneity within pts with EGFR mutations

Sensitive mutations

L858R
≃40%

Exon 19 del
≃50%

Resistance mutations

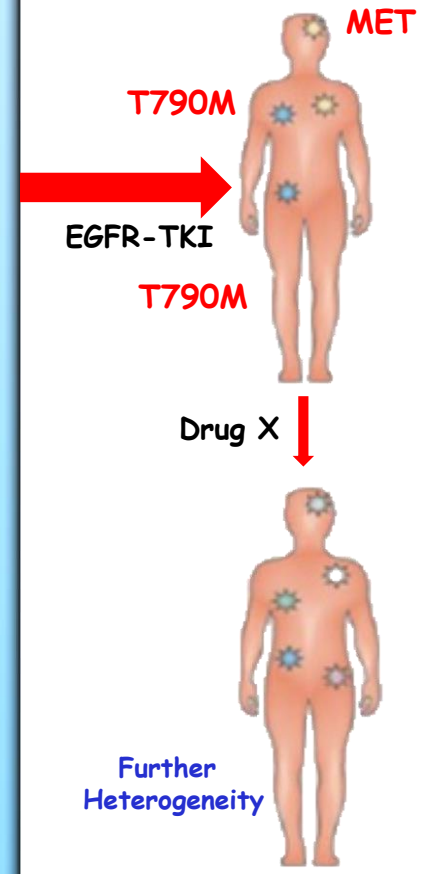
T790M
≃30% by Digital PCR

↓IkB
≃ 30%

↓BIM
20-40%

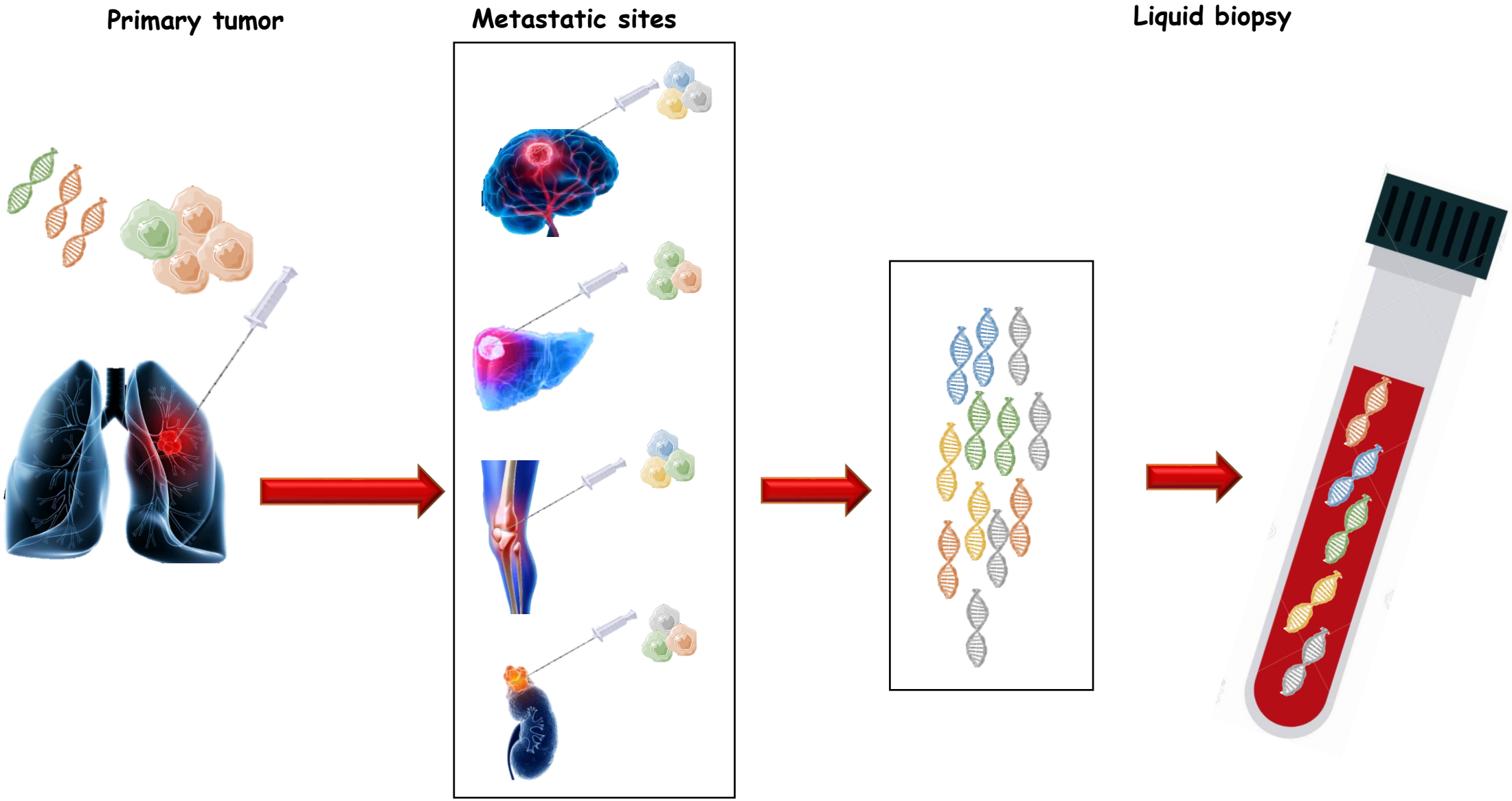
↑CRKL
3%

Heterogeneity in resistance mechanism in one pt



Liquid Biopsy

Representative of the tumor TUMOR HETEROGENEITY



Relevant issues to be considered…

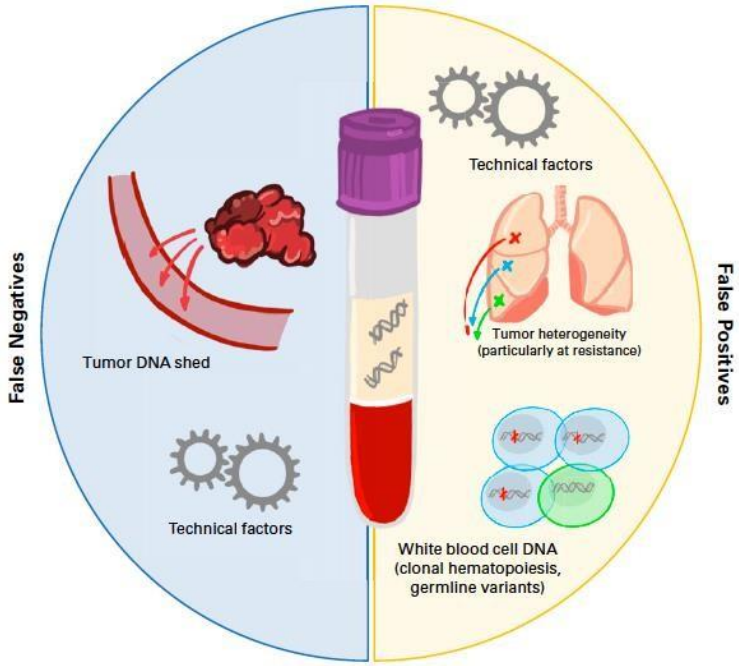
Risk of false negative and false positive results

«False Negatives» in Liquid Biopsy

Insufficient DNA shed into plasma:
(low tumor volume, eliminated by therapy)

cfDNA/ctDNA source:
Plasma, pleuric/peritoneal effusion, CSF

Technical Issues:
Insufficient sensitivity in older assays



«False Positives» in Liquid Biopsy

Technical Factors:
Sample differences
(>6 months from tissue to plasma sampling)

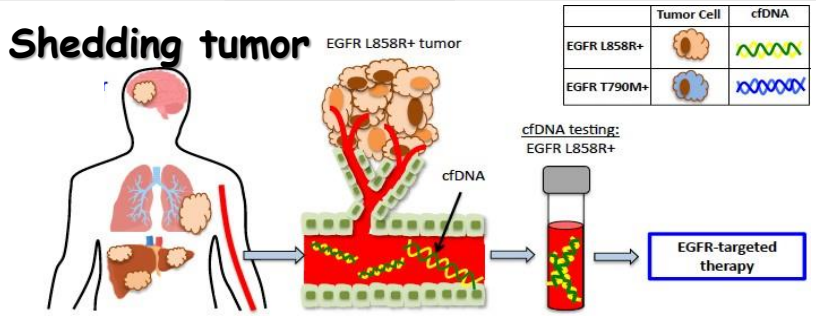
WBC contamination:
Germline Variants
Clonal Hematopoiesis

Tumor Heterogeneity:
Positive Plasma & Negative Tissue
(assumes tissue is **Gold standard**)

Mod from Paweletz CP, et al. JCO Precis Oncol 2019

Liquid Biopsy

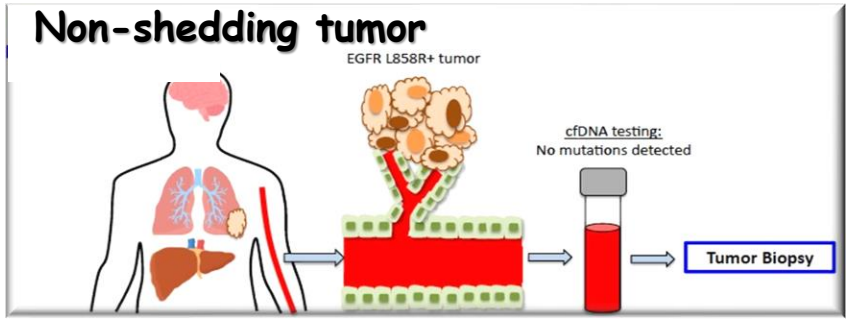
Shedding or non-shedding tumor?

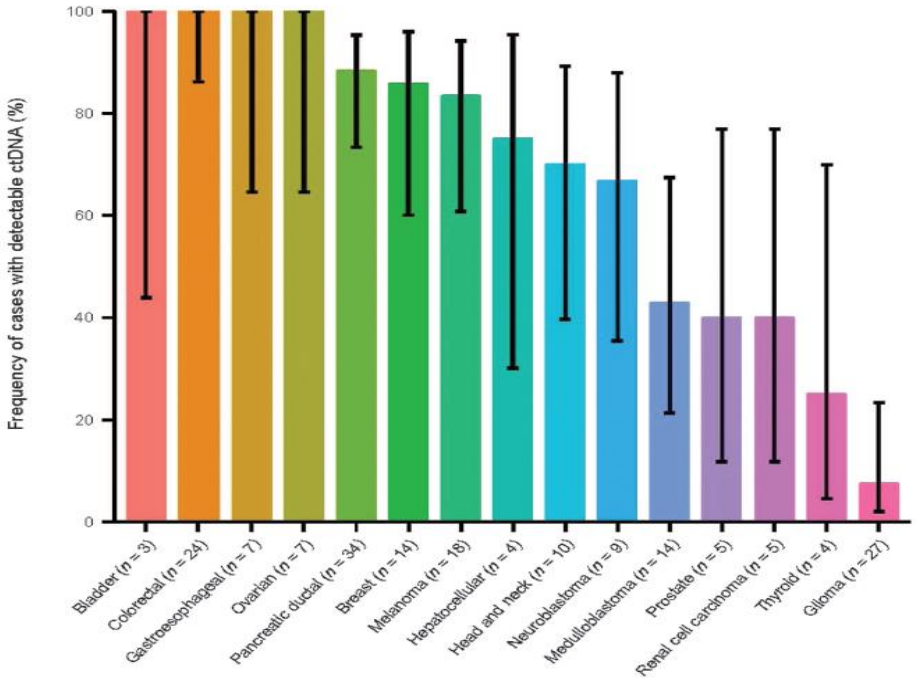


The % (

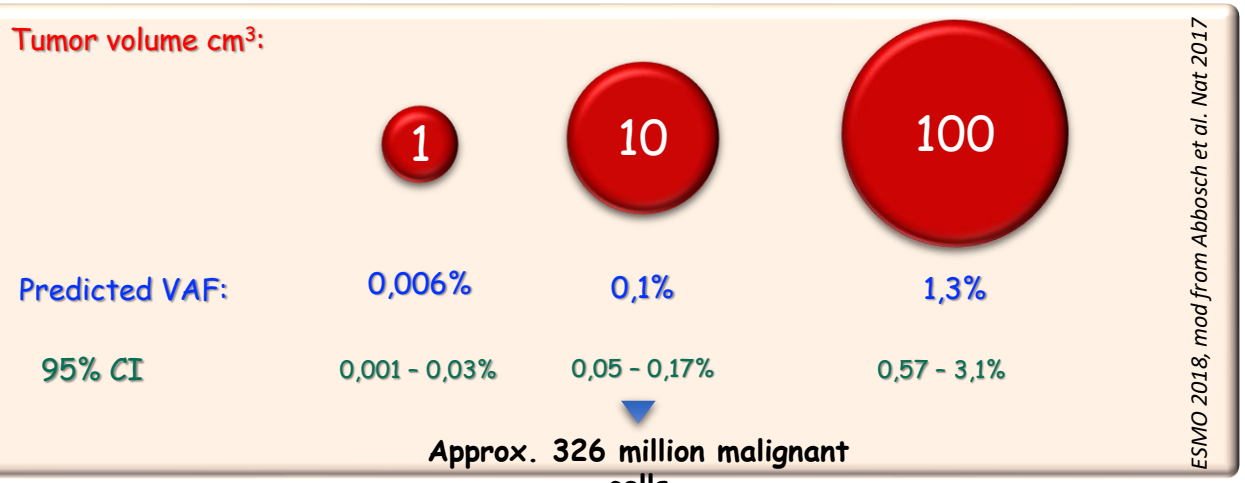
- Tumor stage, volume (Tumor burden)
- Necrosis
- Tumor burden
- Proliferation index
- Histology (adeno K vs squamous for NSCLC)
- Tumor Vascularity
- Anatomical site (i.e. blood-brain barrier for intracranial T., extratoracic vs intrathoracic for NSCLC)
- Pt's renal function
- Type of biological fluid (i.e. cerebrospinal fluid vs plasma for intracranial T.)

A negative biomarker result on ctDNA should be validated using a biomarker test on tissue biopsy





*Sacher AG, et al. JTO 2017; Passiglia F, […] **Russo** Curr Cancer Drug Targ 2018*



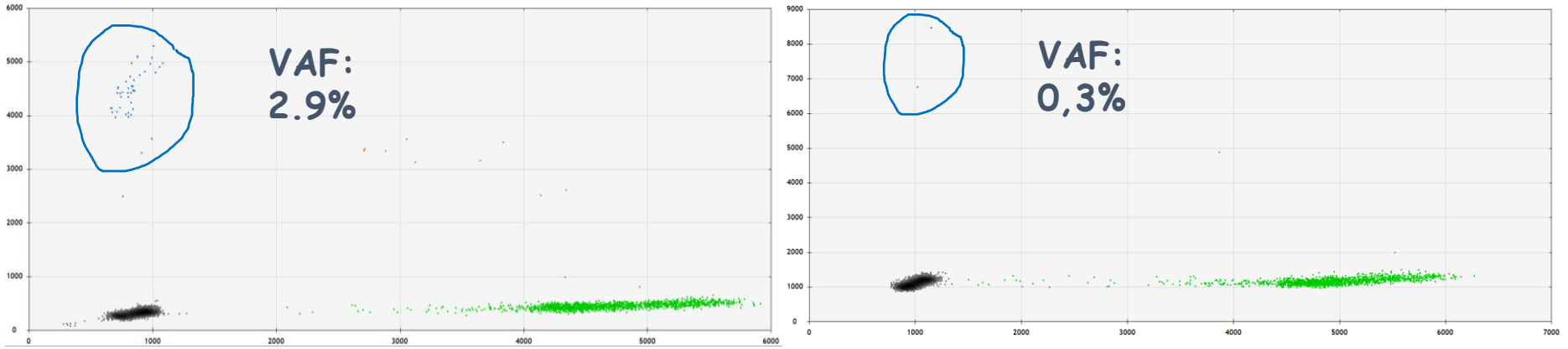
Liquid biopsy: False negative results

Choose the appropriate source of cf/ctDNA: a real-life experience

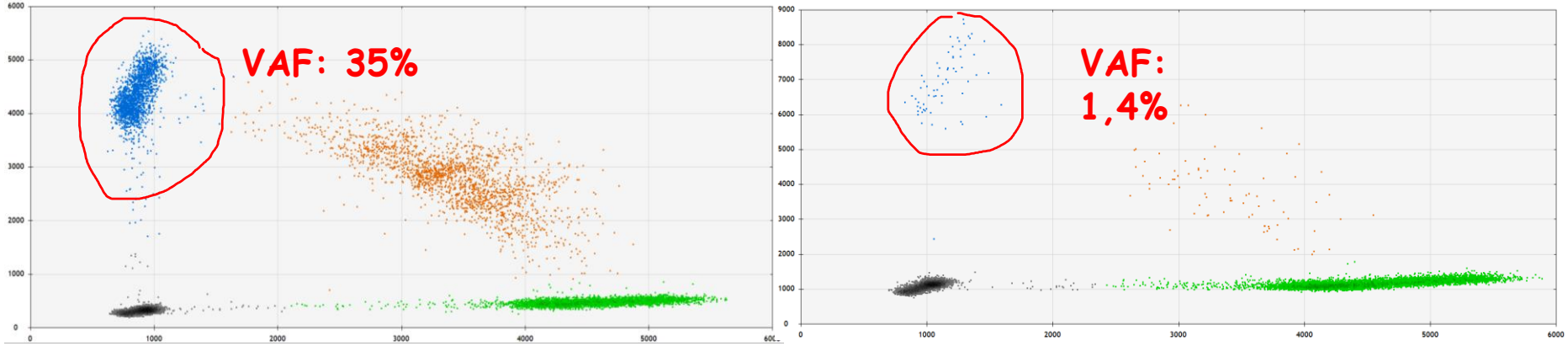
EGFR p.L858R

EGFR p.T790M

Plasma cf/ctDNA



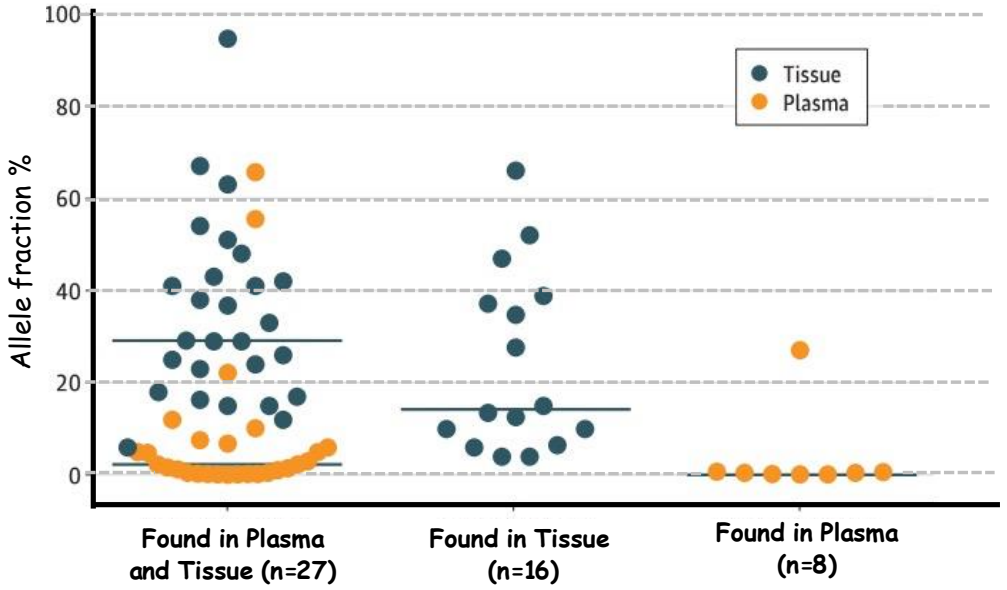
Pleural fluid cf/ctDNA



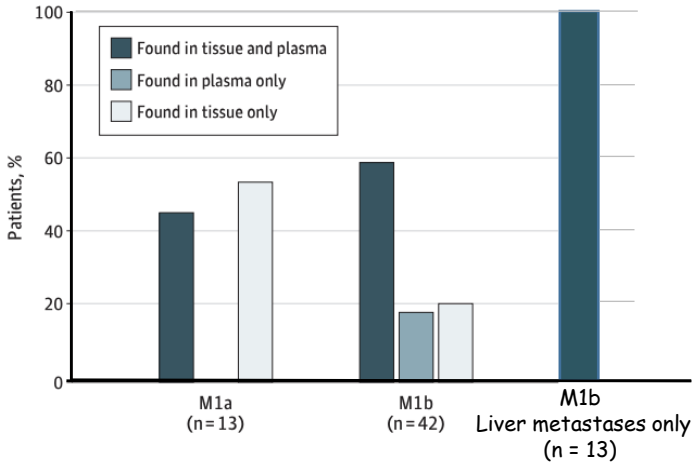
VAF = Variant Allele Frequency

Concordance rates between tissue and ctDNA testing

Allele fraction



Detection of therapeutically targetable mutation



Aggarwal C et Al. JAMA Oncol, 2019

Some considerations

- Main actionable mutations
  Higher concordance rates between tissue and ctDNA T.:
  Range: 80 to ~100%

- All genetic alterations identified
  Lower concordance rates between tissue and ctDNA T.:
  Range: 60 to ~ 70%

- Pts at diagnosis vs pts at progression
  Significantly higher concordance rates between tissue and ctDNA T. in pts at diagnosis

Literature review 2022

Liquid Biopsy

Results of ctDNA testing

Results of Liquid biopsy

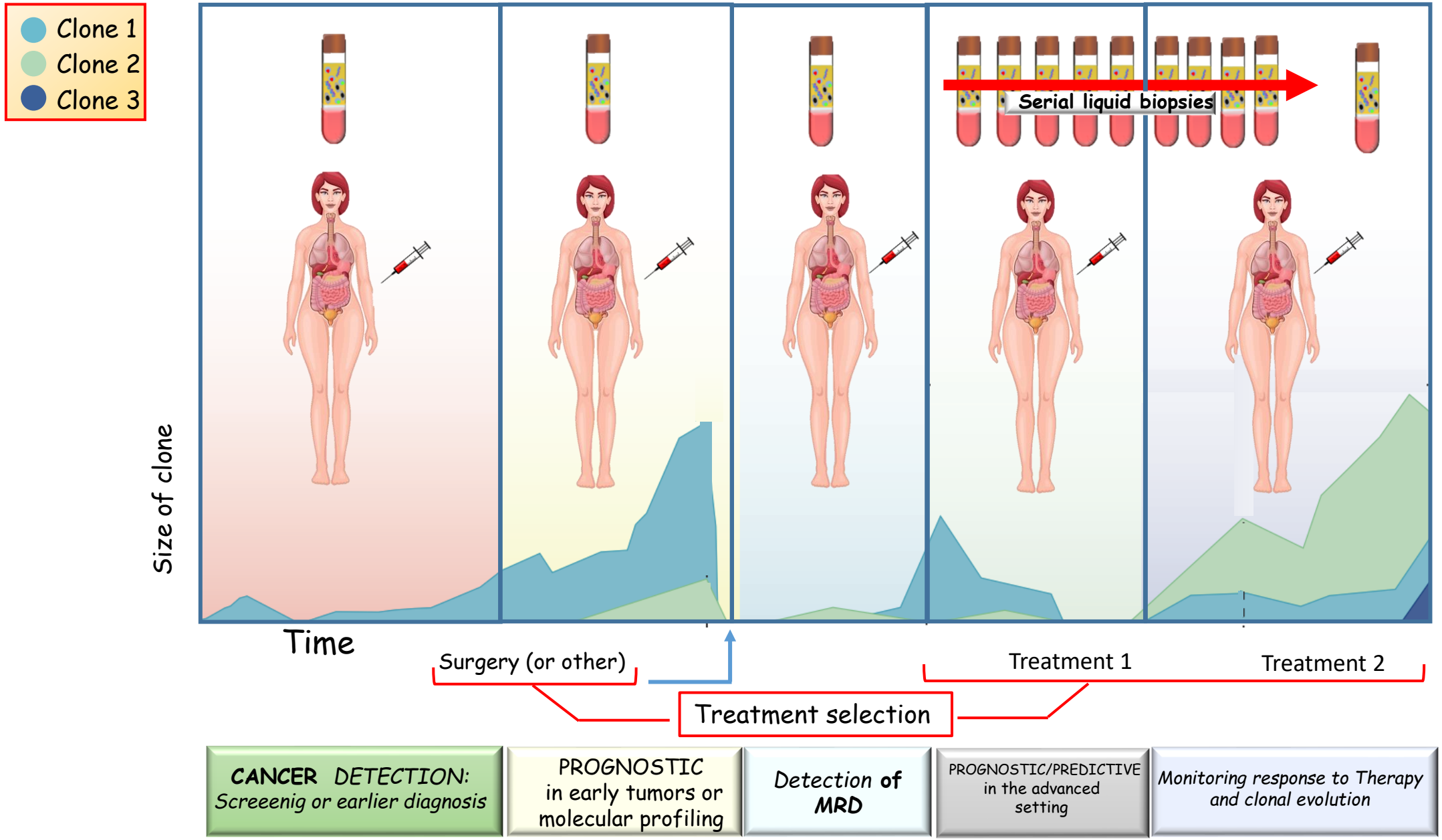
Informative test
(LB test positive)

- TRUE POSITIVE
- TRUE NEGATIVE

Non-informative test
(LB test negative or inconclusive)

Liquid Biopsy

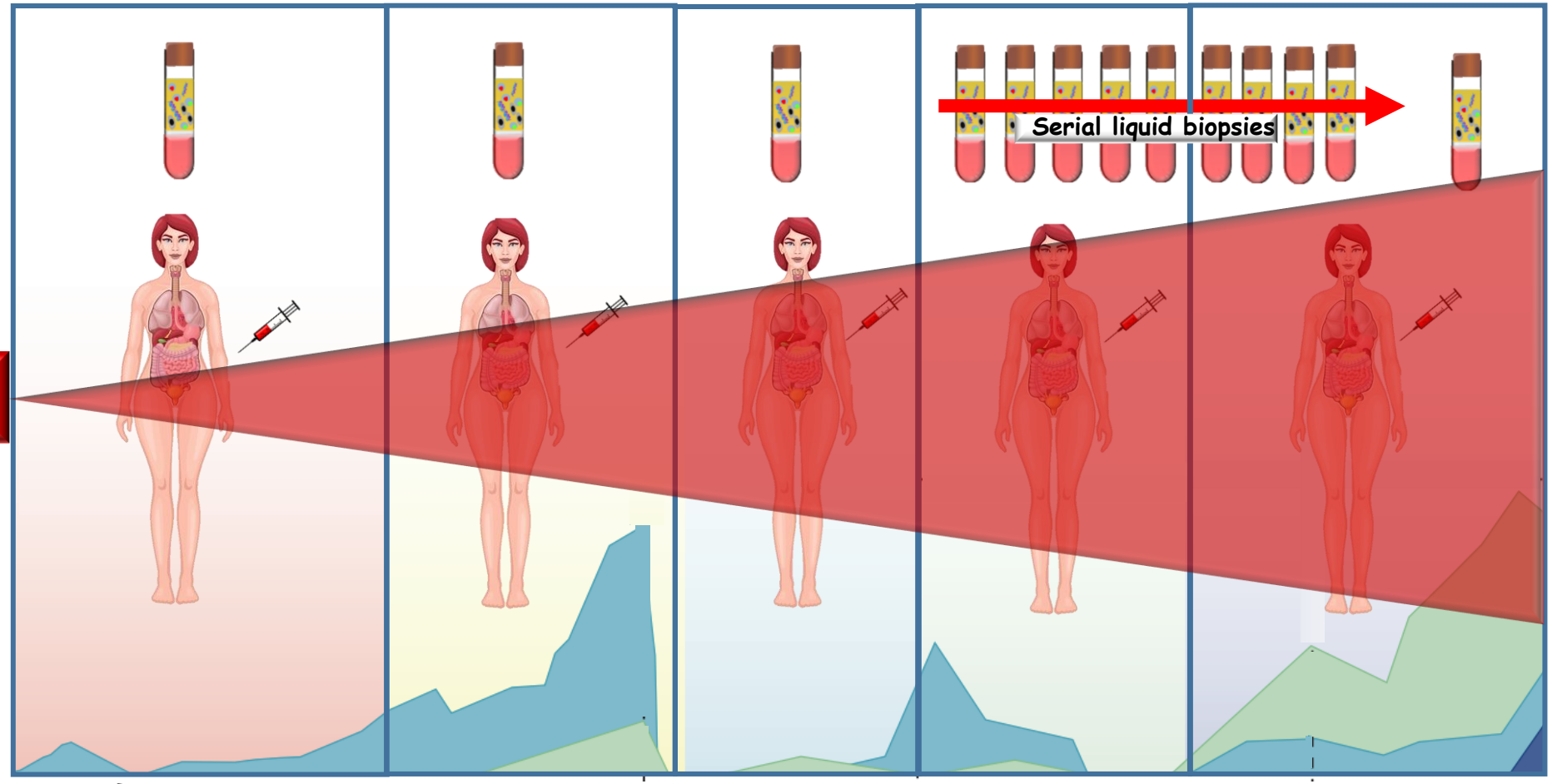
Applications of ctDNA in solid tumors



Mod from Wan et al. Nat Rev Cancer 2017

Liquid Biopsy

Applications of ctDNA in solid tumors



Experimental application

Clinical application

Surgery (or other)

Treatment 1

Treatment 2

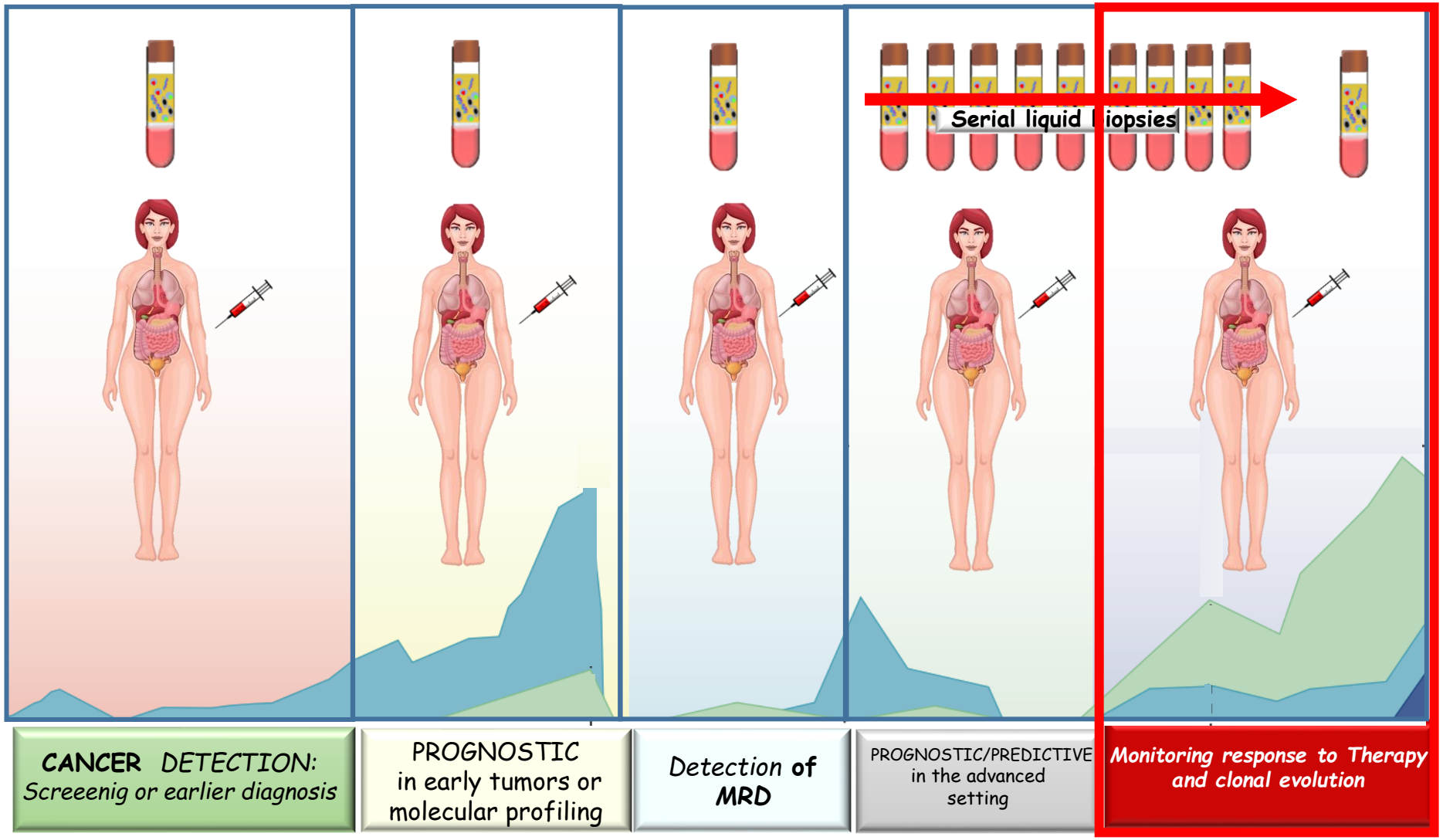
Treatment selection

CANCER *DETECTION:* *Screeenig or earlier diagnosis*	PROGNOSTIC in early tumors or molecular profiling	*Detection* **of** ***MRD***	PROGNOSTIC/PREDICTIVE in the advanced setting	*Monitoring response to Therapy and clonal evolution*

Mod from Wan et al. Nat Rev Cancer 2017

Liquid Biopsy: Application of ctDNA in solid tumors

MONITORING RESPONSE TO THERAPY AND CLONAL EVOLUTION



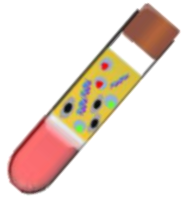
Mod from Wan et al. Nat Rev Cancer 2017

NSCLC

Liquid Biopsy: Recommendations AIOM/SIAPEC

Advanced NSCLC: two clinical scenarios

Liquid biopsy indications



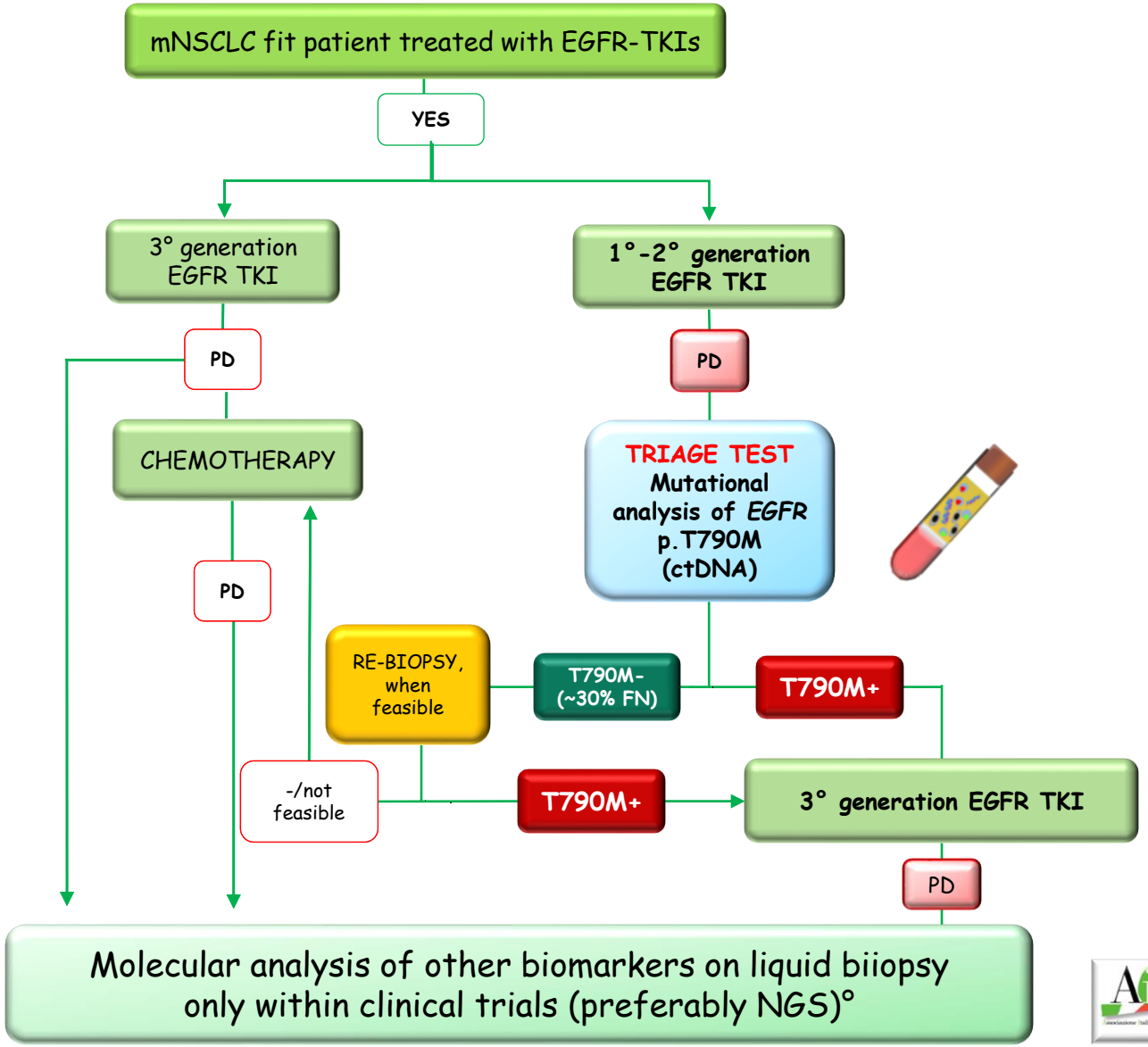
I clinical scenario

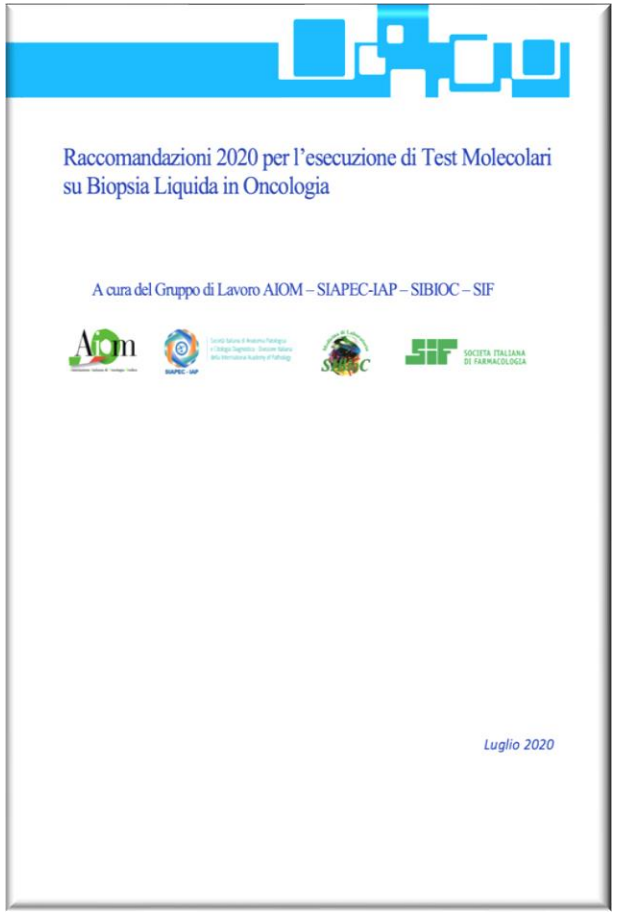
II clinical scenario

NSCLC

Liquid Biopsy: AIOM-SIAPEC-IAP-SIBIOC-SIF 2020

mNSCLC pts pre-treated with *EGFR-TKI*

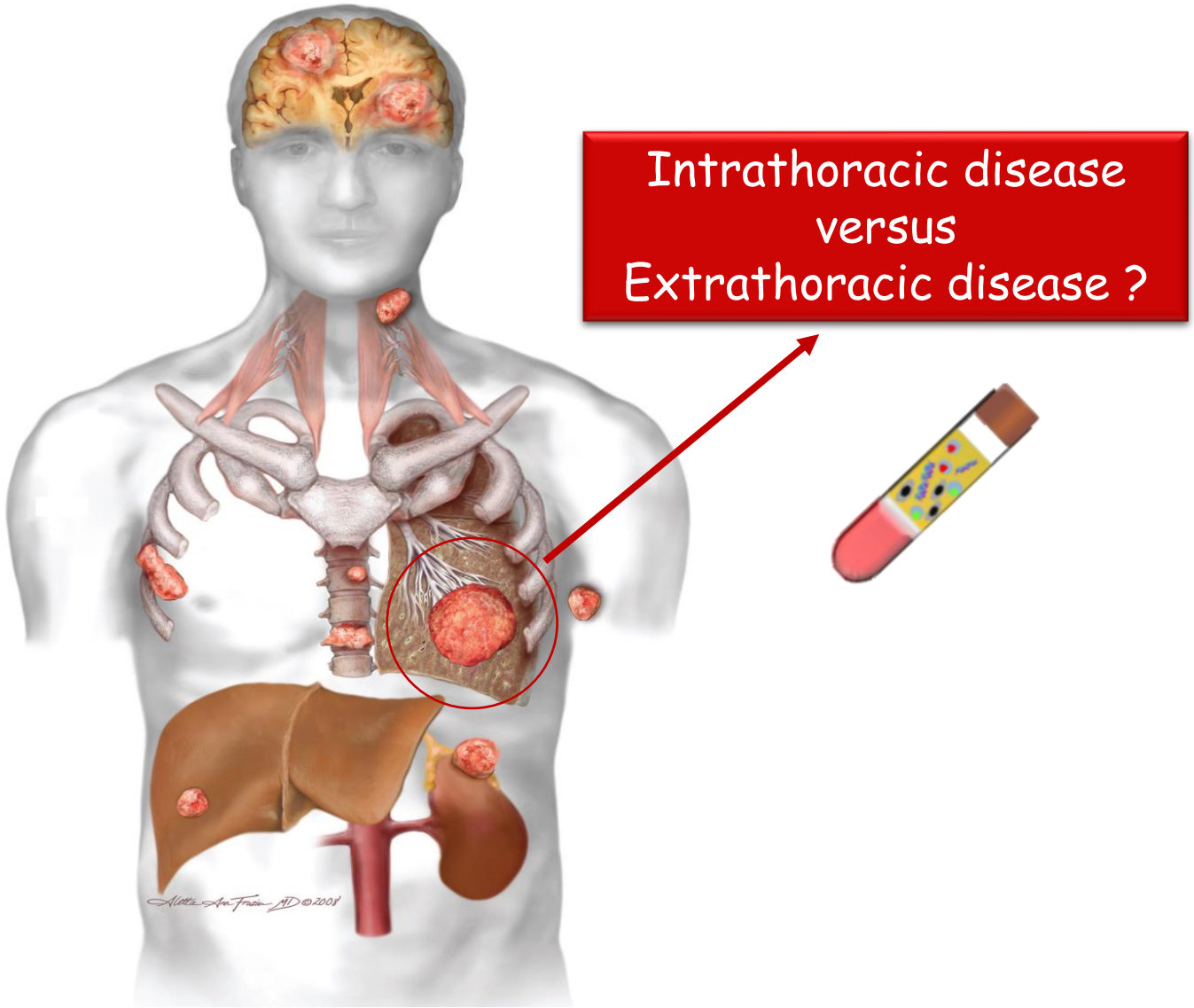




NSCLC

Liquid biopsy: Diagnostic accuracy of ctDNA in NSCLC

Does metastatic site influence ctDNA sensitivity?



NSCLC

NSCLC: EGFR ctDNA sensitivity according to the METASTATIC SITE

Meta-analysis of published trials 10 eligible studies, N= 1425 pts

Metastatic Site Location Influences the Diagnostic Accuracy of ctDNA EGFR- Mutation Testing in NSCLC Patients: a Pooled Analysis

Francesco Passiglia[1,#], Sergio Rizzo[1,#], Christian Rolfo[2], Antonio Galvano[1], Enrico Bronte[1], Lorena Incorvaia[1], Angela Listì[1], Nadia Barraco[1], Marta Castiglia[1], Valentina Calò[1], Viviana Bazan[1,*] and Antonio Russo[1,*]

[1]Department of Surgical, Oncological and Oral Sciences, Section of Medical Oncology, Palermo University Hospital, Palermo, Italy; [2]Phase I- Early Clinical Trials Unit, Oncology Department and Multidisciplinary Oncology Center Antwerp (MOCA), Antwerp University Hospital, Edegem, Belgium

Abstract: **Background:** Recent studies evaluated the diagnostic accuracy of circulating tumor DNA (ctDNA) in the detection of epidermal growth factor receptor (EGFR) mutations from the plasma of NSCLC patients, overall showing a high concordance as compared to standard tissue genotyping. However, it is less clear if the location of the metastatic site may influence the ability to identify EGFR mutations in plasma.

Objective: This pooled analysis aims to evaluate the association between the metastatic site location and the sensitivity of ctDNA analysis in detecting EGFR mutations in NSCLC patients.

Methods: Data from all published studies, evaluating the sensitivity of plasma-based EGFR-mutation testing, stratified by metastatic site location (extrathoracic (M1b) vs intrathoracic (M1a)) were collected by searching in PubMed, Cochrane Library, American Society of Clinical Oncology, and World Conference of Lung Cancer, meeting proceedings. Pooled Odds ratio (OR) and 95% confidence intervals (95% CIs) were calculated for the ctDNA analysis sensitivity, according to metastatic site location.

Results: A total of ten studies, with 1425 patients, were eligible. Pooled analysis showed that the sensitivity of ctDNA-based EGFR-mutation testing is significantly higher in patients with M1b vs M1a disease (OR: 5.09; 95% CIs: 2.93 – 8.84). 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: 5.83, 95% CI: 3.36-9.60) or non-digital PCR technologies (OR: 2.96, 95% CI: 2.24-3.91).

Conclusions: These data suggest that the location of metastatic sites significantly influences the diagnostic accuracy of ctDNA analysis in detecting EGFR mutations in NSCLC patients.

Keywords: EGFR, ctDNA, liquid biopsy, NSCLC, metastasis, intrathoracic, extrathoracic.

1. INTRODUCTION

Targeting the epidermal growth factor receptor (EGFR) by tyrosine-kinase inhibitors (TKIs) has represented a milestone in the treatment of lung cancer. Eight phase III randomized studies have clearly demonstrated that EGFR-TKIs significantly improved response rate (RR), progression-free survival (PFS) and quality of life (QoL) compared to first-line platinum-based chemotherapy in patients with advanced NSCLC harboring EGFR activating mutations [1-8]. Recently a pooled analysis of both LuxLung3 (LL3) and LuxLung6 (LL6) trials showed also an overall survival (OS) benefit in favour of the second generation EGFR-TKI Afatinib in the subgroup of patients with EGFR exon19 deletion [9]. Overall, the results of all such studies convincingly and consistently demonstrated that, for about 40% of Asian and 12% of Caucasian "EGFR-positive" NSCLC patients, the optimal upfront treatment is an EGFR-TKI, as gefitinib, erlotinib, or afatinib. Testing for EGFR mutations in tumor samples DNA is recommended at the time of diagnosis by all the international guidelines for all patients with advanced NSCLC and non-squamous histology in order to decide the proper therapeutic strategy [10]. Even if tissue biopsy remains the current gold-standard, however, it is limited by several features, such as the difficult access to different tumor sites, the invasiveness of procedures, the tumor heterogeneity, and the low patients' compliance. Thus, in the last decade, an alternative approach, known as a liquid biopsy, has been proposed to overcome the aforementioned issues. An increasing number of studies and meta-analysis evaluated the diagnostic accuracy of circulating tumor (ct)DNA in the

*Address correspondence to this author at the Section of Medical Oncology, Department of Surgical, Oncological and Oral Sciences, Palermo University Hospital, Via del Vespro 129, 90127 Palermo, Italy; Tel: + 39-091-6552759; Fax: +39-091-6554529; E-mail: antonio.russo@unipa.it
#These authors equally contributed to this work.
*These authors are both last authors of this work.

1568-0096/18 $58.00+.00

EGFR ctDNA sensitivity according to the metastatic site: Extratoracic (M1b) vs Intrathoracic Disease (M1a)

Study or Subgroup	Extratoracic disease Events	Total	Intraoracic disease Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Jenkins et al. 2017	111	154	123	243	14.8%	2.52 [1.63, 3.88]
Karlovic et al. 2016	52	55	7	18	7.4%	27.24 [6.07, 122.17]
Karlovic et al. 2016*	47	49	4	15	5.9%	64.63 [10.47, 398.82]
Kasahara et al. 2017	26	33	8	16	8.6%	3.71 [1.03, 13.46]
Kumar et al. 2017	21	28	15	27	9.6%	2.40 [0.76, 7.53]
Normanno et al. 2016	52	82	13	57	12.4%	5.87 [2.73, 12.60]
Oxnard et al. 2016	139	161	36	48	12.2%	2.11 [0.95, 4.66]
Thress et al. 2015	21	27	2	11	6.0%	15.75 [2.65, 93.46]
Tseng et al. 2015	32	41	5	21	8.9%	11.38 [3.27, 39.60]
Yi-Long Wu et al. 2016	180	234	63	105	14.4%	2.22 [1.35, 3.65]
Total (95% CI)		864		561	100.0%	5.09 [2.93, 8.84]
Total events	681		276			

Heterogeneity: Tau² = 0.49; Chi² = 32.34, df = 9 (P = 0.0002); I² = 72%
Test for overall effect: Z = 5.79 (P < 0.00001)

Odds Ratio M-H, Random, 95% CI
0.01 0.1 1 10 100
Extratoracic disease Intratoracic disease

Forest plot showing odds ratio for overall sensitivity of plasma ctDNA EGFR-mutation testing by metastatic sites location (M1b vs M1a)

EGFR mutation

Study or Subgroup	Extratoracic disease Events	Total	Intraoracic disease Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Karlovic et al. 2016	52	55	7	18	9.6%	27.24 [6.07, 122.17]
Kasahara et al. 2017	26	33	8	16	11.4%	3.71 [1.03, 13.46]
Kumar et al. 2017	21	28	15	27	12.8%	2.40 [0.76, 7.53]
Normanno et al. 2016	52	82	13	57	17.1%	5.87 [2.73, 12.60]
Oxnard et al. 2016	139	161	36	48	16.8%	2.11 [0.95, 4.66]
Tseng et al. 2015	32	41	5	21	11.8%	11.38 [3.27, 39.60]
Yi-Long Wu et al. 2016	180	234	63	105	20.4%	2.22 [1.35, 3.65]
Total (95% CI)		634		292	100.0%	4.30 [2.35, 7.88]
Total events	502		147			

Heterogeneity: Tau² = 0.41; Chi² = 17.58, df = 6 (P = 0.007); I² = 66%
Test for overall effect: Z = 4.72 (P < 0.00001)

Odds Ratio M-H, Random, 95% CI
0.01 0.1 1 10 100
Extratoracic disease Intraoracic disease

T790M Mutation

Study or Subgroup	Extratoracic disease Events	Total	Intraoracic disease Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Jenkins et al. 2017	111	154	123	243	38.8%	2.52 [1.63, 3.88]
Karlovic et al. 2016*	47	49	4	15	30.4%	64.63 [10.47, 398.82]
Thress et al. 2015	21	27	2	11	30.7%	15.75 [2.65, 93.46]
Total (95% CI)		230		269	100.0%	11.89 [1.45, 97.22]
Total events	179		129			

Heterogeneity: Tau² = 2.91; Chi² = 14.75, df = 2 (P = 0.0006); I² = 86%
Test for overall effect: Z = 2.31 (P = 0.02)

Odds Ratio M-H, Random, 95% CI
0.01 0.1 1 10 100
Extratoracic disease Intraoracic disease

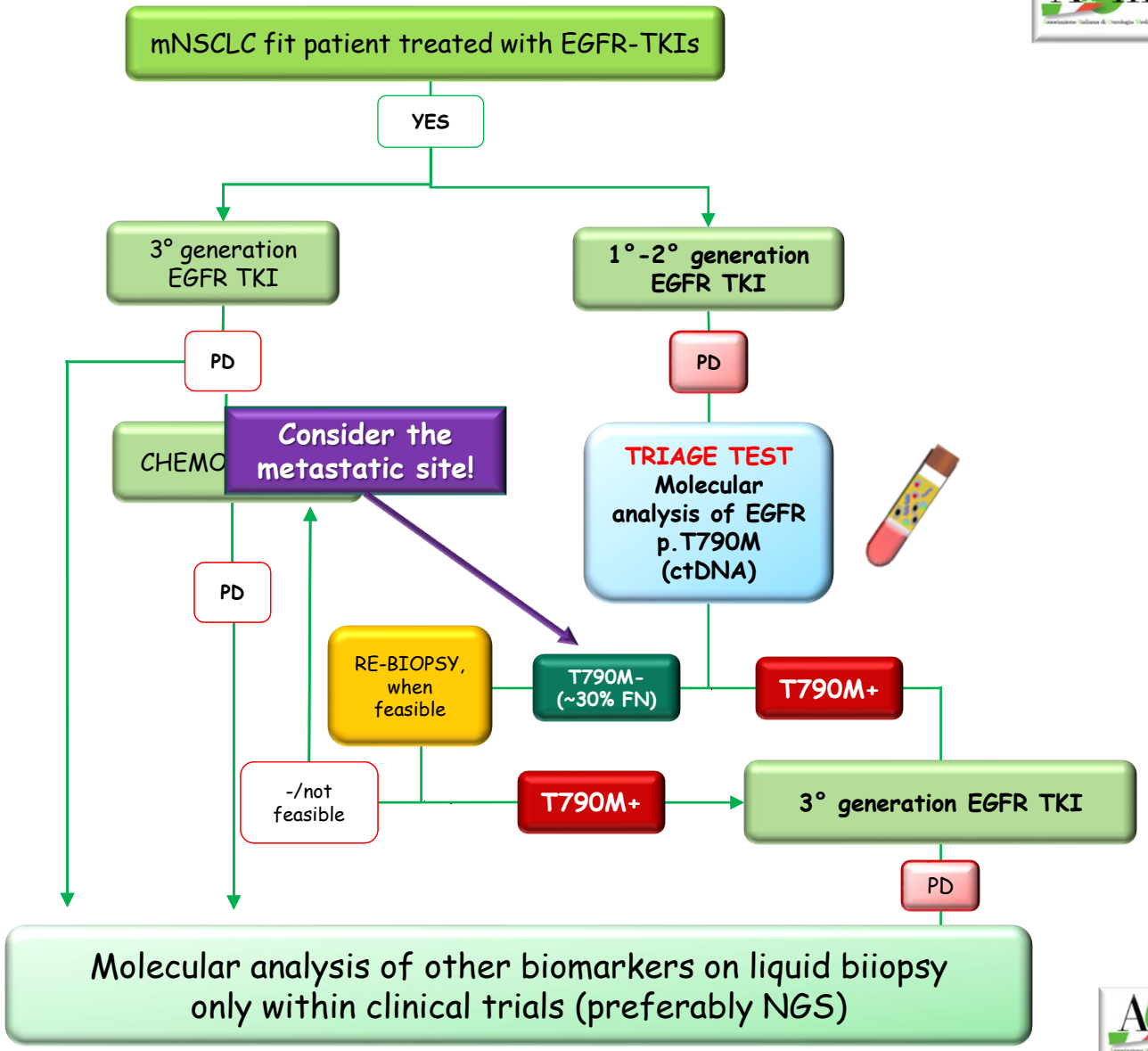
Forest plot showing odds ratio for overall sensitivity of plasma ctDNA EGFR-mutation testing by metastatic sites location (M1b vs M1a) and EGFR or T790M mutation

Passiglia F... Rolfo C [...] A. Russo Current Cancer Drug Target 2018

NSCLC

Liquid Biopsy: AIOM-SIAPEC-IAP-SIBIOC-SIF 2020

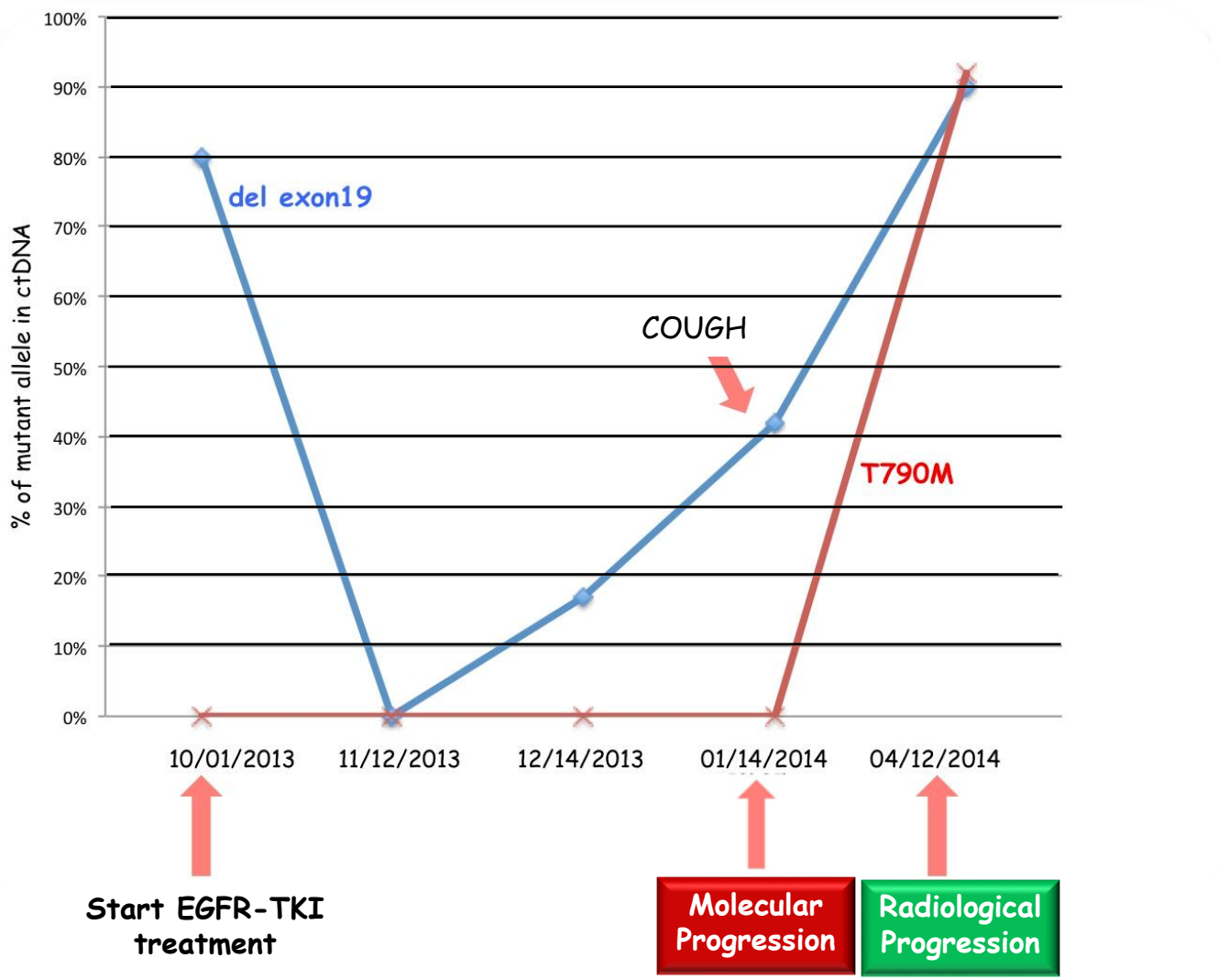
mNSCLC pts pre-treated with *EGFR-TKI*



NSCLC

Liquid biopsy

NSCLC: longitudinal monitoring of EGFR mutations
When switch to 3rd gen TKI: RECIST PD o molecular PD?



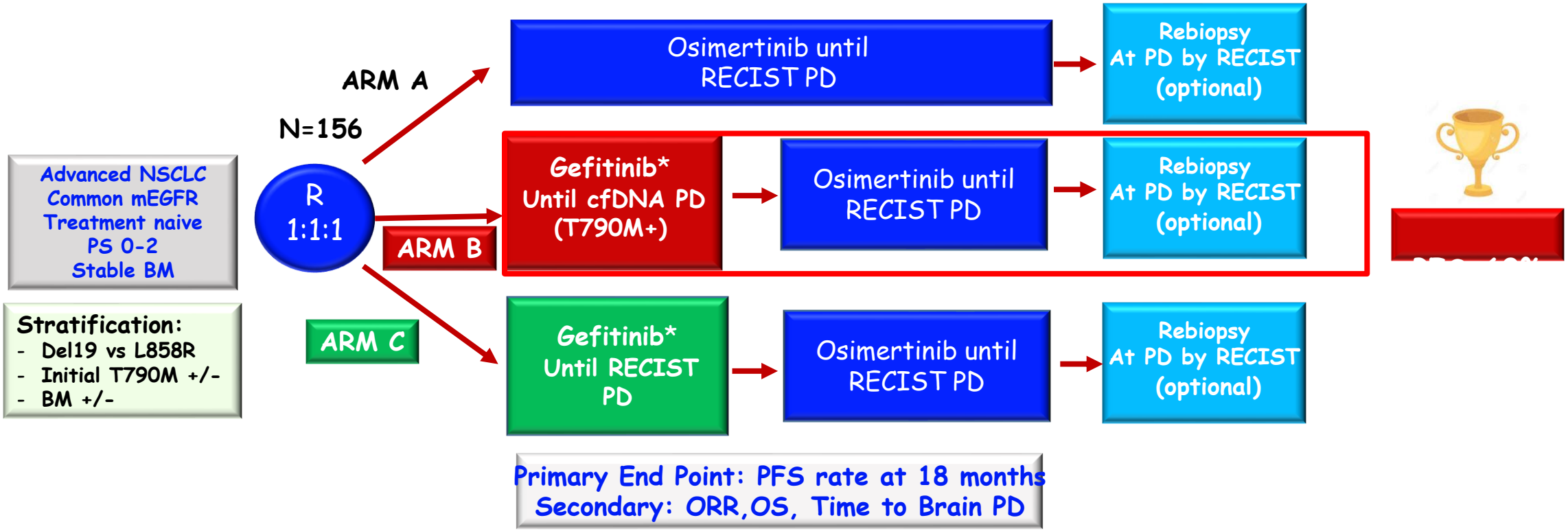
Future perspectives: APPLE trial

When switch to 3rd gen TKI: RECIST PD or molecular PD?

APPLE Trial design

Randomized, open-label, multicenter, phase II trial (NTC02856893)

EORTC European Organisation for Research and Treatment of Cancer

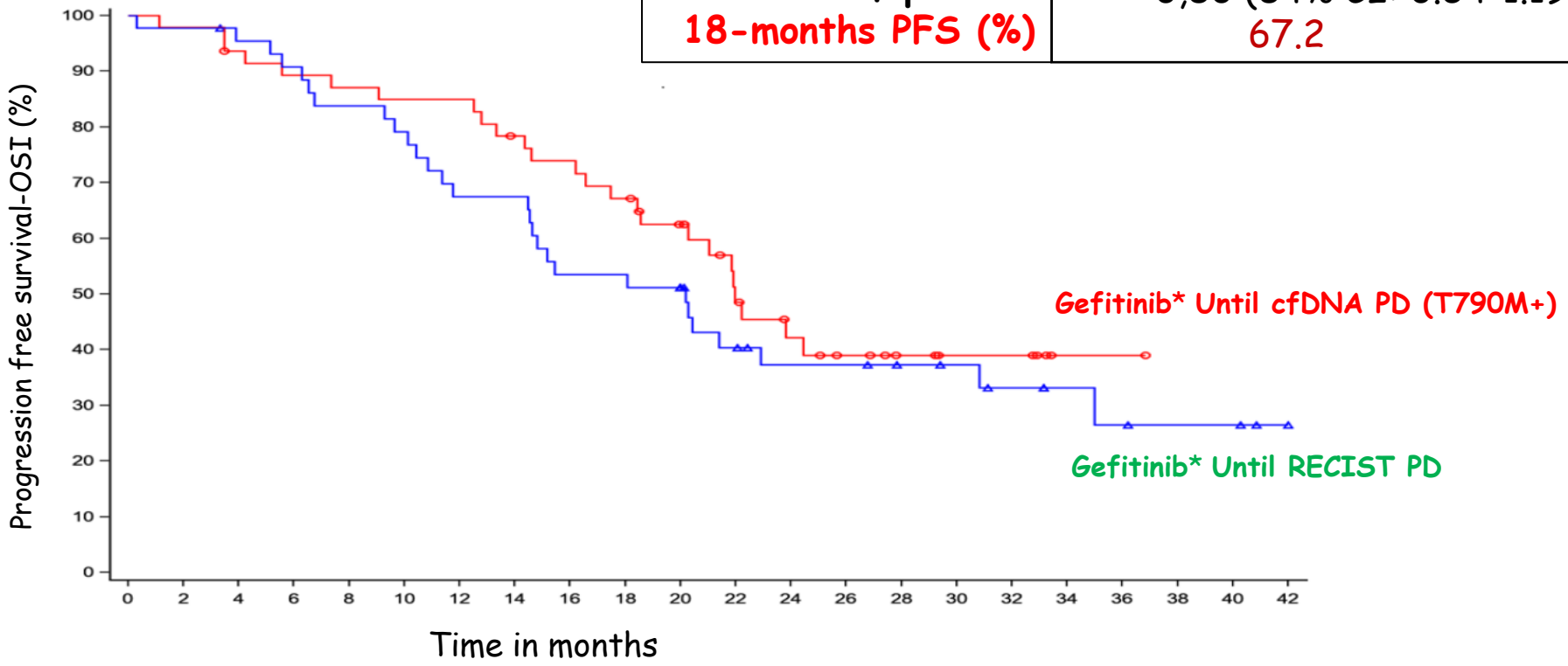


* In case of RECIST PD without T790M+, pts will be switched

APPLE trial: RESULTS

PFS-OSI-18 rate by INV. in arm B vs C, exploratory

	ARM B	ARM C
Median PFS (mo.)	22	20.2
HR; p-value	0,80 (84% CI: 0.54-1.19); p=0.22	
18-months PFS (%)	67.2	53.5

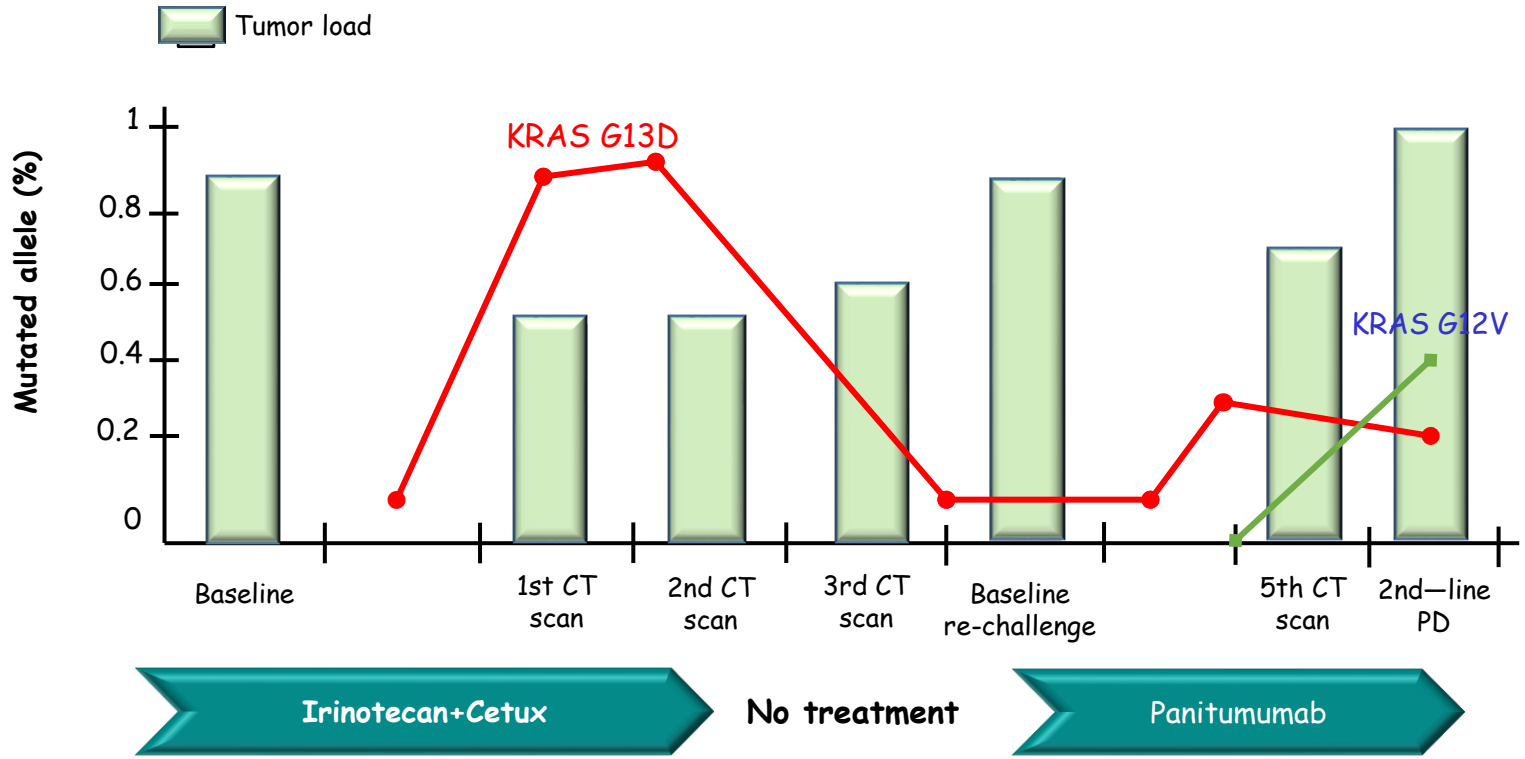


* In case of RECIST PD without T790M+, pts will be switched

Colon Cancer

Liquid biopsy in CRC

Dynamic change of KRAS during intermittent anti-EGFR treatment

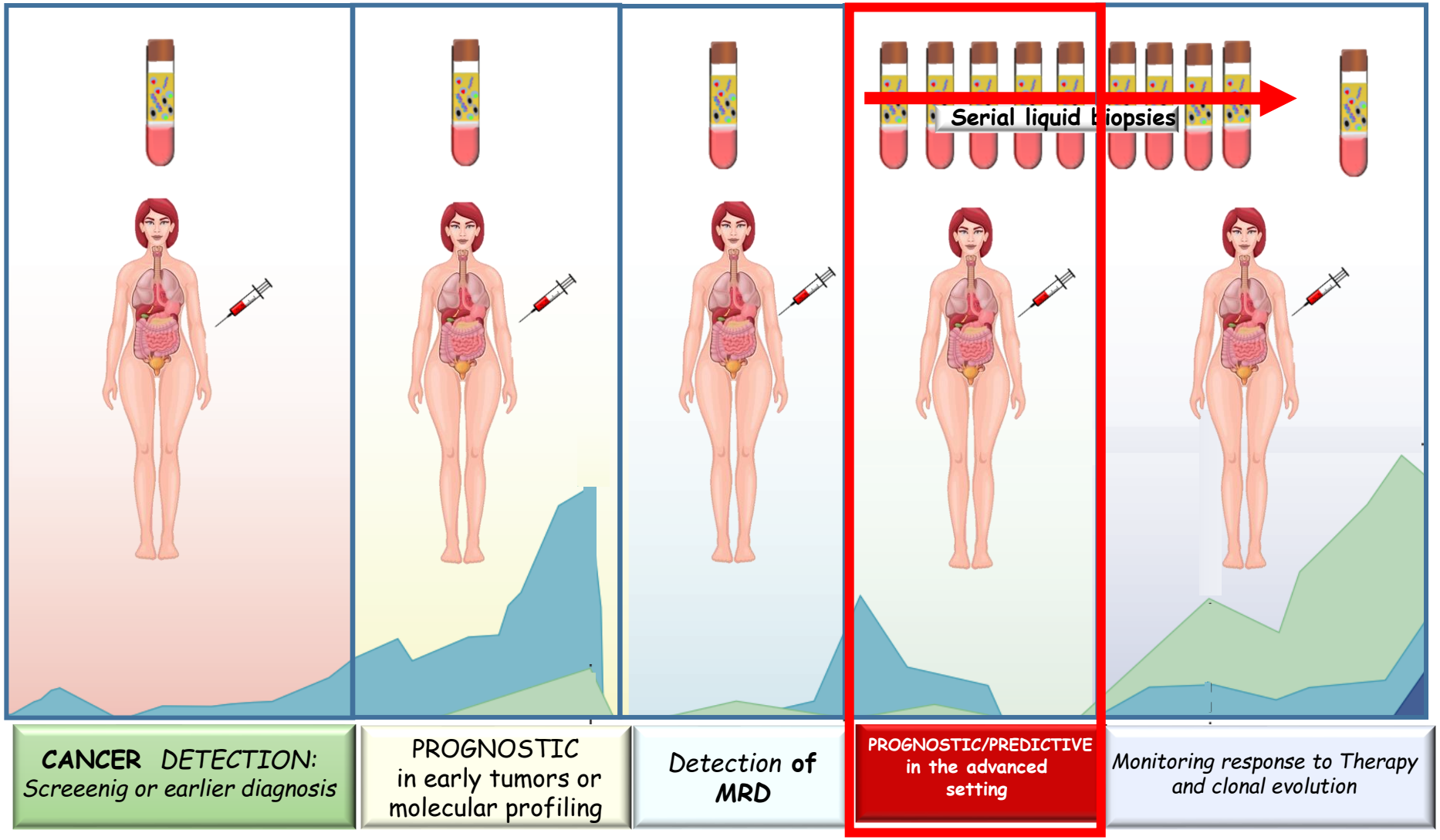


The change of KRAS during intermittent anti-EGFR treatment reflects the dynamic clonal selection → Re-challenge may be a real option?

Adopted from Siravegna G. et al., Nat Med, 2015

Liquid Biopsy: Applications of ctDNA in solid tumors

PROGNOSTIC/PREDICTIVE in the advancedsetting

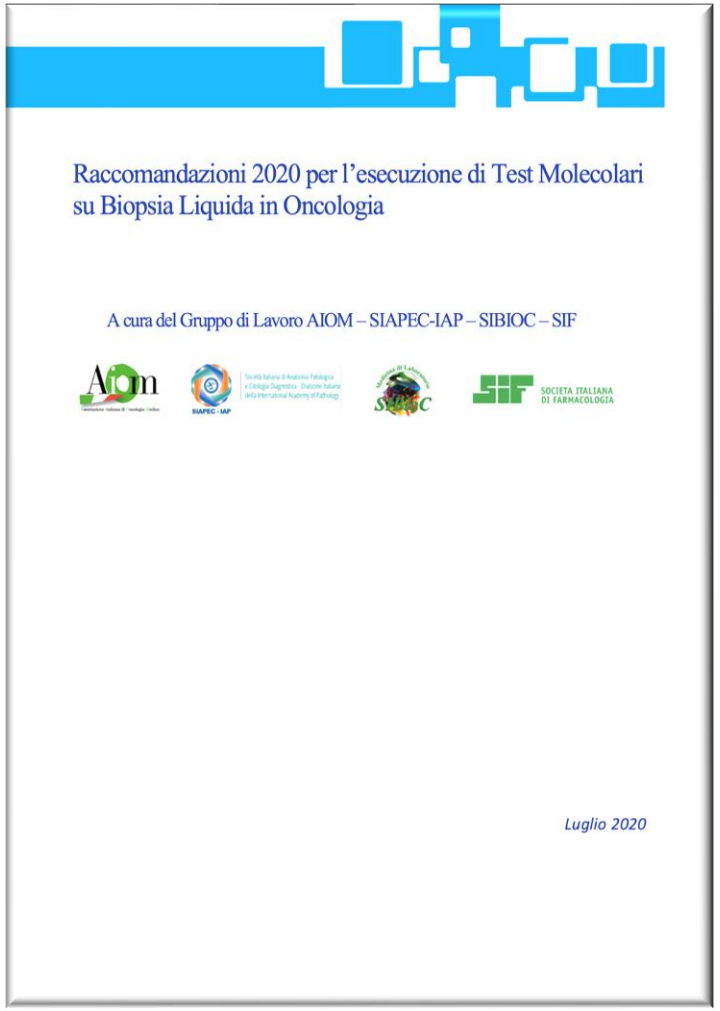


Mod from Wan et al. Nat Rev Cancer 2017

NSCLC

LB: Recommendations AIOM/SIAPEC/SIBIOC-SIF

Current clinical indications

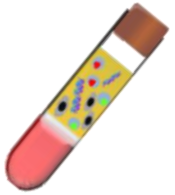


Advanced NSCLC: 2 clinical scenarios

I clinical scenario

Liquid biopsy

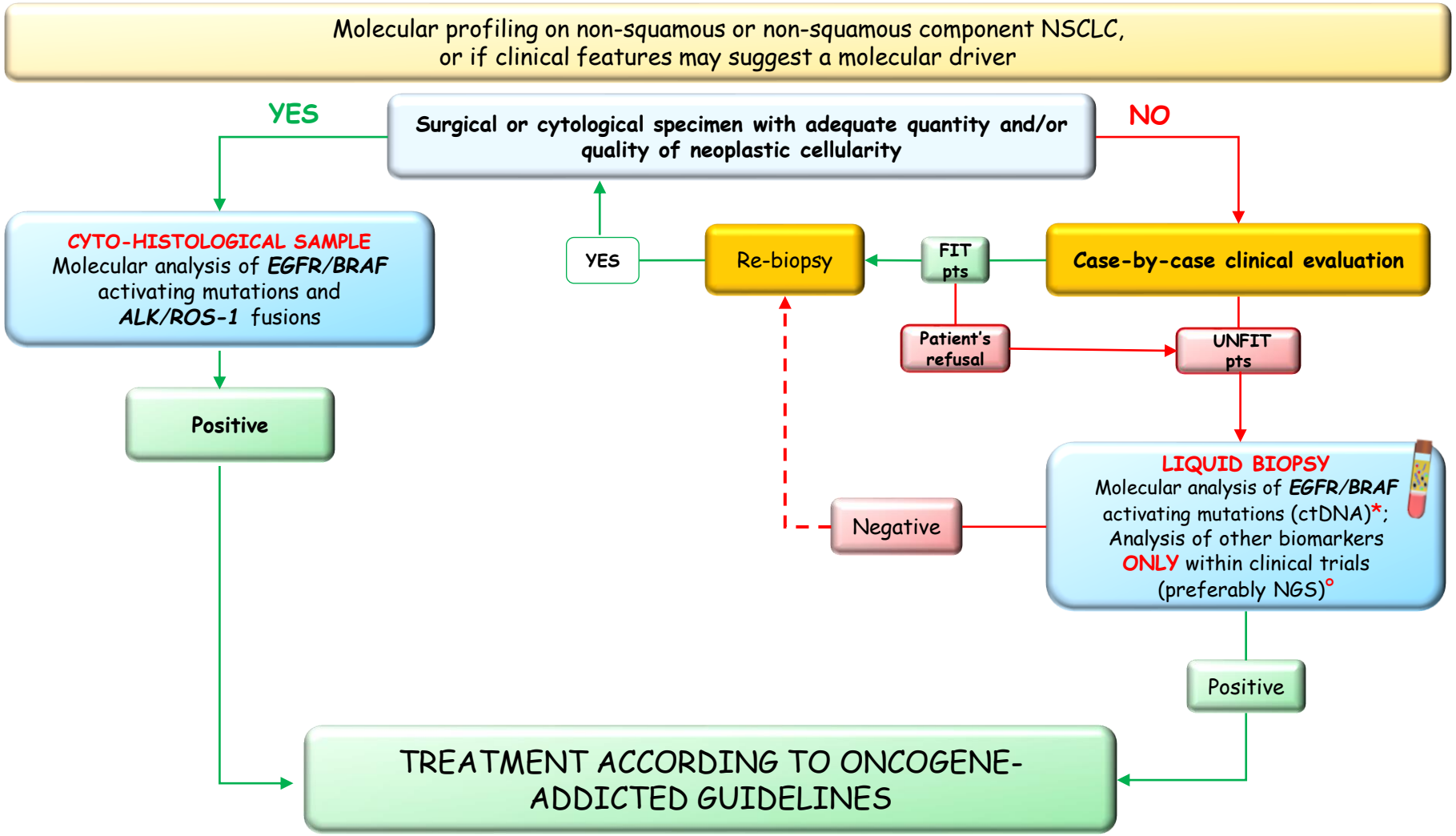
Indications



NSCLC

Liquid Biopsy: AIOM-SIAPEC-IAP-SIBIOC-SIF 2020

Pts with advanced treatment naïve NSCLC (Stage IIIB-C/IV)

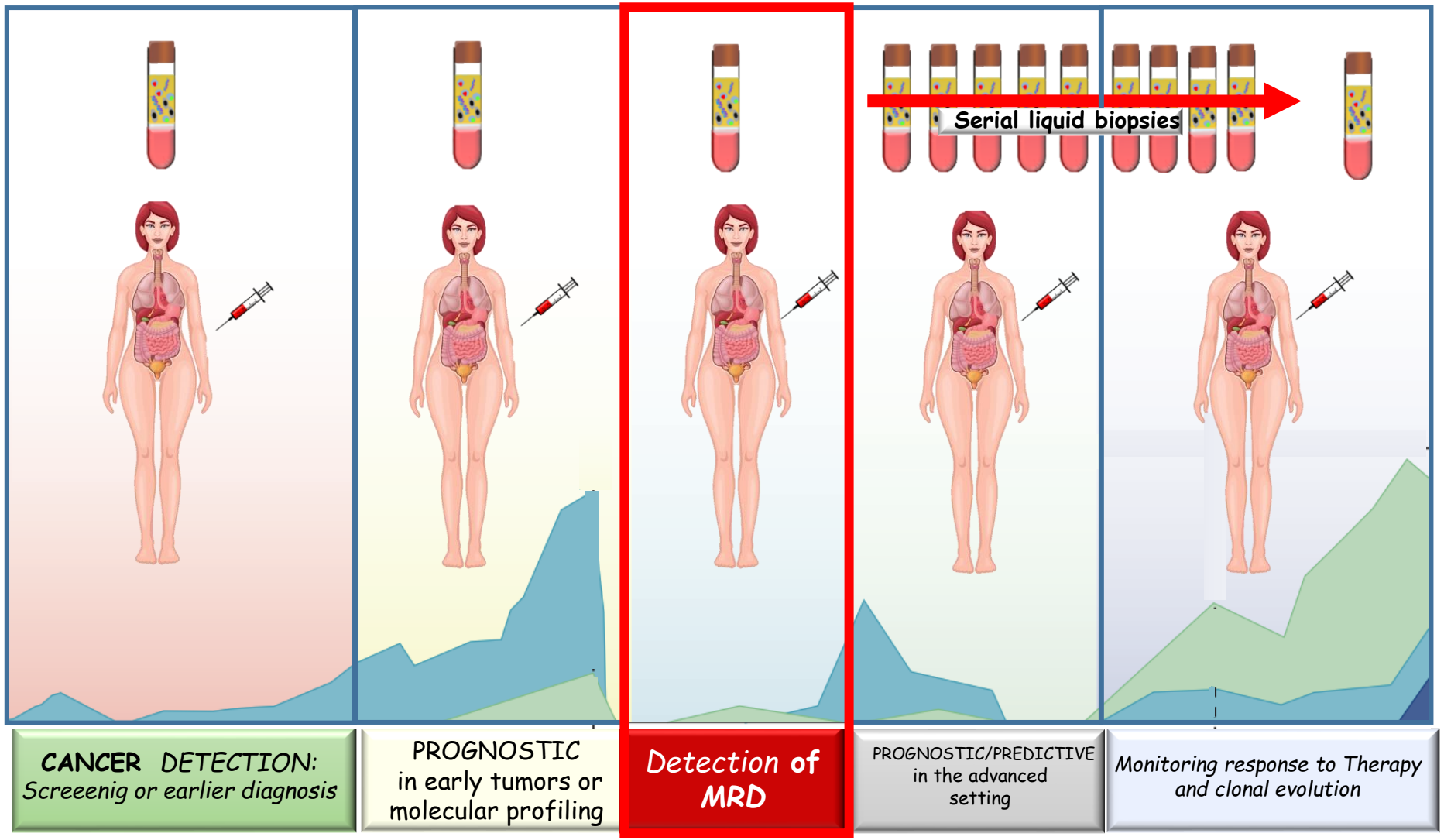


* *EGFR* exon 18, 20, 21 point mutations and exon 19 deletions; *BRAF* p.V600 activating mutations

° *ALK*, *ROS-1*, *RET*, *NTRK* fusions; *MET* exon 14 skipping mutation and *MET* amplification, *HER2* amplification, *KRAS* p.G12C point mutation

Liquid Biopsy: applications of ctDNA in solid tumors

Detection of MRD



Mod from Wan et al. Nat Rev Cancer 2017

DYNAMIC Study

Adjuvant Chemotherapy Guided by ctDNA Analysis in Stage II

DYNAMIC Study design

Multi-centre, randomised, phase II non-inferiority trial (ACTRN12615000381583)

Stage II Colon cancer

- **R0 resection**
- ECOG 0-2
- Staging CT within 8 weeks
- Provision of adequate tumor tissue within 4 weeks post-op
- No synchronus CRC;

Plasma Collections
Week 4 + 7 post-op

N=455

R
2:1

ctDNA-Guided management

- ctDNA-Positive → Adjuvant Chemo (oxaliplatin-based or single agent FP);
- ctDNA-Negative → Observation

ctDNA-Positive = Positive result at week 4 and/or 7

Standard management

Adjunvant treatment decision based on clinico-pathologic criteria

Enpoints

Primary
- RFS rate at 2-yrs

Key Secondary
- Proportion receiving adjuvant chemo

Secondary
- RFS by ctDNA status for ctDNA-guided arm
- TTR
- OS

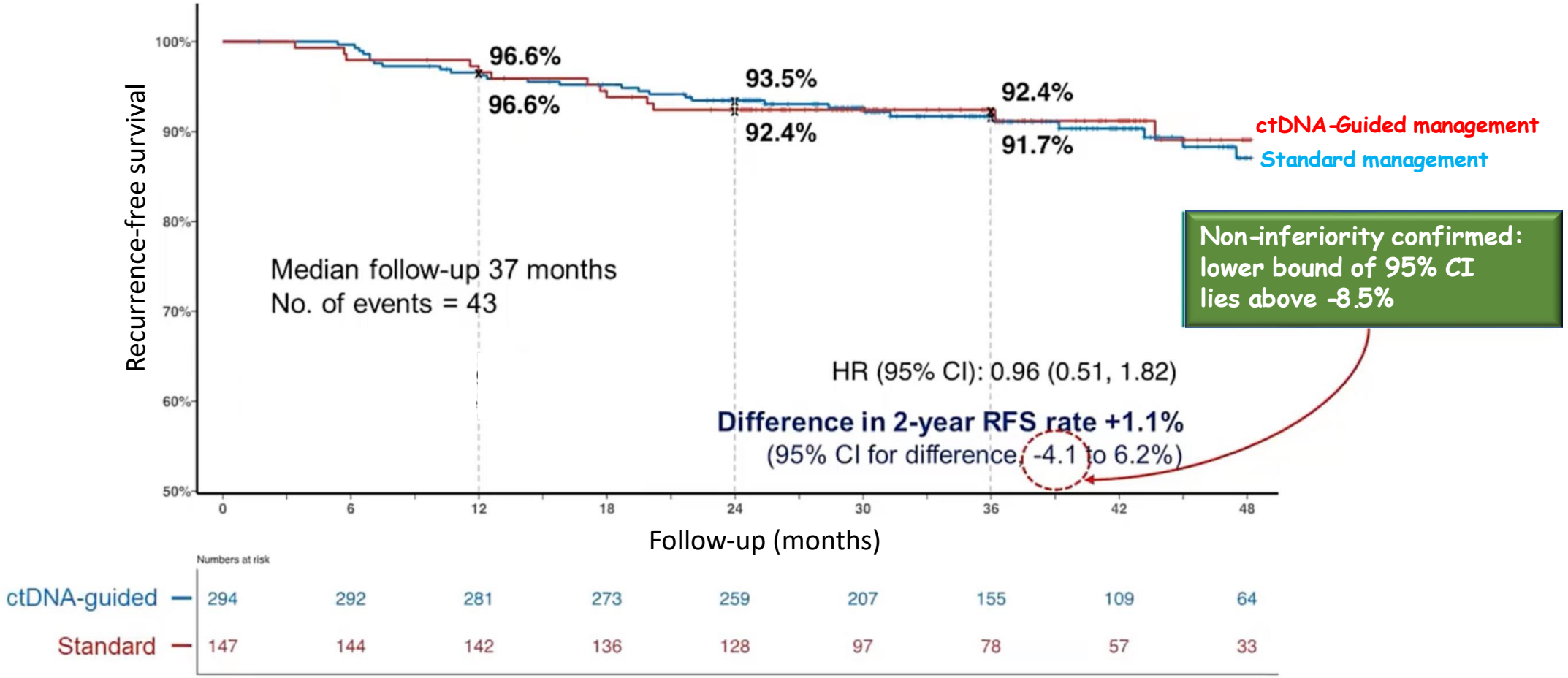
Stratification Factors
- T stage (T3 vs T4)
- Type of participating center (metropolitan vs regional)

Surveillance:
- CEA → 3-monthly for 24M, then 6-monthly for 36M
- CT C/A/P → 6-monthly for 24M, then at 36M

DYNAMIC Study: RESULTS

Post-op ctDNA-Positive: End-of-Treatment ctDNA and RFS

Recurrence-free survival



DYNAMIC Study: RESULTS

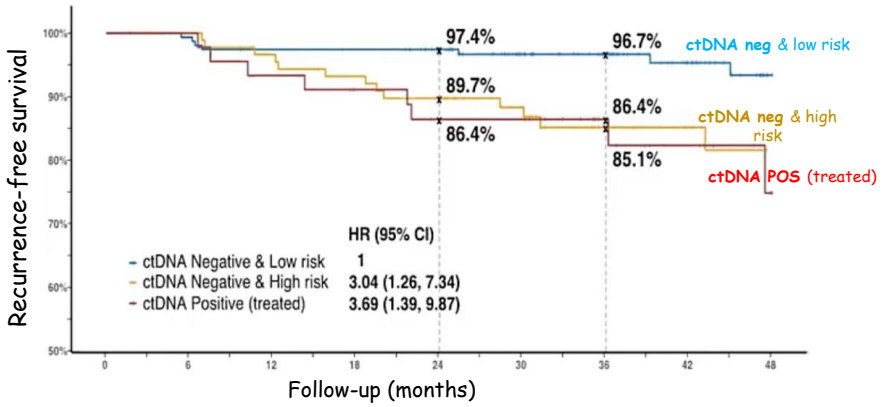
Post-op ctDNA-Positive: End-of-Treatment ctDNA and RFS

Adjuvant treatment delivery

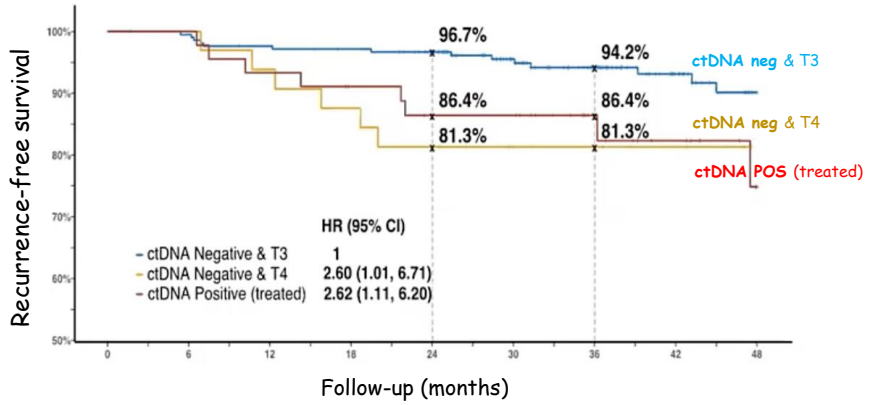
Treatment information	ctDNA-Guided N = 294	Standard management N = 147	P-value
Adjuvant Chemo received, n	45 (15%)	41 (28%)	0.0017
Chemo regimen received, n Oxaliplatin-base doublet Single agent fluoropyrimidine	28/45 (62%) 17/45 (38%)	4/41 (10%) 37/41 (90%)	< 0.001
Time from surgery to commencing Chemo, median (IQR), days	83 (76, 89)	53 (49, 61)	< 0.001
Completed planned treatment, n	38 (85%)	38 (85%)	NS
Percentage of full dose delivered Median (IQR)	78 (56, 100)	84 (64, 100)	NS

RFS: ctDNA, clinical Risk & T Stage

ctDNA and clinical Risk



ctDNA and T Stage



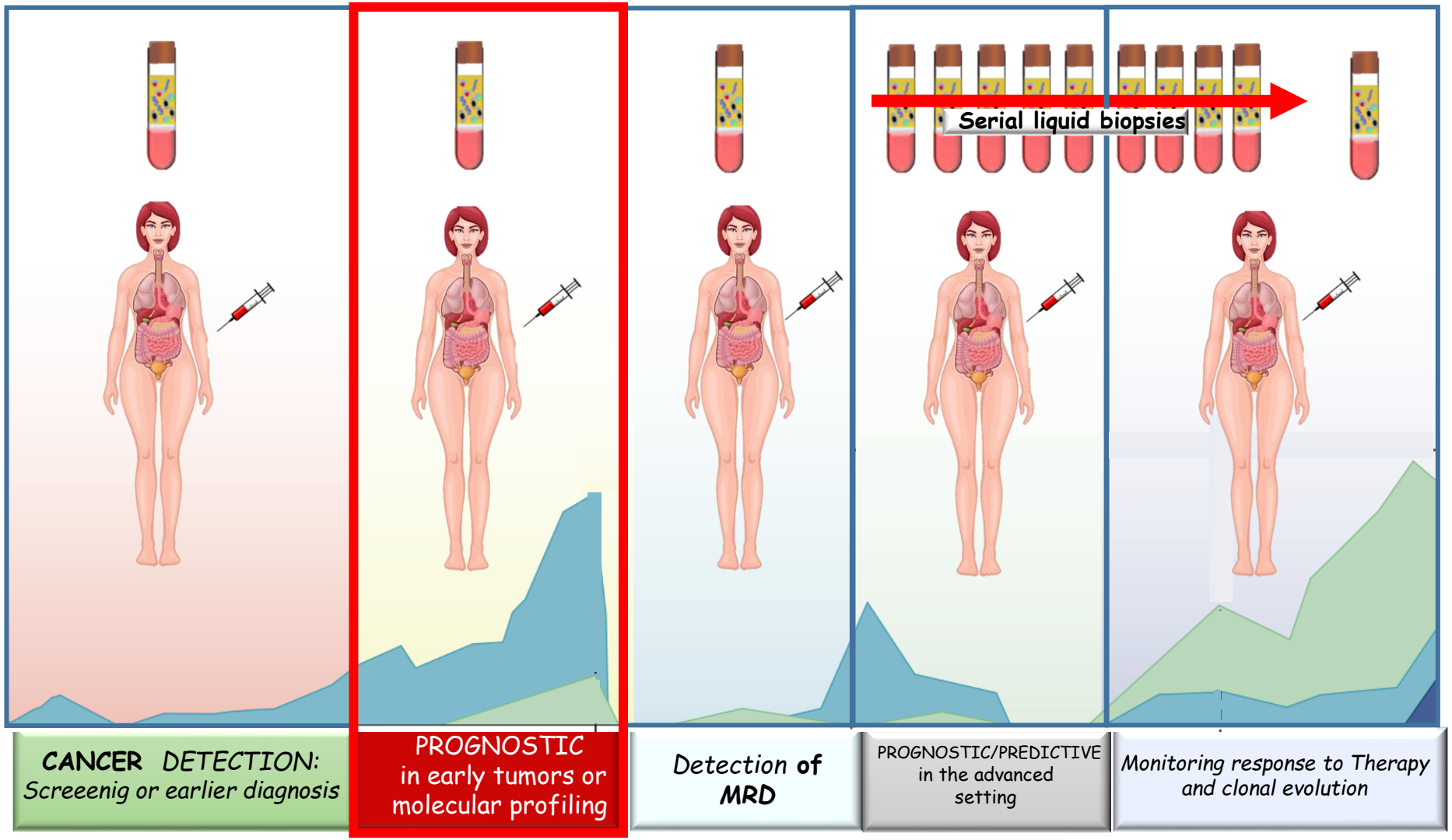
Liquid biopsy

Prospective Studies Ongoing to Evaluate Actionability of ctDNA in Early Stage Colon Canc

- VEGA (ctDNA negative) and ALTAIR (ctDNA pos) trials of CIRCULATE-JAPAN;
- NRG GI-005 (COBRA) – stage IIA colon cancer active surveillance versus assay directed therapy;
- CIRCULATE US – stage II (ctDNA+) or stage III → de-escalate in ctDNA- (CAPOX-FOLFOX *v* obs); escalate in ctDNA+ (CAPOX/FOLFOX *v* FOLFOXIRI);
- SU2C ACT3 trial – stage III ctDNA+ (FOLFIRI *v* obs); biomarker-directed exploratory cohorts.

Liquid Biopsy: Application of ctDNA in solid tumors

PROGNOSTIC in early tumors or molecular profiling

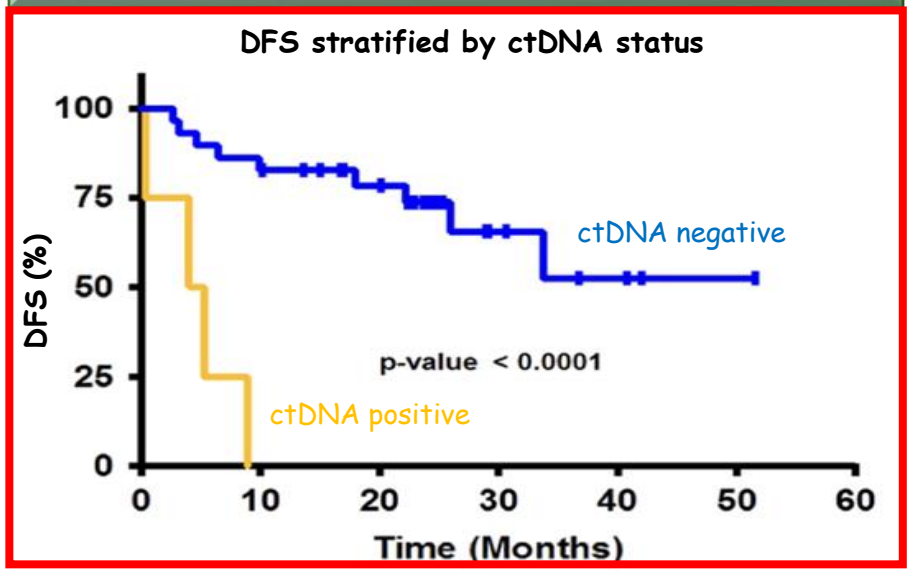


Mod from Wan et al. Nat Rev Cancer 2017

Liquid Biopsy

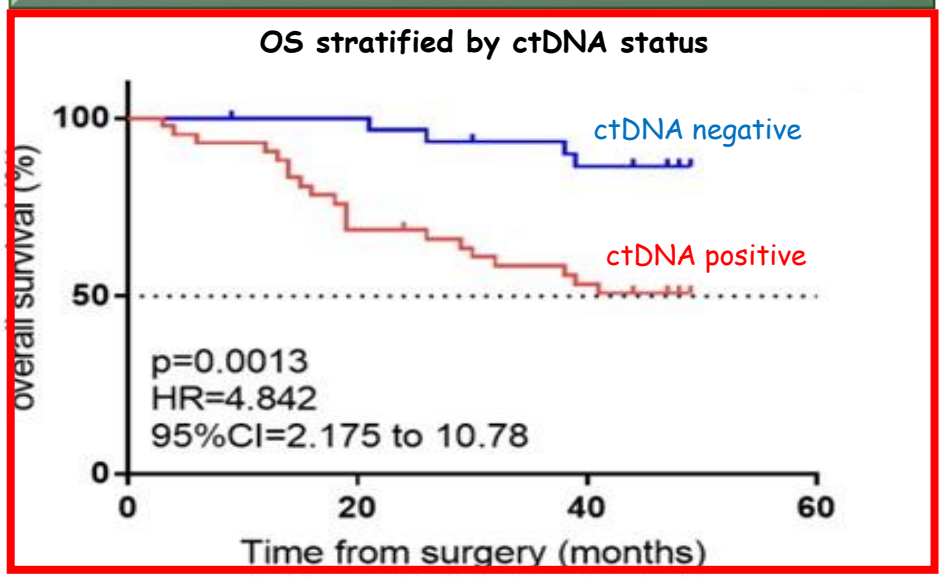
PROGNOSTIC in early tumors or molecular profiling

BC



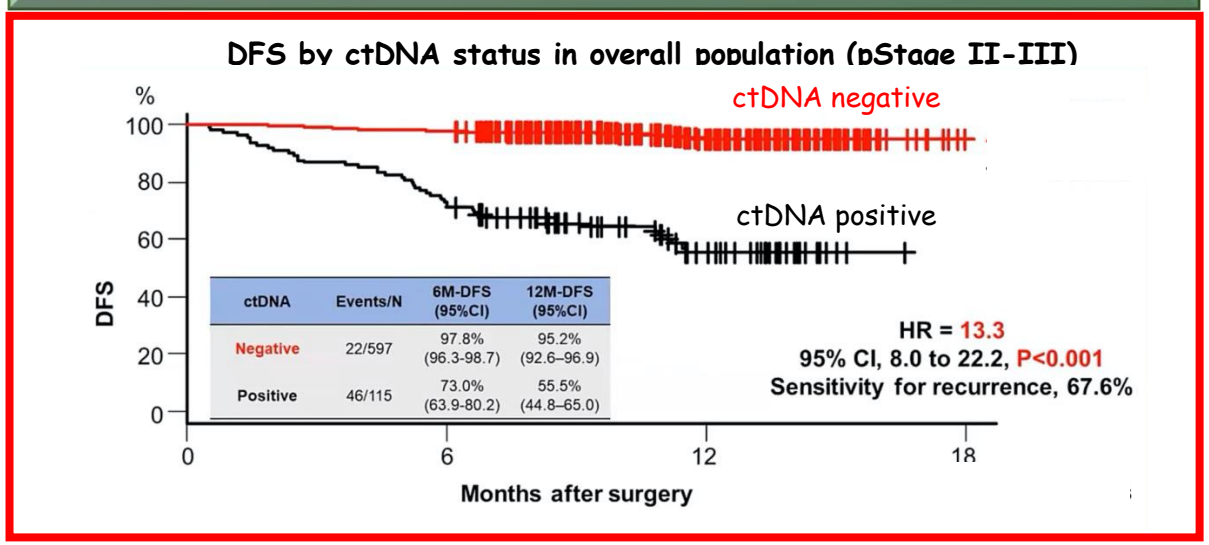
Su-Jin Shin, Plos One 2017

NSCLC



Wang B. et al, CROH 2022

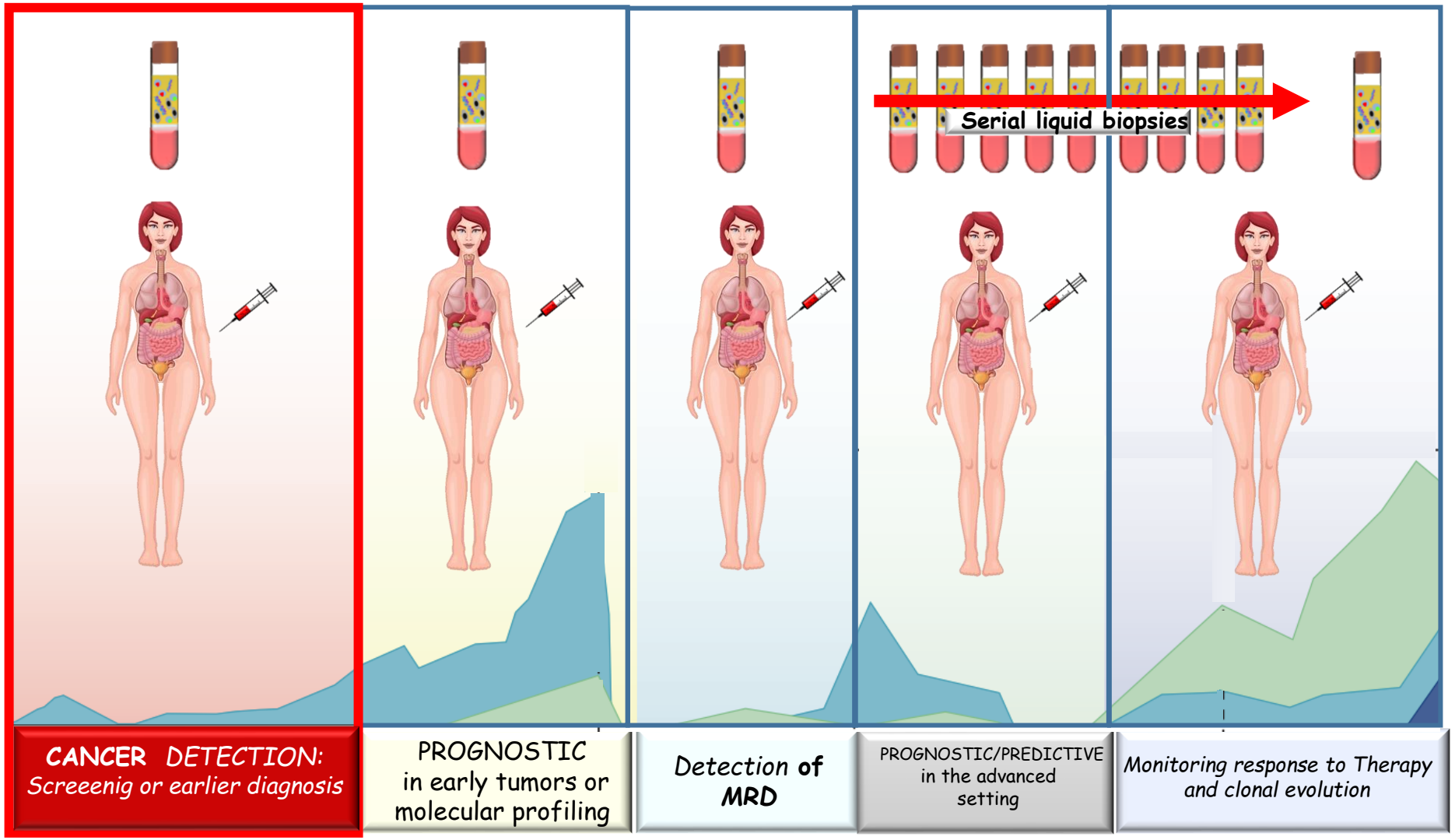
CRC



Masahito Kotaca Et al *ASCO GI*, 2022

Liquid Biopsy: Applications of ctDNA in solid tumors

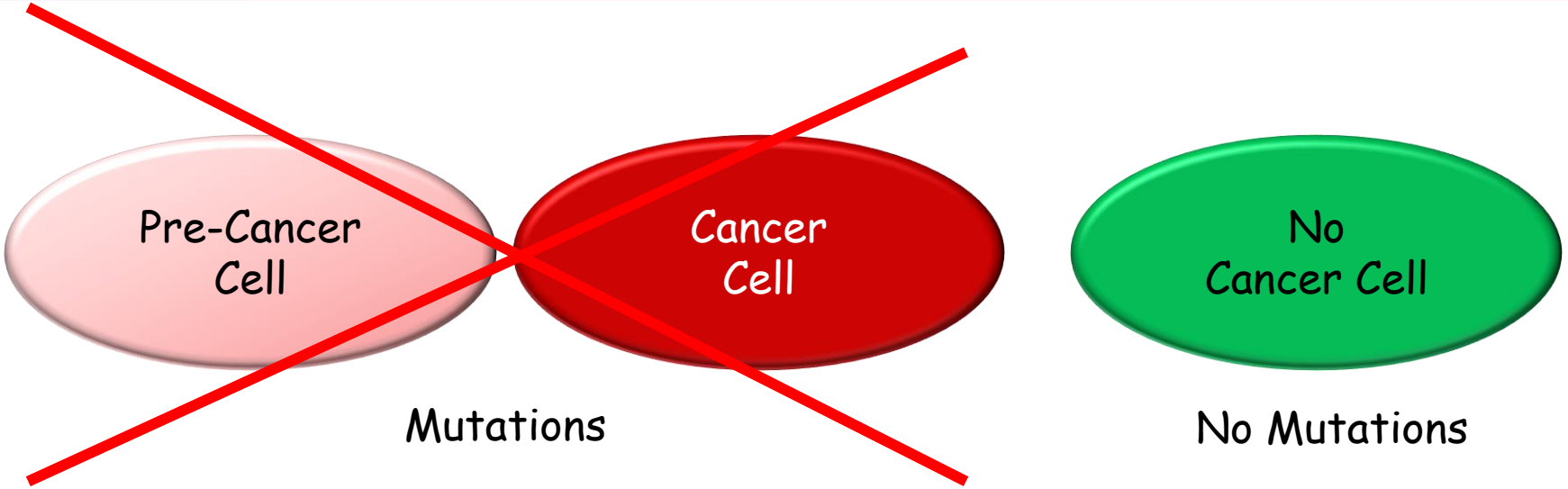
CANCER DETECTION: Screeenig or earlier diagnosis



Mod from Wan et al. Nat Rev Cancer 2017

Liquid biopsy: Screeenig or earlier diagnosis

Risk Not all somatic mutations are cancer…



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Cancer-Associated Mutations in Endometriosis without Cancer

M.S. Anglesio, N. Papadopoulos, A. Ayhan, T.M. Nazeran, M. Noë, H.M. Horlings, A. Lum, S. Jones, J. Senz, T. Seckin, J. Ho, R.-C. Wu, V. Lac, H. Ogawa, B. Tessier-Cloutier, R. Alhassan, A. Wang, Y. Wang, J.D. Cohen, F. Wong, A. Hasanovic, N. Orr, M. Zhang, M. Popoli, W. McMahon, L.D. Wood, A. Mattox, C. Allaire, J. Segars, C. Williams, C. Tomasetti, N. Boyd, K.W. Kinzler, C.B. Gilks, L. Diaz, T.-L. Wang, B. Vogelstein, P.J. Yong, D.G. Huntsman, and I.-M. Shih

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence

Giulio Genovese, Ph.D., Anna K. Kähler, Ph.D., Robert E. Handsaker, B.S., Johan Lindberg, Ph.D., Samuel A. Rose, B.S., Samuel F. Bakhoum, M.D., Ph.D., Kimberly Chambert, M.S., Eran Mick, B.S., Benjamin M. Neale, Ph.D., Menachem Fromer, Ph.D., Shaun M. Purcell, Ph.D., Oscar Svantesson, M.S., Mikael Landén, Ph.D., Martin Höglund, M.D., Ph.D., Sören Lehmann, M.D., Ph.D., Stacey B. Gabriel, Ph.D., Jennifer L. Moran, Ph.D., Eric S. Lander, Ph.D., Patrick F. Sullivan, M.D., Pamela Sklar, M.D., Ph.D., Henrik Grönberg, M.D., Ph.D., Christina M. Hultman, Ph.D., and Steven A. McCarroll, Ph.D.

The NEW ENGLAND JOURNAL of MEDICINE

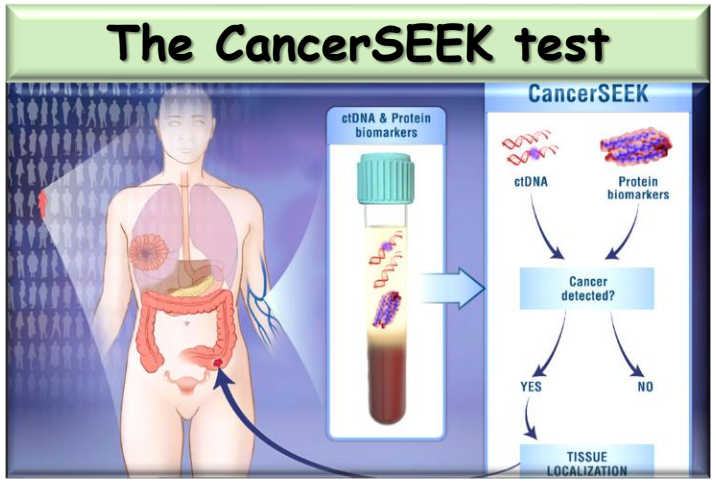
ORIGINAL ARTICLE

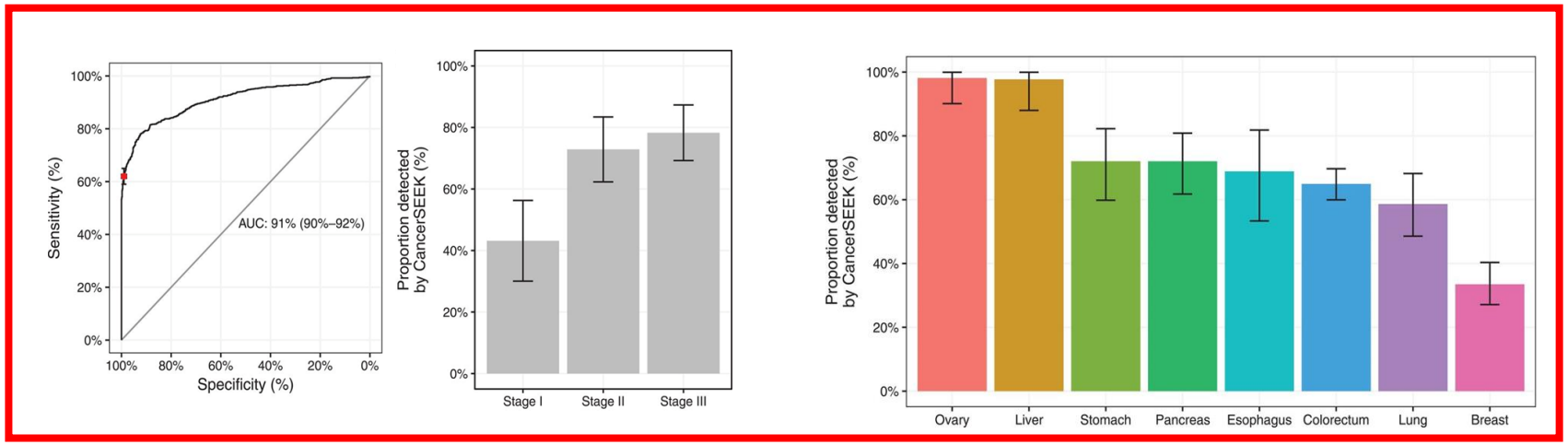
Aneurysm Syndromes Caused by Mutations in the TGF-β Receptor

Bart L. Loeys, M.D., Ph.D., Ulrike Schwarze, M.D., Tammy Holm, M.D., Bert L. Callewaert, M.D., George H. Thomas, Ph.D., Hariyadarshi Pannu, Ph.D., Julie F. De Backer, M.D., Gretchen L. Oswald, M.S., Sofie Symoens, B.S., Sylvie Manouvrier, M.D., Ph.D., Amy E. Roberts, M.D., Francesca Faravelli, M.D., M. Alba Greco, M.D., Reed E. Pyeritz, M.D., Ph.D., Dianna M. Milewicz, M.D., Ph.D., Paul J. Coucke, Ph.D., Duke E. Cameron, M.D., Alan C. Braverman, M.D., Peter H. Byers, M.D., Anne M. De Paepe, M.D., Ph.D., and Harry C. Dietz, M.D.

Liquid biopsy: cancer screening and early detection

The CancerSEEK test





The CancerSEEK test

Study test: it can detect 8 common cancer types

Methods: assessment of ***circulating proteins*** and ***mutations in cfDNA***

Materials: 1005 pts with nonmetastatic cancers (ovary, liver, stomach, pancreas, esophagus, colorectum, lung, or breast) and 812 healthy controls

SOME CONSIDERATIONS AND LIMITS

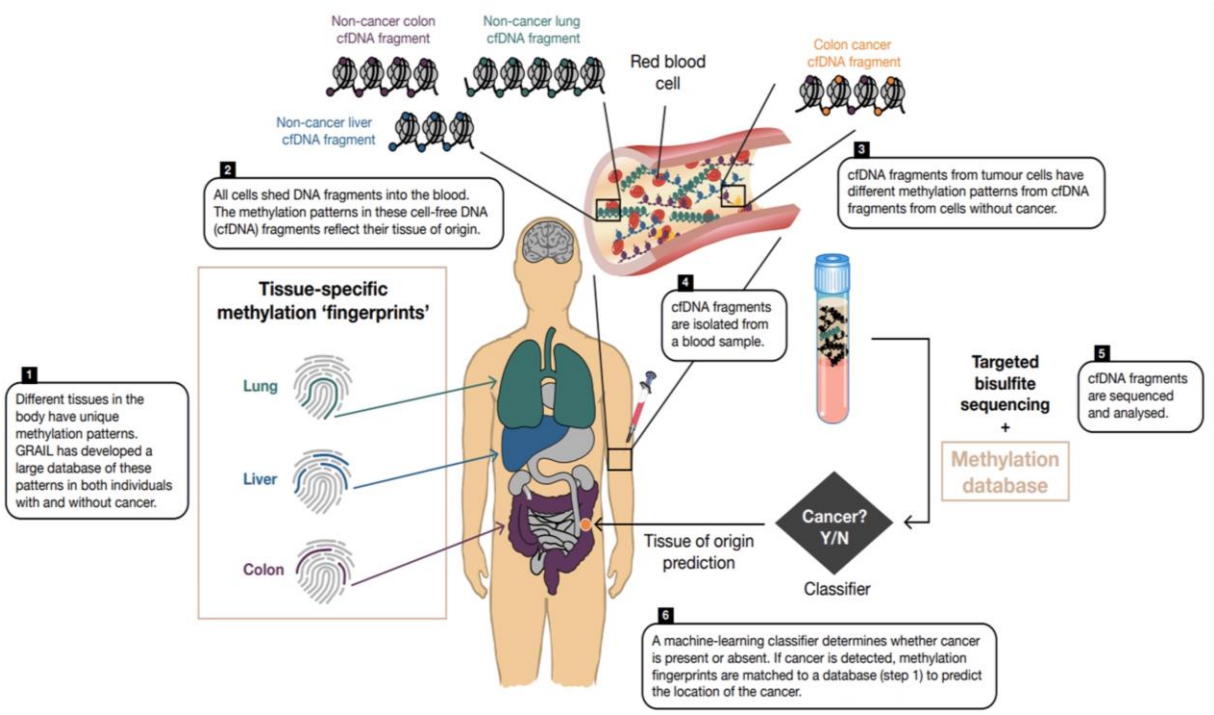
- Median performance: 70% (ranging from 98% in OCs to 33% in BCss)
- **Specificity: > 90%**
- **Proportion of detected cancer**: higher in more advanced stage (stage III)
- **Technique: currently useful only on pts diagnosed with cancer**
- **The proteins used are not cancer-specific (arthritis)**

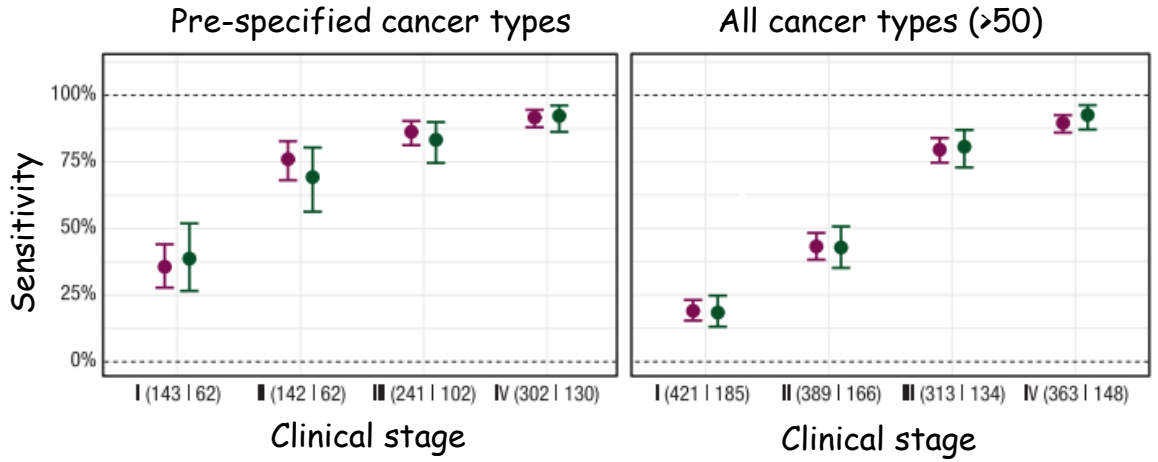
Cohen JD et al, Science 2018

Liquid biopsy: cancer screening and early detection

Multi-cancer detection using cfDNA-methylation signatures

- Prospective, case-control, sub-study to assess the performance of **cfDNA-targeted methylation** analysis to detect and localize multiple cancer types
- N= 6689; 2482 cancer and 4207 non-cancer
- **Sensitivity in all cancer types**: 18% in stage I, 43% in stage II; 81% in stage III and 93% in stage IV





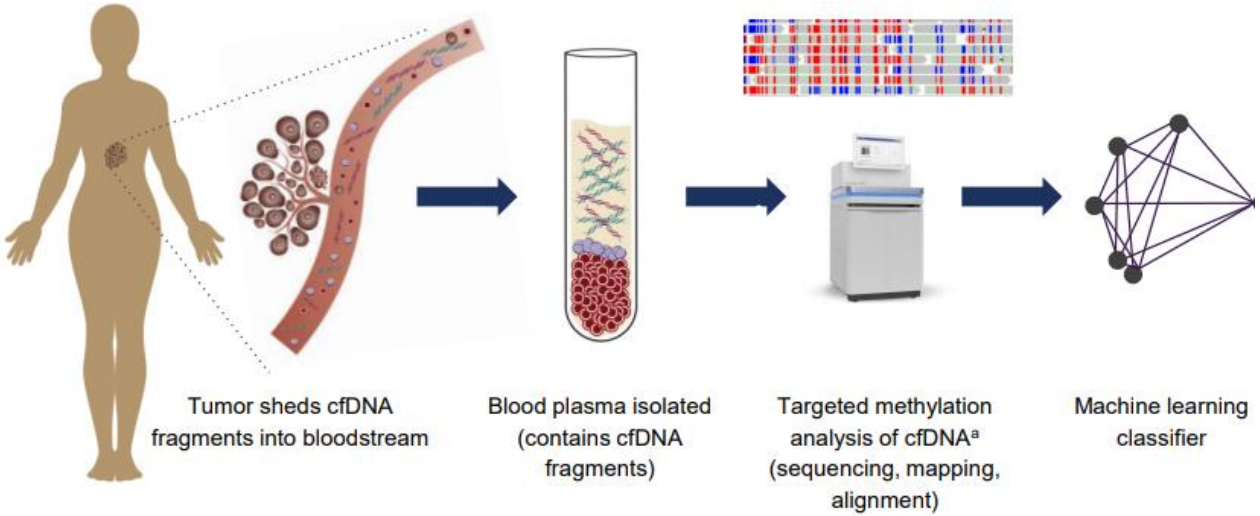
MC Liu, GR Oxnard et al Annals of Oncology 2020

Liquid biopsy: cancer screening and early detection

PATHFINDER: Multi-Cancer Early Detection (MCED)

PARIS 2022 ESMO congress

- Prospective, cohort study to assess MCED using targeted methylation NGS
- **N= 6621 asymptomatic pts**; 3681 with additional risk and 2940 without additional risk
- **Cancer signal origin detected in 1.4%, Specificity 99.1%, PPV 38.0%, NPV 98.6%**

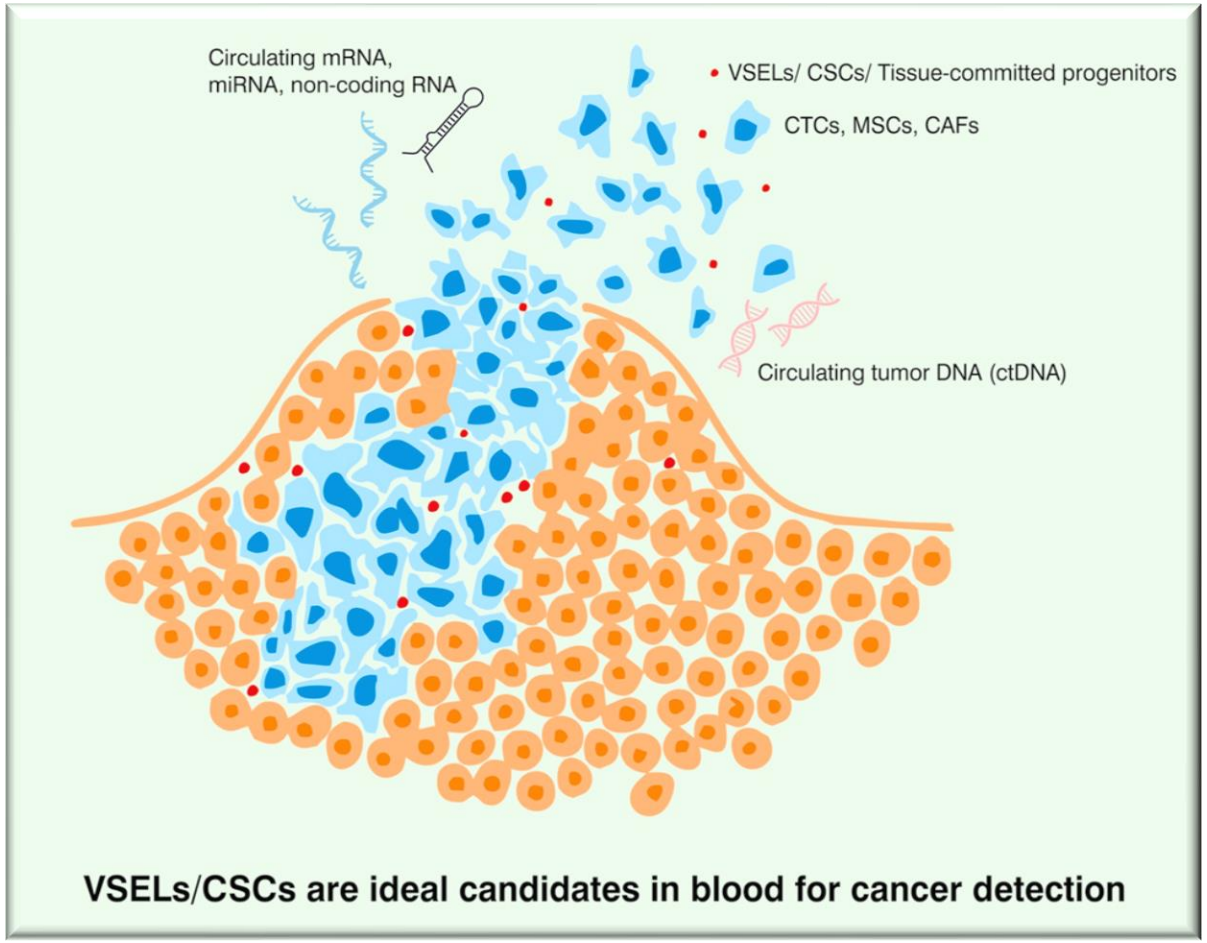


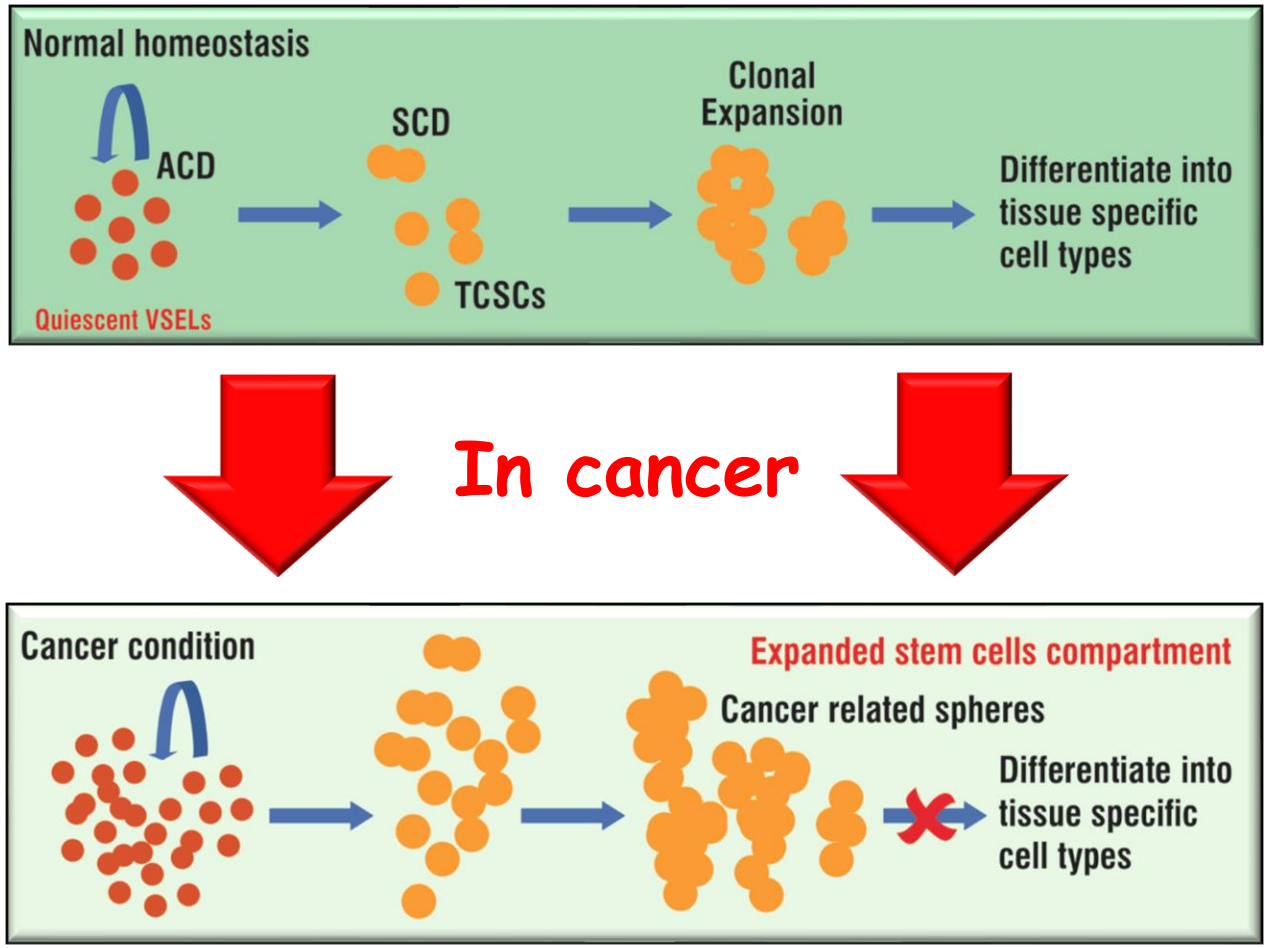
Results: Multi-Cancer Early Detection (MCED)			
	TP (n)	**FP (n)**	**Total (N)**
	35	57	92
Extent of Diagnostic Testing (Primary)	33	57	90
>1 Imaging Test (%)	90.9	93.0	92.2
>1 Invasive Procedure (%)	81.8	29.8	48.9
Time to Resolution [median days (IQR)]	57 (33, 143)	162 (44, 248)	79 (37, 219)
Test Performance (Secondary)	**n/N**	**% (95% CI)**	
PPV	35/92	38.0 (28.8, 48.3)	
NPV	6235/6321	98.6 (98.3, 98.9)	
CSO Prediction Accuracy	33/34	97.1 (85.1, 99.8)	

Schrag, D et al ESMO annual congress 2022

Liquid biopsy: cancer screening and early detection

Very Small Embryonic-Like Stem Cells: Novel Candidates for Detecting/Monitoring Cancer by LB

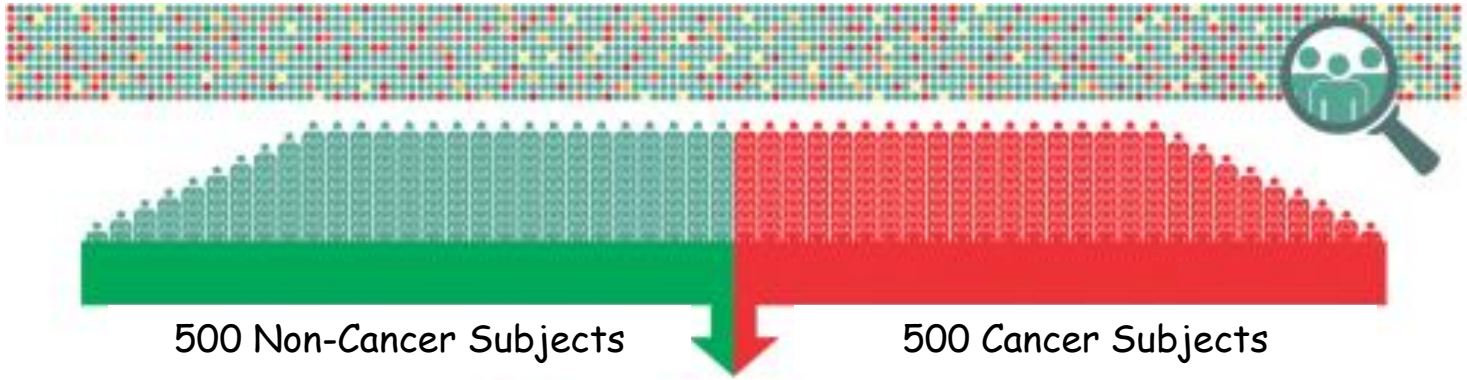




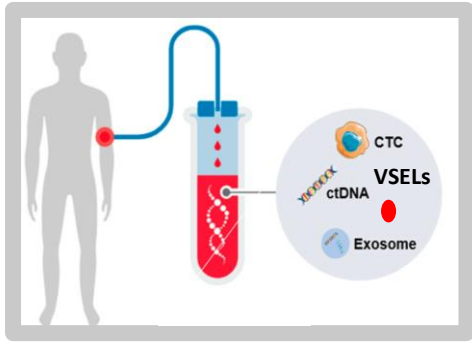
Bhartiya et al., Stem Cells (2023)

Liquid biopsy: cancer screening and early detection

Very Small Embryonic-Like Stem Cells: Novel Candidates for Detecting/Monitoring Cancer by LB



Non-Cancer	0-2
Organ Inflammation	2-6
High Risk	6-10
Stage I Cancer	10-20
Stage II Cancer	20-30
Stage III Cancer	30-40
Stage IV Cancer	40-50



What question could diagnostic technique answer?

- Is cancer present? Is cancer imminent?
- What treatments might work?
- Is there cancer left after the treatment?
- Which type of cancer is developing? And which organ is at risk?

Tripathi et al., Stem Cell Reviews and Reports (2021)

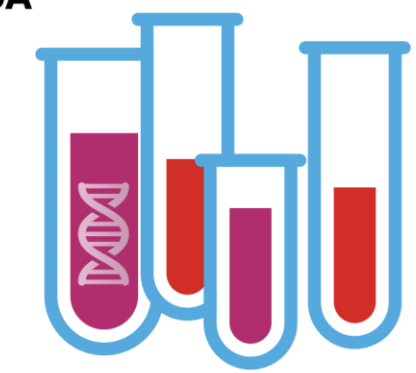
Raccomandazioni 2020 per l'esecuzione di Test Molecolari su Biopsia Liquida in Oncologia

A cura del Gruppo di Lavoro AIOM – SIAPEC-IAP – SIBIOC – SIF

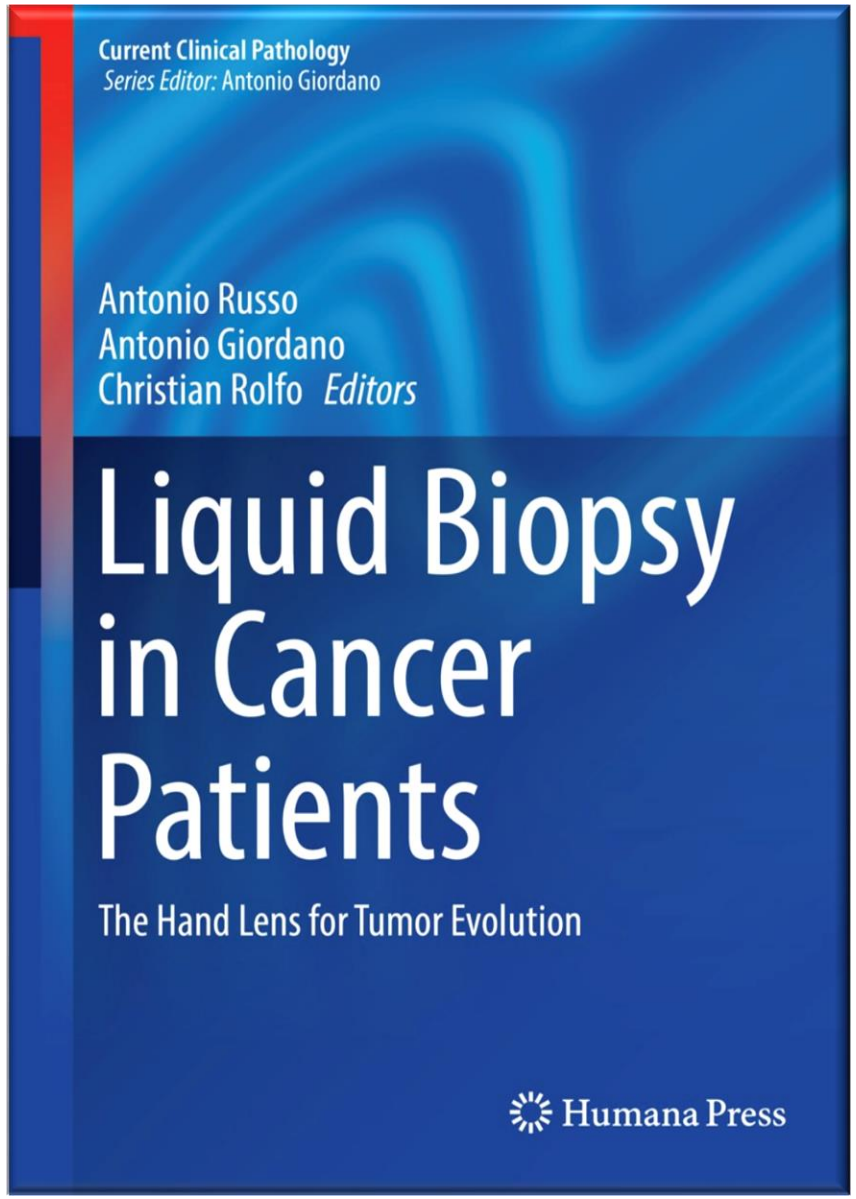
Giordano Beretta, Ettore Capoluongo, Romano Danesi, Marzia Del Re, Matteo Fassan, Giuseppe Giuffrè, Stefania Gori, Valerio Gristina, Lorena Incorvaia, Umberto Malapelle, Antonio Marchetti, Nicola Normanno, Carmine Pinto, Giulio Rossi, Andrea Sartore Bianchi, Nicola Silvestris, Pierosandro Tagliaferri, Giancarlo Troncone e Antonio Russo.

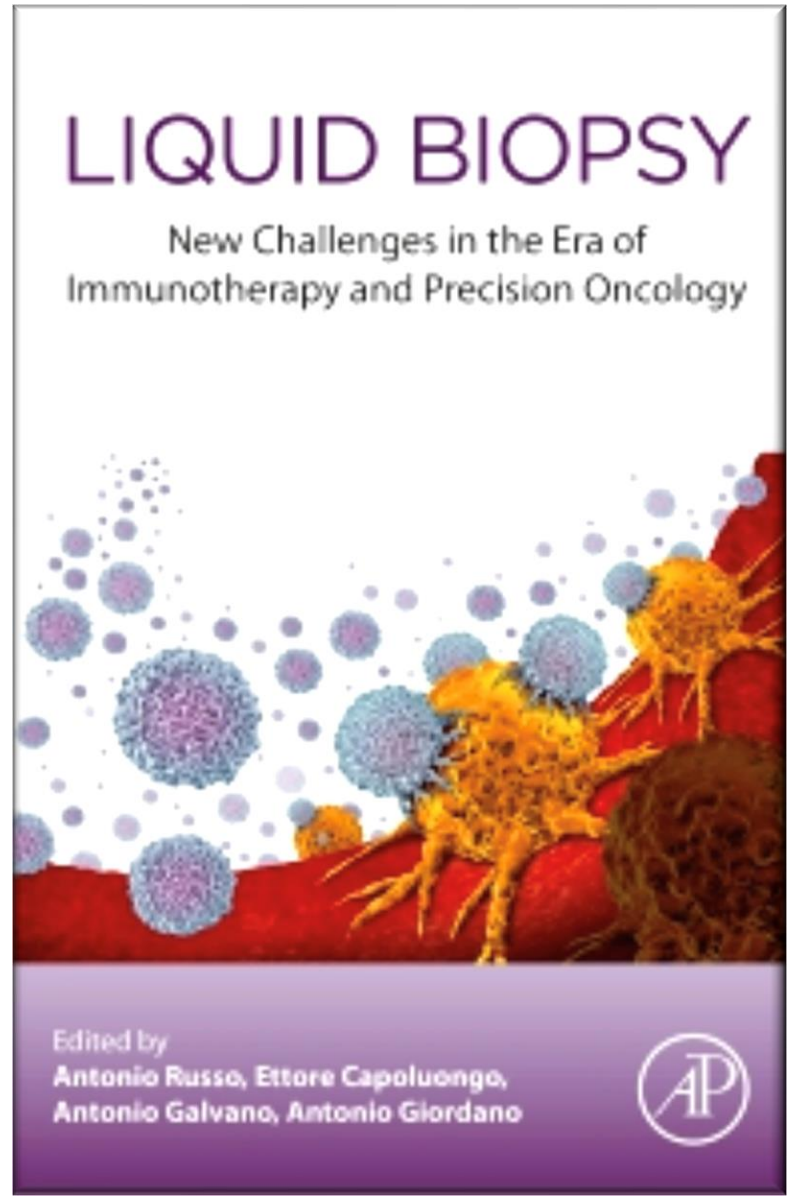
Luglio 2020

RACCOMANDAZIONI PER L'ESECUZIONE DI TEST MOLECOLARI SU **BIOPSIA LIQUIDA** IN ONCOLOGIA



LUGLIO 2018


Current Clinical Pathology
Series Editor: Antonio Giordano
Antonio Russo
Antonio Giordano
Christian Rolfo Editors
Liquid Biopsy in Cancer Patients
The Hand Lens for Tumor Evolution
Humana Press


LIQUID BIOPSY
New Challenges in the Era of Immunotherapy and Precision Oncology
Edited by
Antonio Russo, Ettore Capoluongo,
Antonio Galvano, Antonio Giordano
AP

Liquid biopsy: Future Perspectives

Potential detection and monitoring of IO biomarkers

Therapeutic Advances in Medical Oncology 2019

Original Research

Monitoring blood biomarkers to predict nivolumab effectiveness in NSCLC patients

Francesco Passiglia, Antonio Galvano [...], Viviana Bazan and Antonio Russo

Ther Adv Med Oncol
2019, Vol. 11, 1–11
DOI: 10.1177/1758835919839928

ONCOIMMUNOLOGY 2019

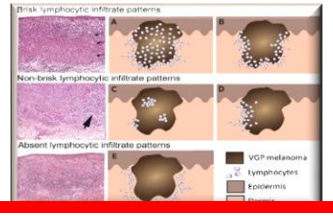
Taylor & Francis
Taylor & Francis Group

ORIGINAL RESEARCH

OPEN ACCESS Check for updates

Prognostic significance of circulating PD1, PD-L1, pan-BTN3As, BTN3A1 and BTLA in patients with pancreatic adenocarcinoma

Benjamin Bian, Daniele Fanale [...], Antonio Russo and Juan Iovanna



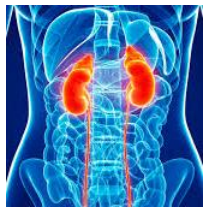
Therapeutic Advances in Medical Oncology 2019

Original Research

Can the plasma PD-1 levels predict the presence and efficiency of tumor-infiltrating lymphocytes in patients with metastatic melanoma?

Lorena Incorvaia, Giuseppe Badalamenti[...], Daniele Fanale and Antonio Russo

Ther Adv Med Oncol
2019, Vol. 11, 1–11
DOI: 10.1177/1758835919839928



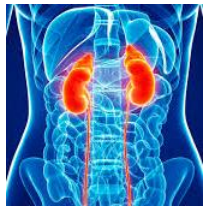
ORIGINAL RESEARCH

Oncoimmunology 2020

OPEN ACCESS Check for updates

Baseline plasma levels of soluble PD-1, PD-L1, and BTN3A1 predict response to nivolumab treatment in patients with metastatic renal cell carcinoma: a step toward a biomarker for therapeutic decisions

Lorena Incorvaia[a†], Daniele Fanale[b†], Giuseppe Badalamenti[b†], Camillo Porta[c], Daniel Olive[d], Ida De Luca[b], Chiara Brando[b], Mimma Rizzo[e], Carlo Messina[f], Mattia Rediti[g], Antonio Russo[b*], Viviana Bazan[a*],



cancers

MDPI

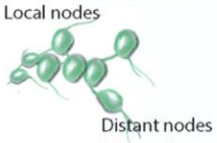
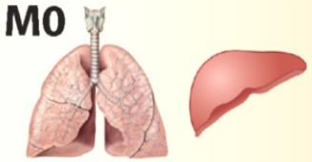
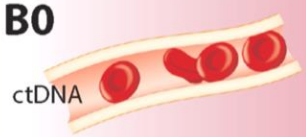
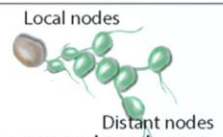
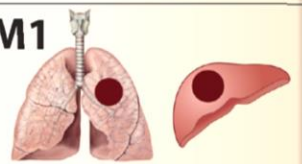
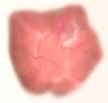
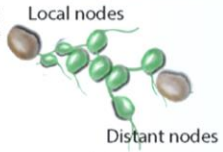
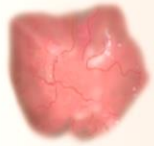
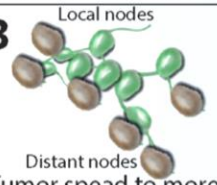
Article

A "Lymphocyte MicroRNA Signature" as Predictive Biomarker of Immunotherapy Response and Plasma PD-1/PD-L1 Expression Levels in Patients with Metastatic Renal Cell Carcinoma: Pointing towards Epigenetic Reprogramming

Lorena Incorvaia [1,†], Daniele Fanale [2,†], Giuseppe Badalamenti [2,†], Chiara Brando [2], Marco Bono [2], Ida De Luca [2], Laura Algeri [2], Annalisa Bonasera [2], Lidia Rita Corsini [2], Salvatore Scurria [3], Juan Lucio Iovanna [4], Antonio Russo [2,*,‡] and Viviana Bazan [1,‡]

Liquid biopsy

Is this the future?

T Tumor Size	N Lymph Node	M Metastasis	B Blood
T1  Tumor size/local invasion	**N0**  No regional lymph node invasion	**M0**  No distant metastasis	**B0**  No ctDNA mutations in blood
T2  Tumor size/local invasion	**N1**  Tumor spead to closest or small number of regional lymph nodes	**M1**  Distant metastasis	**B1**  ctDNA mutations in blood (can be further defined with more detailed quantification in the future)
T3  Tumor size/local invasion	**N2**  Tumor spead to an extent between N1 and N3		
T4  Tumor of any size that invades to other organs	**N3**  Tumor spead to more distant or regional numerous lymph nodes		

Ann Oncol. 2017;29(2):311-323

UNIVERSITÀ DEGLI STUDI DI PALERMO

"P. GIACCONE" - UOC DI ONCOLOGIA MEDICA - AOUP

RETE TUMORI RARI Sicilia

A.O.U.P. "P. Giaccone" University Hospital

DIPARTIMENTO DI DISCIPLINE CHIRURGICHE, ONCOLOGICHE E STOMATOLOGICHE

MEDICAL ONCOLOGY UNIT

(Dir.: Prof. Antonio Russo)

GRAZIE!!!

antonio.russo@unipa.it