The clinical value of liquid biopsy in Lung Cancer: Now and in the future

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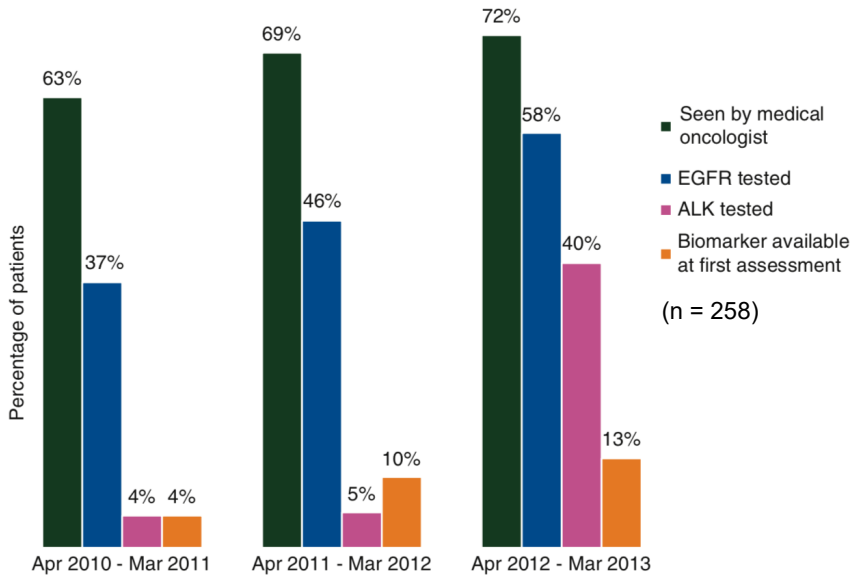
INTERNATIONAL SOCIETY OF LIQUID BIOPSY

Disclosures

Research grants	Lung Cancer Research Foundation-Pfizer Grant 2019 NHI U54 grant (Project co-leader)
Personal financial interests	Speaker: MSD, Astra Zeneca, Roche,
	Advisory board: Inivata, ArcherDx, MD Serono, BMS,Novartis
Non-financial interests	Research Collaboration: GuardantHealth
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).

Biomarker testing and time to treatment decision

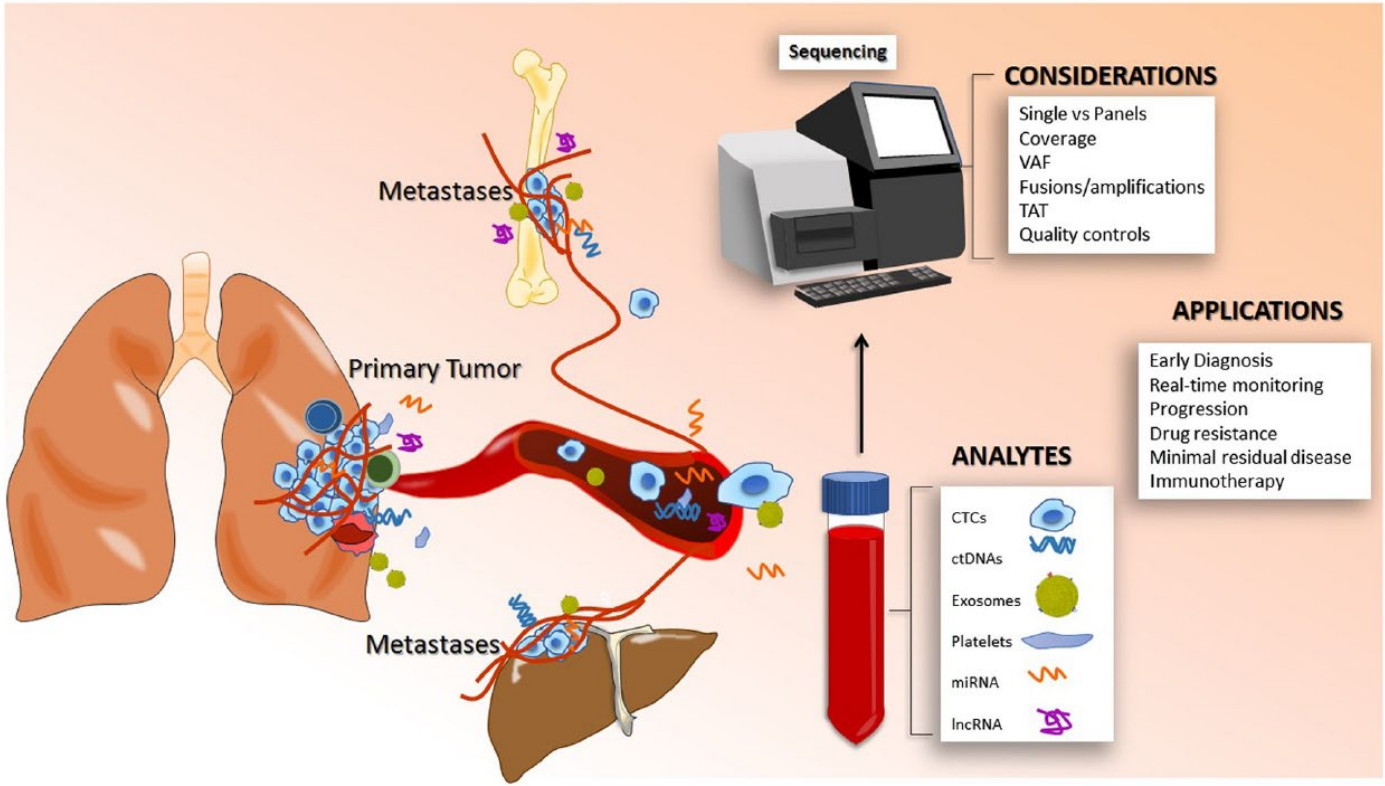


First problem after tissue quantity not sufficient!

Only 21% of patients with biomarker testing had results available at their initial oncology consultation

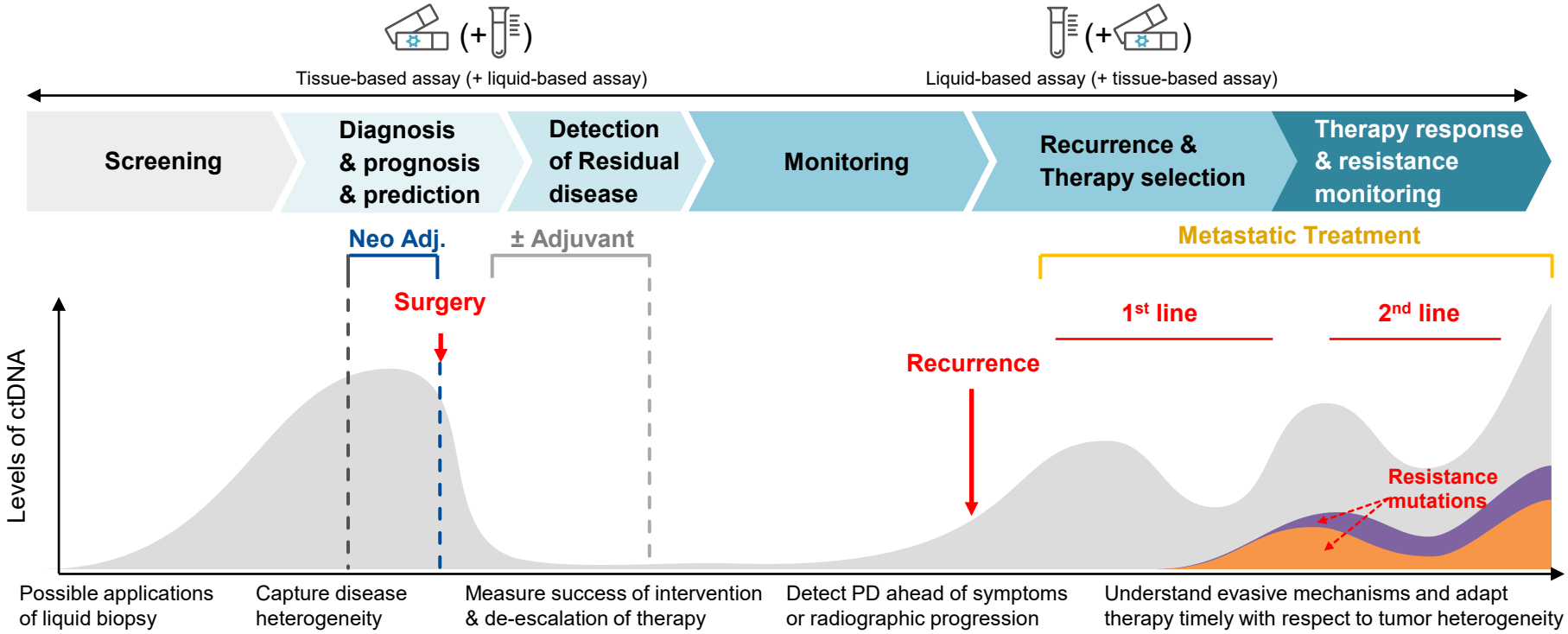
Lim, C., et al (2015) *Ann Oncol* 26:1415–21.

Liquid biopsy components



Scilla, K.A. and Rolfo, C. (2019) *Curr Treat Option On* 20:61.

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



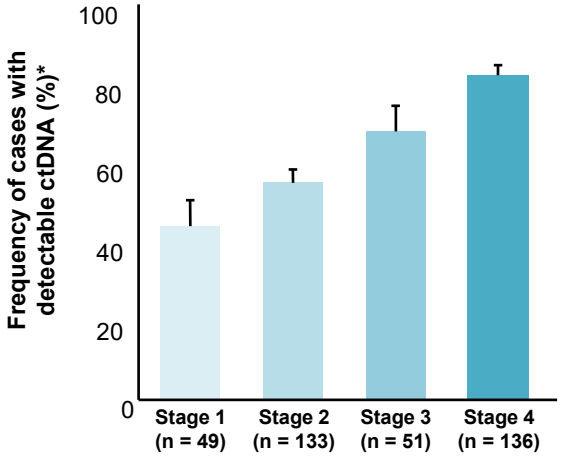
PD: progressive disease.
Adapted from Wan, J.C.M., et al., (2017) *Nat Rev Cancer* 17:223-38.

Analysis of circulating tumour DNA (ctDNA) poses distinct challenges

ctDNA

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

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



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

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

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

Comprehensive genomic profiling by liquid and tissue builds on the strengths of each type of assay

	Blood	Tissue
Strengths	• Less invasive / less morbidity[1,2] • Simpler to obtain / faster results[1,2] • Less biased detection of genomic alterations versus single tissue biopsy site[1,2] • Makes a repeat biopsy more feasible[1,2] • Could allow for real-time monitoring[1,2]	• Remains the standard of care[2] • More confidence in negative results[1] • Higher sensitivity for certain types of alterations[1]
Limitations	• Not all patients have ctDNA[1,2] • Negative result should be confirmed with tissue testing[1]	• Invasive procedure with potential complications[2] • Tumour heterogeneity may not be captured[1] • Finite resource in many patients[2]

ctDNA: circulating tumour DNA.

1. Saarenheimo, J., et al. (2019) *Front Oncol* 9:129; 2. Corcoran, R.B. and Chabner, B.A. (2018) *N Engl J Med* 379:1754-65.

Liquid biopsy: Guidelines and recommendations

"If there is insufficient tissue to allow testing for all of *EGFR*, *ALK*, *ROS1*, *BRAF*, *MET*, and *RET*, repeat biopsy and/or plasma testing should be done"
"Testing should be conducted as part of broad molecular profiling"

NCCN 2020 NSCLC Practice Guidelines[1]

"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"

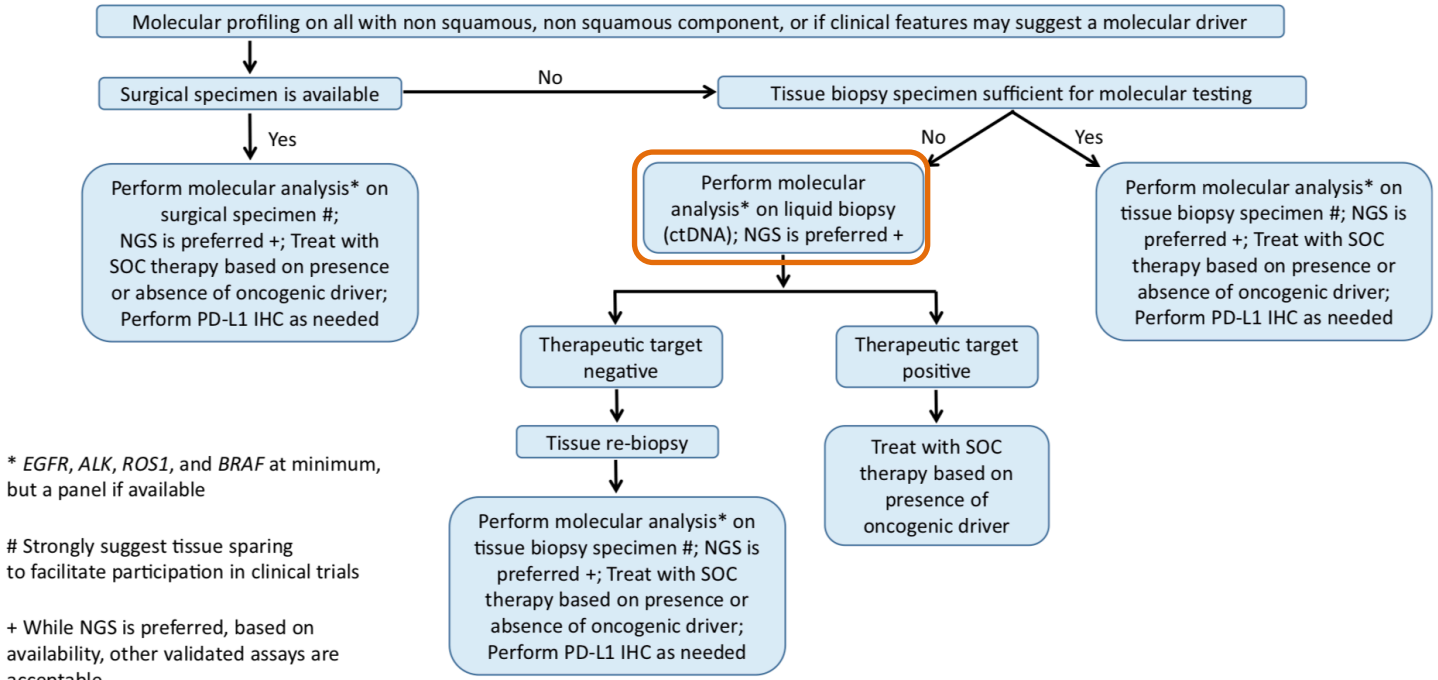
2017 ASCO Clinical Cancer Advances[2]

"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[3]

AMP: Association for Molecular Pathology; ASCO: American Society of Clinical Oncology; CAP: College of American Pathologists;
IASLC: International Association for the Study of Lung Cancer; NCCN: National Comprehensive Cancer Network; NSCLC: non-small cell lung cancer.
1. NCCN Clinical Practice Guidelines in Oncology: NSCLC (Version 2.2020); 2. Burstein, H.J., et al. (2017) *J Clin Oncol* 35:1341-67;
3. Lindeman, N.I., et al. (2018) *J Thorac Oncol* 5:323-7.

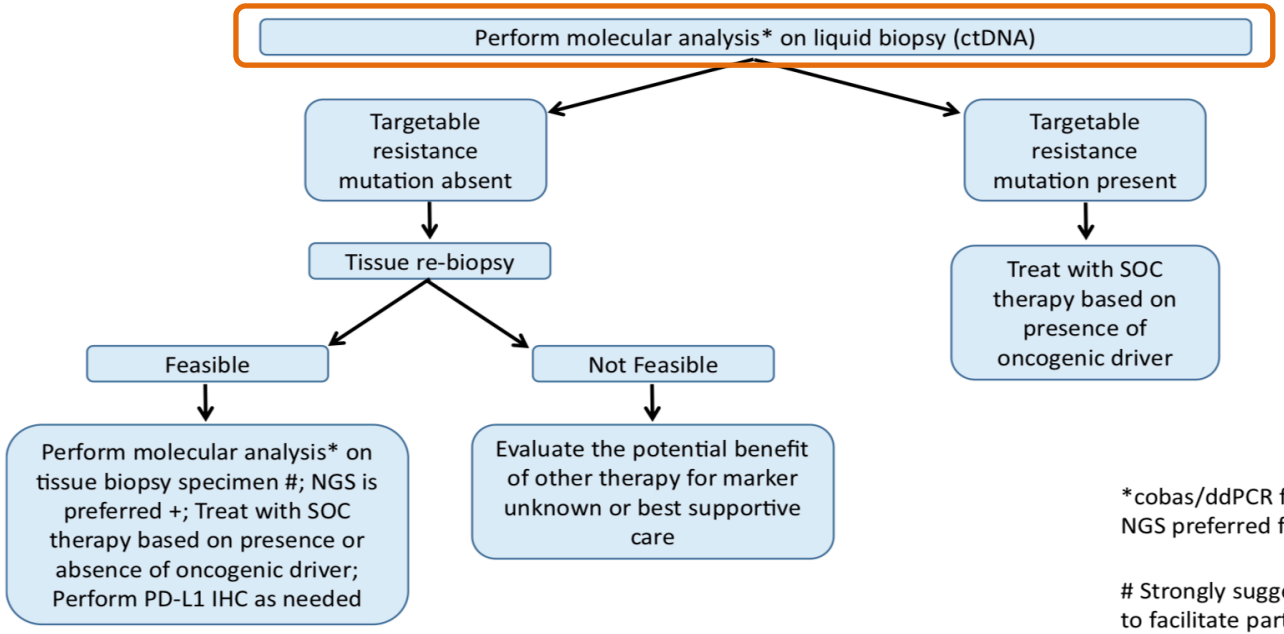
Patients with advanced treatment-naive NSCLC



ctDNA: circulating tumour DNA; IHC: immunohistochemistry; NGS: next-generation sequencing; SOC: standard of care.

Rolfo, C., et al. (2018) *J Thorac Oncol* 9:1248-68.

Patients with progressive or recurrent NSCLC during treatment with TKI



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

Strongly suggest tissue sparing to facilitate participation in clinical trials

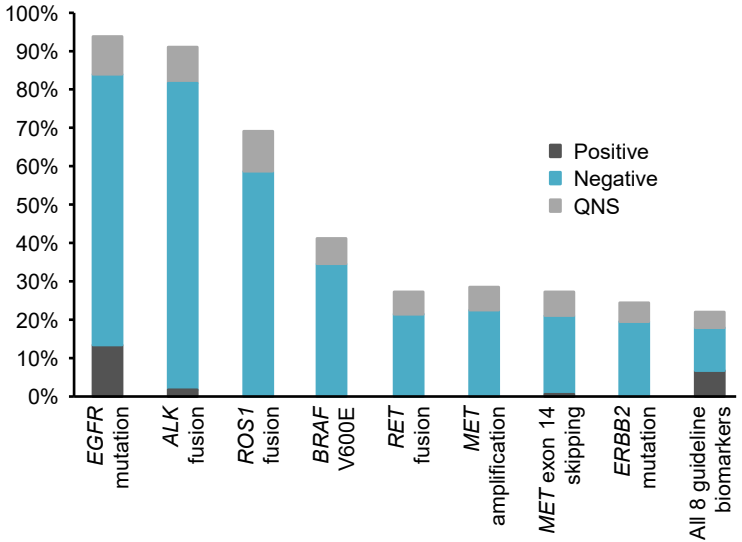
+ While NGS is preferred, based on availability, other validated assays are acceptable

ctDNA: circulating tumour DNA; IHC: immunohistochemistry; NGS: next-generation sequencing; SOC: standard of care; TKI: tyrosine kinase inhibitor.

Rolfo, C., et al. (2018) *J Thorac Oncol* 9:1248-68.

NILE study: Plasma NGS vs SOC tissue genotyping

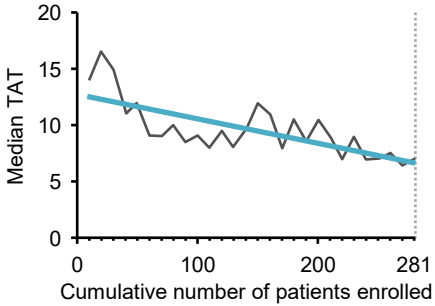
Only 18% of patients had complete tissue genotyping for all 8 guideline-recommended genomic biomarkers



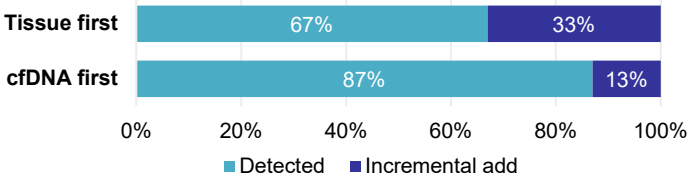
One of eight guideline-recommended biomarkers was identified in 21.3% patients using tissue vs. 27.3% using cfDNA (n = 282; p<0.0001 for non-inferiority)

- **80% cfDNA clinical sensitivity** (relative to tissue) for any of 8 guideline-recommended biomarkers
- For FDA-approved targets (*EGFR, ALK, ROS1, BRAF*) **concordance was >98.2% with 100% positive predictive value** for cfDNA vs tissue (34/34 *EGFR-, ALK-, or BRAF*-positive patients)

cfDNA median turnaround time was significantly faster than tissue
(9 vs. 15 days; p < 0.0001)



Utilising cfDNA in addition to tissue increased detection by 48%

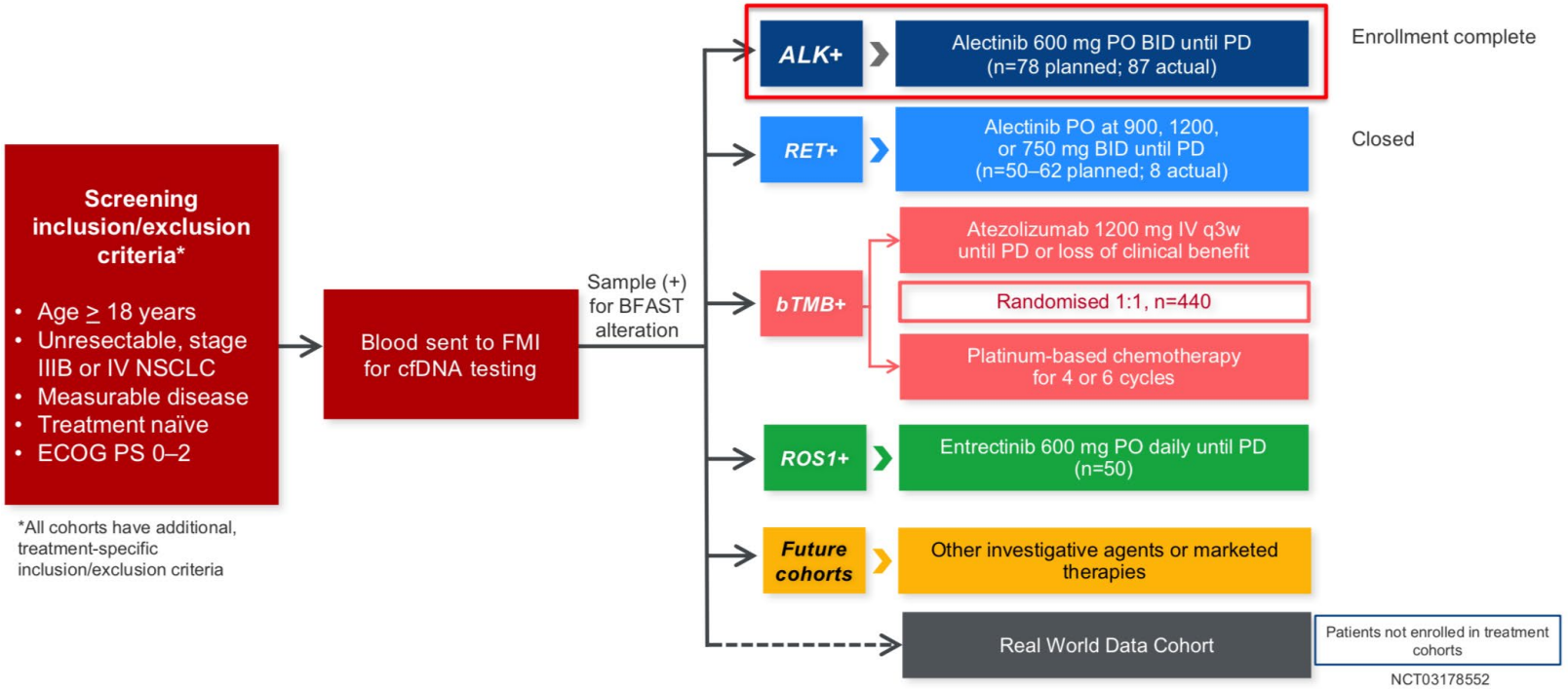


Blood first?

cfDNA: cell-free DNA; FDA: US Food and Drug Administration; NGS: next-generation sequencing; QNS: quantity not sufficient; SOC: standard of care.

Leighl, N.B., et al. (2019) *Clin Cancer Res* 25:4691-700.

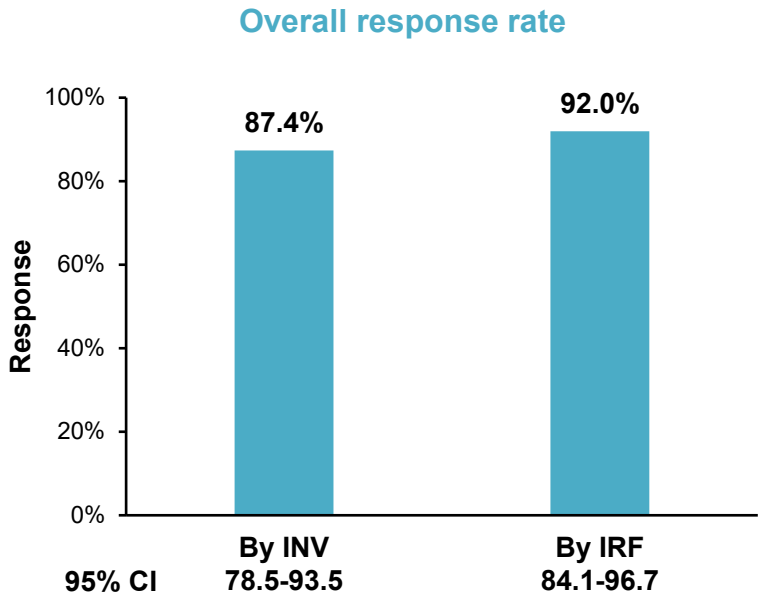
Phase II/III BFAST trial in treatment-naive NSCLC: Initial results from the *ALK*+ cohort



BID: twice daily; cfDNA: cell-free DNA; ECOG PS: Eastern Cooperative Oncology Group Performance Status; FMI: Foundation Medicine, Inc.; IV: intravenous; NSCLC: non-small cell lung cancer; PD: progressive disease; PO: oral administration; q3w: every 3 weeks.

Gadgeel, S., et al. (2019) Slide presentation at ESMO 2019:abstract LBA81_PR.

High response rate to ALK-targeted therapy after blood-based NGS testing in BFAST



	INV (n = 87)	IRF (n = 87)
Complete response, n (%)	**0**	**11 (12.6)**
95% CI	(0.00-4.15)	(6.48-21.50)
Partial response, n (%)	**76 (87.4)**	**69 (79.3)**
95% CI	(78.50-93.52)	(69.29-87.25)
Progressive disease, n (%)	**1 (1.1)**	**1 (1.1)**
95% CI	(0.03-6.24)	(0.03-6.24)

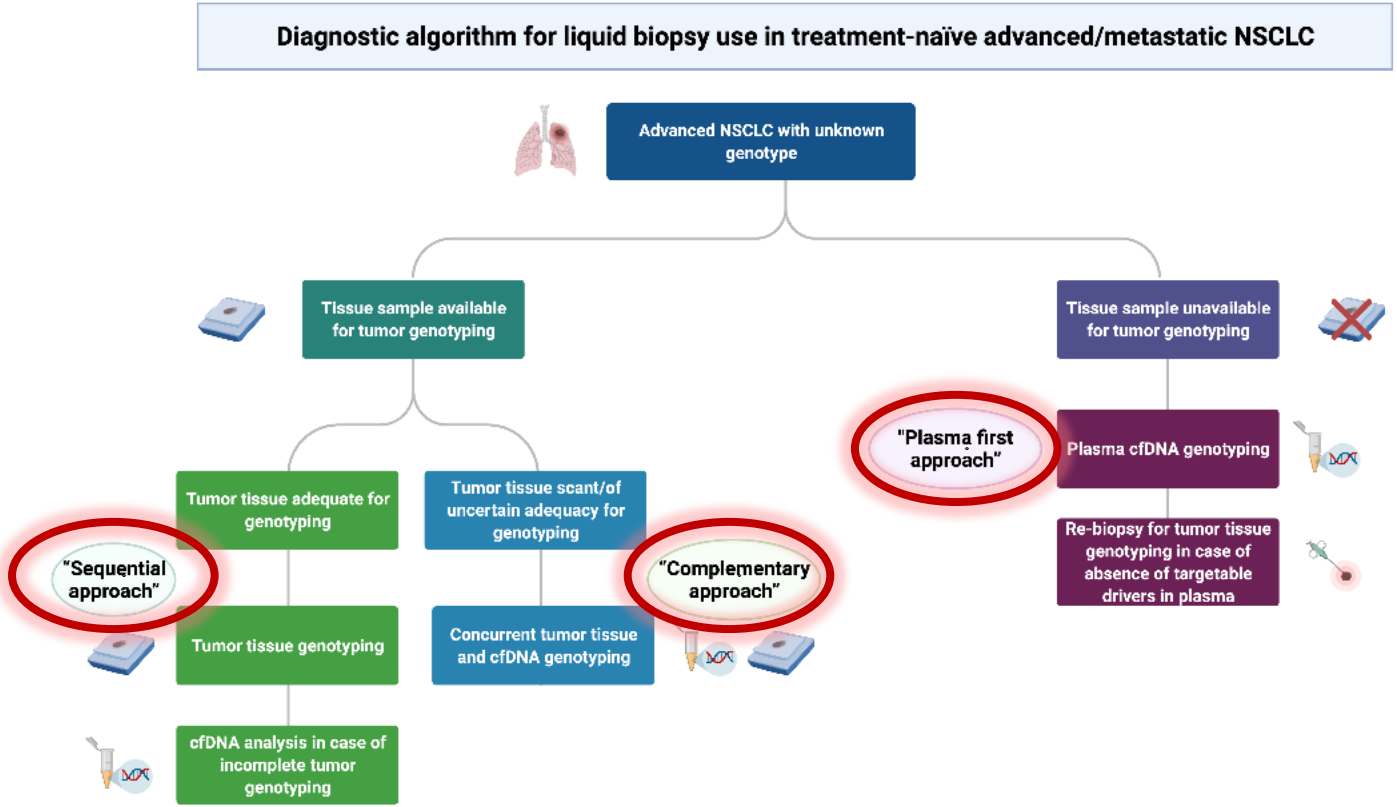
Median duration of follow-up: 12.58 months

ALEX trial confirmed ORR = 71.7% (95% CI 63.8-78.7)

CI: confidence interval; INV: investigator-assessed; IRF: independent review facility-assessed; ORR: overall response rate.

Gadgeel, S., et al. (2019) Slide presentation at ESMO 2019:abstract LBA81_PR.

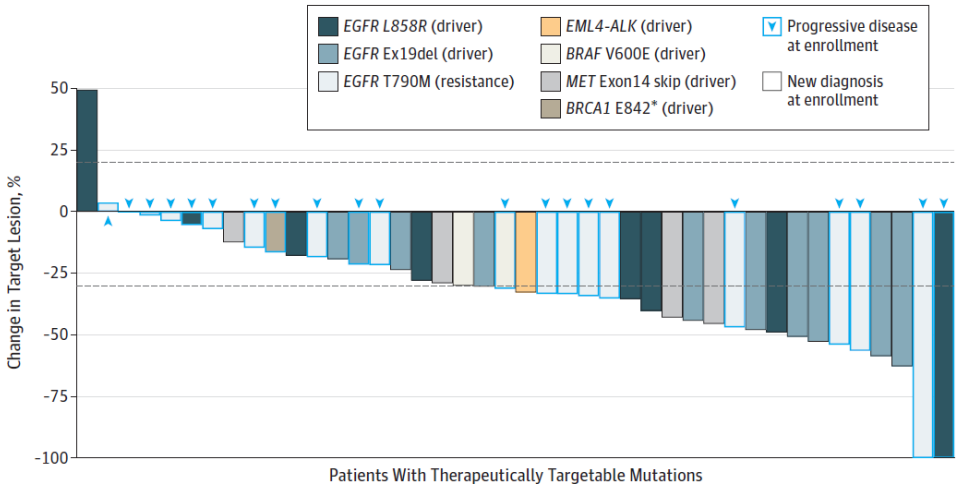
Don´t post



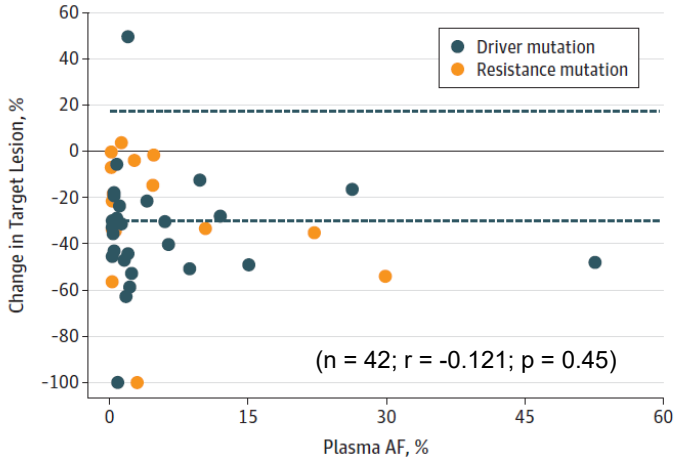
Rolfo et al (Gandara) submitted to JTO 2021

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

Responses to plasma-indicated targeted therapy by RECIST



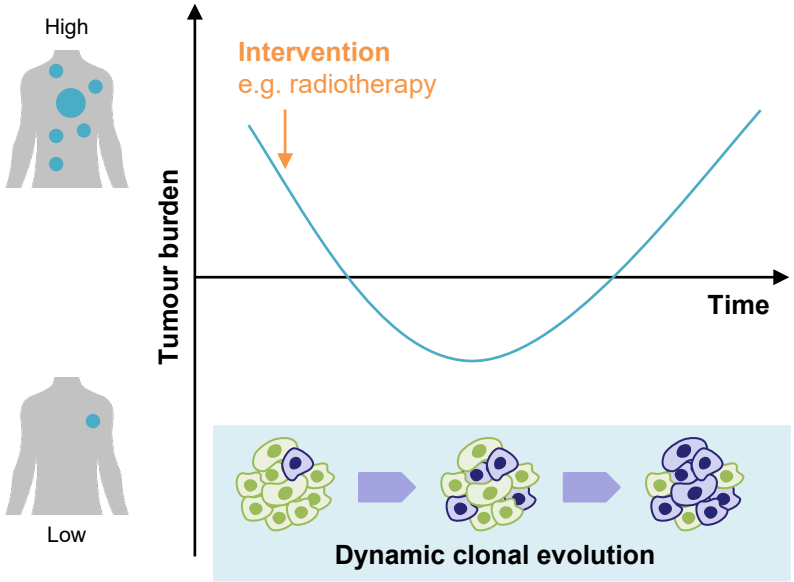
Correlation of RECIST and allele frequency



AF: allele frequency; RECIST: Response Evaluation Criteria in Solid Tumours.
Aggarwal, C., et al. (2019) *JAMA Oncol* 5:173-80.

Timing of sample draw is important

Correlation between tumour burden and dynamic clonal evolution of the tumour[1]



Increasing number of metastatic sites (p = 0.001) and presence of bone (p = 0.007) and hepatic (p = 0.001) metastases significantly associated with assay sensitivity[2]

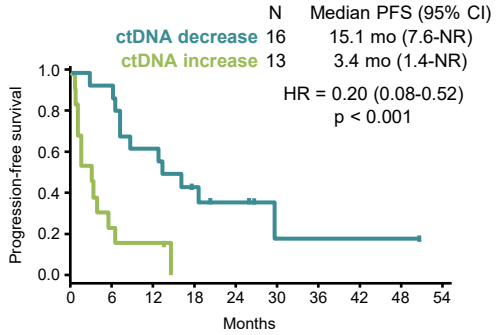
1. Pisapia, P., et al. (2017) in *Liquid Biopsy in Cancer Patients – Clinical Practice Implications: Monitoring Drug Response and Resistance*. Springer;
2. Sacher, A.G., et al. (2016) *JAMA Oncol* 2:1014-22.

ctDNA kinetics as predictive marker for treatment response or resistance

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

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

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

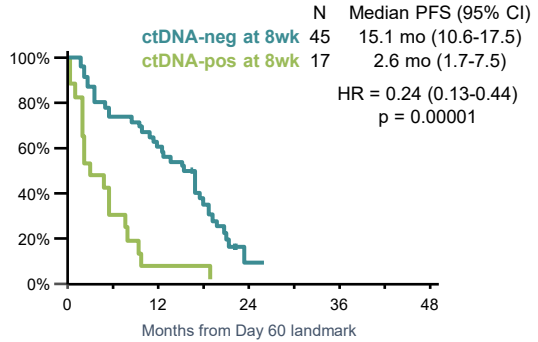


Rapid **decrease of ctDNA** correlated with **clinical benefit**, while **increase** correlated with **PD**

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

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

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



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

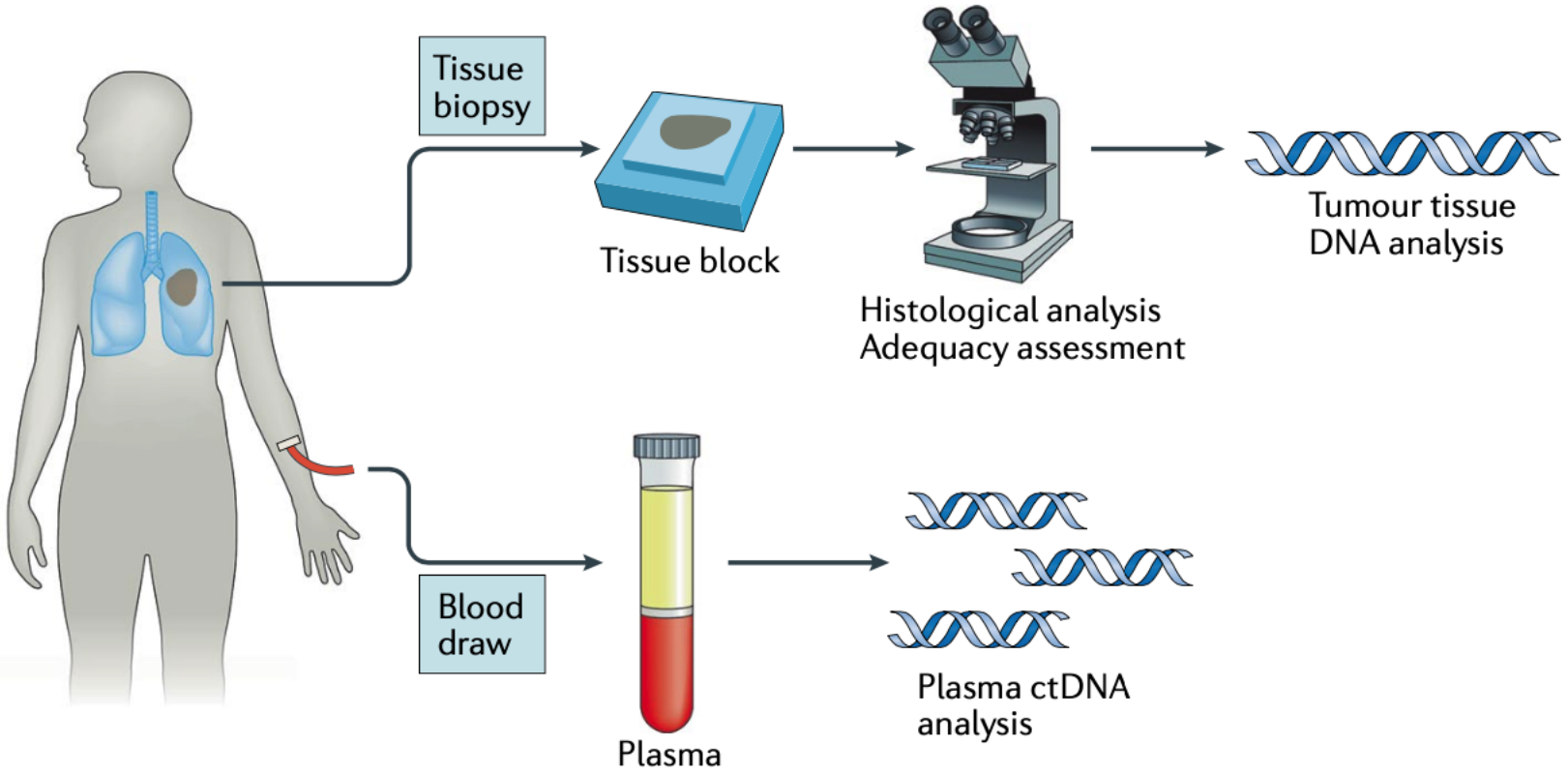
In both studies PFS is significantly longer in NSCLC patients with early ctDNA decrease / clearance

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

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

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

Convenience of plasma ctDNA genotyping



Aggarwal & Rolfo et al, Nature Reviews in Clinical Oncology Jan 2021

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

Intratumour heterogeneity

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

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

Intrapatient heterogeneity

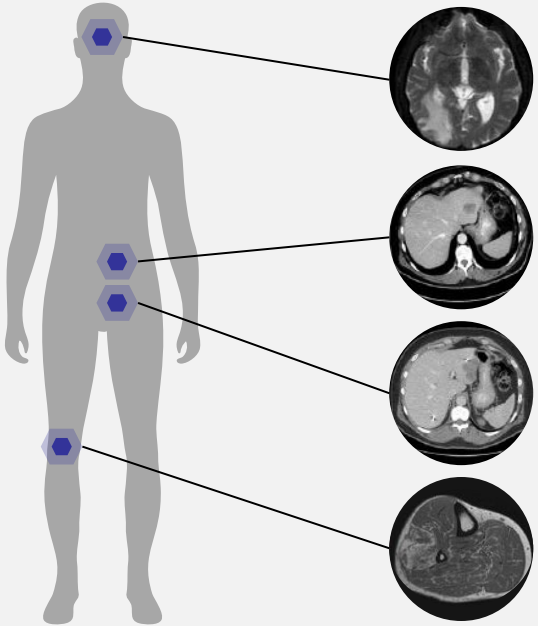
The genomic landscape may **differ between tumour sites** within a patient

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

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

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

Tumour heterogeneity: ctDNA can capture multiple mechanisms of acquired resistance in mCRC



Brain lesion
BRAF V600E, AF 54.7%
EGFR amp
KRAS G12S not detected
NRAS Q61R not detected

Liver biopsy 1
BRAF V600E, AF 36.4%
EGFR amp, not detected
KRAS G12S, AF 6.4%
NRAS Q61R, AF 3.1%

Liver biopsy 2
BRAF V600E, AF 61.6%
EGFR amp, not detected
KRAS G12S, AF 22.4%
NRAS Q61R, not detected

Subcutaneous lesion
BRAF V600E, AF 45.4%
EGFR amp, not detected
KRAS G12S, AF 0.2%
NRAS Q61R, not detected

Multiple solid tumour biopsies show diverging resistance mechanisms in different metastases in a patient with advanced *BRAF* V600E CRC

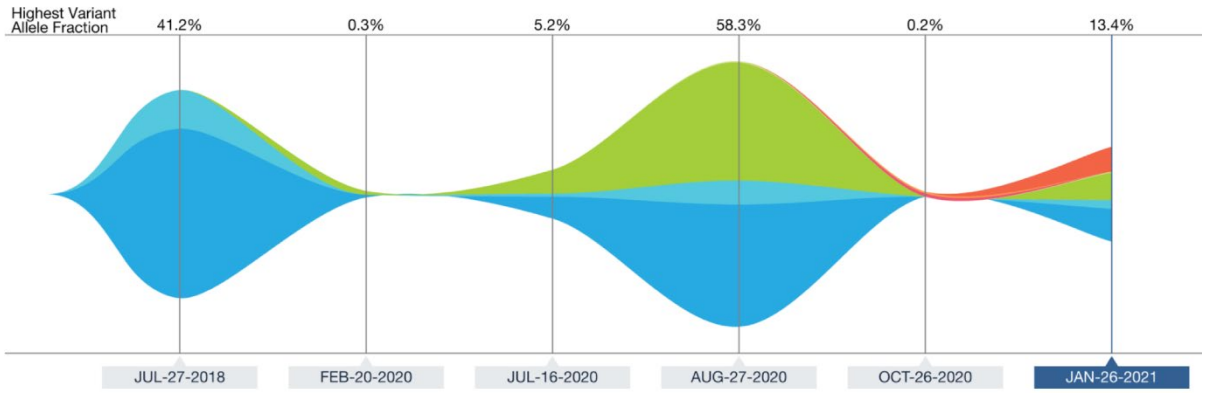
Liquid biopsy captured all resistance mechanisms

cfDNA

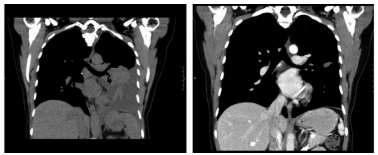
BRAF V600E, AF 24%
EGFR amp
KRAS G12S, AF 2.1%
NRAS Q61R AF 0.6%

AF: allele frequency; cfDNA: cell-free DNA; mCRC: metastatic colorectal cancer.
Parikh, A.R., et al. (2019) *Nat Med* 25:1415-21.

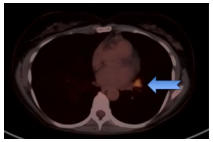
Courtesy of G. Oxnard



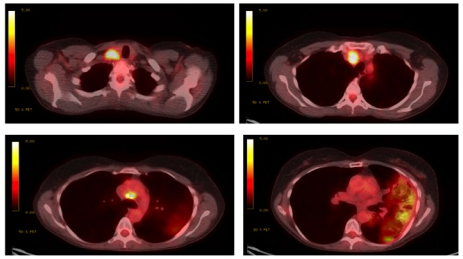
EGFR E746_A750del (Exon 19 deletion)	13.4%	41.2% 0.2% 4.7% 58.3% ND 13.4%
EGFR C797S	10.7%	ND 0.3% 5.2% 55.6% ND 10.7%
EGFR T790M	9.6%	ND ND ND ND ND 9.6%
TP53 S127F	2.6%	6.5% ND 0.4% 7.6% ND 2.6%
ARID1A Q456Q	0.2%	ND ND ND 0.2% ND 0.2%
BRAF Amplification	ND	2.2 ND ND ND ND ND
CDK6 Amplification	ND	2.2 ND ND ND ND ND
EGFR Amplification	ND	3.4 ND ND 4.2 ND ND
NTRK2 L699L	ND	0.2% ND
EGFR N338N	ND	ND ND ND ND 0.1% ND



Osimertinib



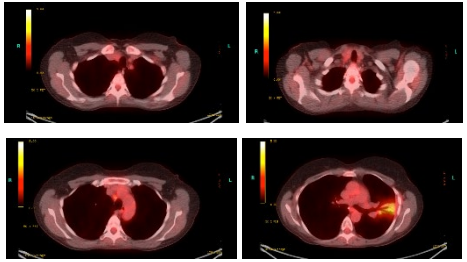
Radiotherapy



Erlotinib

PFS 24 months

PR 2 months



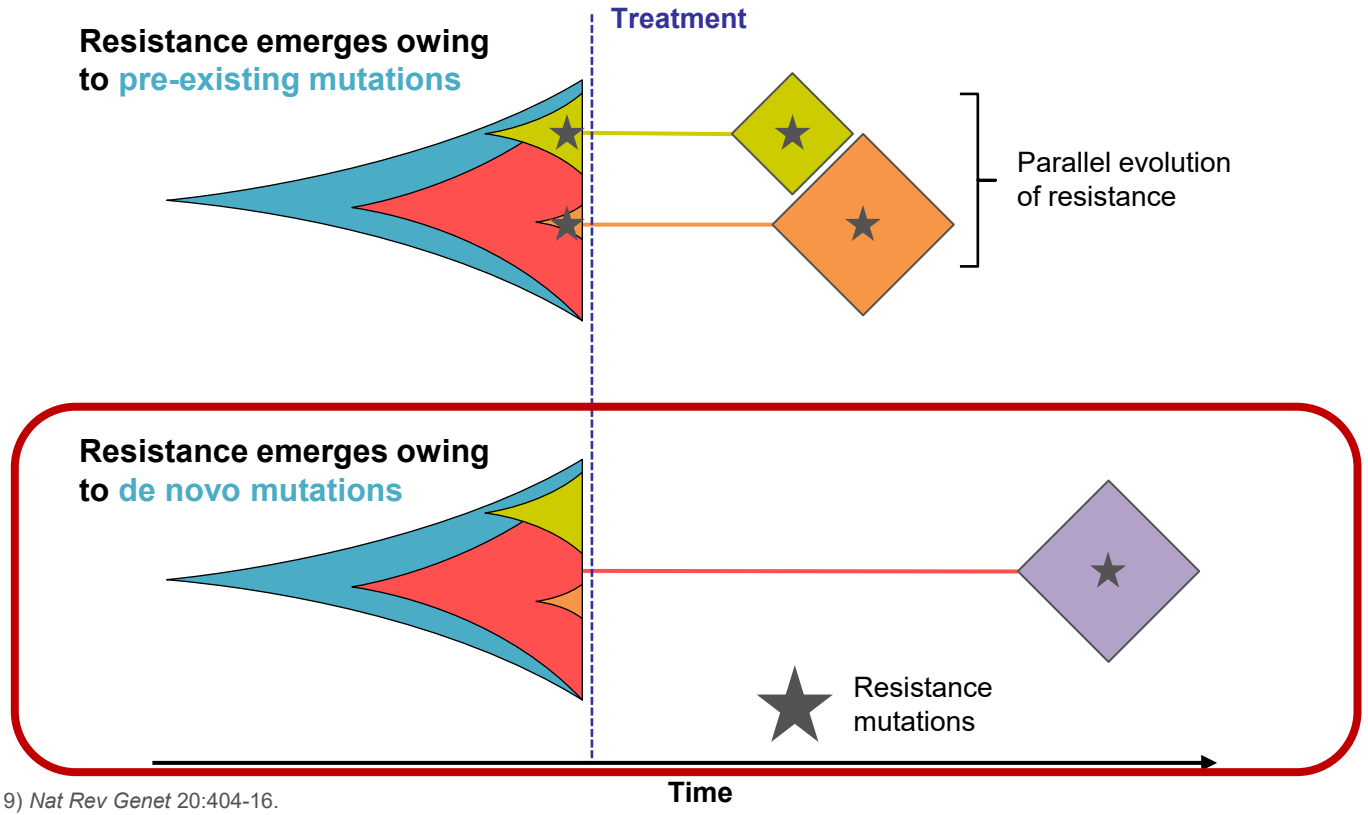
PFS 5 months

3 mm lesion

Chemotherapy

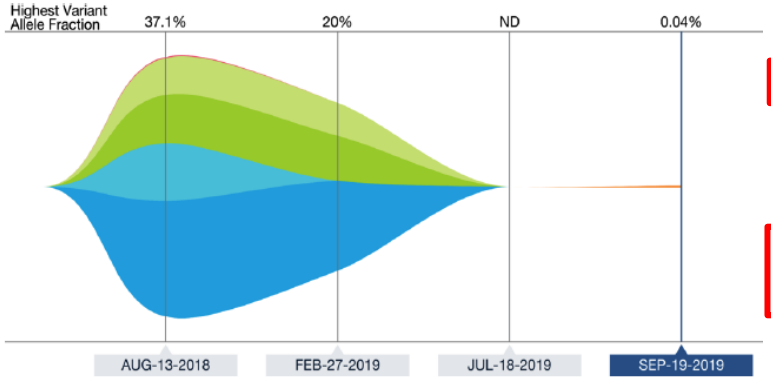
Rolfo Unpublished data @ChristianRolfo

Clonal evolution of treatment resistance



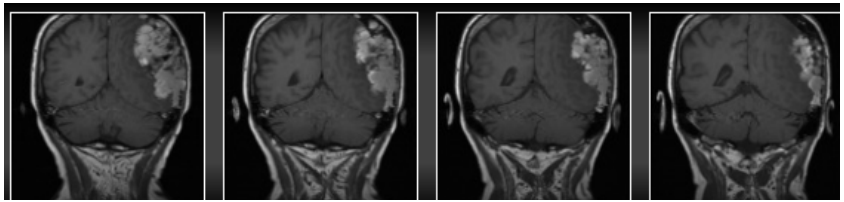
Turajlic S, et al. (2019) *Nat Rev Genet* 20:404-16.

Case #2: 71 year old NSCLC patient



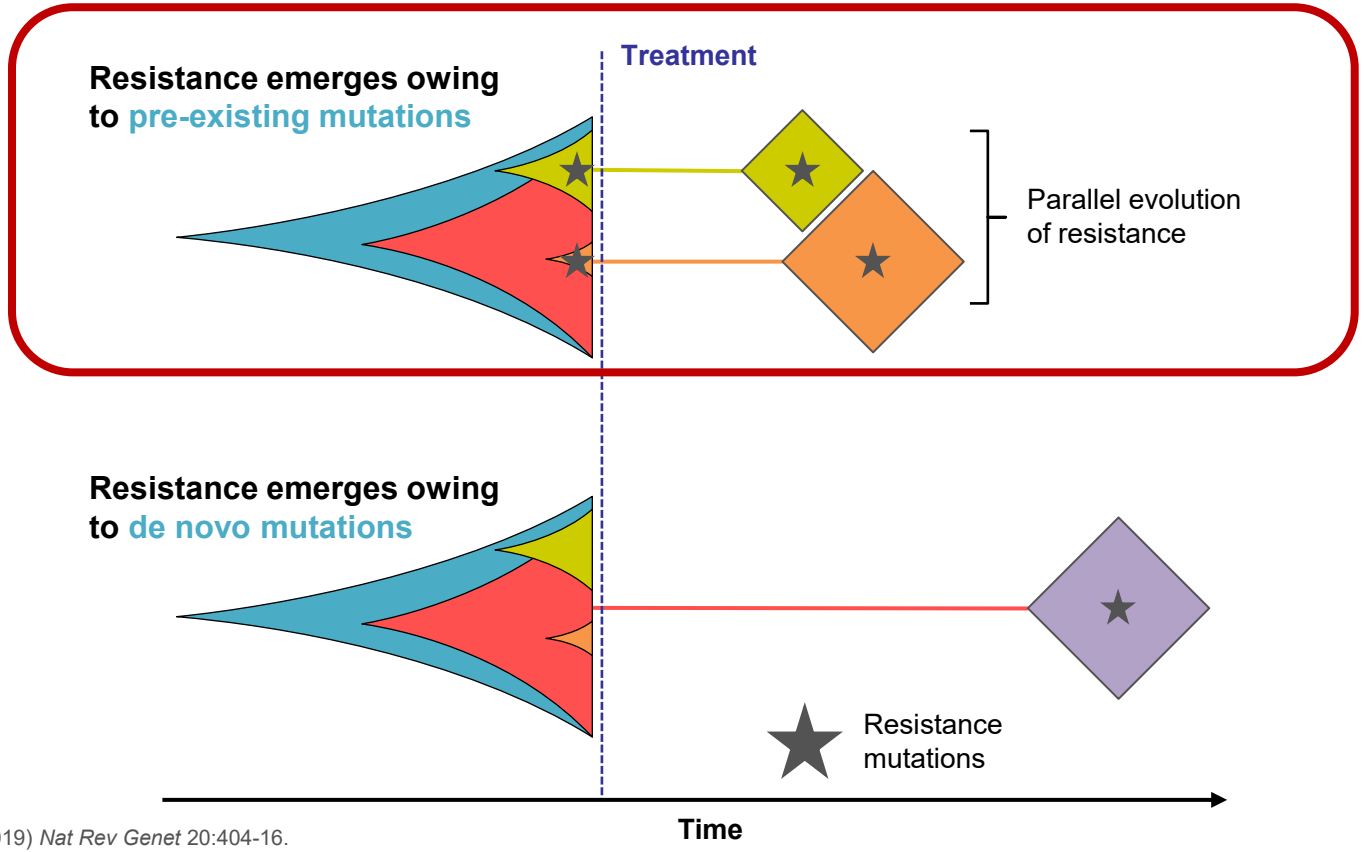
	Aug 18	Feb 19	Jul 19	Sep 19
EGFR L858R	37.1%	20.0%	ND	ND
EGFR T790M	15.8%	ND	ND	ND
TP53 Splice Site SNV	12.5%	8.4%	ND	ND
BRCA1 D2N	8.9%	5.7%	ND	ND
TP53 V272M	0.1%	ND	ND	ND
EGFR Amplification	++	+	ND	ND
MET Amplification	++	+++	ND	ND
AR Amplification	+	ND	ND	ND

osimertinib start → PFS 6 months → osimertinib + crizotinib start



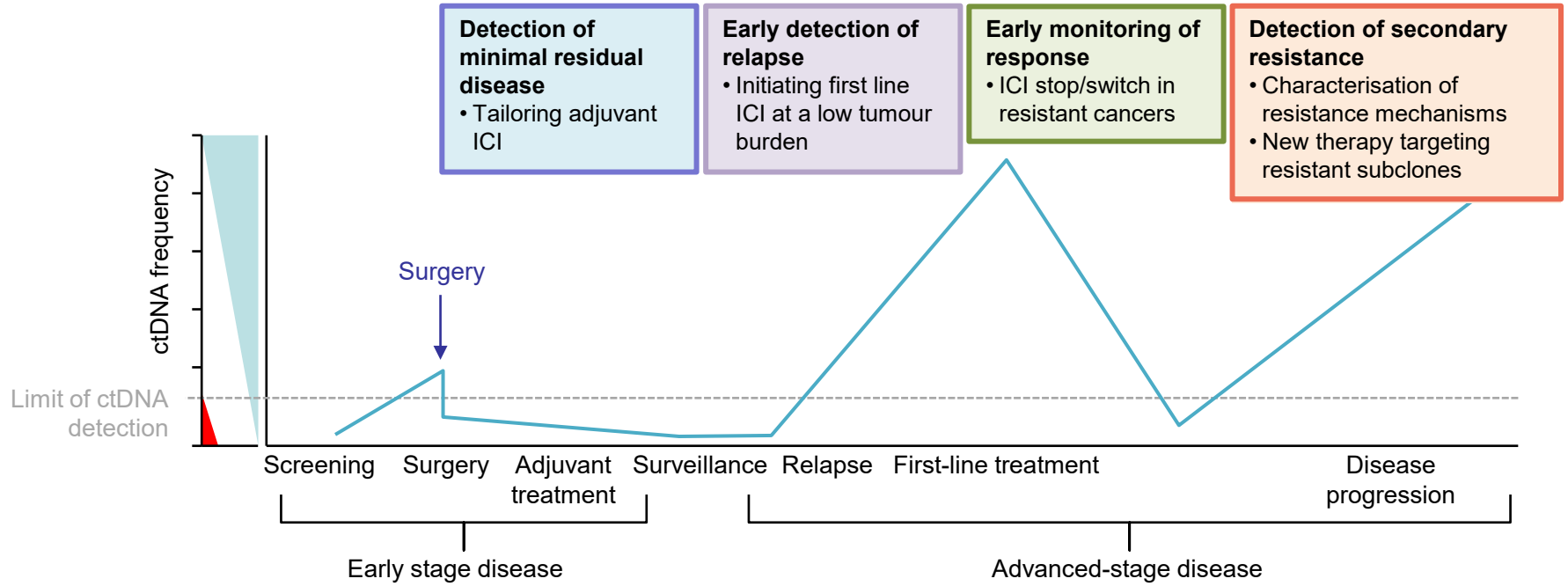
NSCLC: non-small cell lung cancer; PFS: progression-free survival.
Case courtesy of Dr Rolfo, University of Maryland School of Medicine.

Clonal evolution of treatment resistance



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

Clinical application of liquid biopsy in immunotherapy



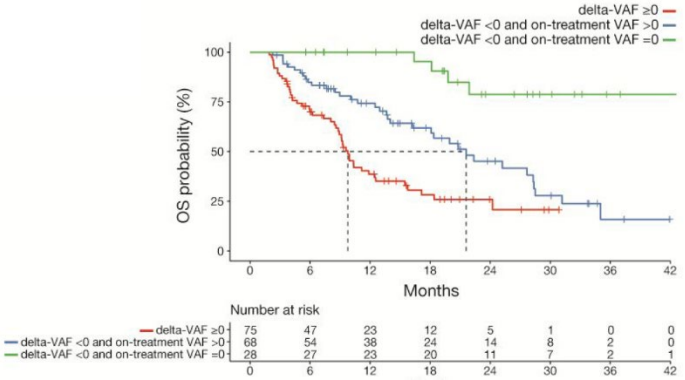
Not so easy!!

ctDNA: circulating tumour DNA; ICI: immune checkpoint inhibitor.

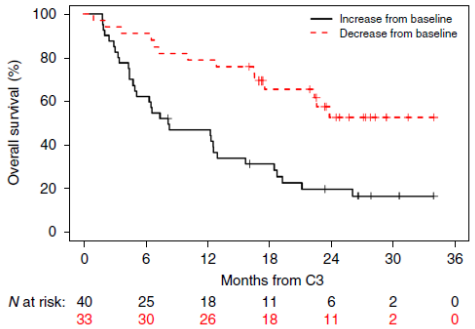
Cabel, L., et al. (2018) *Nat Rev Clin Oncol* 15:639-50.

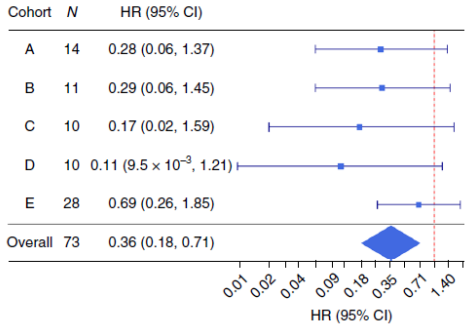
“ctDNA dynamics”: On-treatment change in ctDNA predicts OS on immunotherapy in advanced cancers

On-treatment changes in **ctDNA VAF** across 16 advanced tumour types in three phase I/II trials of durvalumab (± tremelimumab)[1]



On-treatment changes in **ctDNA concentration** across advanced solid tumours in a prospective phase II trial of pembrolizumab[2]





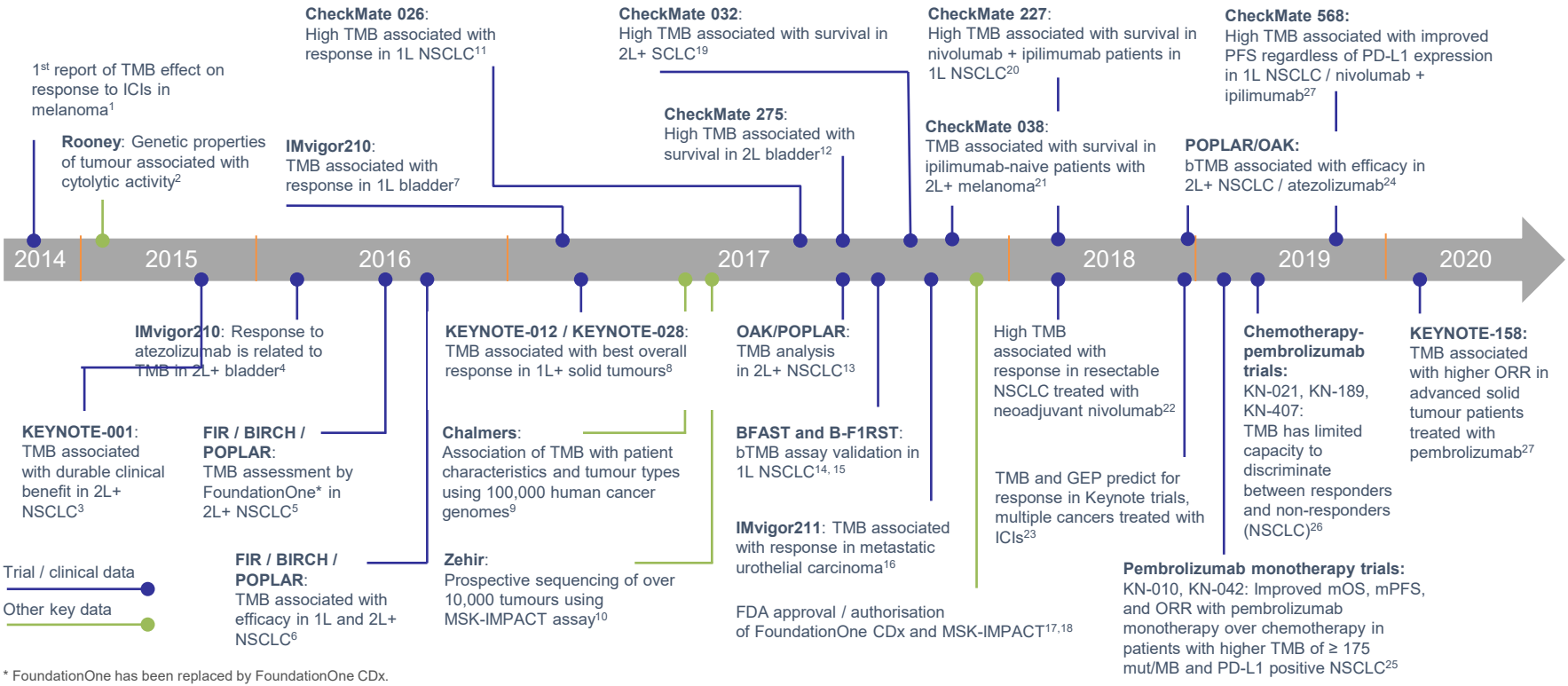
On-treatment reductions in VAF and lower on-treatment VAF are independently associated with longer PFS and OS, and increased ORR[1]

Baseline ctDNA concentration correlates with PFS, OS, clinical response and clinical benefit. This association becomes stronger when considering ctDNA kinetics during treatment[2]

C3: cycle 3; CI: confidence interval; ctDNA: circulating tumour DNA; delta-VAF: mean change in VAF; HR: hazard ratio; ORR: objective response rate; OS: overall survival; PFS: progression-free survival; VAF: variant allele frequency.

1. Zhang, Q., et al. (2020) *Cancer Discov* doi:10.1158/2159-8290.CD-20-0047 [Epub ahead of print];
2. Bratman, S.V., et al. (2020) *Nat Cancer* doi:10.1038/s43018-020-0096-5 [Epub ahead of print].

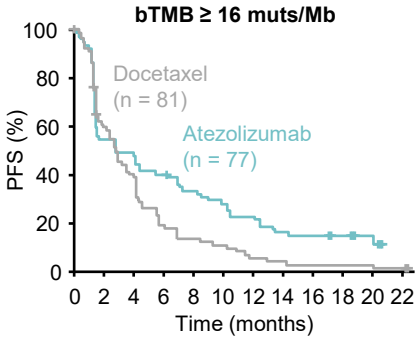
Evolution of TMB as an immunotherapy biomarker over the last several years



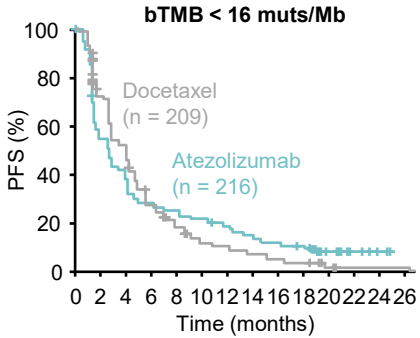
* FoundationOne has been replaced by FoundationOne CDx.
1L: first line; 2L: second line; +: including others; CDx: companion diagnostic; FDA: Food and Drug Administration; GEP: gene expression profile;
ICI: immune checkpoint inhibitor; NSCLC: non-small cell lung cancer; ORR: objective response rate, SCLC: small cell lung cancer; TMB: tumour mutational burden.
Timeline adapted from Chan, T.A., et al. (2019) *Ann Oncol* 30:44-56 (full referencing in notes).

High bTMB may predict survival benefit from immunotherapy in NSCLC patients

Phase III OAK trial[1]
FoundationOne®Liquid assay (394 genes)

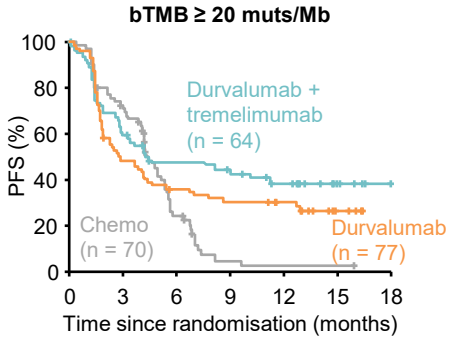


A vs Do:
HR 0.65 (0.47-0.92)

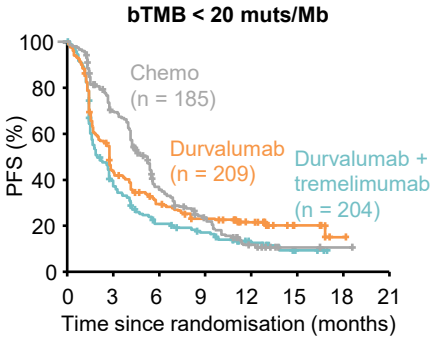


A vs Do:
HR 0.98 (0.80-1.20)

Phase III MYSTIC trial[2]
GuardantOMNI™ assay (500 genes)[3]



D vs Ch:
HR 0.77 (0.52-1.13)
D + T vs Ch:
HR 0.53 (0.34-0.81)
D + T vs D:
HR 0.76 (0.50-1.15)

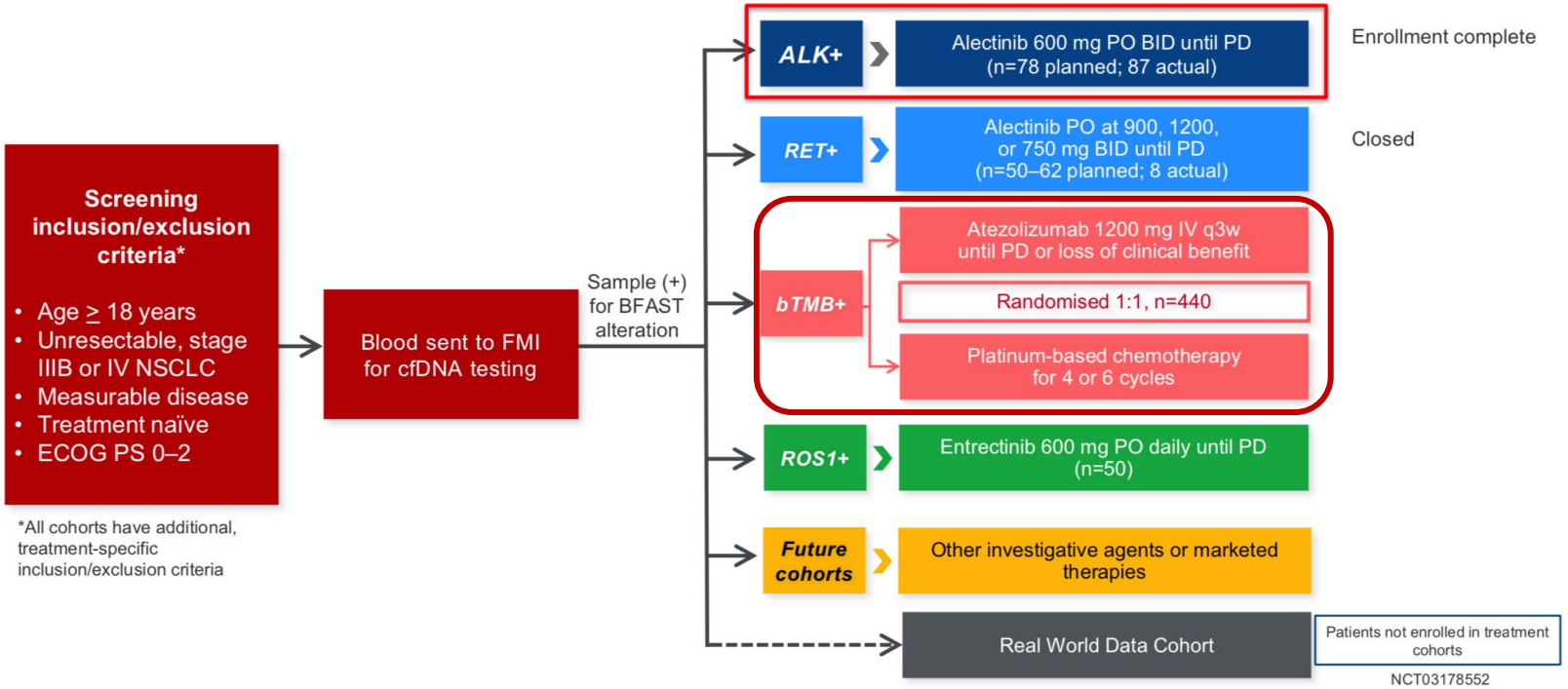


D vs Ch:
HR 1.19 (0.94-1.50)
D + T vs Ch:
HR 1.55 (1.23-1.94)
D + T vs D:
HR 1.26 (1.02-1.57)

High bTMB scores in both OAK and MYSTIC trials were also associated with improved OS on immunotherapy vs chemotherapy*

* In OAK, low bTMB score was also associated with favourable OS on immunotherapy vs chemotherapy.
A: atezolizumab; bTMB: blood-based tumour mutational burden; Chemo/Ch: chemotherapy; D: durvalumab; D + T: durvalumab + tremelimumab; Do: docetaxel; HR: hazard ratio; muts/Mb: mutations per megabase; NSCLC: non-small cell lung cancer; OS: overall survival; PFS: progression-free survival.
1. Gandara, D.R., et al. (2018) *Nat Med* 24:1441-8; 2. Rizvi, N.A., et al. (2020) *JAMA Oncol* 6:661-74; 3. Rossi, G., et al. (2020) *Cancers (Basel)* 12:1125.

Phase II/III blood-first assay screening trial (BFAST) in treatment-naïve NSCLC



BID: twice daily; cfDNA: cell-free DNA; ECOG PS: Eastern Cooperative Oncology Group Performance Status; FMI: Foundation Medicine, Inc.; IV: intravenous; NSCLC: non-small cell lung cancer; PD: progressive disease; PO: oral administration; q3w: every 3 weeks.

Gadgeel, S., et al. (2019) Slide presentation at ESMO 2019:abstract LBA81_PR.

There are some concerns about the use of TMB

Key concerns

- No correlation with PD-L1 status
- Lack of standardisation across different platforms and cut-off
- TMB could be a useful predictive biomarker but are we making good use of it?
- Do we need to know just a number?

These concerns are due to factors such as:

- Non-academic-driven research
- Technology challenges, and
- Bad trial design

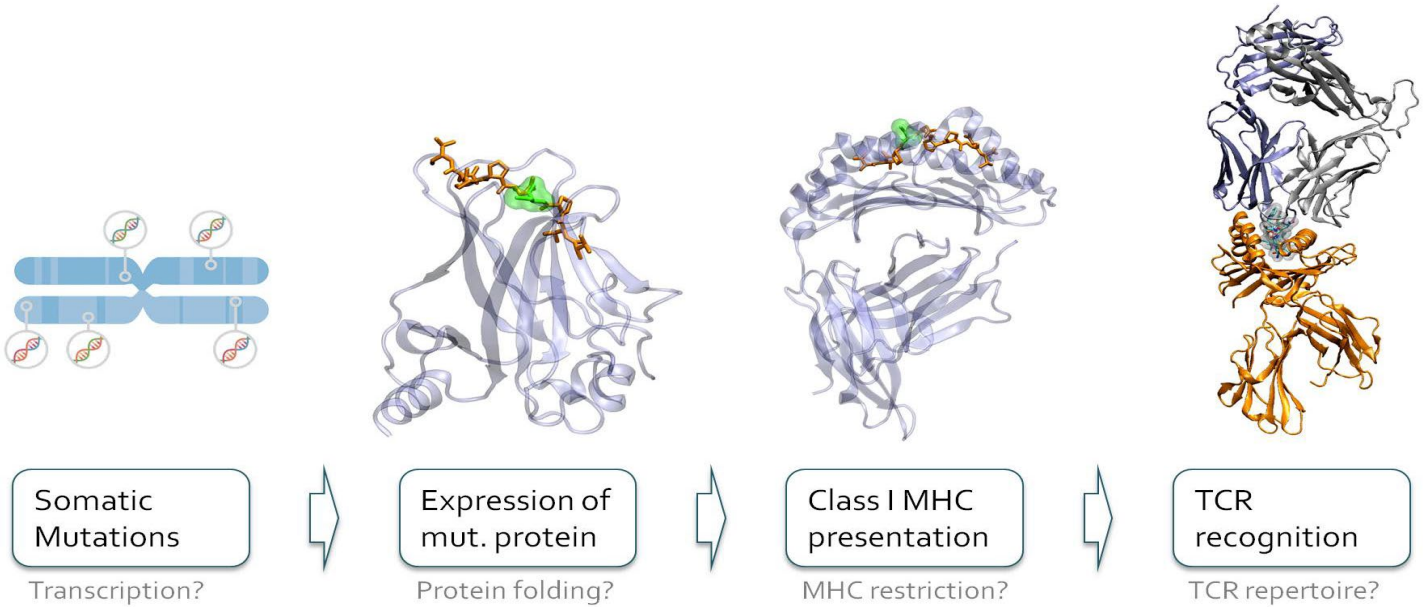
We need to take more responsibility in trial design using TMB
and promote more academic research efforts rather than industry-driven research

TMB is not dead yet!

PD-L1: programmed death-ligand 1; TMB: tumour mutational burden.
Based on the personal thoughts of Dr Rolfo.

Quantity or quality of mutations?

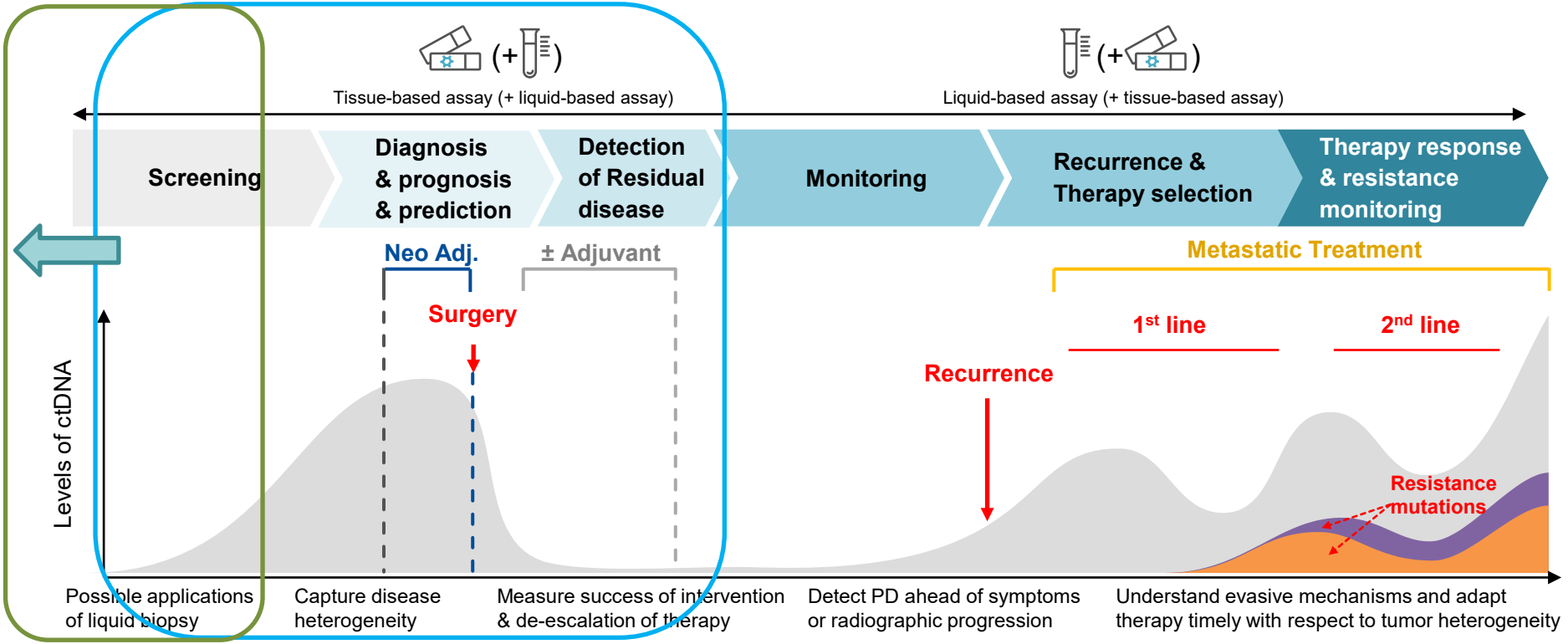
Only a minority of mutations produce neoantigens



MHC: major histocompatibility complex; TCR: T-cell receptor.

Peters, S. (2018) Education session at ASCO 2018: Biomarkers, Sequence, and Duration of Immunotherapy in Non-Small Cell Lung Cancer.

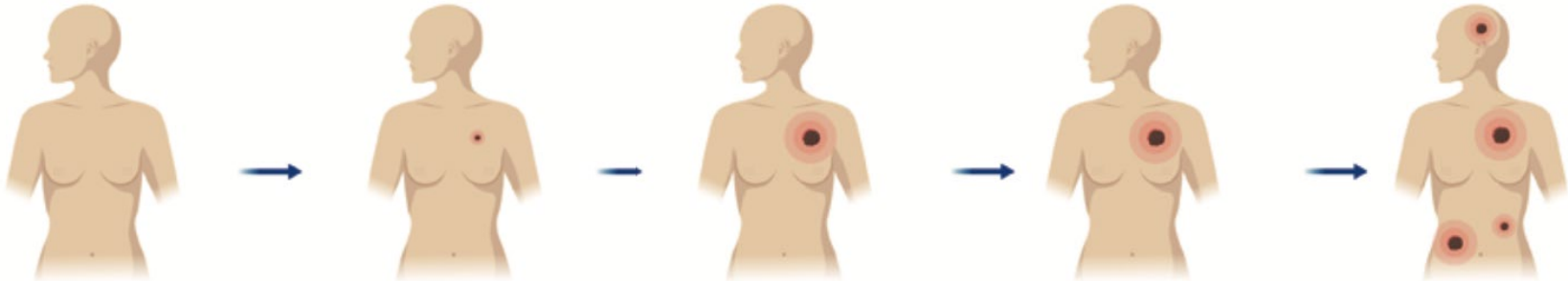
Liquid biopsy can provide clinically-valuable information along the whole patient journey



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

Liquid vs. tissue biopsies in cancer interception

Model 1 for cancer interception (Avoiding cancer)	Model 2 for mestasis interception (Avoiding dissemination: metastasis interception)



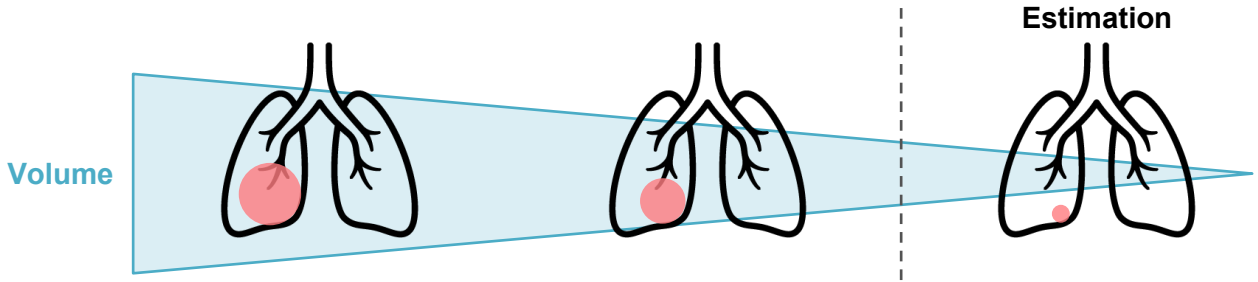
Predisposition	Early diagnosis	Late detection	Monitoring	Dissemination
Liquid biopsy	Liquid biopsy	Tissue biopsy	Liquid biopsy	Tissue biopsy

Cancer interception

Survival rates

Mj. Serrano, Critofanilli et al (Rolfo) Cancer Discov 2020;10:1635–44

Mutant allele frequency (MAF) in early stage NSCLC



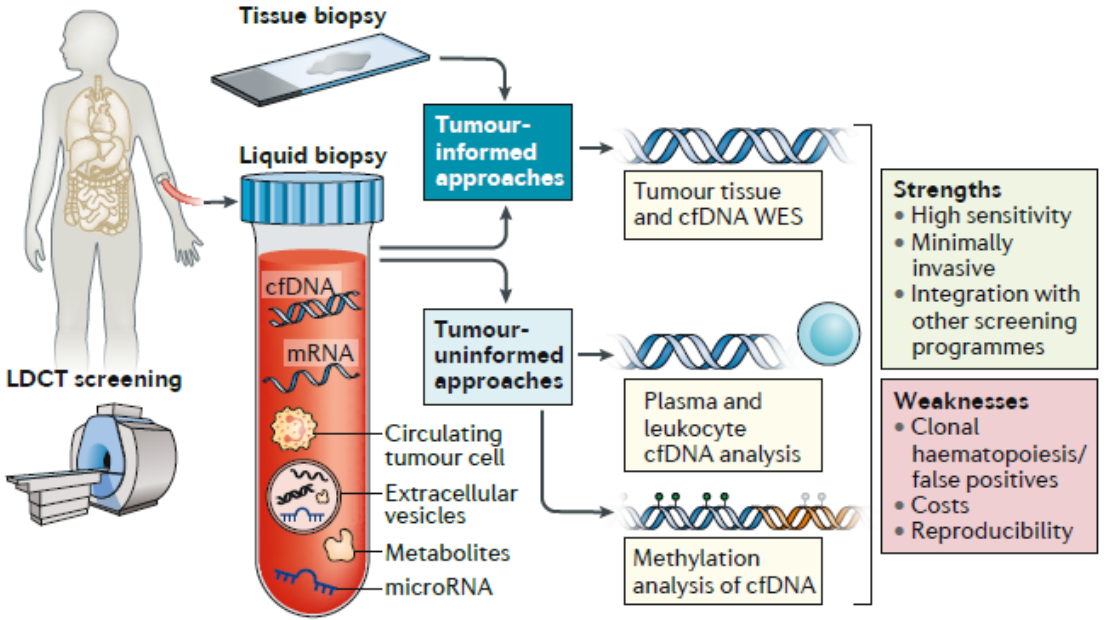
MAF	1.4% (0.62-3.1%)	0.1% (0.06-0.18%)	0.008% (0.002-0.03%)
Nodule diameter	5.8 cm	2.6 cm	1.2 cm
Nodule volume	100 cm^3	10 cm^3	1 cm^3
T stage	T3	T1c	T1b

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%)

ctDNA: circulating-tumour DNA; MAF: mutant allele frequency; NSCLC: non-small cell lung cancer.

Abbosh, C., et al. (2018) *Nat Rev Clin Oncol* 15:577-86.

Liquid biopsy for early lung cancer detection



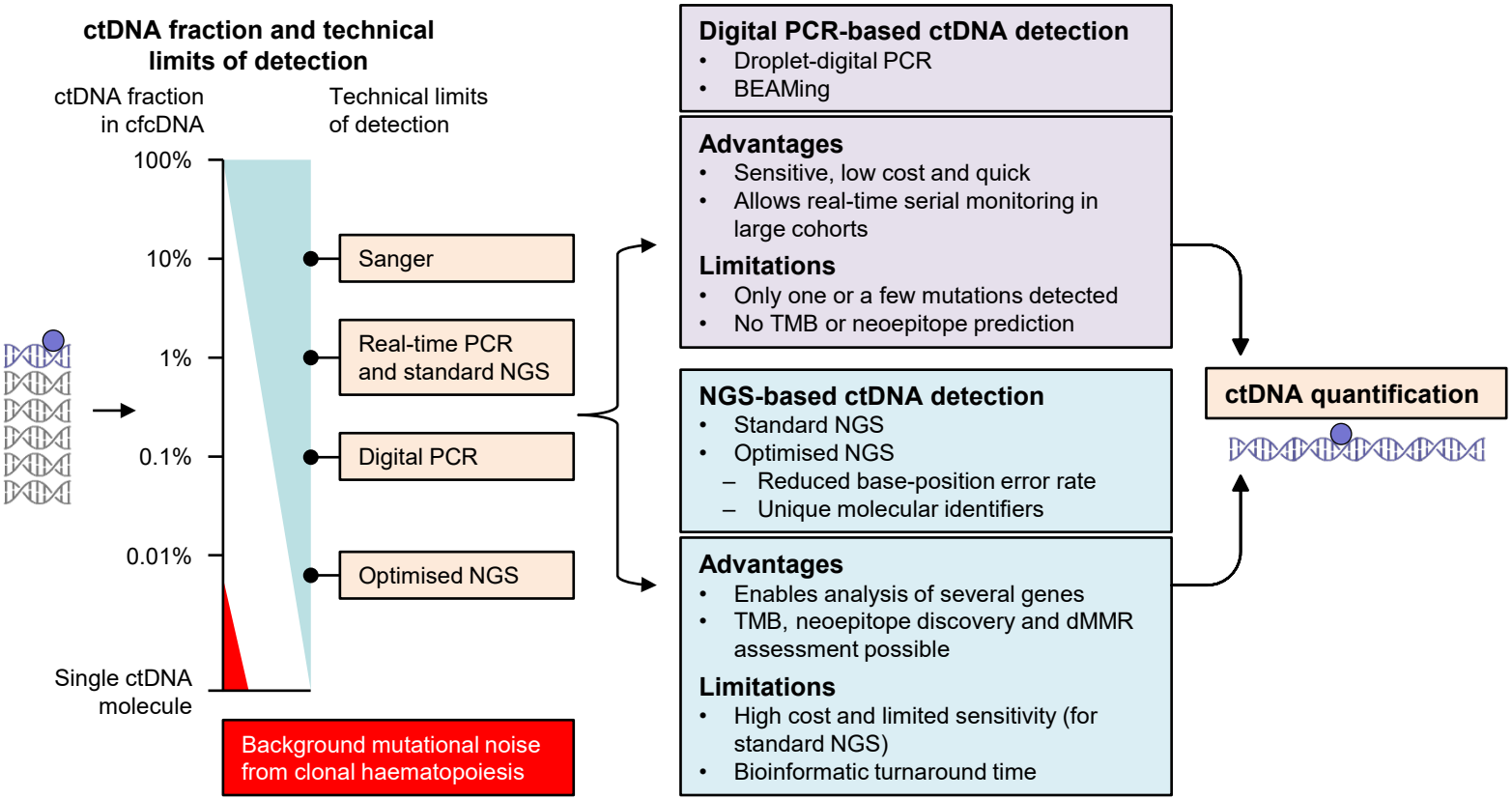
cfDNA: cell-free DNA; LDCT: low-dose computed tomography; WES: whole exome sequencing.
Rolfo, C., and Russo, A. (2020) *Nat Rev Clin Oncol* 17:523-4.

Important considerations for NGS 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!**

CLIA: Clinical Laboratory Improvement Amendments; NGS: next-generation sequencing; PCR: polymerase chain reaction; TAT: turnaround time; VAF: variant allele frequency.
Based on the personal thoughts of Dr Rolfo.

Advantages and limitations of ctDNA detection methods



BEAMing: beads, emulsions, amplification and magnetics; cfcDNA: cell-free circulating DNA; ctDNA: circulating tumour DNA; dMMR: deficient mismatch repair; NGS: next-generation sequencing; PCR: polymerase chain reaction; TMB: tumour mutational burden.

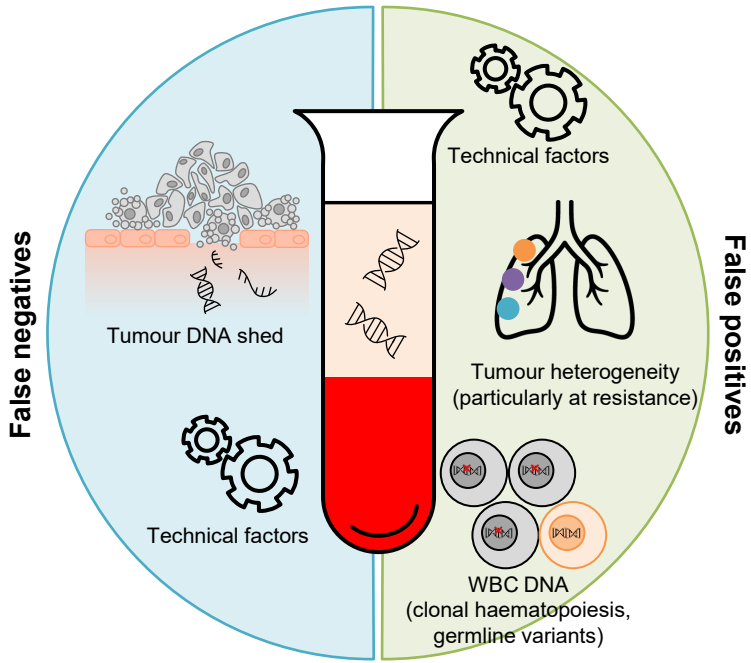
Cabel, L., et al. (2018) *Nat Rev Clin Oncol* 15:639-50.

Sources of false positive and false-negative results in plasma NGS

"False negatives" in liquid biopsy

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

Technical issues
(insufficient sensitivity in older assays)



"False positives" in liquid biopsy

Technical factors
(sample differences
e.g. > 6 months from tissue
to plasma sampling)

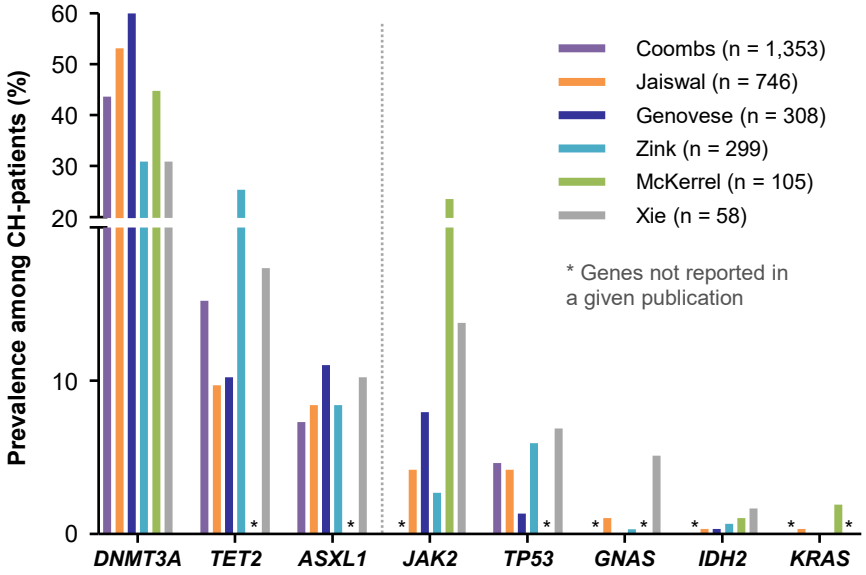
WBC contamination
(germline variants, clonal haematopoiesis)

Tumour heterogeneity
(positive plasma and negative tissue [assumes tissue is the "gold standard"])

NGS: next-generation sequencing; WBC: white blood cell.
Slide courtesy of Dr Gandara and based on the personal thoughts of Dr Rolfo.
Figure adapted from Paweletz, C.P., et al. (2019) *JCO Precis Oncol* doi:10.1200/PO.18.00408.

A new problem: Clonal haematopoeisis

Genes commonly mutated in clonal haematopoiesis[1]



Clonal haematopoiesis (CH) is the somatic acquisition of genomic alterations in haematopoietic stem and/or progenitor cells, leading to clonal expansion[2]

- A **large proportion of cfDNA is derived from peripheral blood cells** - somatic mutations within non-malignant haematopoietic cells is known as clonal haematopoiesis[1]
- CH might be a **recurring source of discordance** between tumour genotyping and plasma cfDNA genotyping[1]

cfDNA: cell-free DNA; CH: clonal haematopoiesis.

1. Hu, Y., et al. (2018) *Clin Cancer Res* 24:4437-43; 2. Ptashkin, R.N., et al. (2018) *JAMA Oncol* 4:1589-93.

Liquid biopsy take home message

- Clinical implementation of liquid biopsy is hampered by several **biological, technical and socio-economic challenges**
- **NGS panels preferred**
- The **exact knowledge of the limits of different liquid biopsy techniques** is essential for correct interpretation of test results and choice of the optimal methodology
- **”Blood first”** approach is almost here
- Immunotherapy and liquid biopsy are on the right pathway, **but we are still beginning this journey**

Based on the personal thoughts of Dr Rolfo.

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OF LIQUID BIOPSY

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Thanks

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