The clinical value of liquid biopsy: Indications in Oncology

Christian Rolfo, MD, PhD, MBA, Dr.h.c.
Marlene and Steward Greenebaum Comprehensive Cancer Center
University of Maryland School of Medicine
Baltimore, MD, United States

UNIVERSITY of MARYLAND
MARLENE AND STEWART GREENEBAUM
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SCHOOL OF MEDICINE

ISLB
INTERNATIONAL SOCIETY OF LIQUID BIOPSY

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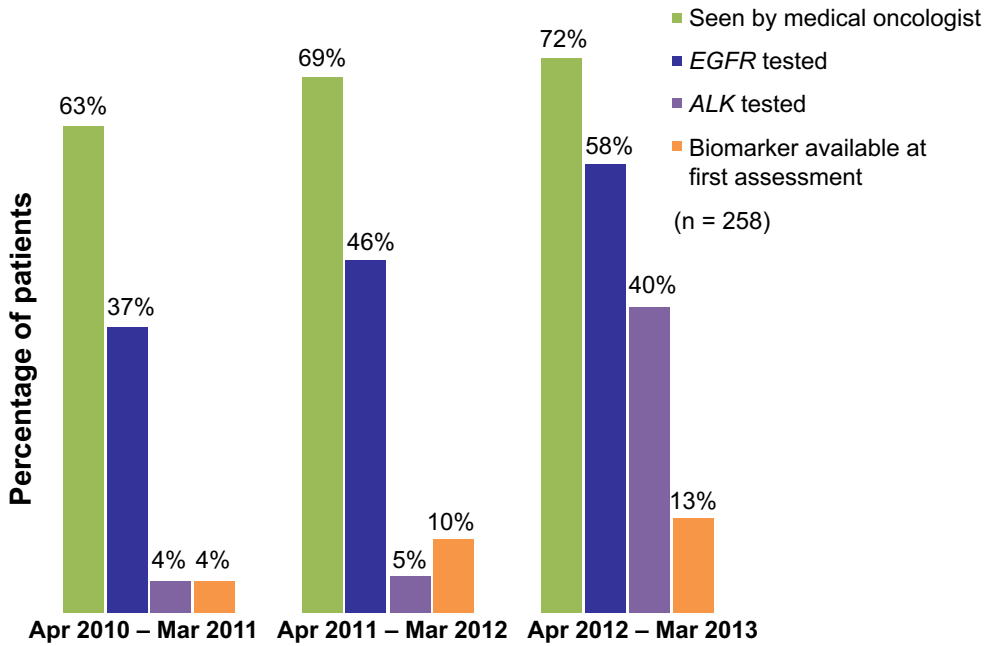
Non-financial interests	Research Collaboration: GuardantHealth
Leadership roles	Educational Committee Member: IALSC - Vice President : ISLB (International Society of Liquid Biopsy) - Educational Chair: OLA Oncology Latin American Association - Faculty for ASCO International Scientific Committee Member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

Biomarker testing and time to treatment decision

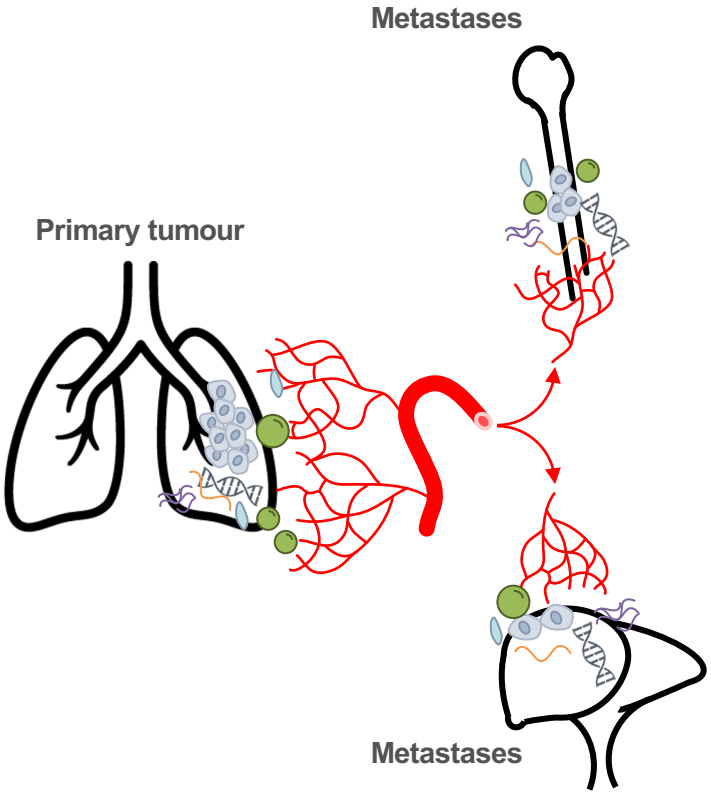


First problem after tissue quantity not sufficient!

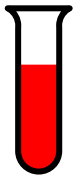
Only 21% of patients with biomarker testing had results available at their initial oncology consultation

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

Liquid biopsy components



Liquid biopsy



Analytes

- CTCs
- ctDNAs
- Exosomes
- Platelets
- miRNA
- lncRNA

Sequencing

Considerations

- Single vs Panels
- Coverage
- VAF
- Fusions / amplifications
- TAT
- Quality controls

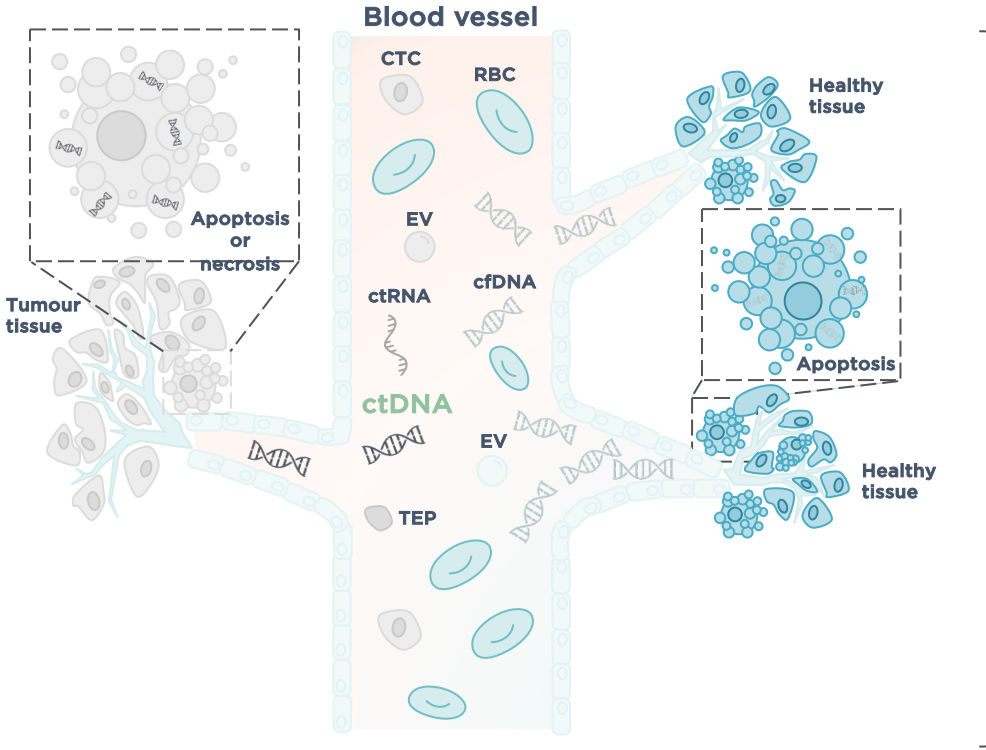
Applications

Early diagnosis	Real-time monitoring
Progression	Drug resistance
Minimal residual disease	Immunotherapy

CTC: circulating tumour cell; ctDNA: circulating tumour DNA; lncRNA: long non-coding RNA; miRNA: microRNA: TAT: turnaround time; VAF: variant allele fractions

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

Liquid biopsy depends upon shed of ctDNA



Circulating tumours cells (CTCs) are shed into circulation from primary tumour and metastases[3,4]

Healthy and tumour tissue release **cell-free DNA and RNA** into circulation through apoptosis, necrosis and lysis of circulating cells. ctDNA comprises the fraction of cfDNA originating from cancer cells[3,5,6]

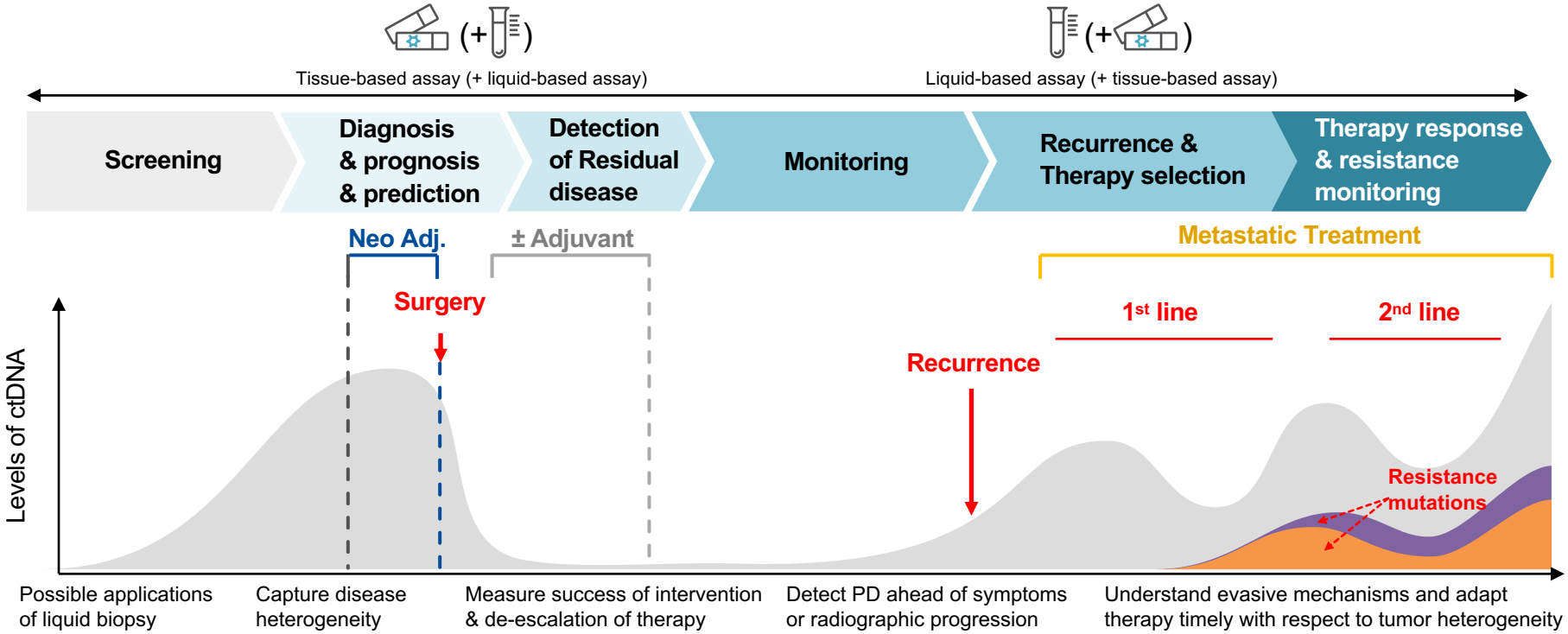
Membrane-encapsulated **extracellular vesicles** (EVs) are released from healthy and tumour cells[3]

Tumour-educated platelets (TEPs) may contain tumour-derived RNA and alternatively spliced transcripts[3]

cfDNA: cell-free DNA; ctDNA: circulating tumour DNA; ctRNA: circulating tumour RNA;
CTC: circulating tumour cell; EV: extracellular vesicle; TEP: tumour-educated platelet; RBC: red blood cell.
Figure adapted from references 1-3.

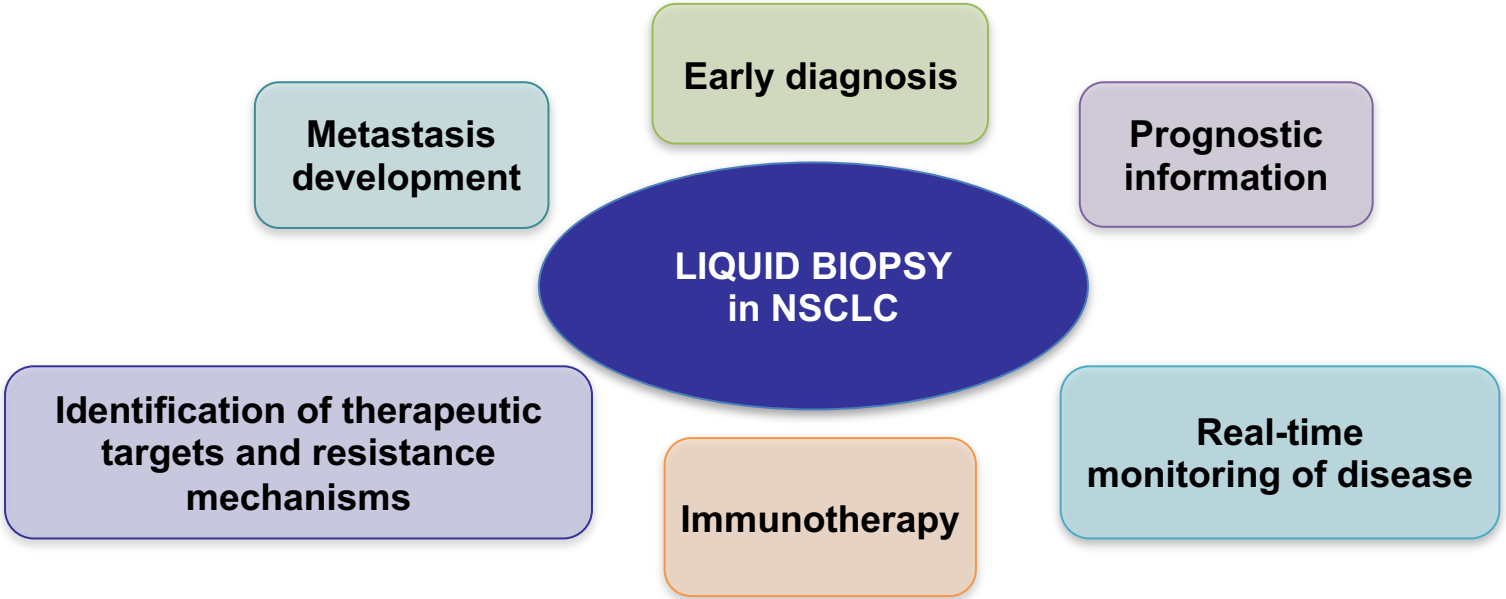
1. Dahl et al. (2015) *Pathologe* 36:572-8; 2. Crowley, E., et al. (2013) *Nat Rev Clin Oncol* 10:472-84;
3. De Rubis, G., et al. (2019) *Trends Pharmacol Sci* 40:172-86; 4. Yu, M., et al. (2011) *J Cell Biol* 192:373-82;
5. Francis, G. & Stein, S. (2015) *Int J Mol Sci* 16:14122-42; 6. Bettegowda, C., et al. (2014) *Sci Transl Med* 6:224ra24.

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 biopsy: Clinical applications in NSCLC



NSCLC: non-small cell lung cancer.

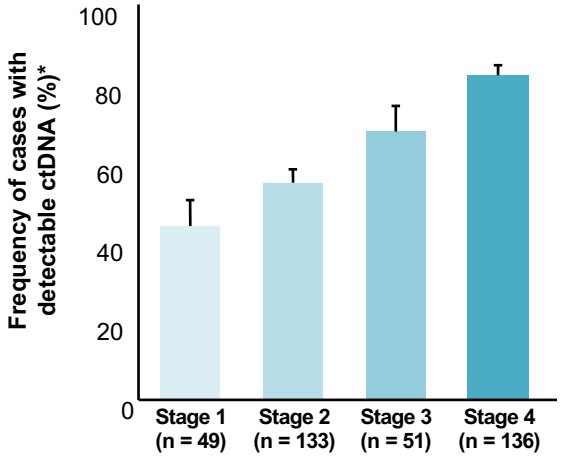
Rolfo, C., et al. (2014) *Biochim Biophys Acta* 1846:539-46; Cabel, L., et al. (2018) *Nat Rev Clin Oncol* 15:639-50.

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]

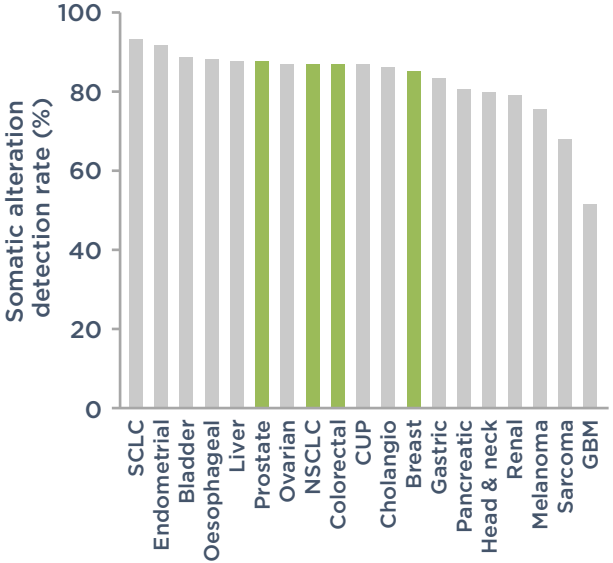


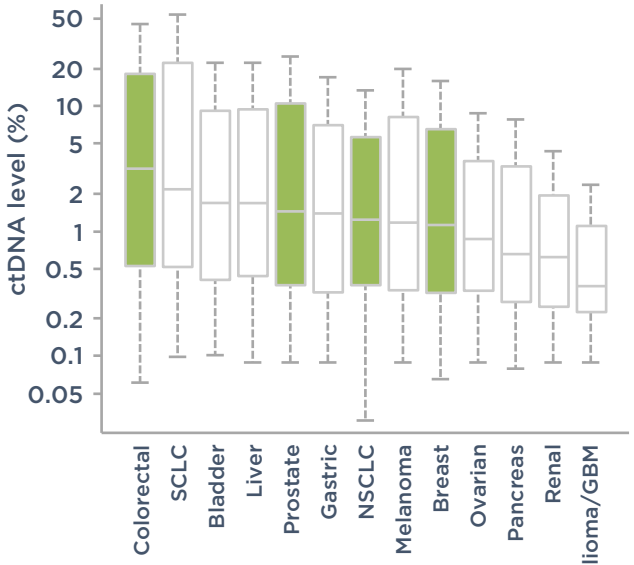
Somatic cfDNA alterations were detected in 85% (18,503 / 21,807) of patients across various cancer types[9]

* 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.

ctDNA is detectable but variable in patients across tumour types





Somatic cfDNA alterations were detected in **85% of patients** (n = 18,503 / 21,807) across all cancer types

CRC had the highest average ctDNA fraction while **pancreas, renal and brain cancers had the lowest**

Alteration-positive samples had **average of 3-4 alterations** including copy number amplifications

Cholangio: cholangiocarcinoma; CRC: colorectal cancer; cfDNA: cell-free DNA; ctDNA: circulating tumour DNA; CUP: carcinoma of unknown primary; GBM: glioblastoma; NSCLC: non-small cell lung cancer; SCLC: small cell lung cancer.

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

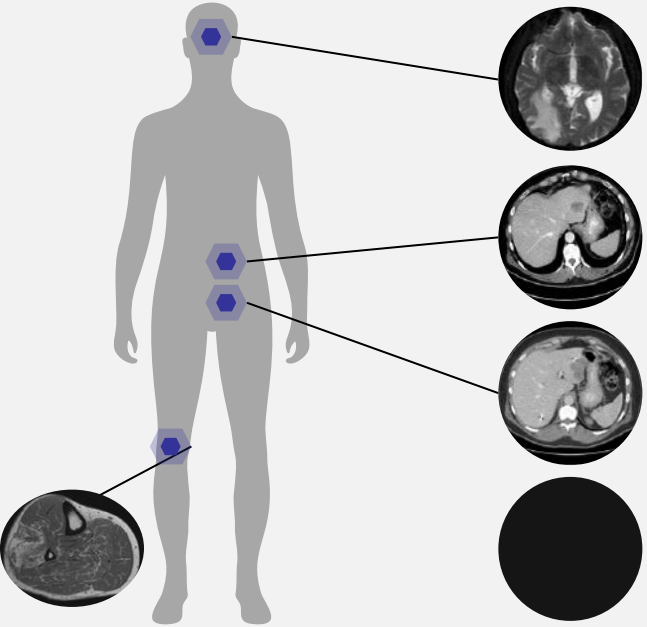
	Blood	Tissue
Strengths	• Less invasive / less morbidity[1,2] • Simpler to obtain / faster results[1,2] • Less biased detection of genomic alterations versus single tissue biopsy site[1,2] • Makes a repeat biopsy more feasible[1,2] • Could allow for real-time monitoring[1,2]	• Remains the standard of 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.

Courtesy G. Oxnard

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 G. Oxnard

Patients who may benefit from liquid biopsy

1

Patients in whom traditional biopsy is inaccessible or impractical

- Anatomically inaccessible / unacceptable risk[1,2]
- Settings where tissue biopsy results may be delayed[1]

2

Patients in whom traditional biopsy is insufficient

- Tissue exhausted by other pathology analyses[1]
- Sample inadequate for successful molecular testing (few tumour cells or inflamed, fibrotic and necrotic tissue)[3,4]

3

Patients who have disease progression or relapse on targeted therapies

- Detection of suspected resistance mutations[1,2]
- To consider new therapy options including clinical trials[1]

1. Rolfo, C., et al. (2018) *J Thorac Oncol* 13:1248-68; 2. Francis, G. and Stein, S. (2015) *Int J Mol Sci* 16:14122-42;
3. Stevenson, M., et al. (2014) *Cancer Invest* 32:291–8; 4. Al-Kateb, H., et al. (2015) *Mol Oncol* 9:1737-43.

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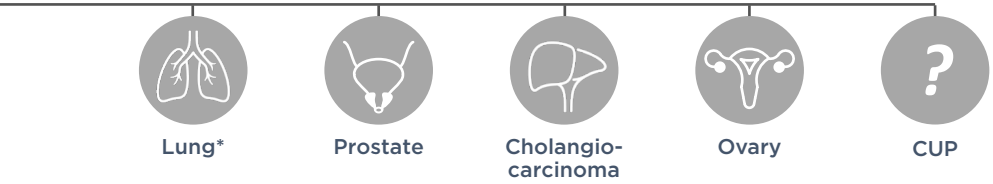
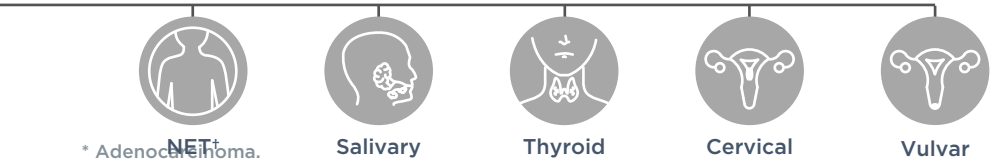
The ESMO Precision Medicine Working Group recommend NGS testing for a range of solid tumours in daily practice

*Multigene NGS testing **recommended***



*Multigene NGS testing **may be considered**, taking into account relative cost*

***TMB testing recommended** (pending drug access)*



> " It is highly recommended that **clinical research centres perform multigene sequencing** in the context of molecular screening programmes in order to increase access to innovative drugs and to speed-up clinical research. This is particularly relevant in **breast, pancreatic and hepatocellular cancers** [...]"
>
> ESMO Precision Medicine Working Group

* Adenocarcinoma.
† Well-to-moderately differentiated.
CUP: cancer of unknown primary; NET: neuroendocrine tumour; NGS: next-generation sequencing; TMB: tumour mutational burden.
Mosele, F., et al. (2020) *Ann Oncol* doi.org/10.1016/j.annonc.2020.07.014 [Epub ahead of print].

Courtesy G. Oxnard

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]

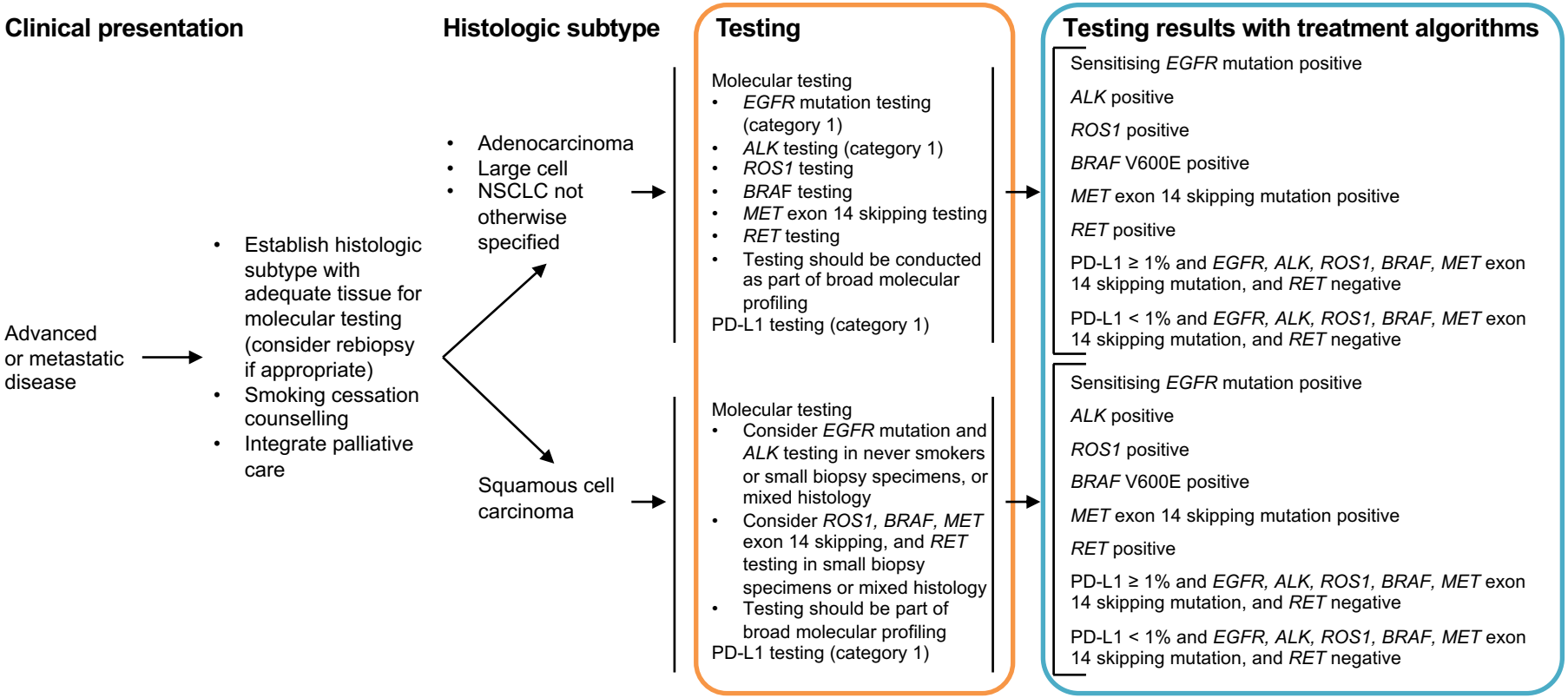
"Key new recommendations include […] the inclusion of additional genes (*ERBB2*, *MET*, *BRAF*, *KRAS*, and *RET*) […] and the use of cell-free DNA to "rule in" targetable mutations when tissue is limited or hard to obtain"

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.

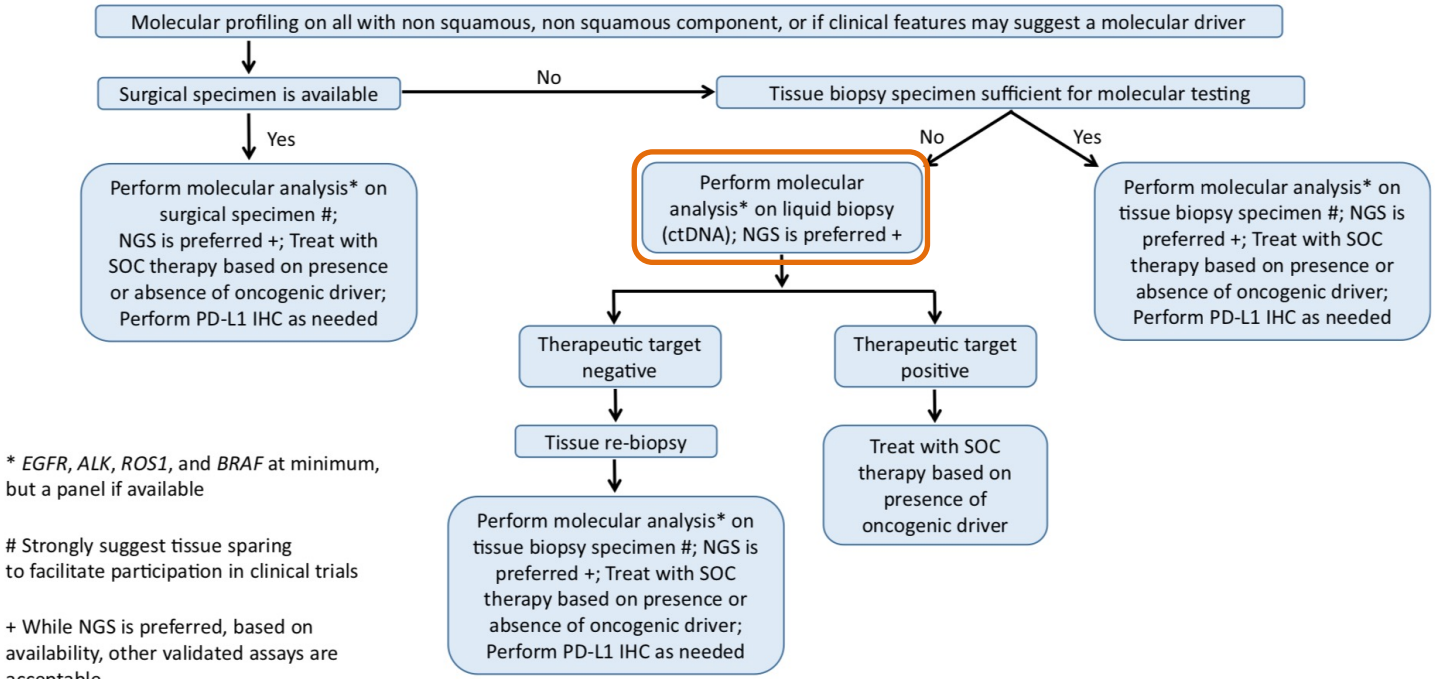
1. NCCN Clinical Practice Guidelines in Oncology: NSCLC (Version 2.2020); 2. Burstein, H.J., et al. (2017) *J Clin Oncol* 35:1341-67;
3. Lindeman, N.I., et al. (2018) *J Thorac Oncol* 5:323-7.

NCCN Guidelines Version 6.2020 NSCLC



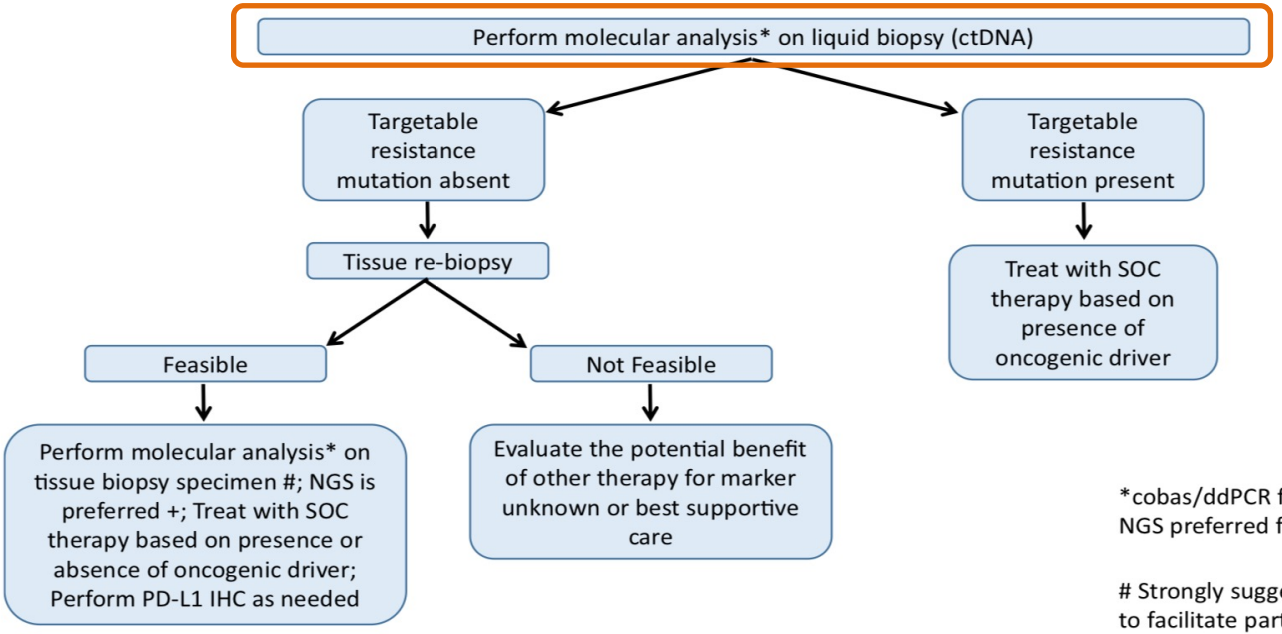
NCCN Clinical Practice Guidelines in Oncology: NSCLC (Version 2.2020).

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

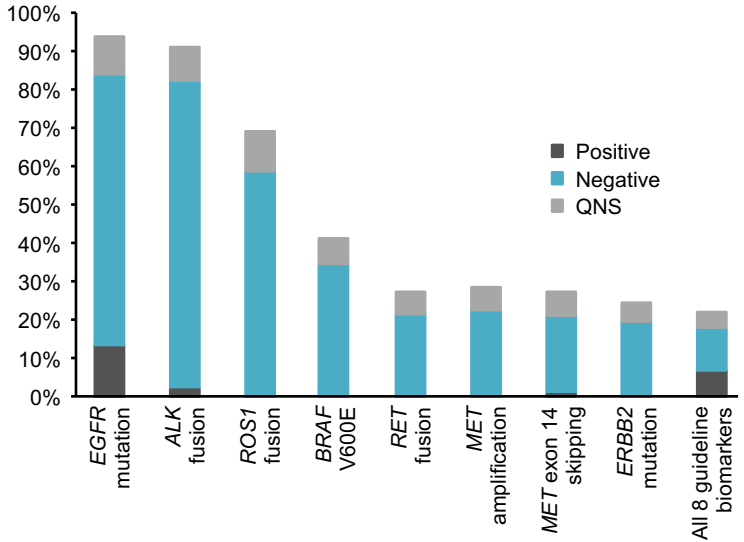
+ While NGS is preferred, based on availability, other validated assays are acceptable

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

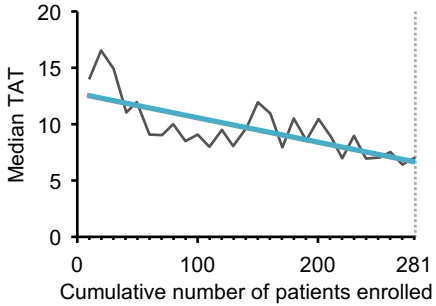
Only 18% of patients had complete tissue genotyping for all 8 guideline-recommended genomic biomarkers



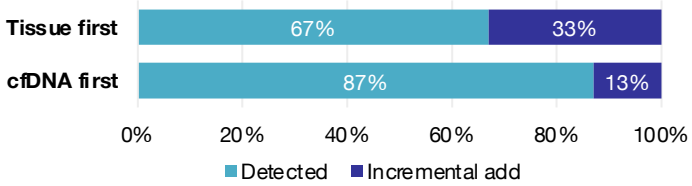
One of eight guideline-recommended biomarkers was identified in 21.3% patients using tissue vs. 27.3% using cfDNA (n = 282; p<0.0001 for non-inferiority)

- **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%

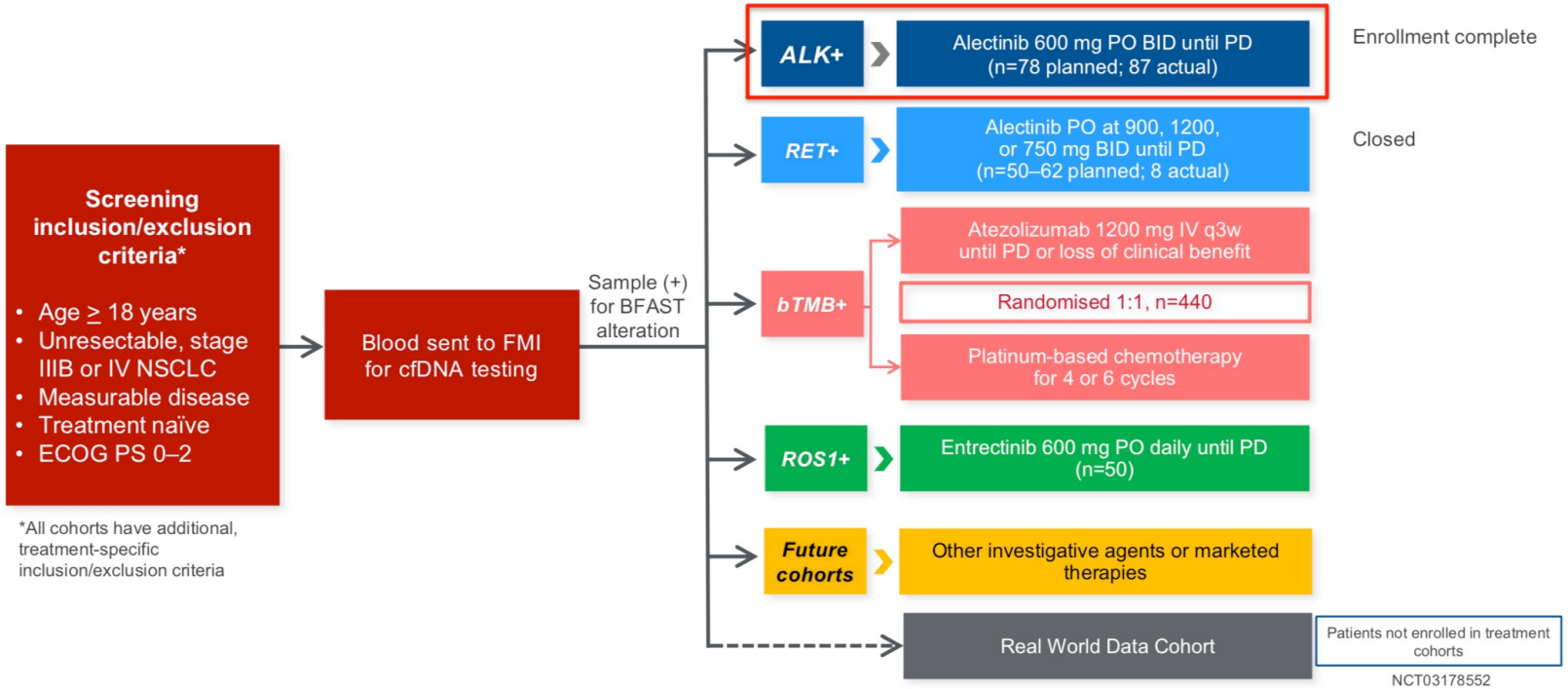


Blood first?

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.

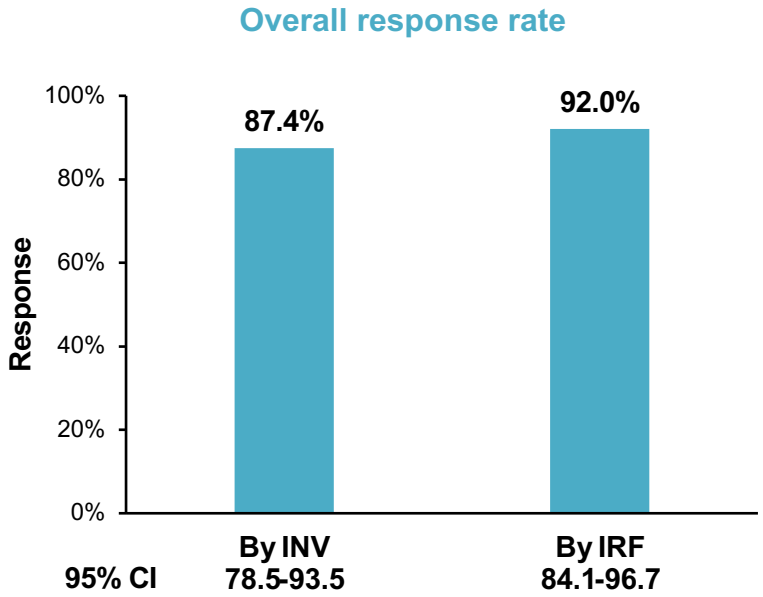
Phase II/III BFAST trial in treatment-naive NSCLC: Initial results from the *ALK*+ cohort



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



Median duration of follow-up: 12.58 months

	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)

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.

VISION
Phase 2 trial

Single-arm study of tepotinib in stage IIIB/IV NSCLC (all histologies) with *MET* ex14 skipping mutations (Cohort A)

First-, second- and third-line therapy patients included, unless prior anti-MET therapy was used

Patients with active brain metastases excluded

Tepotinib in NSCLC with *MET* exon 14 skipping mutations (*MET* ex14)

***MET* ex14 positive by:**	**Liquid biopsy** (n=48)	**Tissue biopsy** (n=51)
Best overall response by RECIST 1.1 (independent review committee), n (%)		
Complete response	0 (0)	0 (0)
Partial response	24 (50.0)	23 (45.1)
Stable disease	8 (16.7)	14 (27.5)
Progressive disease	7 (14.6)	8 (15.7)
Not evaluable	9 (18.8)	6 (11.8)
ORR* n (%) [95% CI]	24 (**50.0**) [35.2, 64.8]	23 (**45.1**) [31.1, 59.7]

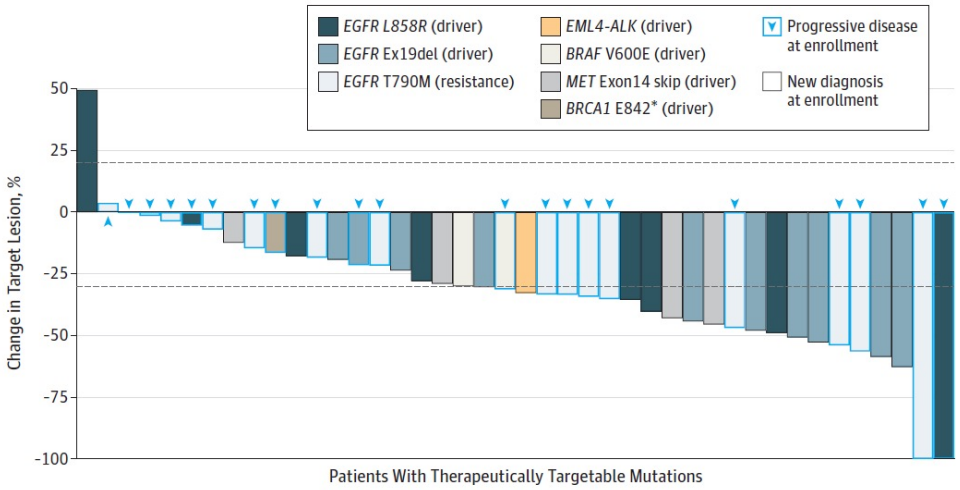
*ORR = Complete response + partial response

CI: confidence interval;
NSCLC: non-small cell lung cancer; ORR: overall response rate.
Paik, P.K., et al. (2019) Slide presentation at ASCO 2019:abstract 9005.

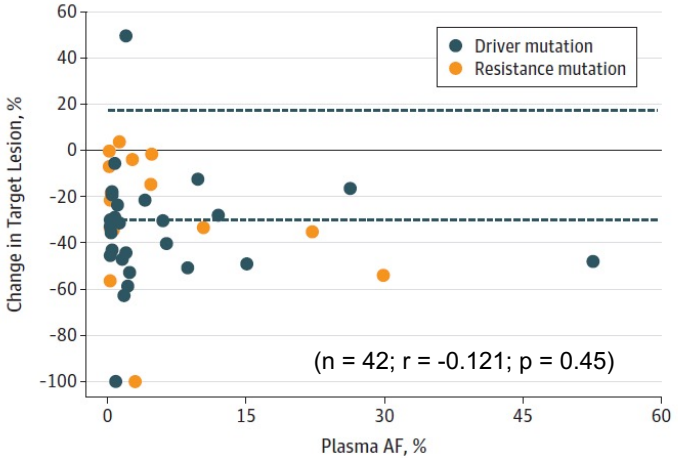
Courtesy G. Oxnard

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

Responses to plasma-indicated targeted therapy by RECIST



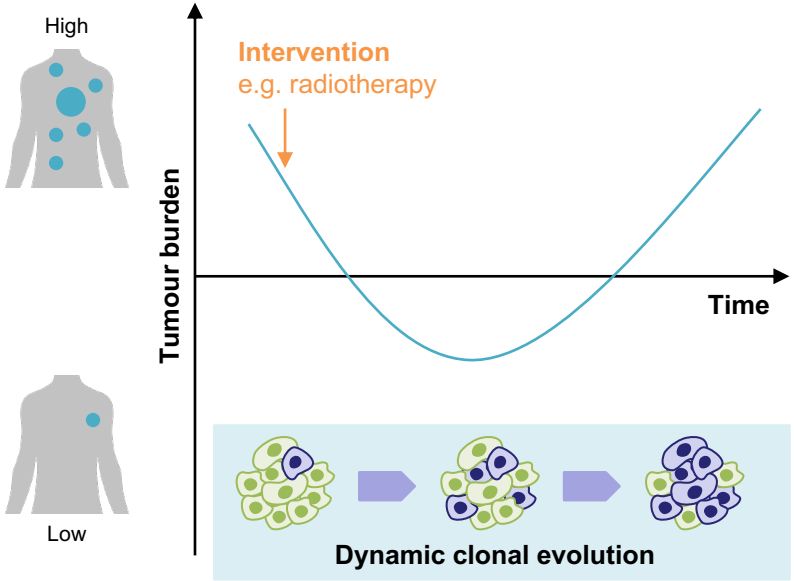
Correlation of RECIST and allele frequency



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]

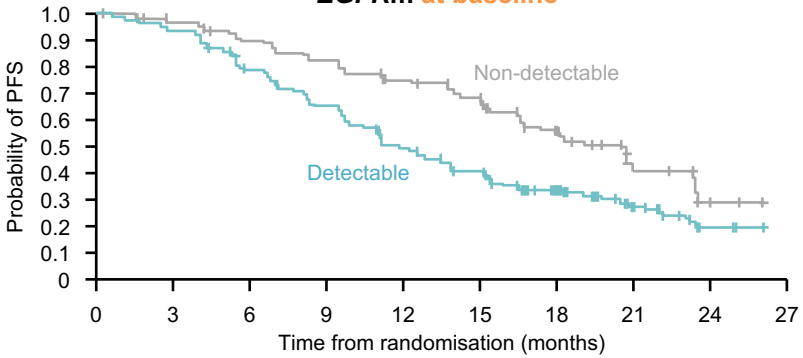


Increasing number of metastatic sites (p = 0.001) and presence of bone (p = 0.007) and hepatic (p = 0.001) metastases significantly associated with assay sensitivity[2]

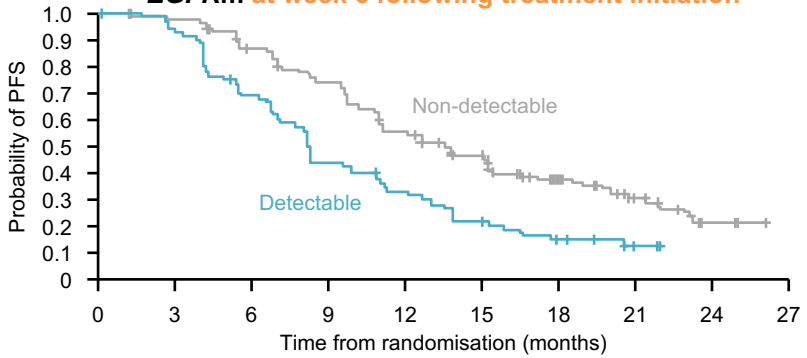
1. Pisapia, P., et al. (2017) in *Liquid Biopsy in Cancer Patients – Clinical Practice Implications: Monitoring Drug Response and Resistance*. Springer;
2. Sacher, A.G., et al. (2016) *JAMA Oncol* 2:1014-22.

FLAURA study: Early clearance of plasma *EGFR* mutation predicts PFS on EGFR-targeted therapies

PFS based on detection of plasma *EGFR*m at baseline

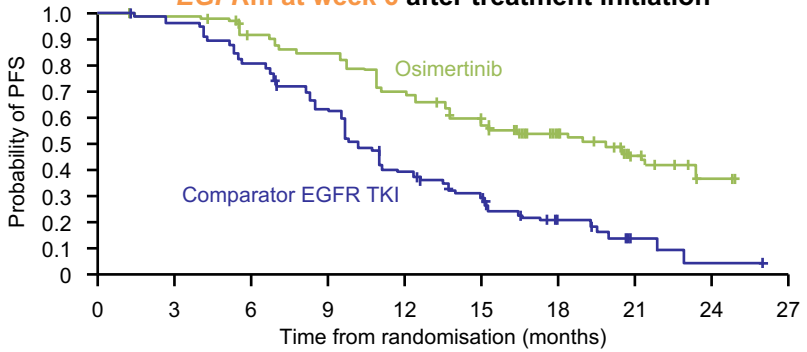


PFS based on detection of plasma *EGFR*m at week 6 following treatment initiation



- FLAURA analysis confirms prior studies showing that **presence of *EGFR* mutation in ctDNA** at baseline is a **poor prognostic factor**
- Patients with **plasma *EGFR* mutation clearance** have **improved PFS**

PFS in patients with clearance of plasma *EGFR*m at week 6 after treatment initiation



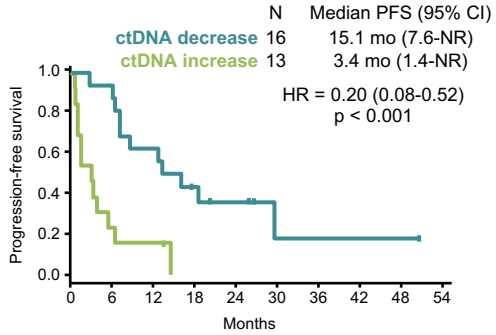
ctDNA: circulating tumour DNA; *EGFR*m: *EGFR* mutation;
PFS: progression-free survival; TKI: tyrosine kinase inhibitor.
Zhou C. et al (2019) Poster presentation at ASCO 2019:abstract 9020.

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

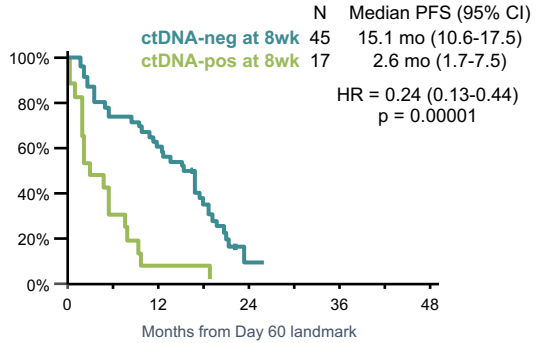


Rapid **decrease of ctDNA** correlated with **clinical benefit**, while **increase** correlated with **PD**

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.

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

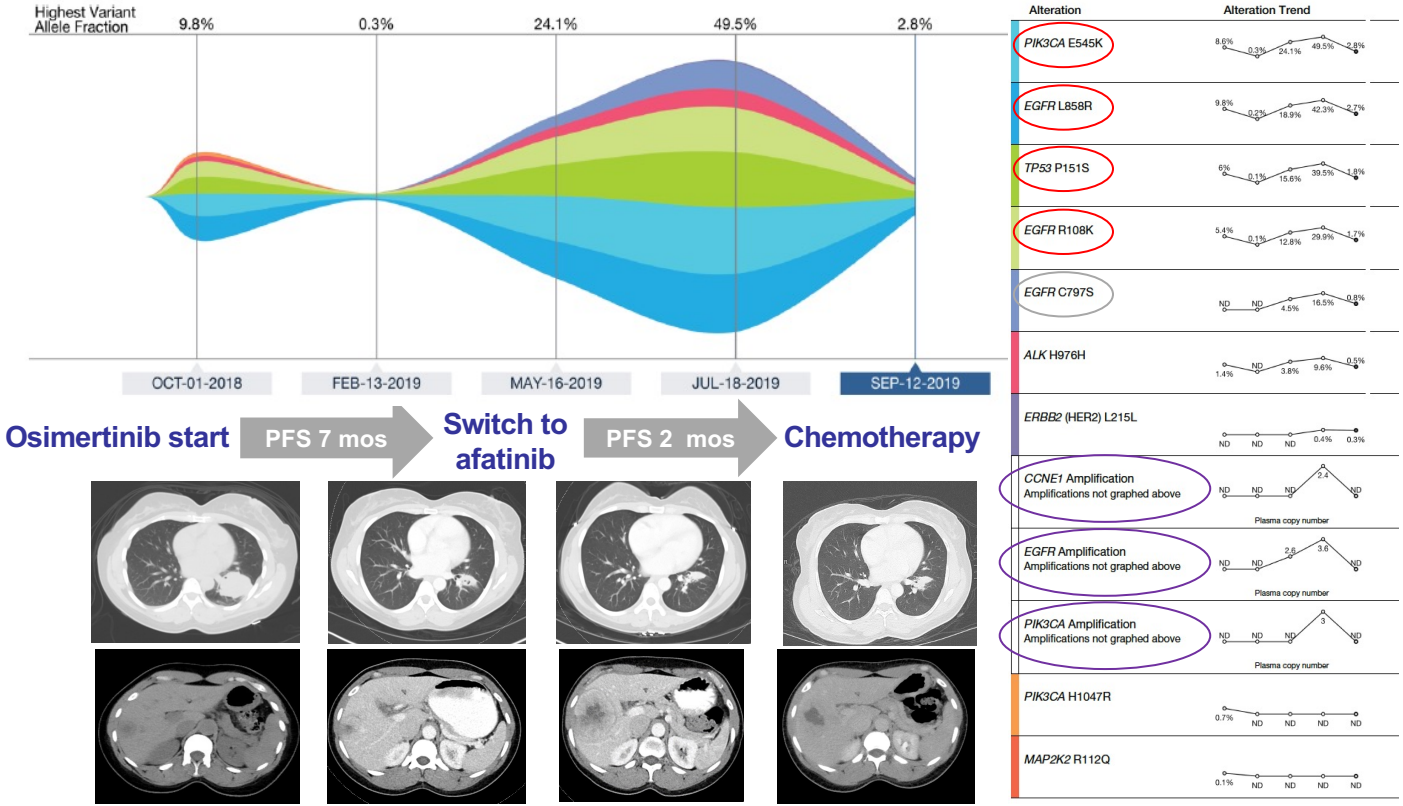
The genomic landscape may **differ between tumour sites** within a patient

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.

Case #1: 47 year old female with NSCLC

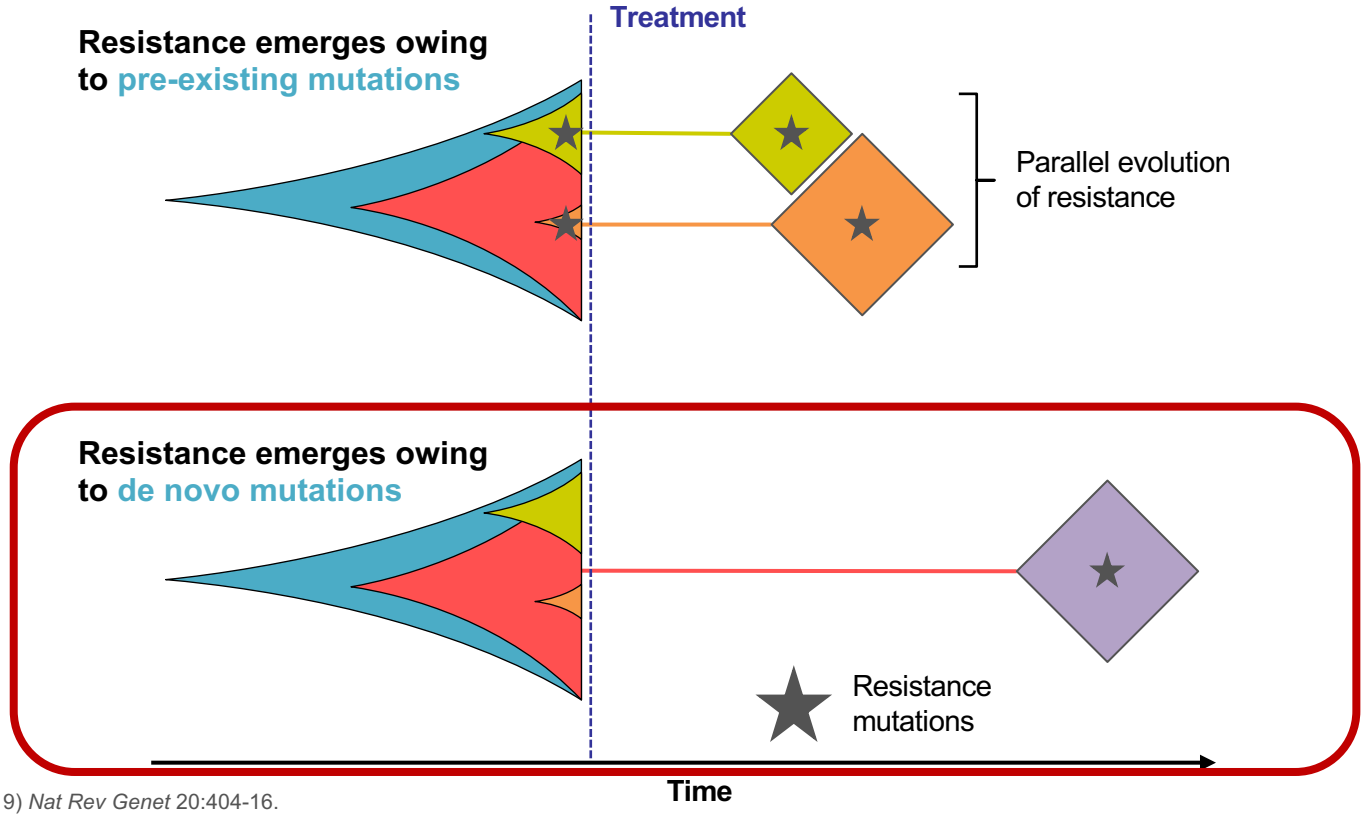


NSCLC: non-small cell lung cancer; PFS: progression-free survival.

Malapelle, U. and Rolfo, C. (2020) *Cancer* 126:22-5.

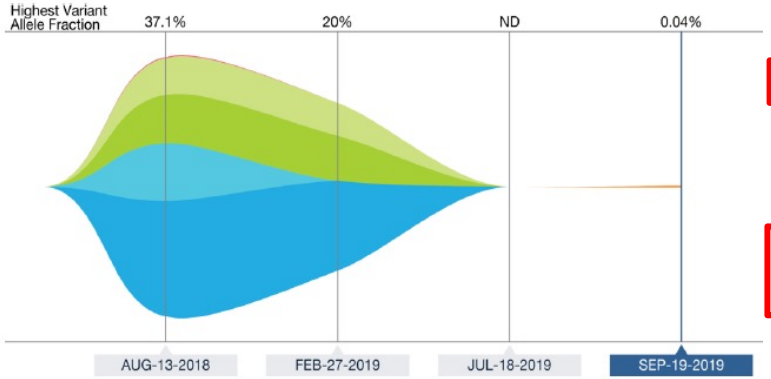
Updated case data courtesy of Dr Rolfo, University of Maryland School of Medicine.

Clonal evolution of treatment resistance



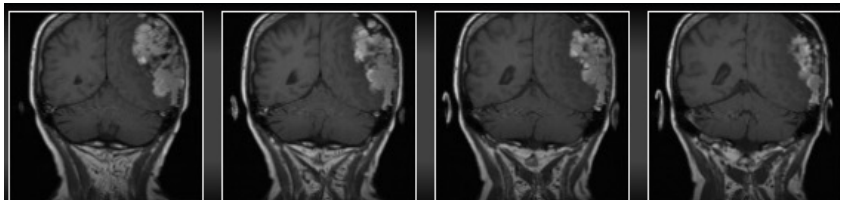
Turajlic S, et al. (2019) *Nat Rev Genet* 20:404-16.

Case #2: 71 year old NSCLC patient



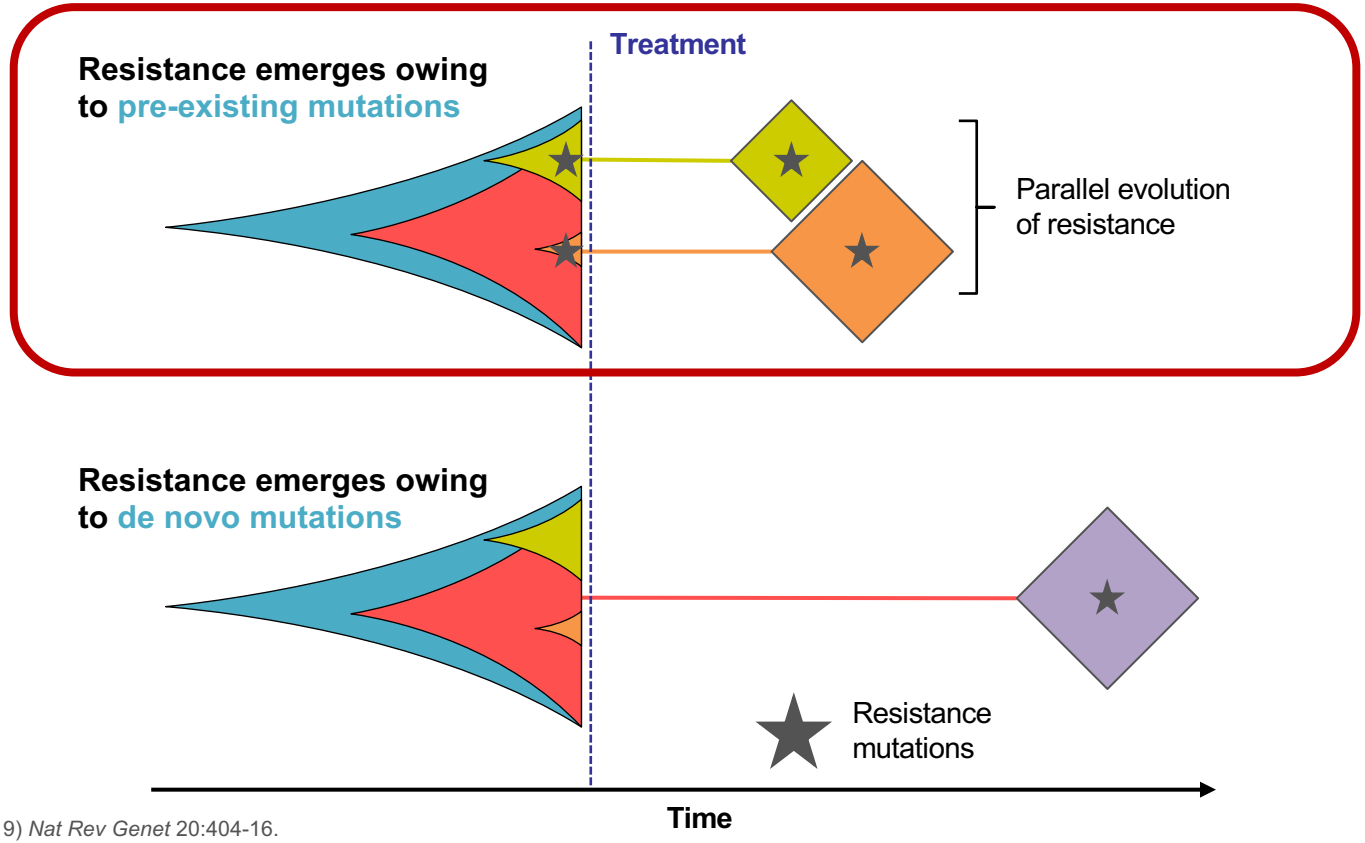
	Aug 18	Feb 19	Jul 19	Sep 19
EGFR L858R	37.1%	20.0%	ND	ND
EGFR T790M	15.8%	ND	ND	ND
TP53 Splice Site SNV	12.5%	8.4%	ND	ND
BRCA1 D2N	8.9%	5.7%	ND	ND
TP53 V272M	0.1%	ND	ND	ND
EGFR Amplification	++	+	ND	ND
MET Amplification	++	+++	**ND**	**ND**
AR Amplification	+	ND	ND	ND

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.

TRITON-2*
Phase 2 trial

Single arm study of rucaparib in mCRPC with deleterious germline or somatic *BRCA1/2* and other pre-specified DDR gene alterations

Central NGS screening of tumour tissue or plasma (Foundation Medicine testing*)

Disease progression on AR-directed therapy and 1 prior taxane-based chemotherapy

Rucaparib in men with mCRPC and *BRCA1/2* alterations

Characteristics, n (%)	Gene		All (n=45)
	BRCA1 (n = 5)	*BRCA2* (n = 40)	
Gene alteration status			
Germline	2 (40.0)	13 (32.5)	15 (33.3)
Somatic	3 (60.0)	27 (67.5)	30 (66.7)
Types of *BRCA1/2* alterations			
Frameshift	NR	NR	22 (48.8)
Homozygous loss	NR	NR	12 (26.6)

Efficacy, n/N (%)	*BRCA1/2*		All
	Germline	Somatic	
Confirmed investigator-assessed objective response	5 / 10 (50.0)	6 / 15 (40.0)	11 / 25 (**44.0**) (95% CI, 24.4-65.1)
Confirmed PSA response	10 / 15 (66.7)	13 / 30 (43.3)	23 / 45 (**51.1**) (95% CI, 35.8-66.3)

* This study used previous versions of FoundationOne®CDx and FoundationOne®Liquid CDx.

DDR: DNA damage repair; mCRPC: metastatic castration resistant prostate cancer; NGS: next-generation sequencing; NR: not reported; PSA: prostate-specific antigen.

Abida, W., et al. (2019) *Ann Oncol* 30 (suppl):v327-8; Abida, W., et al. (2019) Poster presentation at ASCO 2019:abstract 5031.

Courtesy G. Oxnard

SOLAR-1

Phase 3 trial

Alpelisib + fulvestrant
vs
placebo + fulvestrant
in advanced HR-positive / HER2-negative breast cancer with *PIK3CA* mutations

PIK3CA testing by hotspot PCR in exons 7, 9, and 20

Disease progression on / after aromatase inhibitor therapy, but no prior chemotherapy or PI3K pathway-targeted therapy

CI: confidence interval; ctDNA: circulating tumour DNA; HR: hormone receptor; PCR: polymerase chain reaction; PFS: progression-free survival.
André, F., et al. (2019) *N Engl J Med* 380:1929-40; Juric, D., et al. (2018) Slide presentation at SABCS 2018:abstract GS3-08.

Alpelisib in HR-positive / HER2-negative breast cancer with *PIK3CA* mutations

	Fulvestrant + Alpelisib		Fulvestrant + Placebo		Hazard ratio
	Event n/N (%)	Median PFS*	Event n/N (%)	Median PFS*	
PIK3CA-altered by **tissue biopsy**[1]	103 / 169 (60.9)	11.0	129 / 172 (75.0)	5.7	**0.65**
PIK3CA-altered by **liquid biopsy**[2]	57 / 92 (62.0)	10.9	75 / 94 (79.8)	3.7	**0.55**

* Primary endpoint, in months

Improved PFS on alpelisib was seen in patients with PIK3CA mutations in ctDNA, similar to those with PIK3CA mutations in tissue

Courtesy G. Oxnard

BEACON

Phase 3 trial

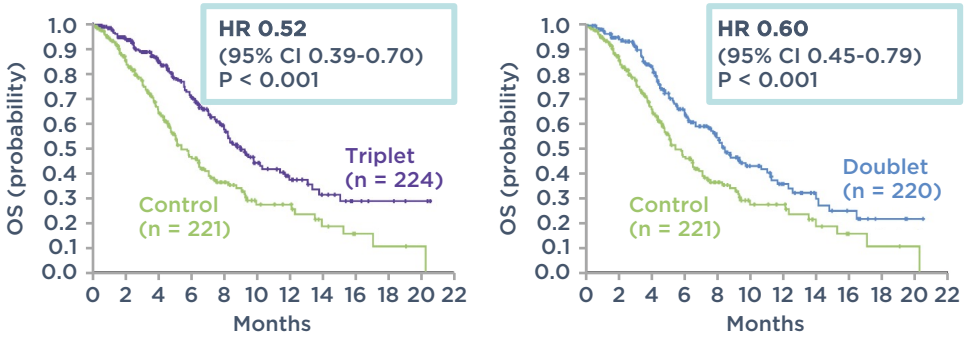
Encorafenib + binimetinib + cetuximab (triplet regimen) *or* encorafenib + cetuximab (doublet regimen)
vs
cetuximab + irinotecan *or* cetuximab + FOLFIRI (investigator's choice)
in *BRAF* V600E mCRC

Disease progression after 1-2 prior regimens

CI: confidence interval; FOLFIRI: folinic acid, fluorouracil, and irinotecan; mCRC: metastatic colorectal cancer; mo: months; ORR: objective response rate; OS: overall survival.

1. Kopetz, S., et al. (2019) *N Engl J Med* 381:1632-43;
2. Kato, S., et al. (2019) *JCO Precis Oncol* doi:10.1200/PO.18.00158.

Encorafenib + binimetinib + cetuximab in *BRAF* V600E mCRC[1]



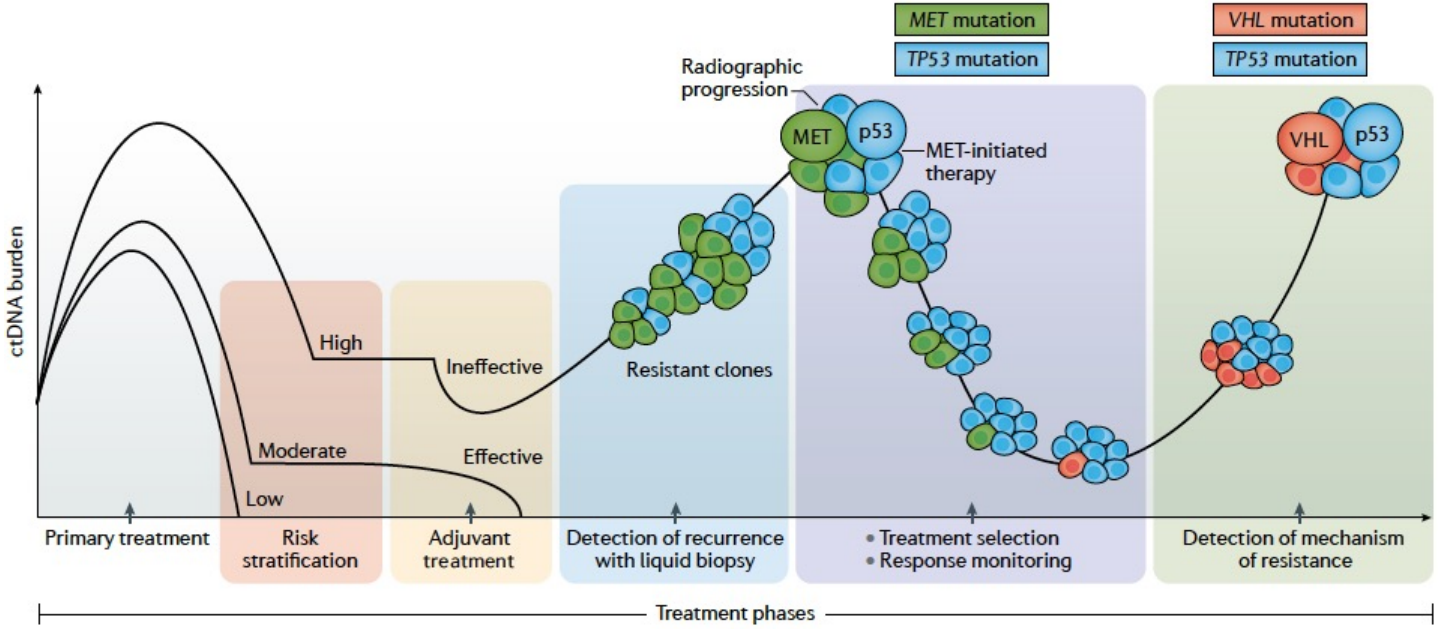
BRAF mutations are important targets in mCRC – liquid biopsy detects more BRAF mutations than tissue, with high concordance overall[2]

BRAF mutations* (n = 76)	Positive in tissue	Negative in tissue	Concordance
Positive in ctDNA	8	9	85.5%
Negative in ctDNA	2	57	

* Based on Kato et al. (2019); data not from BEACON.

Courtesy G. Oxnard

Clinical applicability of ctDNA across genitourinary malignancies



Caitano Maia M, et al. Nat Rev Urol 2020

August 2020

FDA Approves Two Liquid Biopsy Tests

Companion Diagnostic Labels for Guardant360 CDx and FoundationOne Liquid CDx			
Blood Test	**Cancer Type**	**Genetic Change**	**Corresponding Drug**
Guardant360 CDx	Non-small cell lung cancer	*EGFR* exon 19 deletions L858R mutation T790M mutation	Osimertinib
FoundationOne Liquid CDx	Non-small cell lung cancer	*EGFR* exon 19 deletions L858R mutation	Osimertinib Gefitinib Erlotinib
FoundationOne Liquid CDx	Prostate cancer	*BRCA1* and *BRCA2* alterations	Rucaparib

Novel FDA approved indications in multiple solid tumor:

FDA approves liquid biopsy NGS companion diagnostic test for multiple cancers and biomarkers

On October 26 and November 6, 2020, the Food and Drug Administration approved the liquid biopsy next-generation sequencing-based FoundationOne Liquid CDx test (Foundation Medicine, Inc.) as a companion diagnostic device for multiple additional biomarkers detected in cell free-DNA isolated from plasma specimens.

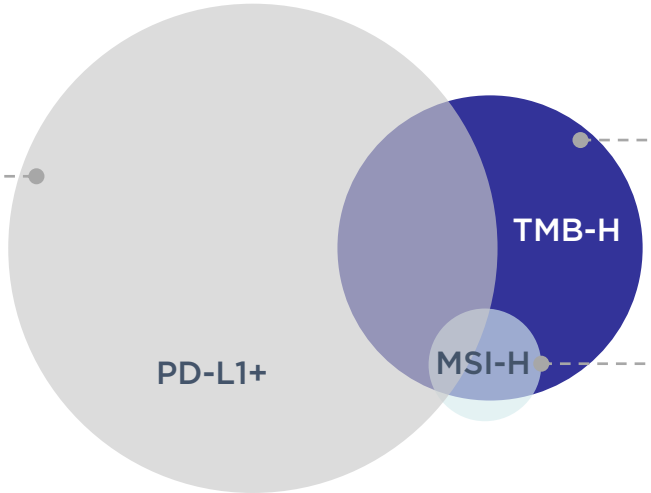
The companion diagnostic indications in the October 26 approval are 1) to identify mutations in *BRCA1* and *BRCA2* genes in patients with ovarian cancer eligible for treatment with rucaparib (RUBRACA, Clovis Oncology, Inc.), 2) to identify *ALK* rearrangements in patients with non-small cell lung cancer (NSCLC) eligible for treatment with alectinib (ALECENSA, Genentech USA, Inc). and 3) to identify mutations in the *PIK3CA* gene in patients with breast cancer eligible for treatment with alpelisib (PIQRAY, Novartis Pharmaceutical Corporation).

On November 6, FDA approved the FoundationOne Liquid CDx test as a companion diagnostic device to identify mutations in *BRCA1, BRCA2* and *ATM* genes in patients with metastatic castration resistance prostate cancer (mCRPC) eligible for treatment with olaparib (LYNPARZA, AstraZeneca Pharmaceuticals LP).

The list of predictive biomarkers is expanding

Complementary biomarkers that may indicate eligibility for immunotherapy across cancer types[1-4]

PD-L1 expression by IHC is an approved biomarker (on-label) for several indications and may inform eligibility for immunotherapies across cancer types*[,5,6]



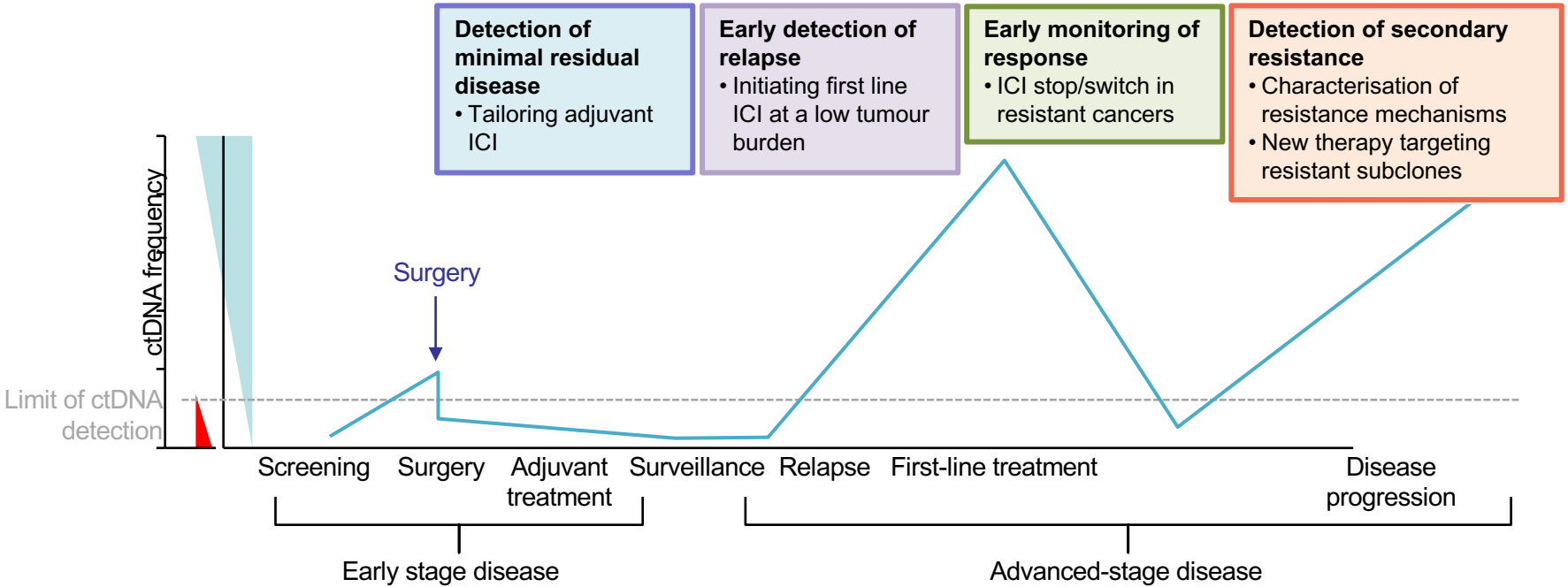
Tumour mutational burden (TMB) acts as a proxy for neoantigen load to allow more informed immunotherapy use[5,6]

Microsatellite instability (MSI) is characterised by high rates of alterations to repetitive DNA sequences and has emerged as a surrogate for increased TMB[5,7]

* "may inform" refers to complementary Dx status.
GEP: gene expression profile; IHC: immunohistochemistry; MSI(-H): (high) microsatellite instability;
PD-L1: programmed cell death ligand 1; TMB(-H): (high) tumour mutational burden.
1. Vanderwalde, A., et al. (2018) *Cancer Med* 7:746-56; 2. Mariathasan, S., et al. (2018) *Nature* 554:544-8;
3. Cristescu, R., et al. (2018) *Science* 362:eaar3593; 4. Ott, P.A., et al. (2018) *J Clin Oncol* 37:318-27; 5. Foundation Medicine website.
FoundationOne CDx. Available at: https://www.foundationmedicine.com/genomic-testing/foundation-one-cdx (Accessed August 2020);
6. Merino, D.M., et al. (2020) *J Immunother Cancer* 8: e000147; 7. Boland, C.R. and Goel, A. (2010) *Gastroenterology* 138:2073-87.

Courtesy G. Oxnard

Clinical application of liquid biopsy in immunotherapy

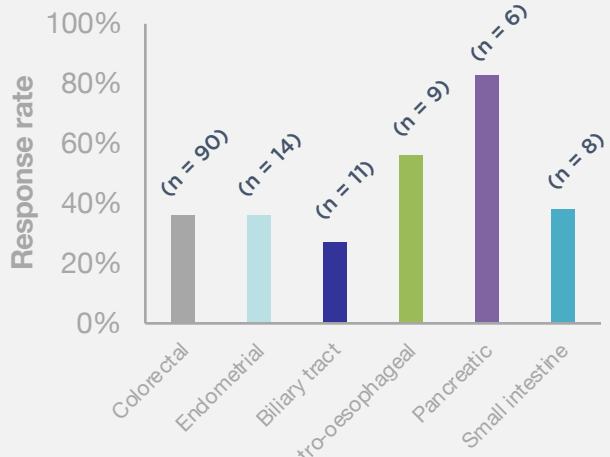


ctDNA: circulating tumour DNA; ICI: immune checkpoint inhibitor.
Cabel, L., et al. (2018) *Nat Rev Clin Oncol* 15:639-50.

MSI-High tumours show high response to immune checkpoint inhibitors

Data led to tumour-agnostic approval of pembrolizumab[1]

Response to pembrolizumab in MSI-H / dMMR cancers by tumour type[2]



Other tumour types	
Breast (n = 2)	PR, PR
Prostate (n = 2)	PR, SD
Bladder (n = 1)	NE
Sarcoma (n = 1)	PD
Thyroid (n = 1)	NE
Retroperitoneal adenocarcinoma (n = 1)	PR
Small cell lung (n = 1)	CR
Renal cell (n = 1)	PD
Oesophageal (n = 1)	PR

Phase 2 trial of pembrolizumab in 86 patients, comprising 12 tumour types, with dMMR tumours[3]

Objective response rate: 53%

2-year overall survival rate: 64%

Tumours with inherited or acquired defects in DNA repair acquire a higher number of somatic mutations, which is thought to lead to higher immunogenicity

CR: complete response; CUP: cancer of unknown primary; dMMR: deficient mismatch repair; FDA: US Food and Drug Administration;

MSI-H: microsatellite instability high; NE: not evaluable; PD: progressive disease; PR: partial response; SD: stable disease.

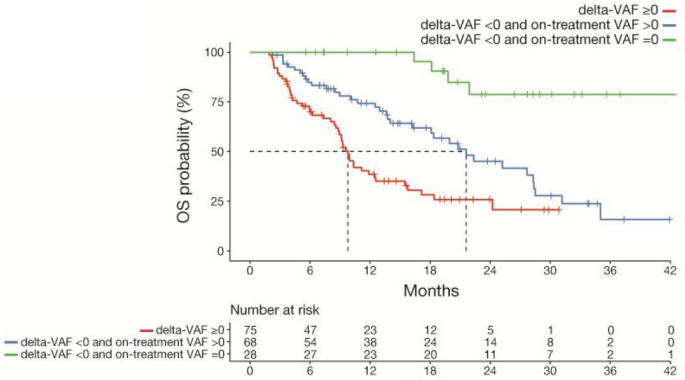
1. FDA website (2017) Available at: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-pembrolizumab-first-tissuesite-agnostic-indication (Accessed August 2020); 2. Pembrolizumab prescribing information (2020) Available at: https://www.merck.com/product/usa/pi_circulars/k/keytruda/keytruda_pi.pdf (Accessed August 2020);

3. Le, D.T., et al. (2017) *Science* 357:409-13.

Courtesy G. Oxnard

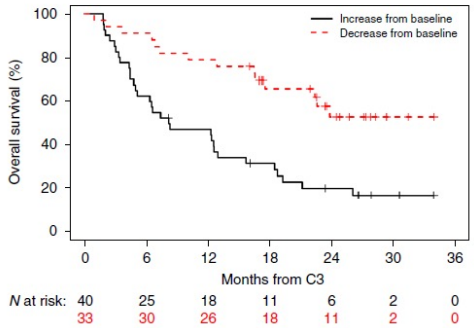
“ctDNA dynamics”: On-treatment change in ctDNA predicts OS on immunotherapy in advanced cancers

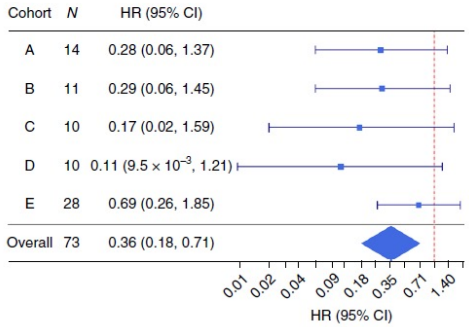
On-treatment changes in **ctDNA VAF** across 16 advanced tumour types in three phase I/II trials of durvalumab (± tremelimumab)[1]



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

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



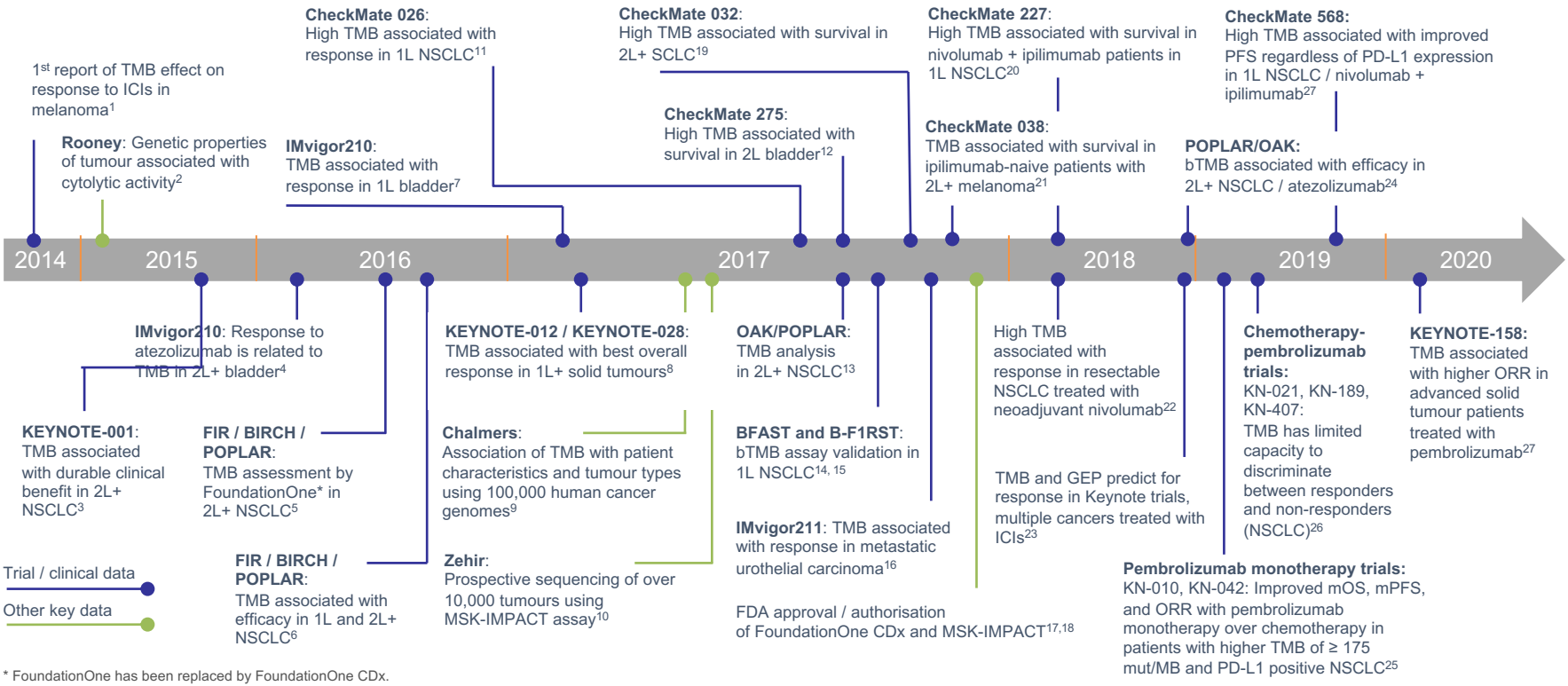


Baseline ctDNA concentration correlates with PFS, OS, clinical response and clinical benefit. This association becomes stronger when considering ctDNA kinetics during treatment[2]

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].

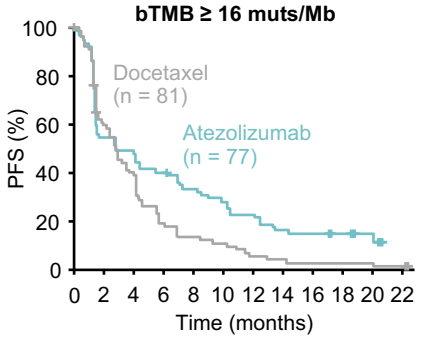
Evolution of TMB as an immunotherapy biomarker over the last several years



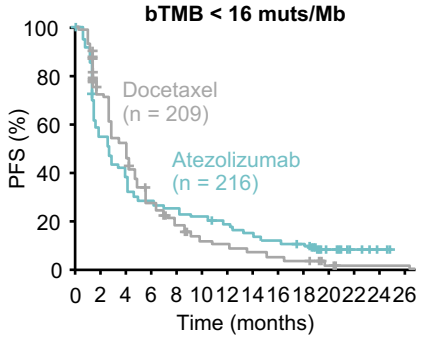
* FoundationOne has been replaced by FoundationOne CDx.
1L: first line; 2L: second line; +: including others; CDx: companion diagnostic; FDA: Food and Drug Administration; GEP: gene expression profile;
ICI: immune checkpoint inhibitor; NSCLC: non-small cell lung cancer; ORR: objective response rate, 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)

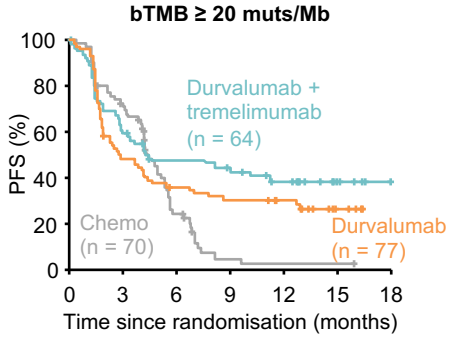


A vs Do:
HR 0.65 (0.47-0.92)

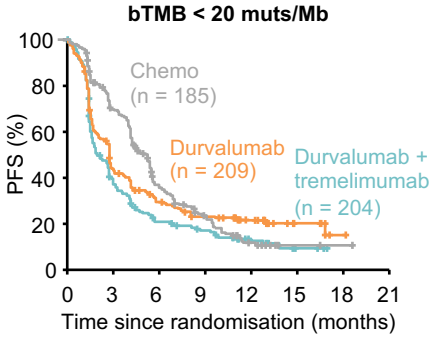


A vs Do:
HR 0.98 (0.80-1.20)

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)

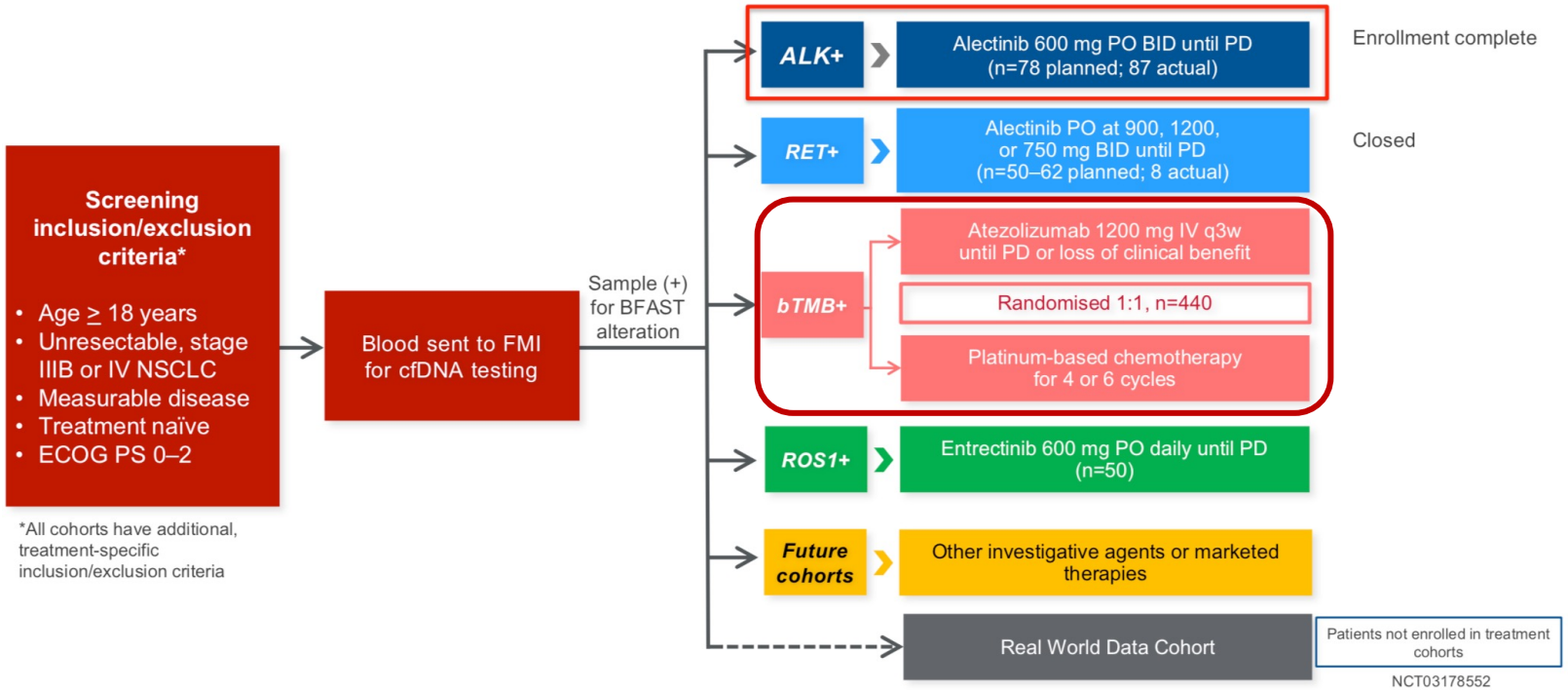


D vs Ch:
HR 1.19 (0.94-1.50)
D + T vs Ch:
HR 1.55 (1.23-1.94)
D + T vs D:
HR 1.26 (1.02-1.57)

High bTMB scores in both OAK and MYSTIC trials were associated with improved PFS and also 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

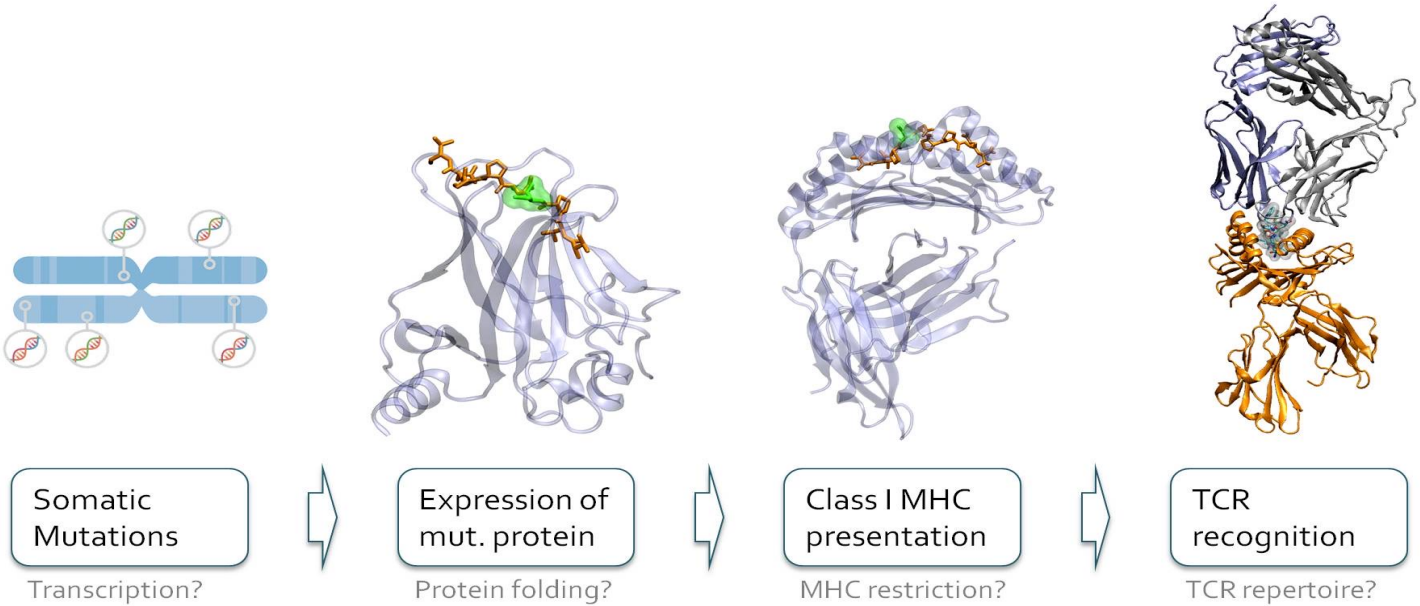
We need to take more responsibility in trial design using TMB
and promote more academic research efforts rather than industry-driven research

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?

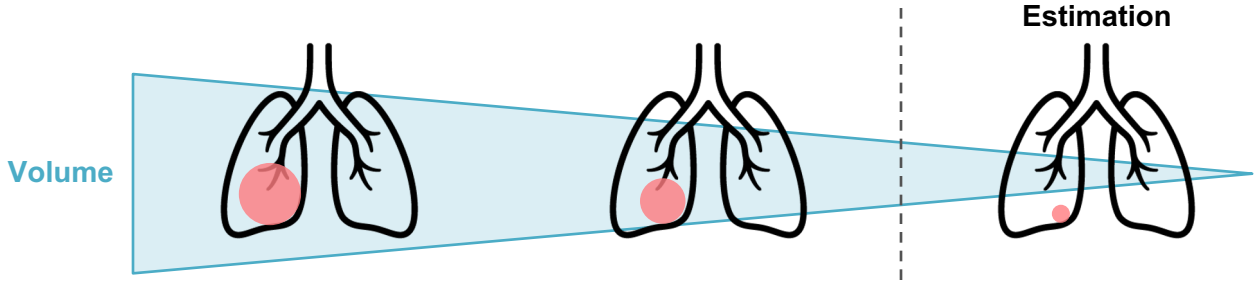
Only a minority of mutations produce neoantigens



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.

Mutant allele frequency (MAF) in early stage NSCLC

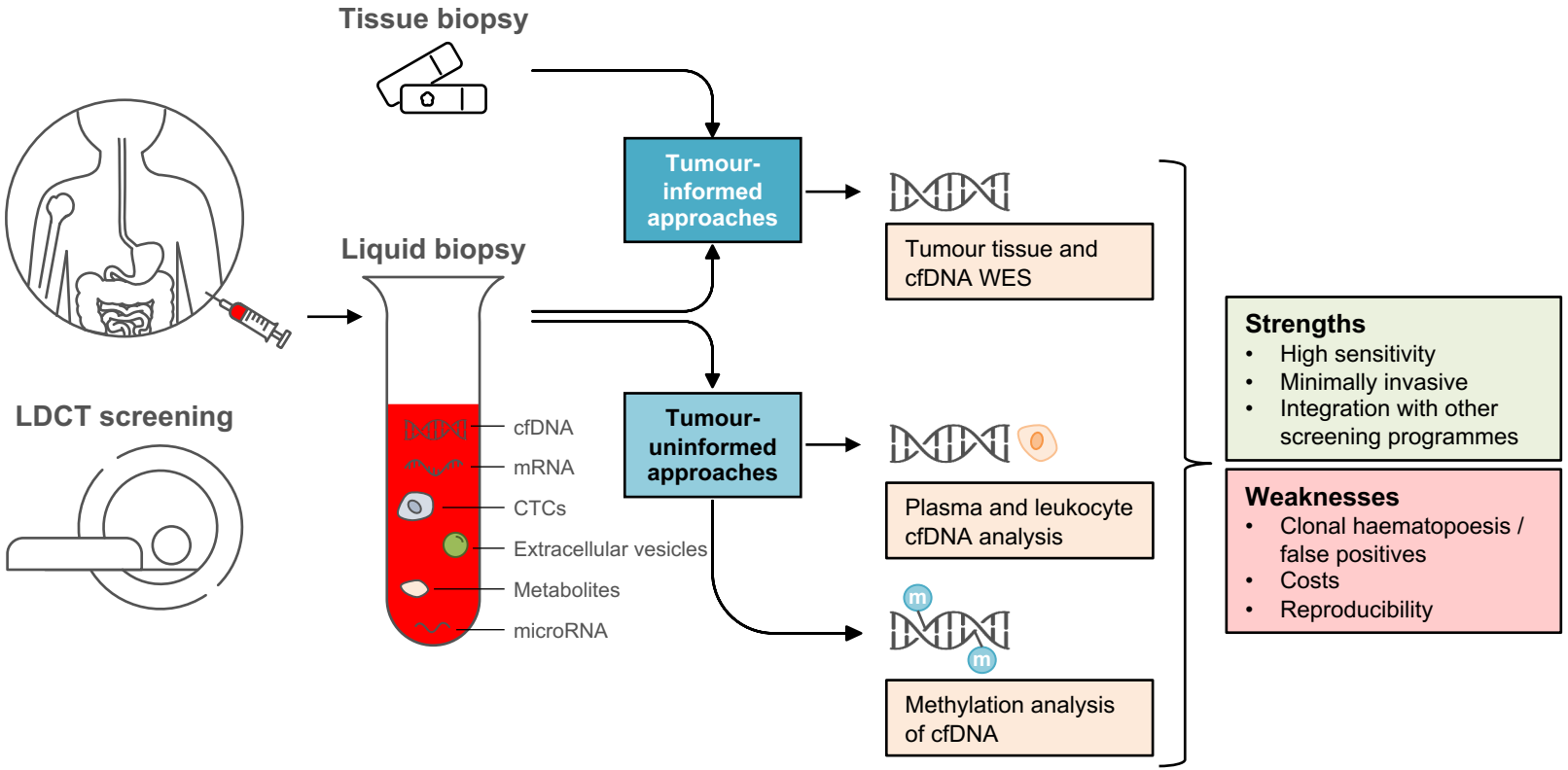


MAF	1.4% (0.62-3.1%)	0.1% (0.06-0.18%)	0.008% (0.002-0.03%)
Nodule diameter	5.8 cm	2.6 cm	1.2 cm
Nodule volume	100 cm^3	10 cm^3	1 cm^3
T stage	T3	T1c	T1b

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



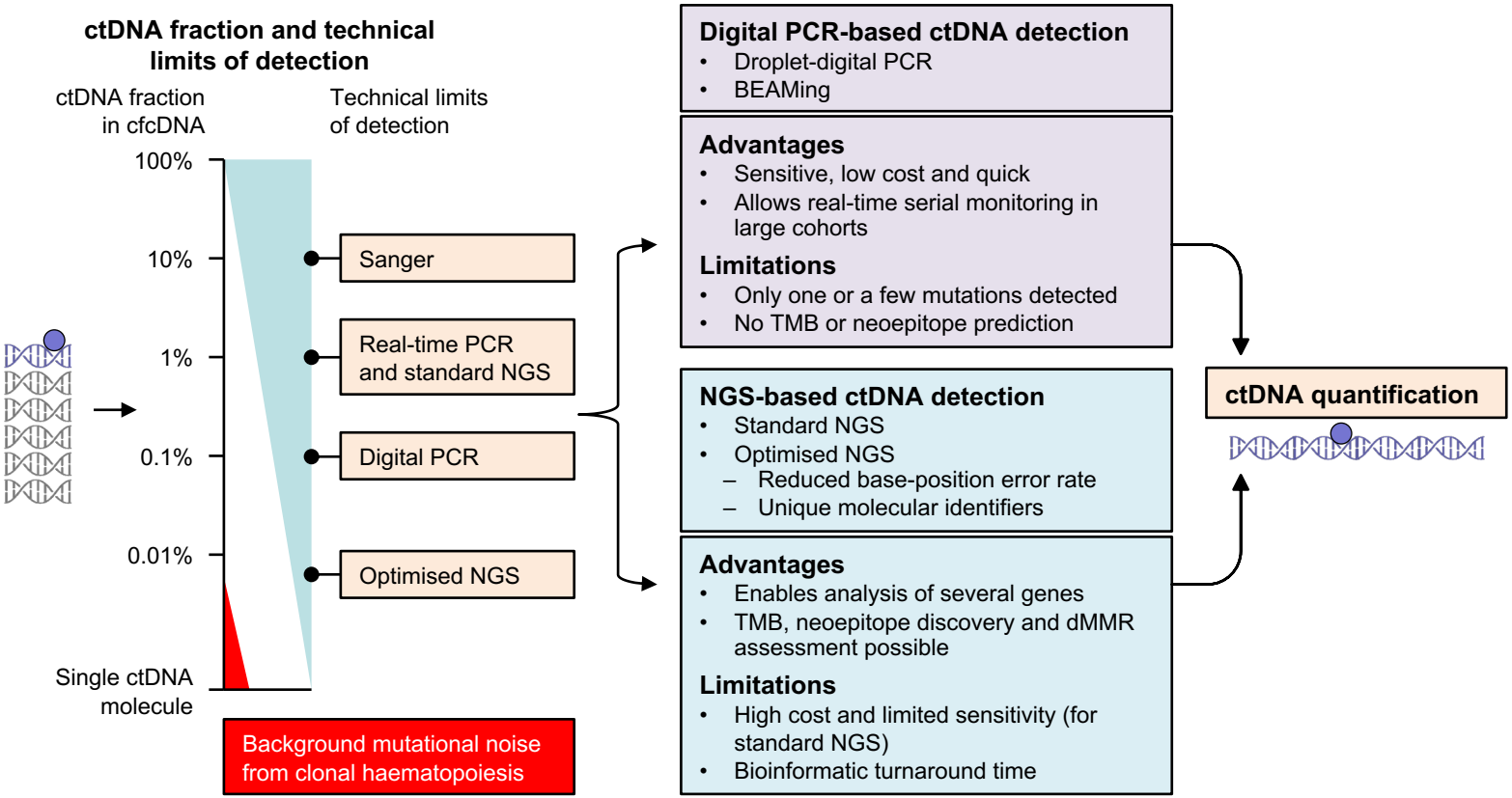
cfDNA: cell-free DNA; CTC: circulating tumour cell; LDCT: low-dose computed tomography; WES: whole exome sequencing.
Rolfo, C., and Russo, A. (2020) *Nat Rev Clin Oncol* 17:523-4.

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.

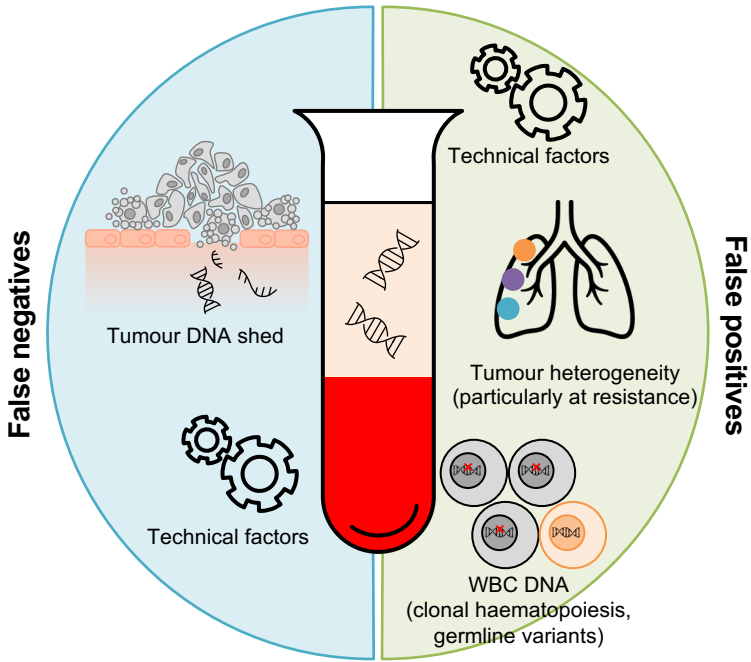
Cabel, L., et al. (2018) *Nat Rev Clin Oncol* 15:639-50.

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)

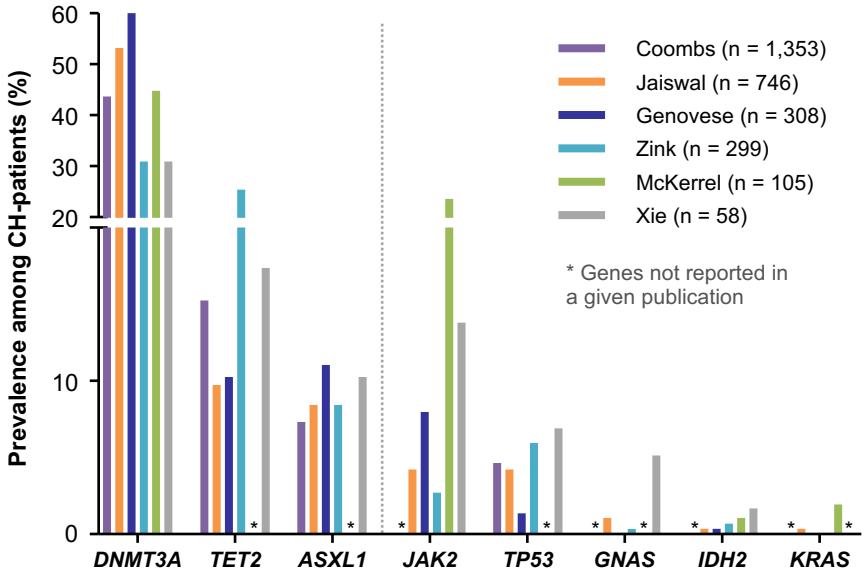
WBC contamination
(germline variants, clonal haematopoiesis)

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**
- Liquid biopsy has shown clinical utility in multiple solid tumors, including **lung, breast, and prostate, as well as in pan-tumour applications**
- 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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Thanks

christian.rolfo@umm.edu

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