Clinical Application of Guideline-Complete Liquid Biopsy

Why sequence the tumor genome?

Clinical → to guide therapy

Which drugs to give?

Osimertinib for *EGFR*-mutated NSCLC

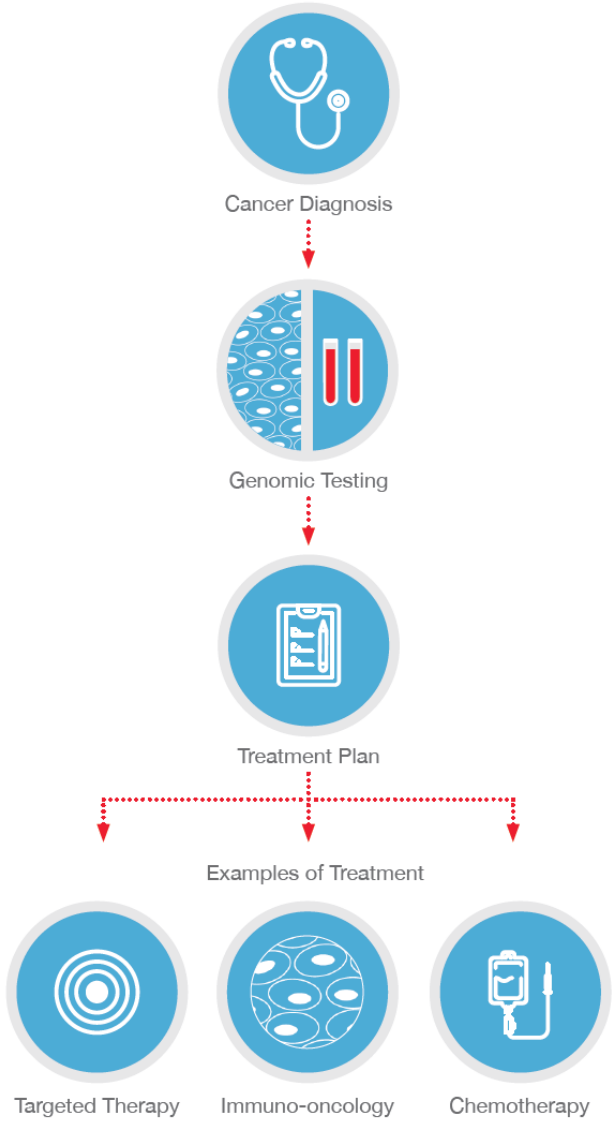
Which drugs not to give?

Cetuximab for *KRAS*-mutated CRC

Research → disease biology and drug discovery

- Pathway analysis to determine new targets
- Clinical trials testing new targeted agents

Genomic testing is critical for patient care



Genomic testing is critical to getting patients on the correct therapy

- Immunotherapies are not very effective in patients who could be treated with targeted therapies

Traditionally, genomic testing is performed on the tissue biopsy that was used for the cancer diagnosis

Tissue samples are often too small to get patients complete genomic testing

- This can lead to patients being given less effective therapies

Clinical case 1

69-year-old woman with with light smoking history admitted with massive stroke

Evaluation showed lung mass with diffuse nodal and bony metastases and endobronchial ultrasound (EBUS) showed lung adenocarcinoma

Guidelines recommend testing for 9 biomarkers in patients with mNSCLC, prior to 1L treatment

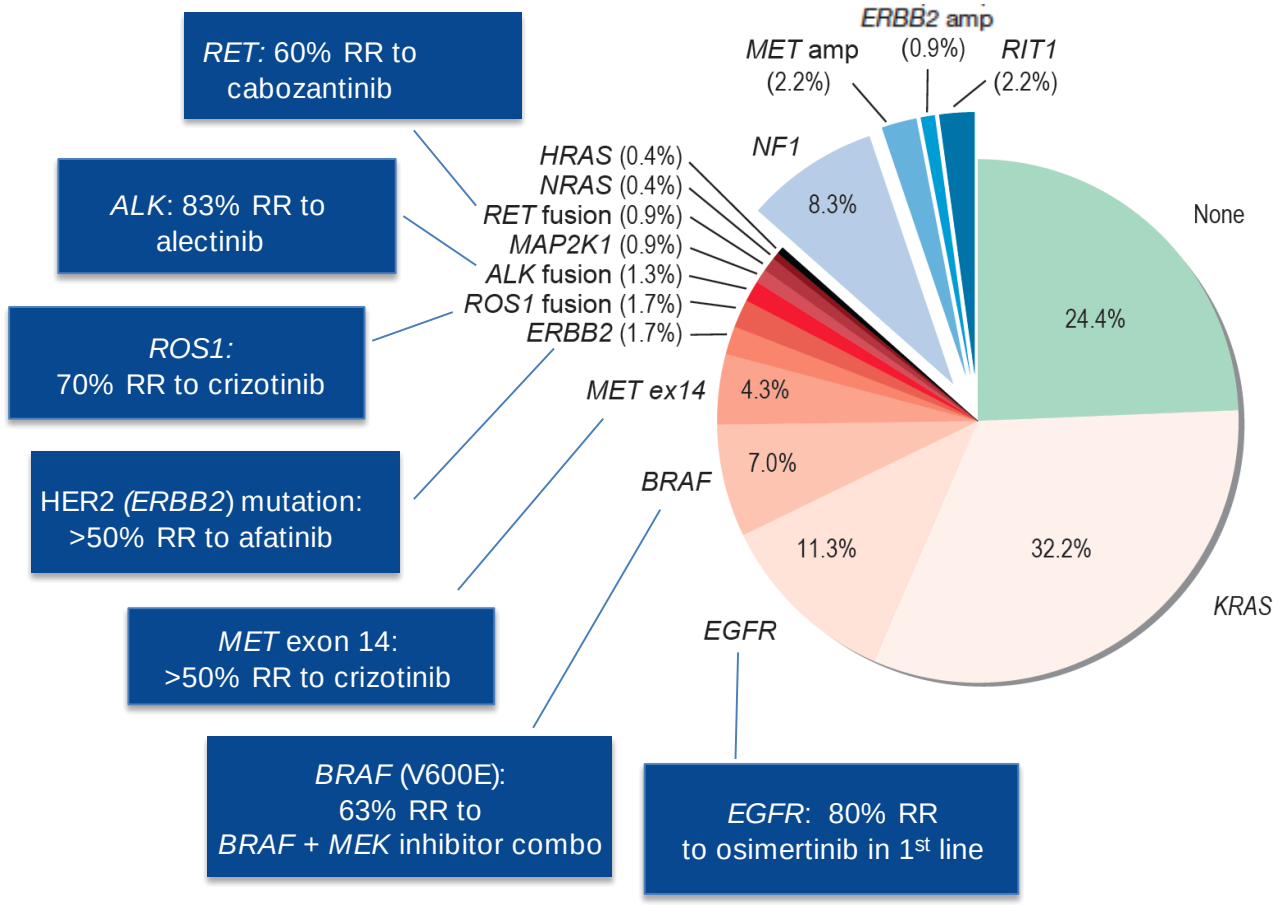
	EGFR	*ALK*	*ROS1*	*BRAF*	*RET*	*MET*	*NTRK*	*KRAS*	*ERBB2* (HER2)
Biomarkers with FDA-approved targeted therapy	●	●	●	●	●	●	●		
Emerging biomarkers with promising clinical data								●	●

Current Guideline-Based Recommendations for Genomic Testing: NCCN and CAP/IASLC/AMP[1,2]

The number of mNSCLC biomarkers with FDA-approved therapies is growing

1. NCCN. NCCN Guidelines Non-Small Cell Lung Cancer. 2018. 2. Lindeman NI et al. J Mol Diagn, 2018.

About 30% of biomarkers in mNSCLC tumors are targetable with a high response rate to targeted therapy



TCGA 2014 *Nature* and references below

Clinical case 1

69-year-old woman with with light smoking history admitted with massive stroke

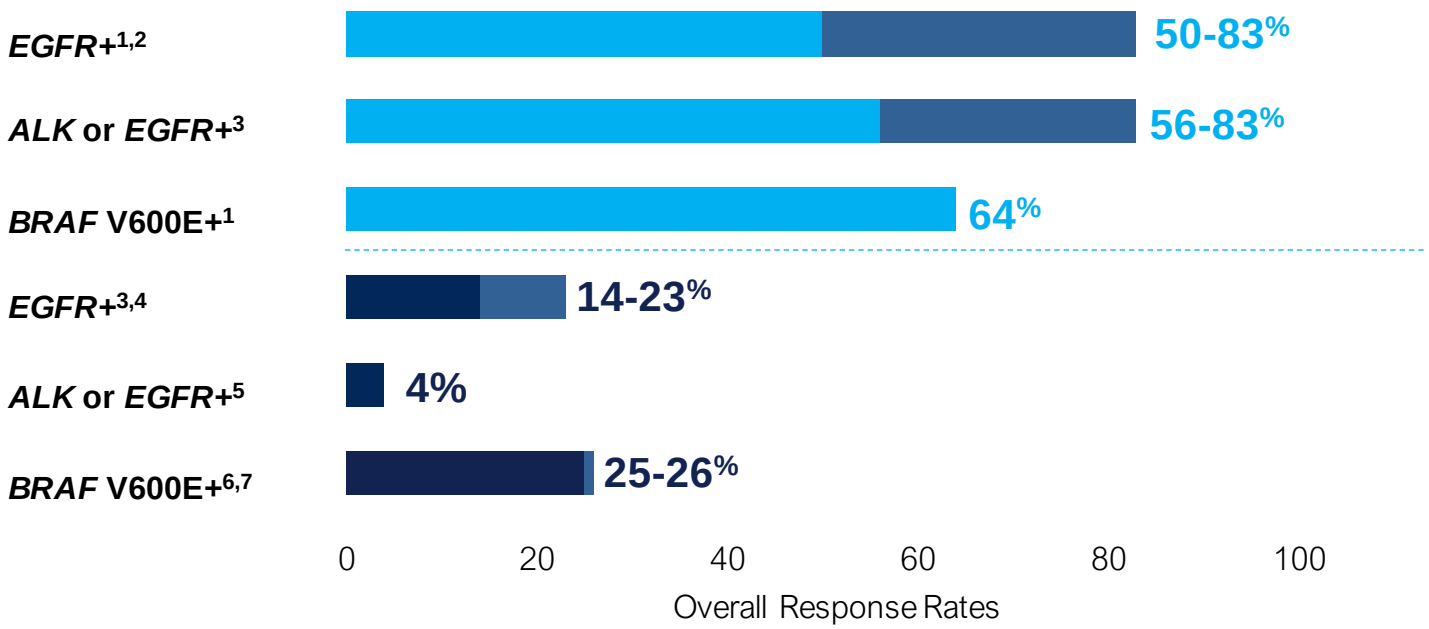
Evaluation showed lung mass with diffuse nodal and bony metastases and endobronchial ultrasound (EBUS) showed lung adenocarcinoma

PD-L1 IHC showed 16% expression

Guideline-recommended genomic testing identified ***EGFR*** **exon 19 deletion**

For some patients with mNSCLC, 1L immunotherapy is suboptimal

Patients with targetable alterations show higher response rates with targeted therapy vs. immunotherapy



TARGETED THERAPY
50-83% Response rates

IMMUNOTHERAPY
4-26% Response rates

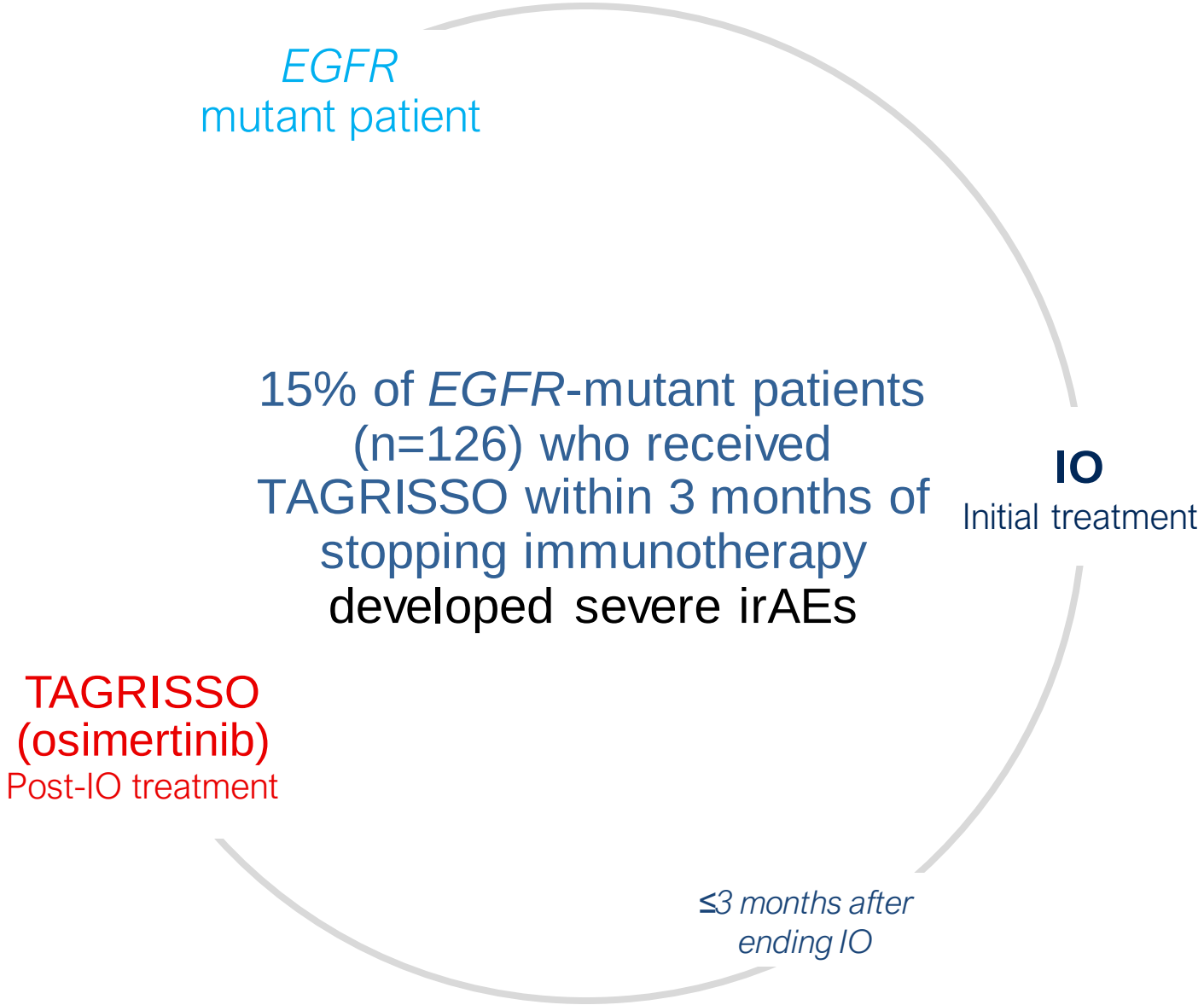
Guidelines and drug labels require genomic results for *EGFR* and *ALK* prior to starting immunotherapy[1]

1. National Comprehensive Cancer Network. Non–Small Cell Lung Cancer Guidelines. 2020. 2. Mack PC et al. Cancer 2020. 3. Gettinger S et al. J Clin Oncol. 2016. 4. Peters S et al. J Clin Oncol. 2017. 5. Gainor JF et al. Clin Cancer Res. 2016. 6. Dudnik E et al. J Thorac Oncol. 2018. 7. Guisier F et al. J Thorac Oncol. 2019.

Starting 1L therapy for mNSCLC without complete results has consequences[1]

- Toxicities included pneumonitis, colitis, and hepatitis
- All patients with severe irAEs required steroids; nearly all required hospitalization

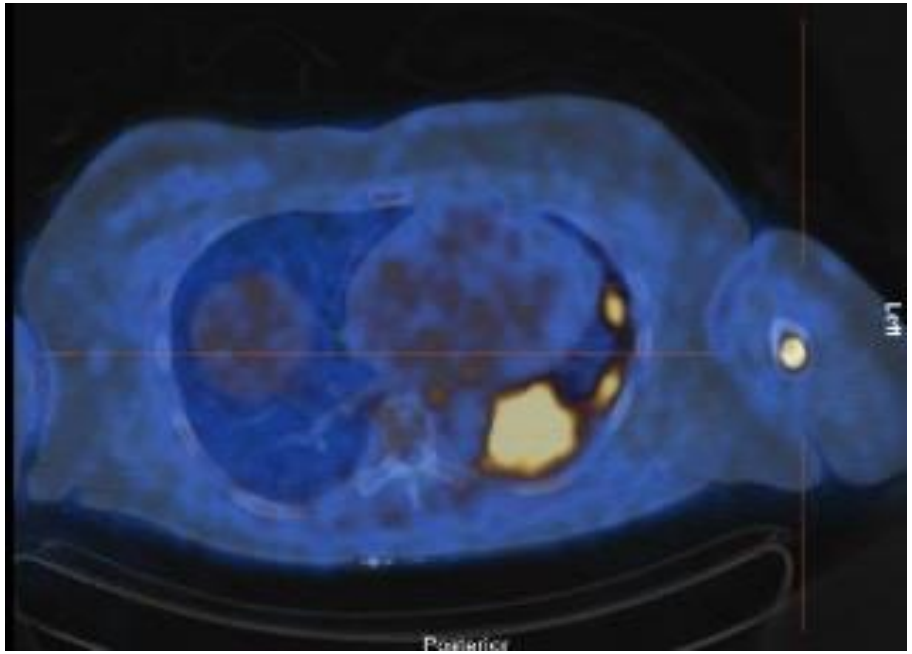
No severe irAEs were identified when patients received TAGRISSO followed by IO



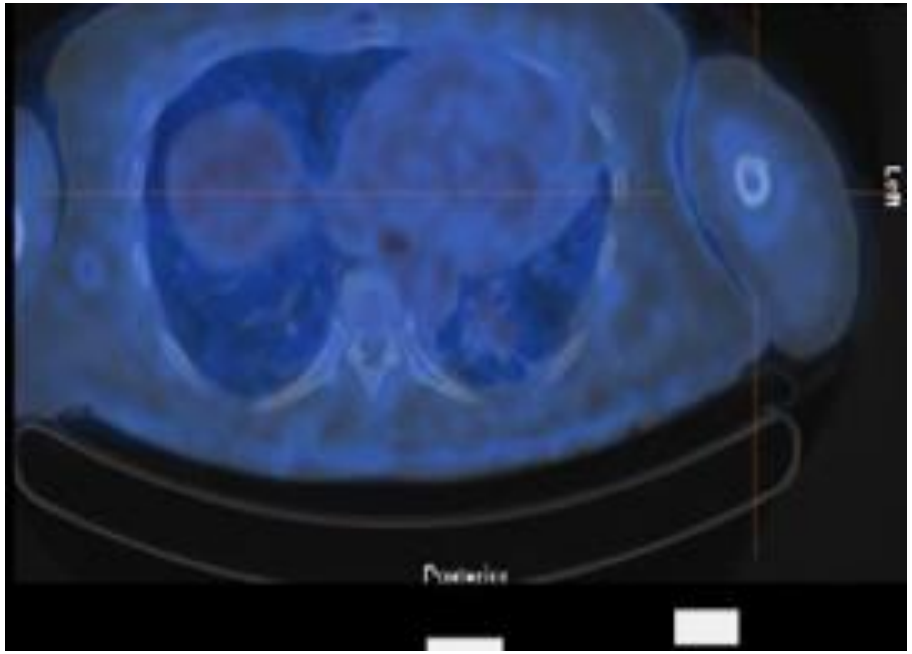
1. Schoenfeld AJ et al. Ann Oncol. 2019.

Clinical case 1

Patient responded to an EGFR TKI for >1 year



Pre-treatment imaging



Post-treatment imaging

Clinical case 2

44-year-old woman presented with persistent cough and weight loss; diagnosed with **stage IV lung adenocarcinoma**

Imaging identified a brain lesion suspected to be metastases

Oncologist ordered SOC tissue testing and liquid biopsy testing at time of first oncology appointment

Clinical case 2

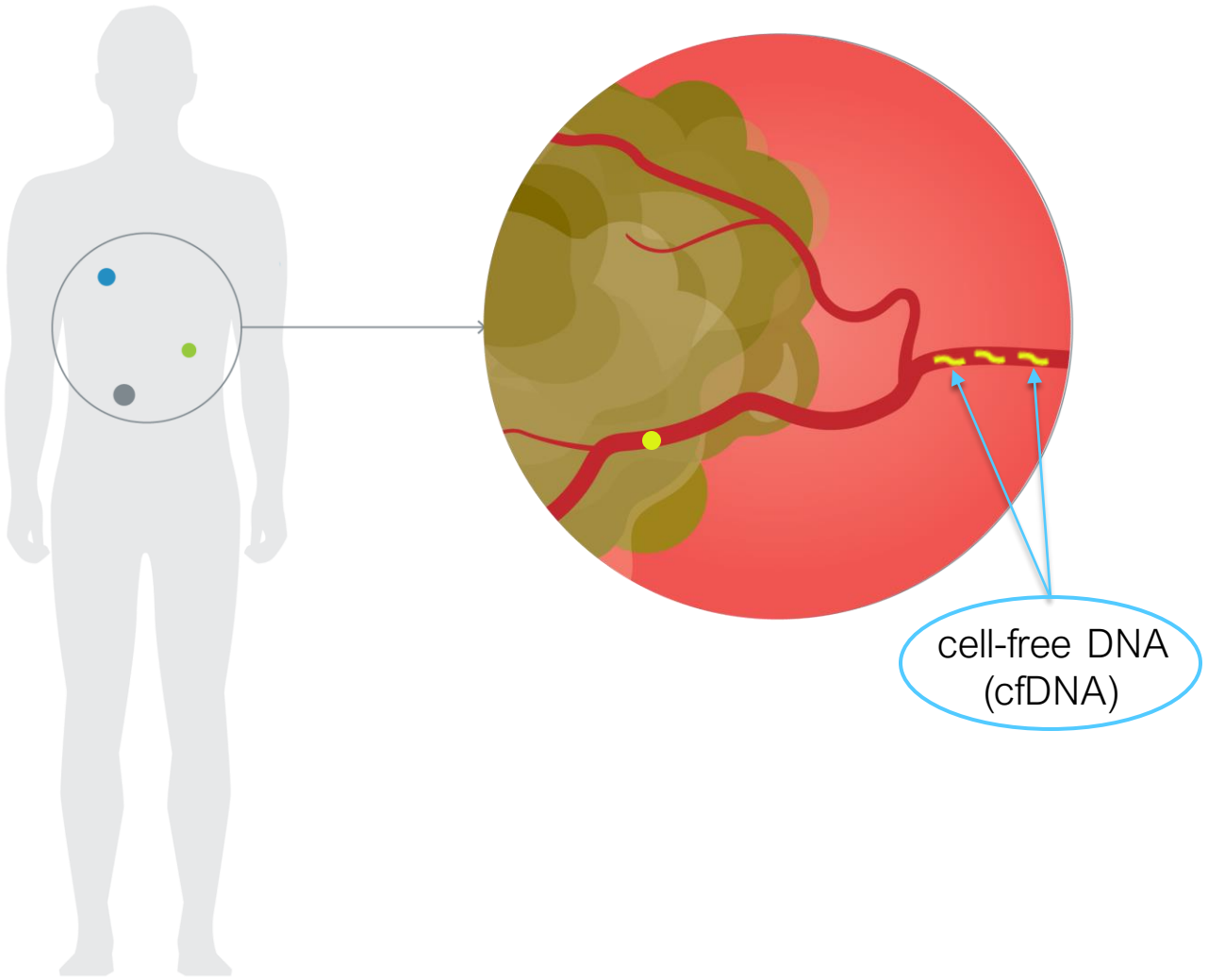
44-year-old woman presented with persistent cough and weight loss; diagnosed with **stage IV lung adenocarcinoma**

Liquid biopsy results reported out in 7 days with ***RET* fusion**

Patient initiated selpercatinib and showed immediate signs of response; continues to improve 2 months later

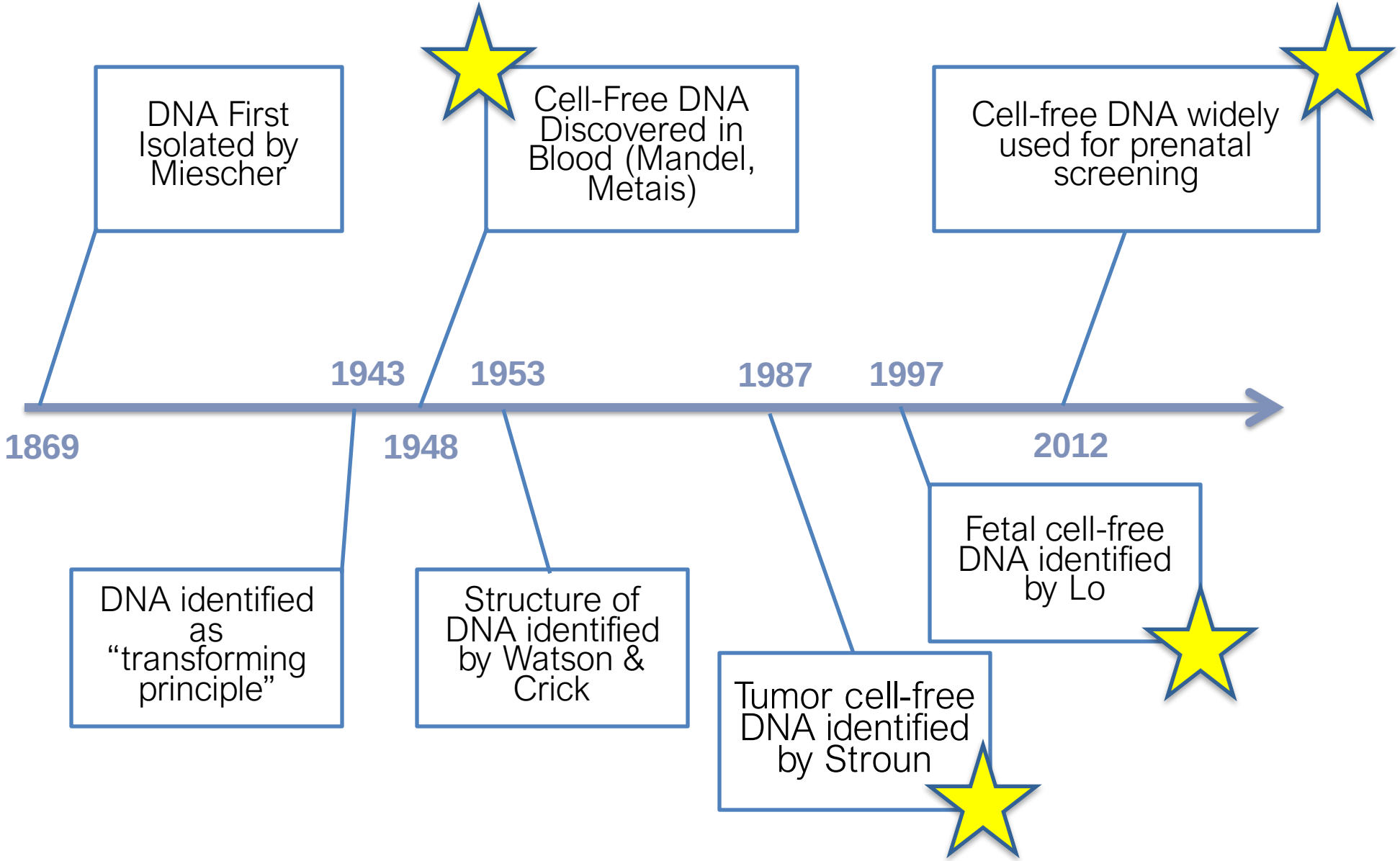
Though tissue results also found the *RET* fusion, the results were not returned until 3 weeks after liquid biopsy

Liquid biopsy captures tumor DNA non-invasively



- Tumors shed circulating tumor DNA into the blood through a number of different mechanisms
- A liquid biopsy uses a blood sample to perform genomic testing
- A simple blood draw allows you to test any advanced cancer patient
- Some liquid biopsies can cover relevant cancer genes to help inform treatment decisions
- Receive results faster than tissue

History of cell-free DNA



Liquid biopsy avoids challenges that prevent guideline-complete genomic profiling

TISSUE COMPLEXITIES

- **LENGTHY PROCESS** — Results can be unpredictable, may take up to a month or longer, and can be incomplete[1-5]
- **FINITE RESOURCE** — Exhausted by histopathology stains and PD-L1 testing[6,7]
- **PATIENT BURDEN** — Repeated invasive tissue biopsies expose patients to potential adverse events[6,7]
- **PRACTICE / STAFF BURDEN** — Significant coordination involving multiple care team members[6,7]

~1 in 2 patients with NSCLC are unable to get complete genomic profiling results from tissue[2,8]

1. Kris et al. JAMA. 2014. 2. Aggarwal C et al. JAMA Oncol. 2019. 3. Thompson et al. Clin Canc Res. 2016. 4. Villaflor et al. Oncotarget. 2016. 5. Hagemann et al. Cancer. 2015. 6. Gutierrez et al. Clin Lung Cancer. 2017. 7. Pennell et al. ASCO Educational Book. 2019. 8. Hagemann IA et al. Cancer. 2015..

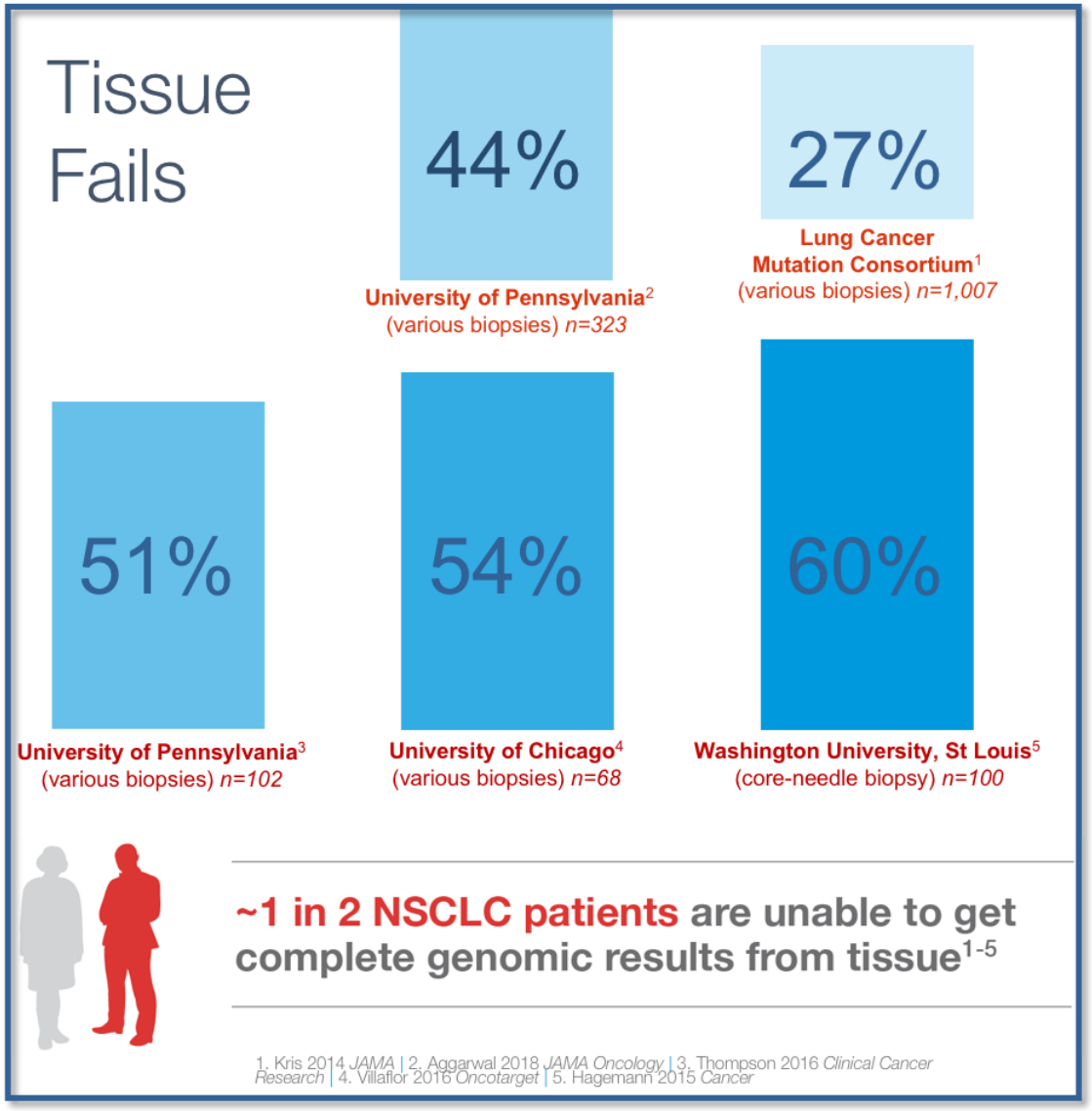
Tissue biopsies cause harm & often fail

Invasive biopsies can cause harm

- **1-2%** of lung cancer biopsies result in death[1]
- **19%** of lung cancer biopsies result in adverse events[2]
- Average cost of a lung cancer biopsy to Medicare is **$14,634** because of complications[2]

Invasive biopsies can miss the target

- Tissue biopsies often fail[3,4]



[1] National Lung Screening Trial Research Team 2011 *NEJM*
[2] Lokhandw ala 2016 *Clinical Lung Cancer*
[3] Meric-Bernstam 2015 *Journal of Clinical Oncology*
[4] Sundaresan 2015 *Clinical Cancer Research*

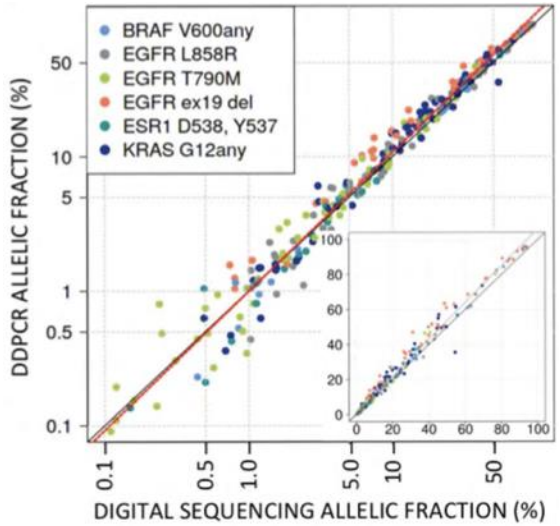
High Concordance (*PPV*) with Tissue

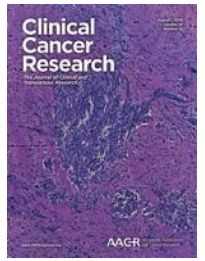
6948 consecutive clinical NSCLC samples

2973 samples with an oncogenic driver mutation detected by liquid biopsy

2430 without tissue genotyping reports

559 samples with tissue genotyping reports





"Validation of a plasma-based comprehensive cancer genotyping assay utilizing orthogonal tissue- and plasma-based methodologies"

181 *EGFR* L858R	291 *EGFR* exon 19 del	16 *EGFR* exon 20 ins	37 *ALK / ROS1* fusion	26 *KRAS* mutations	5 *BRAF* V600E	3 *MET* exon 14 skipping
98%	98%	100%	92%	100%	100%	100%

86% response to erlotinib, afatinib, gefitinib

82% response to crizotinib

No further testing needed

63% response to MEK inhibitor

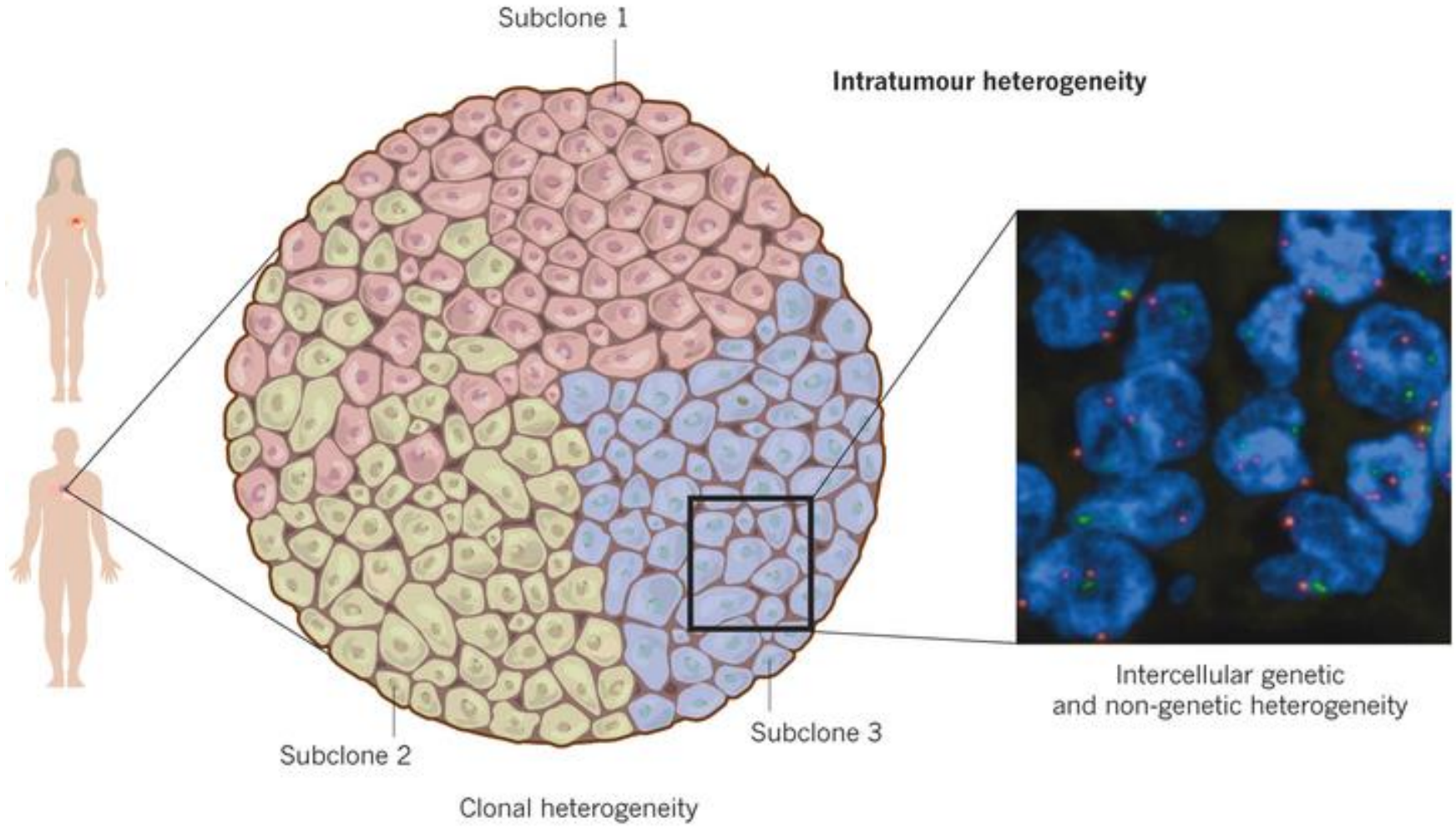
> 50% response to crizotinib

All 3 putative false-positive *ALK* fusions ***responded*** to targeted therapy

So PPV for *ALK/ROS1* fusions was *actually* 100%

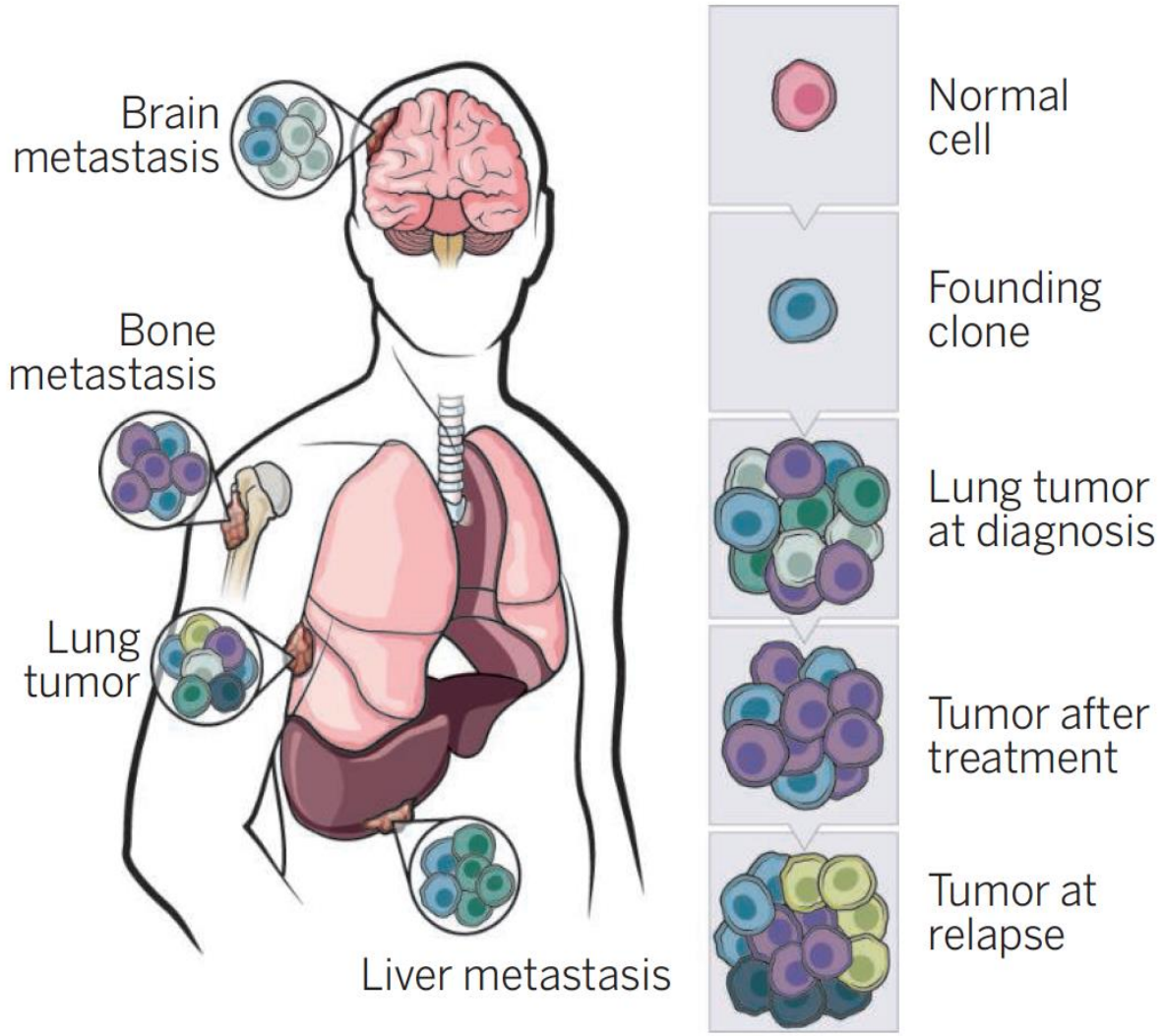
McCoach 2018 *Clinical Cancer Research*
Odegaard 2018 *Clinical Cancer Research*
Campbell 2016 *Nature Genetics* (references in slide notes)

Intratumor Heterogeneity: A Needle or Forceps Biopsy May Not Hit the Right Spot



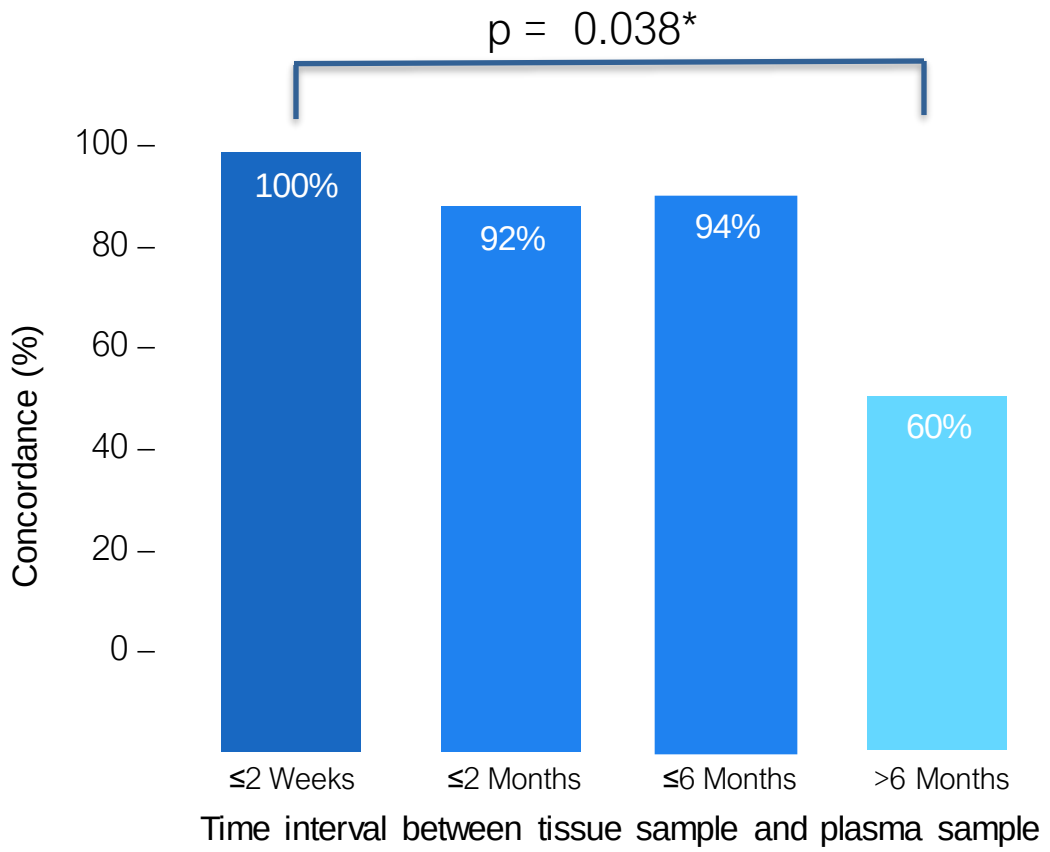
Burrell et al. 2013 *Nature*

Spatial and Temporal Tumor Heterogeneity



Govindan 2014 Science

Temporal Heterogeneity Confounds Plasma to Tissue NGS Concordance: Blinded External Validation Study



Prospective study of 102 Consecutive NSCLC patients

Thompson et al. 2016 *Clinical Cancer Research*

International Association for the Study of Lung Cancer

- Liquid biopsy is particularly recommended when tumor tissue is scarce or unavailable, or a significant delay (>14 days) is expected in obtaining tissue
- Targeted therapy may reliably be predicated upon blood-based results
- If a plasma-based "hot-spot" test is negative, repeat testing via comprehensive profiling (such as liquid biopsy) should be pursued; however, if testing initially performed through comprehensive means is negative, no further testing need be pursued
- The cobas test covers only some of the *EGFR* alterations and its sensitivity is outperformed by NGS, which can reach higher levels of sensitivity without diminishing specificity:

  "Multiplex panels using NGS platforms are reliable and preferred as they detect beyond the common mutations…NGS can reach acceptable levels of sensitivity and optimal levels of specificity"

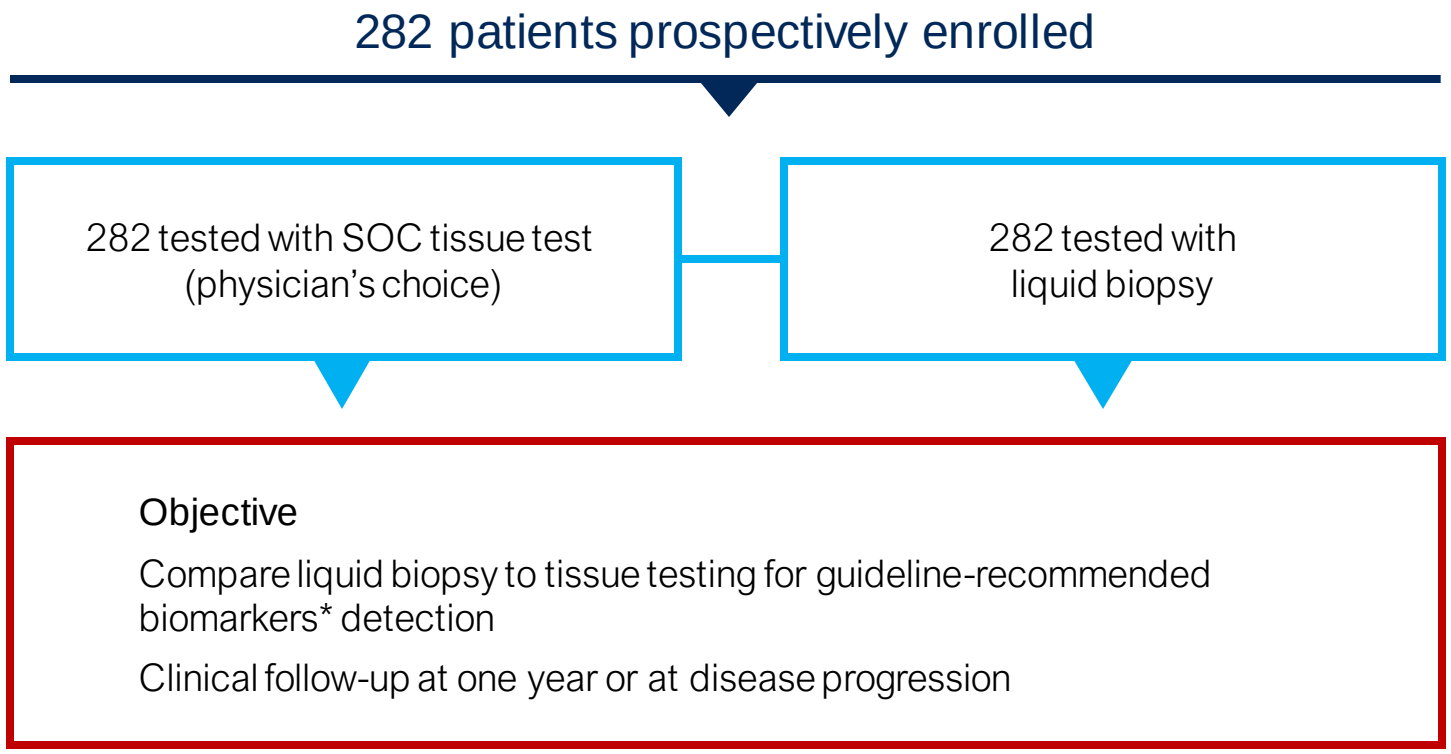
Rolfo 2018 *Journal of Thoracic Oncology*

National Comprehensive Cancer Network

- "if there is insufficient tissue to allow testing for all of *EGFR, ALK, ROS1* and *BRAF*, repeat biopsy and/or plasma testing should be done"
- "Plasma-based testing should be considered at progression on EGFR TKIs for the T790M mutation"

NILE

“Blood-first” benefit found when comparing liquid biopsy directly to SOC tissue testing[1]



Study Endpoints

- **Primary:** Detection of guideline-recommended genomic biomarkers
- **Secondary:** Median turn around time (TAT)

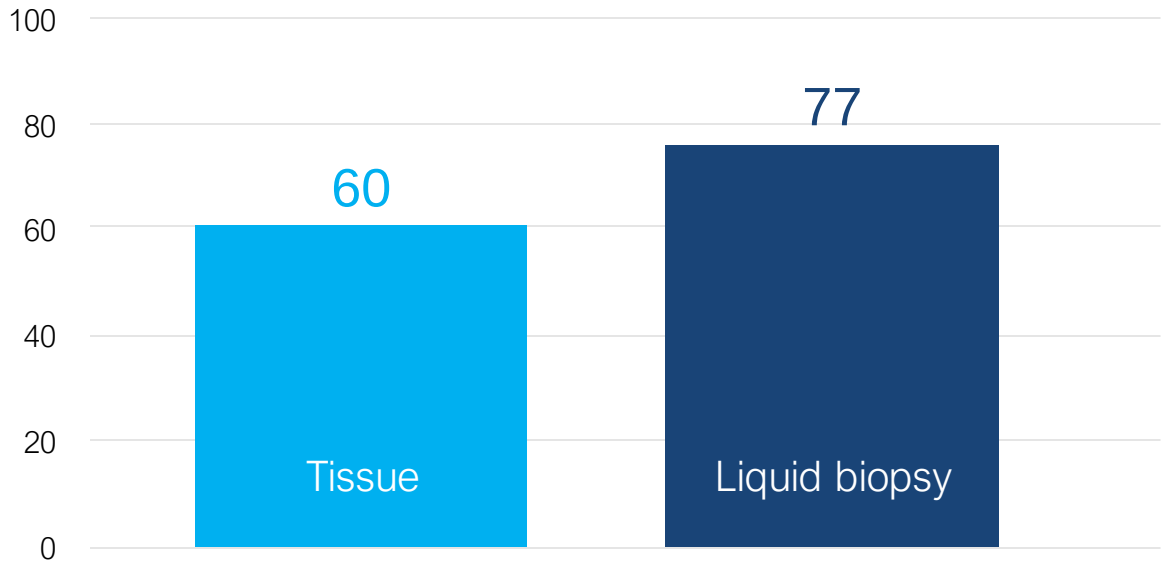
*8 Guideline-recommended biomarkers: EGFR, ALK, BRAF, ROS1, RET, ERBB2, and MET (amplification and exon 14 skipping)

1. Leighl et al. Clin Cancer Res. 2019.

NILE

Liquid biopsy as effective as SOC tissue testing[1]

Number of patients with an identified guideline-recommended biomarker by testing modality



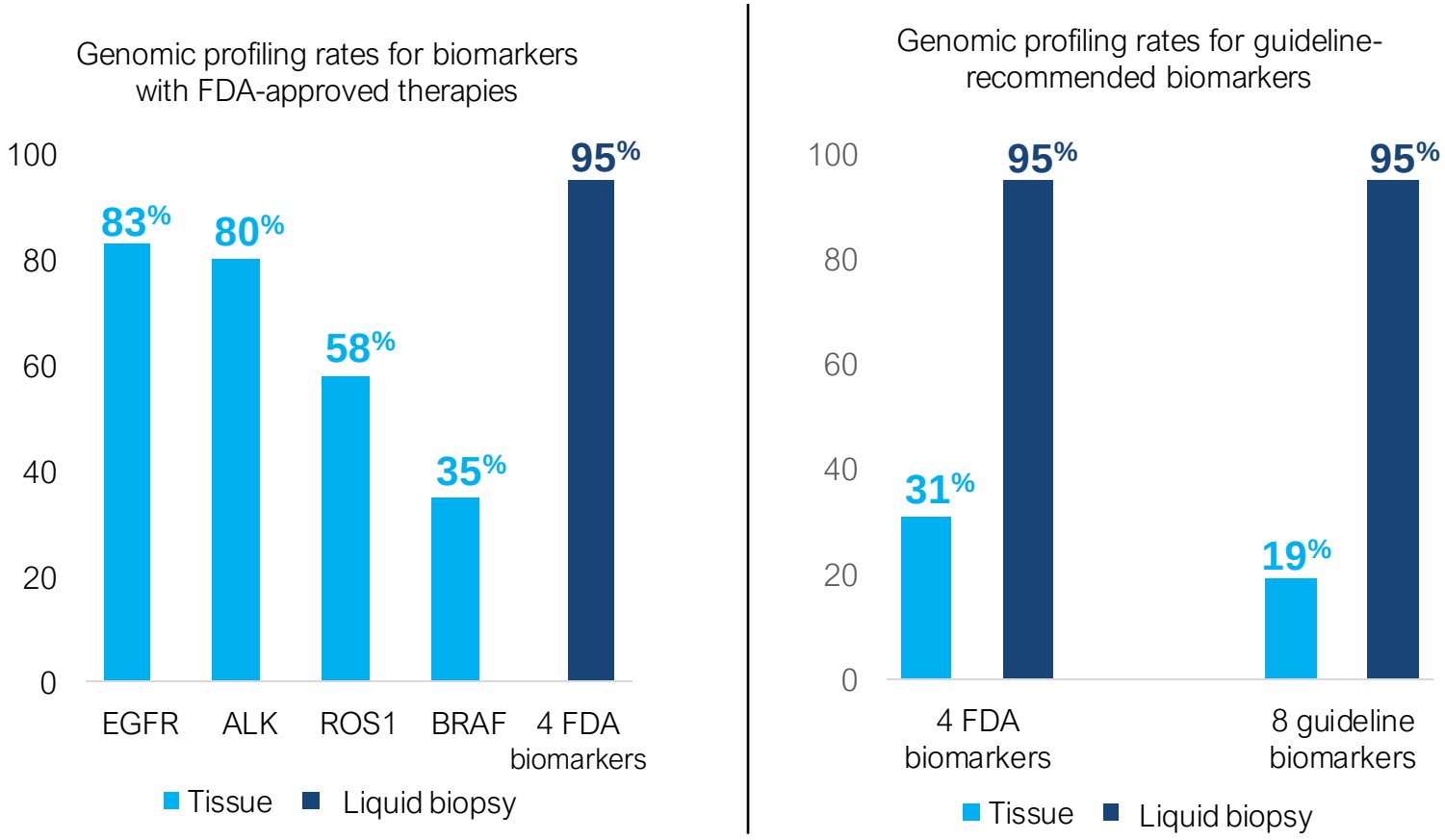
- Liquid biopsy and tissue testing performed similarly in the detection of guideline-recommended biomarkers (27.3% versus 21.3%; p<0.0001, non-inferiority)
- 98% concordance between liquid biopsy and tissue testing for *EGFR*, *ALK*, *ROS1*, and *BRAF**

* At the time of this study, EGFR, ALK, ROS1 and BRAF were the only biomarkers with FDA-approved targeted therapies for NSCLC

1. Leighl et al. Clin Cancer Res. 2019.

NILE

More mNSCLC patients receive guideline-complete testing with liquid biopsy[1]



~3x as many patients tested for *EGFR*, *ALK*, *ROS1*, and *BRAF* mutations* with liquid biopsy (95%) vs. tissue testing (31%)

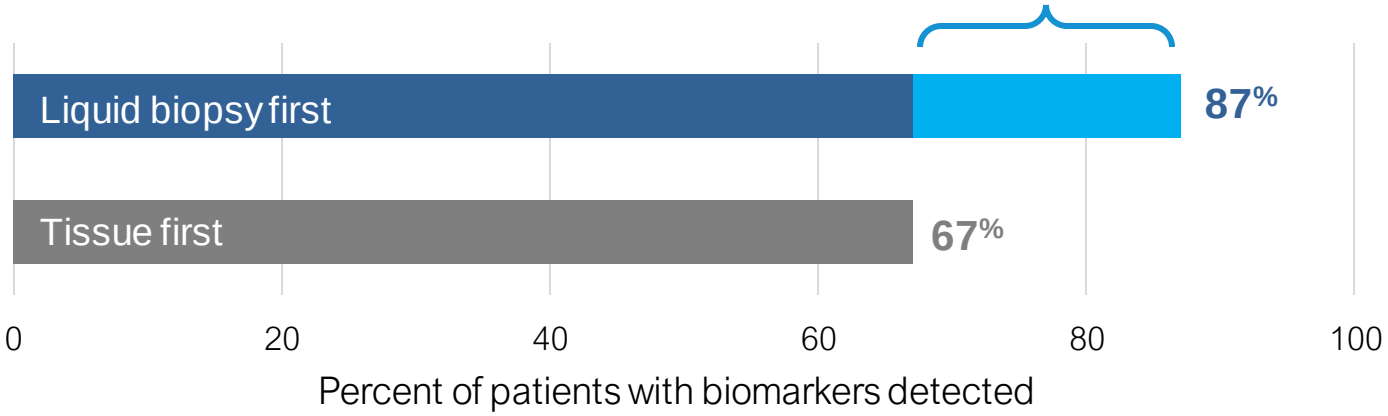
* At the time of this study, EGFR, ALK, ROS1 and BRAF were the only biomarkers with FDA-approved targeted therapies for NSCLC

1. Leighl et al. Clin Cancer Res. 2019.

NILE

Testing with liquid biopsy identifies more patients with actionable biomarkers - faster[1]

20% more patients with biomarkers detected when using **liquid biopsy** first

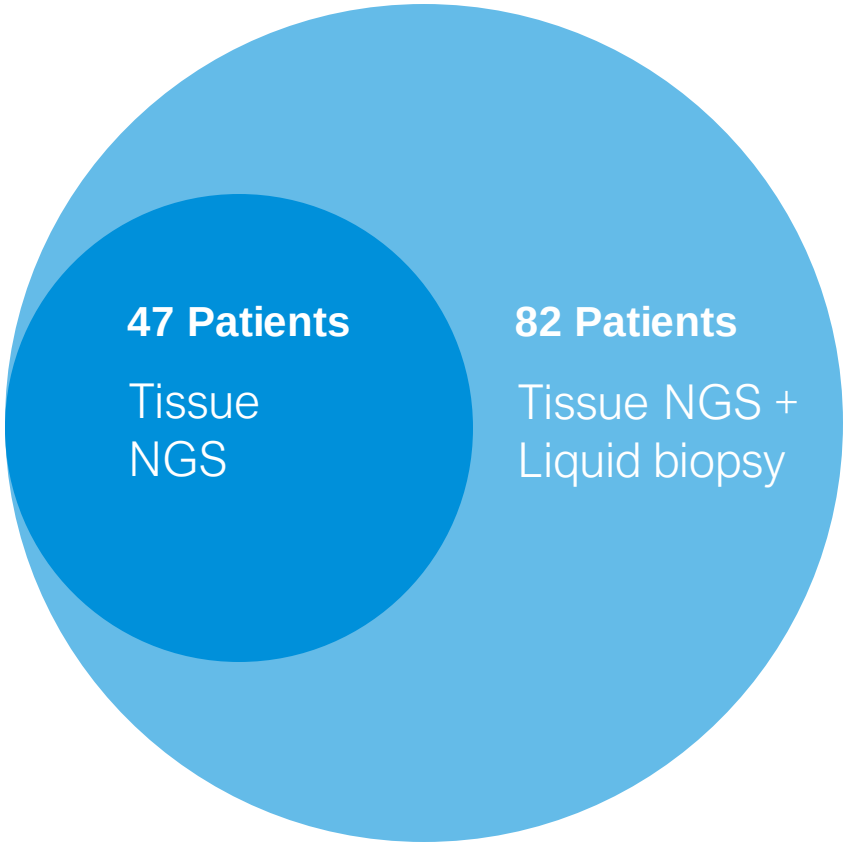


- More patients with biomarkers identified
  - Due to the limitations of tissue testing, only 31% of patients received complete testing
- Median turn-around-time was significantly faster
  (9 vs. 15 days; p<0.0001)
  - Liquid biopsy median TAT improved to 7 days by the end of the study

1. Leighl et al. Clin Cancer Res. 2019.

Liquid biopsy + tissue testing nearly doubles number of patients identified with targetable mutations[1]

Independent (single site) prospective study of 323 patients comparing liquid biopsy + tissue NGS vs. tissue NGS



44% of patients who were eligible for tissue biopsy were unable to get tissue results due to tissue insufficiency

1. Aggarwal C et al. JAMA Oncol. 2018.

Testing with Liquid biopsy first improves 1L treatment selection for more mNSCLC patients

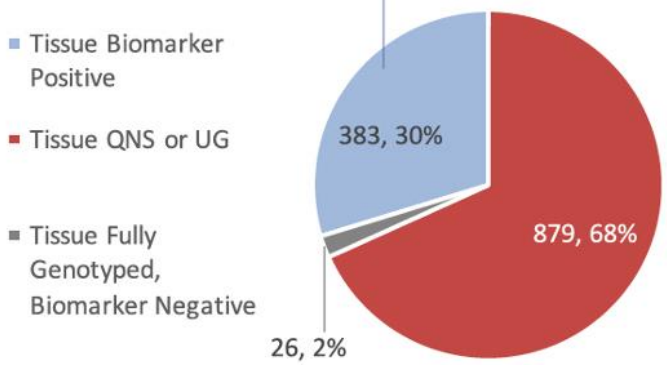
"These results (Aggarwal et al.), combined with the patient satisfaction with the relative ease of providing blood rather than a solid tissue sample, suggest a clinical strategy of pursuing plasma NGS first, then tissue NGS if plasma NGS cannot detect relevant mutations."[1]

- Drs. Bishal Gyawali and Jack West (*JAMA Onc* editorial)

1. Gyawali B, West HJ. JAMA Onc. 2018.

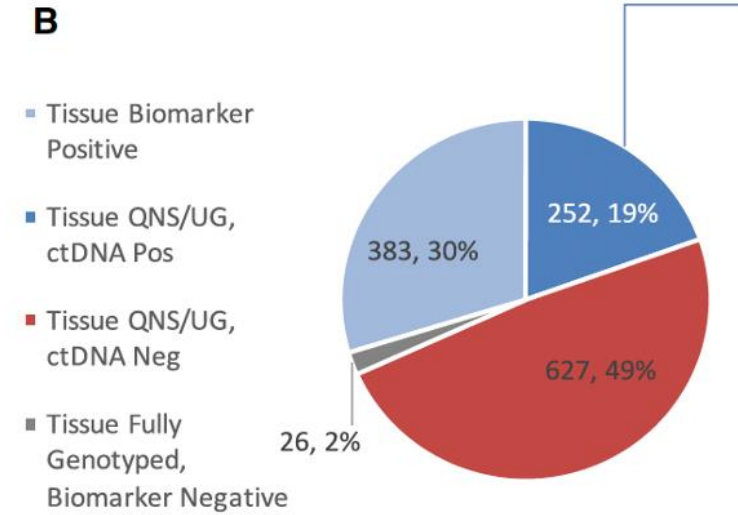
Expanded mutation detection at diagnosis

A



Biomarker	N in Tissue
EGFR	256
KRAS	61
ALK fusion	27
ROS1 fusion	9
RET fusion	4
BRAF V600E	10
MET amp	10
MET E14	4
HER2 mutation	1
FGFR3 fusion	1
TOTAL	**383**

B



Biomarker	Additional N from ctDNA in Tissue QNS/UG Cases
EGFR	42
KRAS	127
ALK fusion	3
ROS1 fusion	2
RET fusion	14
BRAF V600E	13
MET amp	23
MET E14	7
HER2 mutation	21
TOTAL	**252**

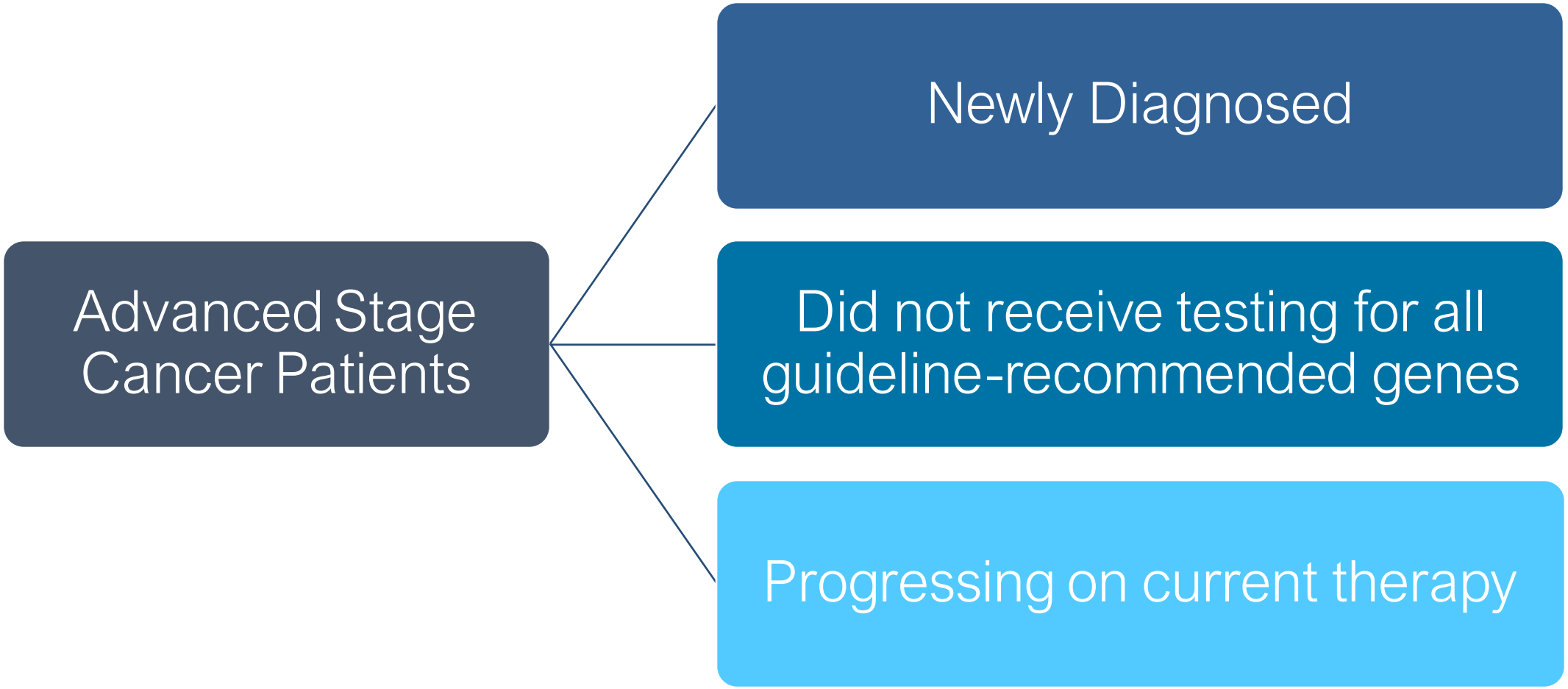
Chart review of 1288 patients – 68% undergenotyped

Genotyping of ctDNA increases biomarker identification by 65%

Mack(Gandara) Cancer 2020

Liquid for patients with advanced solid tumors

Who can benefit from liquid biopsy?



Liquid biopsy identifies actionable alterations across cancers

FDA-approved therapies are available for **solid tumor patients** with *NTRK* fusions or MSI-High status

Lung

EGFR •

ALK •

ROS1 •

BRAF •

MET •

RET •

ERBB2 (HER2)

KRAS

Breast

PIK3CA •

ERBB2 (HER2) •

BRCA1 •

BRCA2 •

NTRK •

MSI •

Colorectal

MSI •

KRAS

NRAS

BRAF •

NTRK•

ERBB2 (HER2)

Prostate

MSI •

BRCA1 •

BRCA2 •

NTRK •

• FDA-approved matched therapy

Clinical case 3

63-year-old man initially diagnosed with Stage 2 **CRC**, referred to a large cancer center after **liver metastases** were detected

No previous genomic testing was recorded for the patient

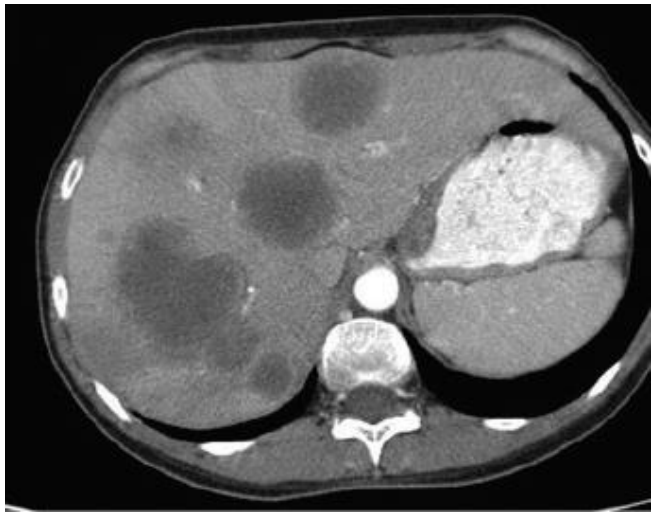
Oncologist **ordered liquid biopsy** to receive guideline-recommended results within 7 days **while locating archival tissue sample**

Liquid biopsy found a ***KRAS* exon 4 mutation**

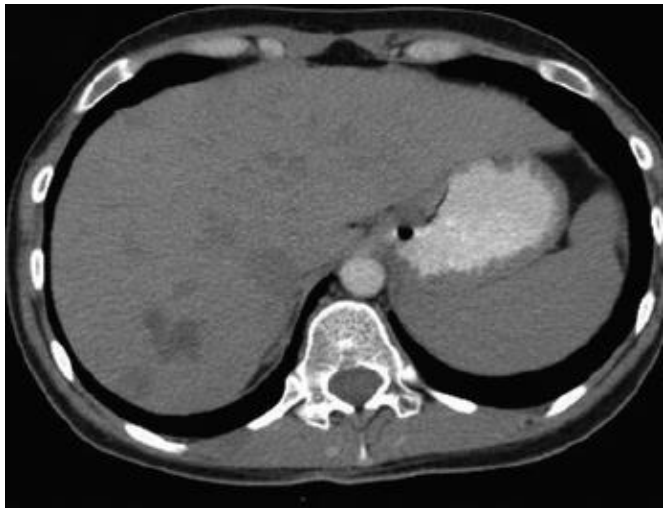
Clinical case 3

The oncologist **excluded anti-*EGFR* therapy** from the patient's treatment plan

The patient was put on **chemo + anti-VEGF therapy** and has had a **strong response for 6+ months**



Pre-treatment imaging



Post-treatment imaging

Clinical case 4

63-year-old woman non-smoker **diagnosed with stage IV breast cancer**
Progressed through several lines of therapy, “out of options”

Patient had ER/PR/HER2 testing performed at diagnosis (several years ago)

No comprehensive genomic testing performed at that time; archival tissue at different institution
Liquid biopsy was ordered and in 7 days, identified ***PIK3CA* mutation**

The patient was **started on PIQRAY® (alpelisib)** May 2020 and **continues to respond today**

Case-based discussion and Q&A

Patient case template

Clinical History

- *Brief description of patient's clinical history*
- *x*
- *x*

Testing

- *Description of prior genomic testing, if available*
- *Liquid biopsy order and results*
- *X*

Treatment and Response

- *Treatment decision based on liquid biopsy results*
- *Patient outcomes, if available*

Pre-treatment imaging

Scan images if possible

Post-treatment imaging

Scan images if possible

Additional clinical cases (branded)

Liquid “rescues” a tissue T790M-negative

Initial Presentation

1st Tissue Biopsy–Lung
EGFR L858R

Progressed on Erlotinib After 5 Months

2nd Invasive Biopsy–Lung
EGFR L858R
No resistance alterations found using NGS

Continued Progression

Whole brain radiation and six cycles of Carboplatin, Pemetrexed, Bevacizumab, and Erlotinib, then Nivolumab

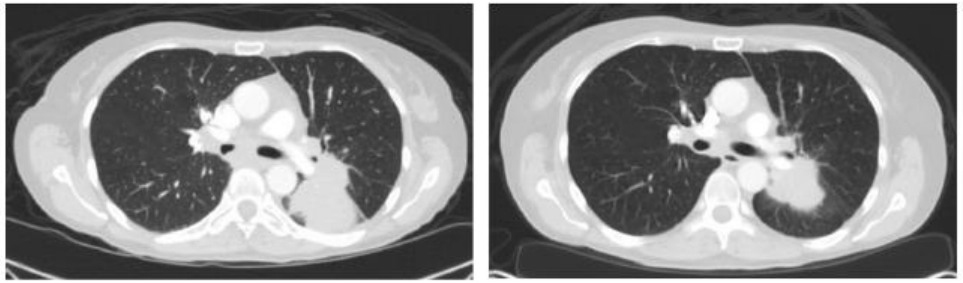
Guardant360

EGFR L858R
EGFR T790M

Response

Clinical and Radiographic Response to 3rd Generation TKI

Baseline Pre-Osimertinib



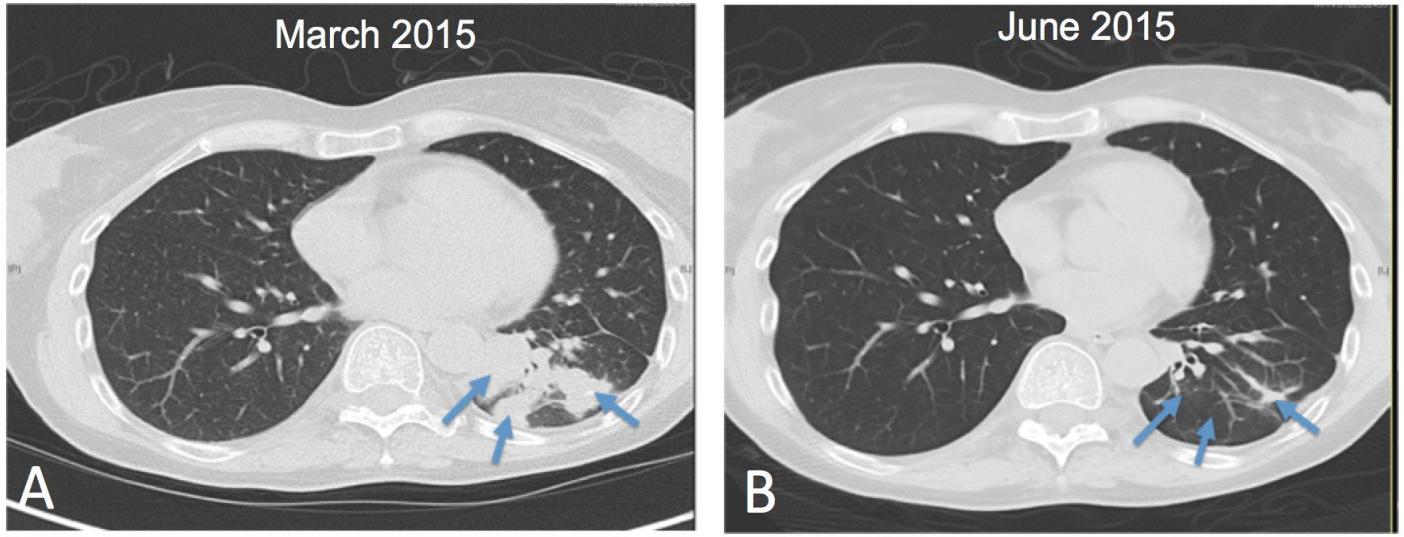
After 4 weeks on Osimertinib

Piotrowska et al. 2016 *Journal of Thoracic Oncology*

NSCLC case 1: tissue insufficient for genotyping

CLINICAL CASE

- 58 y/o F non-smoker presents with metastatic NSCLC
- Tissue is insufficient for genotyping despite three biopsy attempts
- Guardant360 demonstrated *EML4-ALK* fusion at 0.06% variant allele fraction
- Crizotinib initiated with significant and durable response



Guardant360 “rescues” a tissue insufficient case

Courtesy Nisha Mohindra, Northwestern & Jyoti Patel, Univ. Chicago (2015 WCLC/IASLC Abstract)

NSCLC case 2: tissue tested for 3 of 7 NCCN genes

CLINICAL CASE

– 77 y/o F non-smoker presents with advanced lung adenocarcinoma

– Tissue *EGFR*, *ALK*, *ROS1* negative, no additional in-house testing available

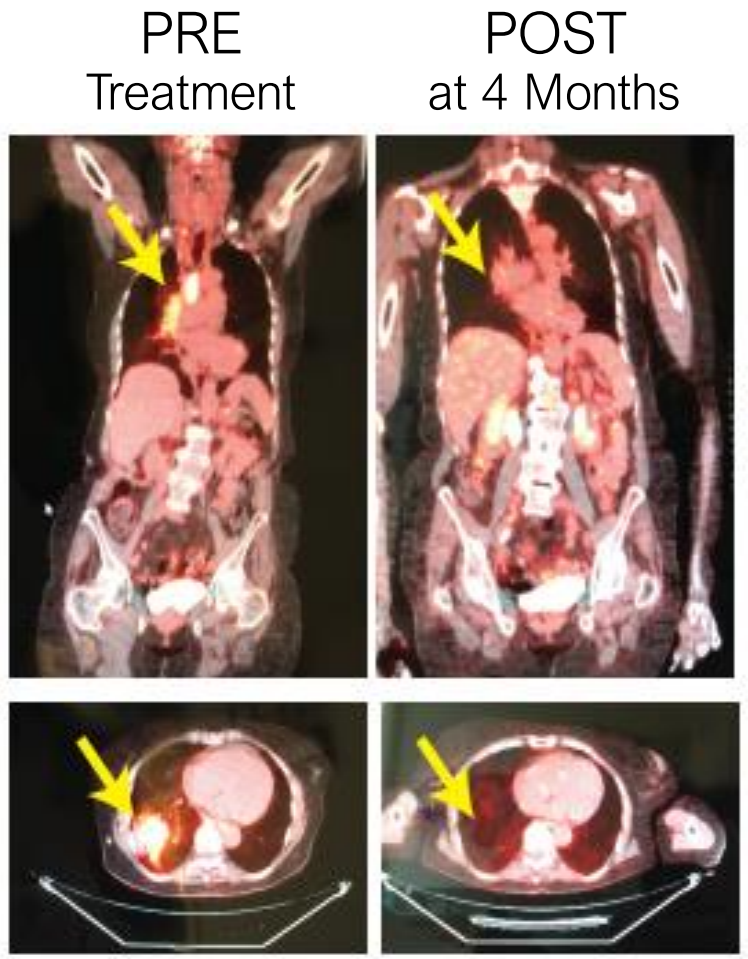
– Guardant360 ordered

– Carboplatin/Taxol initiated

– Guardant360 identified *BRAF* V600E at 2.1%

– Switched to Trametinib + Dabrafenib

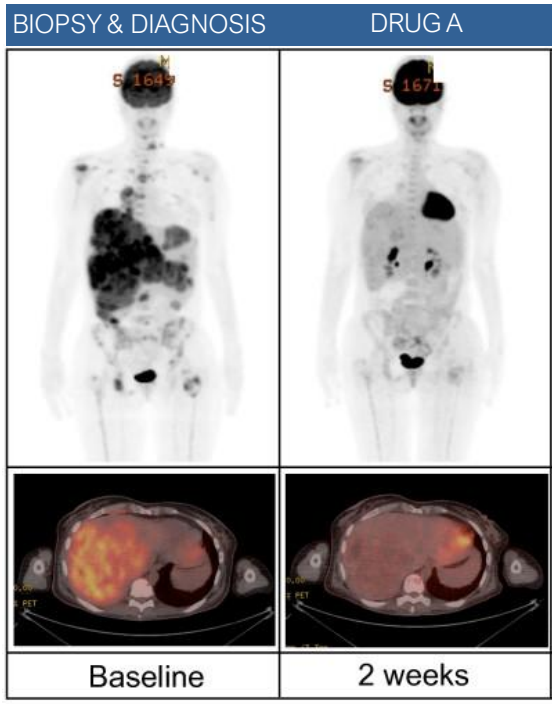
– Near complete response



Guardant360 “rescues” an undergenotyped case

Courtesy of Joseph Ye, MD Vista Oncology

Targeted cancer therapies often work dramatically



But at some point they fail—what to do next?

Patient case #2

Clinical History

- 43 y/o female non-smoker diagnosed with stage IV lung adenocarcinoma
- Progressed through chemotherapy and immunotherapy
- Hospice considered

Genomic Testing

- Tissue testing performed at diagnosis found no targetable alterations – FISH negative for *ALK*
- Guardant360 was ordered and in 7 days, identified *EML4-ALK* fusion

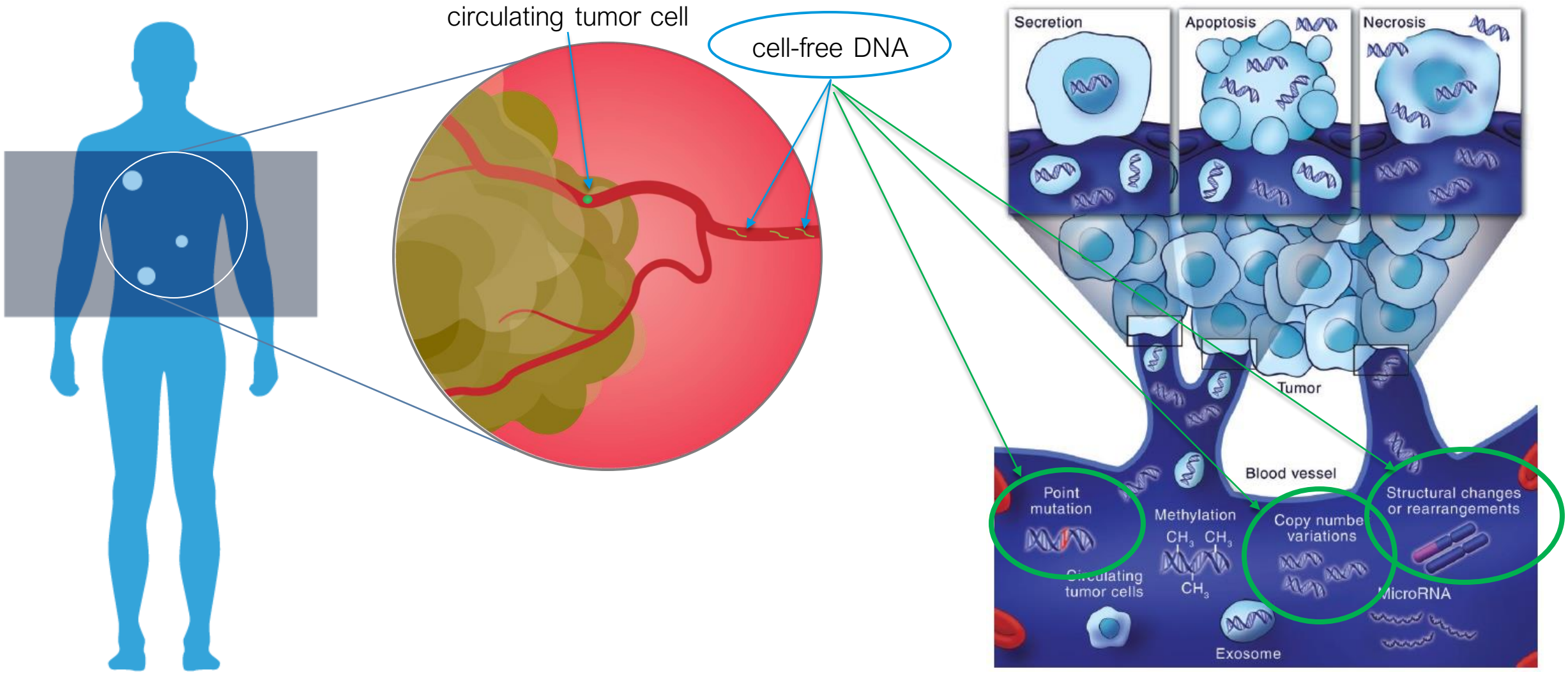
Treatment and Response

- Started on crizotinib in April 2018
- Continues to respond; currently no evidence of disease

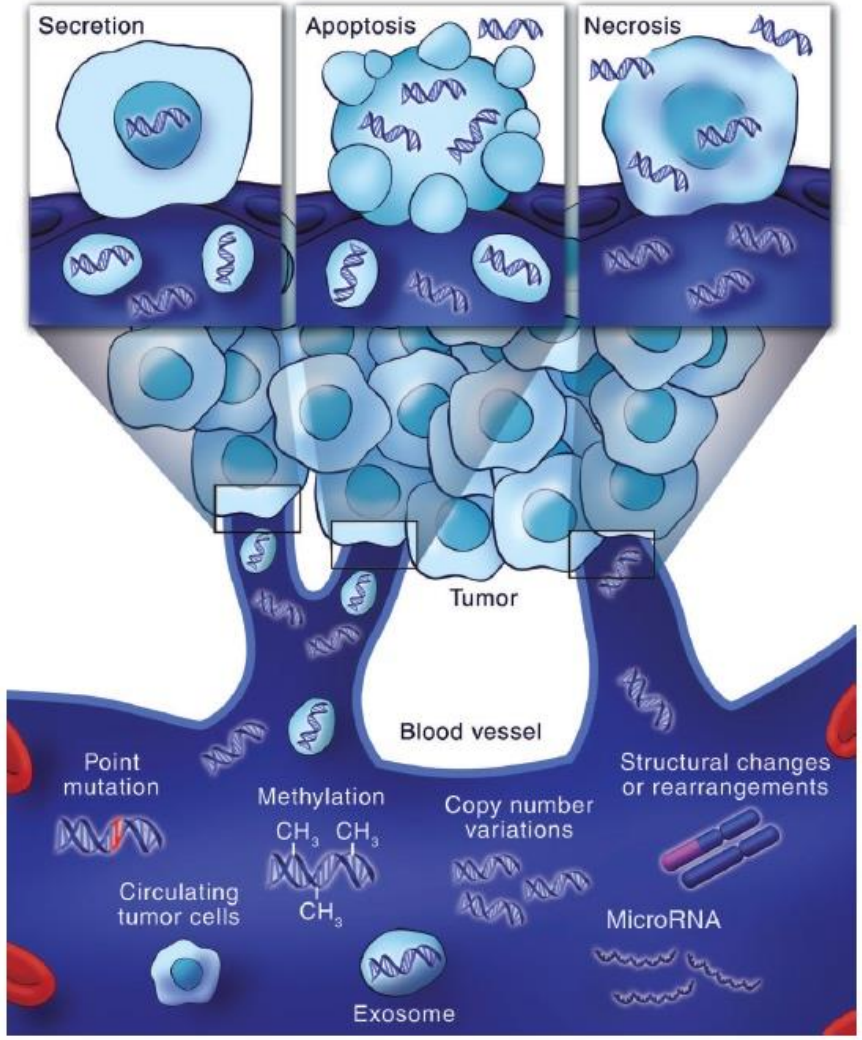
Actual patient case from Guardant360 user

Appendix (includes branded G360 slides)

Liquid Biopsy – capturing tumor DNA non-invasively



Sources of cell-free DNA (cfDNA)



Diaz and Bardelli, 2014 *JCO*

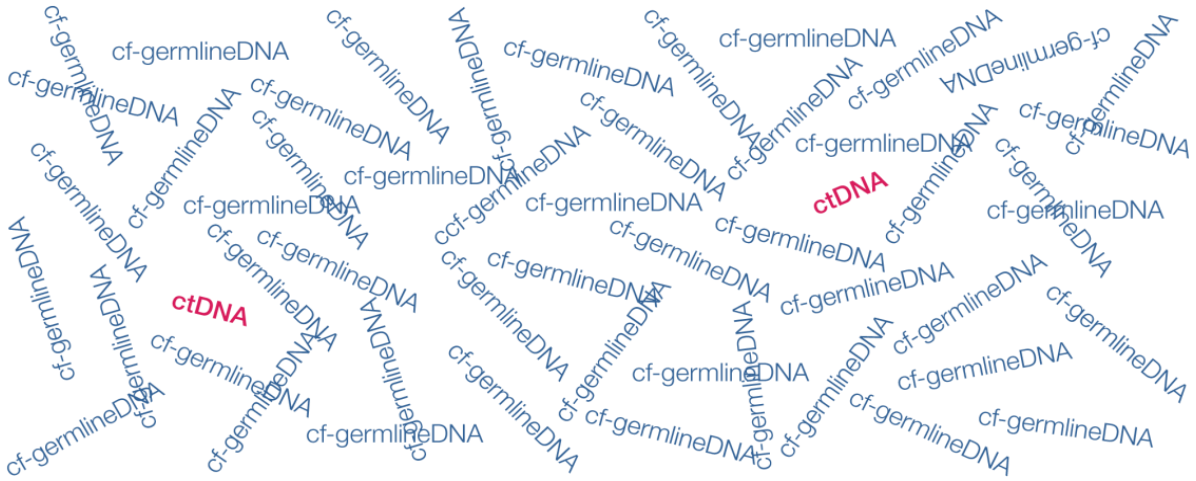
Key facts about cell-free DNA (cfDNA)

Present in circulation at low concentrations in healthy individuals

1-2 hour half-life in circulation

Higher total cfDNA levels in pregnant women and cancer patients

- Mixture of normal (germline) cfDNA and “other” cfDNA
  - Mean fetal fraction in pregnant women: ~12%
  - Median tumor fraction in cancer patients: ~0.4%



Targeted “hotspot” tests

NGS optimized for low fractions of the target DNA

Rava et al. 2013 *Clinical Chemistry* / Bettegowda et al. 2014 *Sci Transl Med* / Lanman, et al. 2015 *PLOS One*

Uncovering a genomic alteration may not always result in the patient receiving appropriate therapy

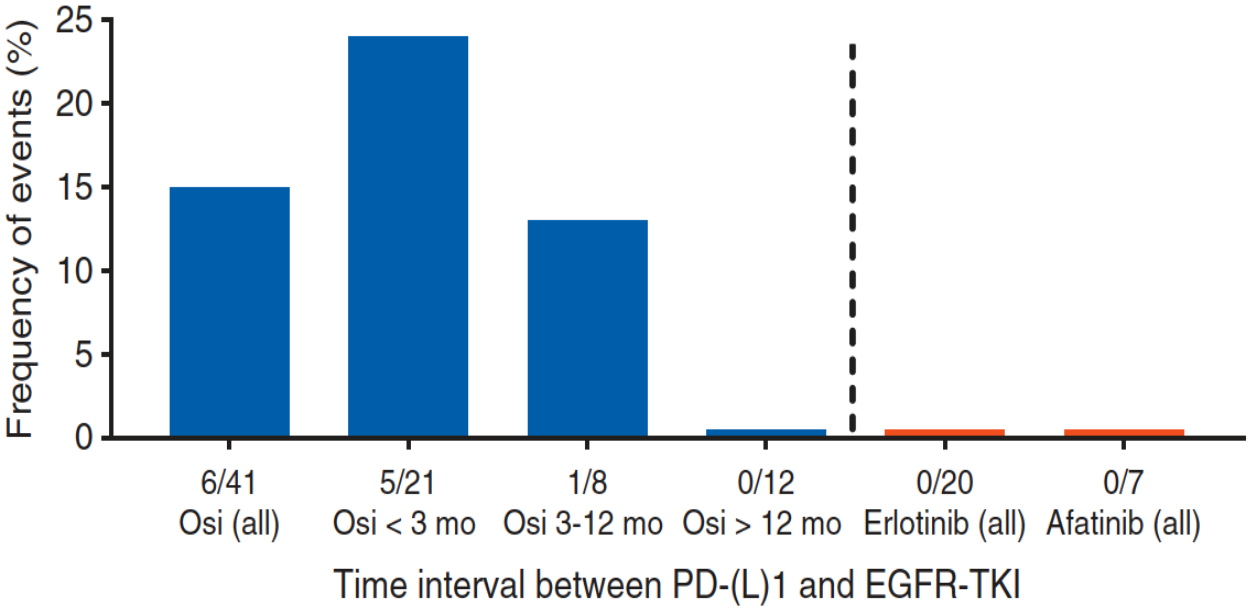
- Retrospective study of 5,688 patients with advanced NSCLC documented in the Flatiron Health Database between Jan 1, 2011 and Jul 31, 2016
- All patients received genomic testing
- All patients' received first-line therapy and the treatment decision was examined

What the results found…

<50% of patients with documented *EGFR* or *ALK* alterations were treated with tyrosine kinase inhibitors in the first line

Presley et al. 2018, *JAMA Onc*

Severe immune-related adverse events are common with sequential PD-(L)1 blockade and osimertinib



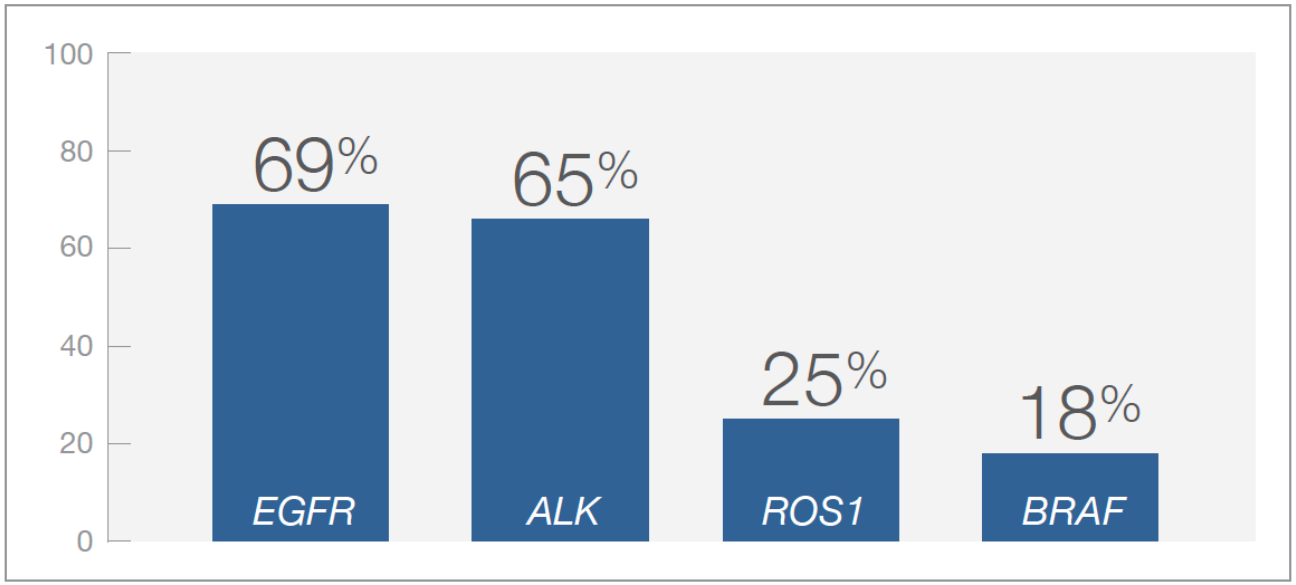
- Identified 126 patients treated with both EGFR TKI and PD-(L)1 blockade.
- **15% of patients treated with sequential PD-(L)1 blockade followed by osimertinib developed a severe irAE.**
- Severe irAEs were most common among those who began osimertinib within 3 months
- No irAEs were observed when osimertinib preceded PD-(L)1 blockade or when PD-(L)1 was followed by other EGFR-TKIs.

Schoenfeld (Hellman) et al. 2019 *Annals of Oncology*
* Pneumonitis > Colitis > Hepatitis, 83% required hospitalization

Real-world issues of undergenotyping in community practices

Results from a review of 814 consecutive NSCLC patients treated at 15 community practices

Genotyping rates for genomic alterations with FDA-approved therapies in NSCLC[6]

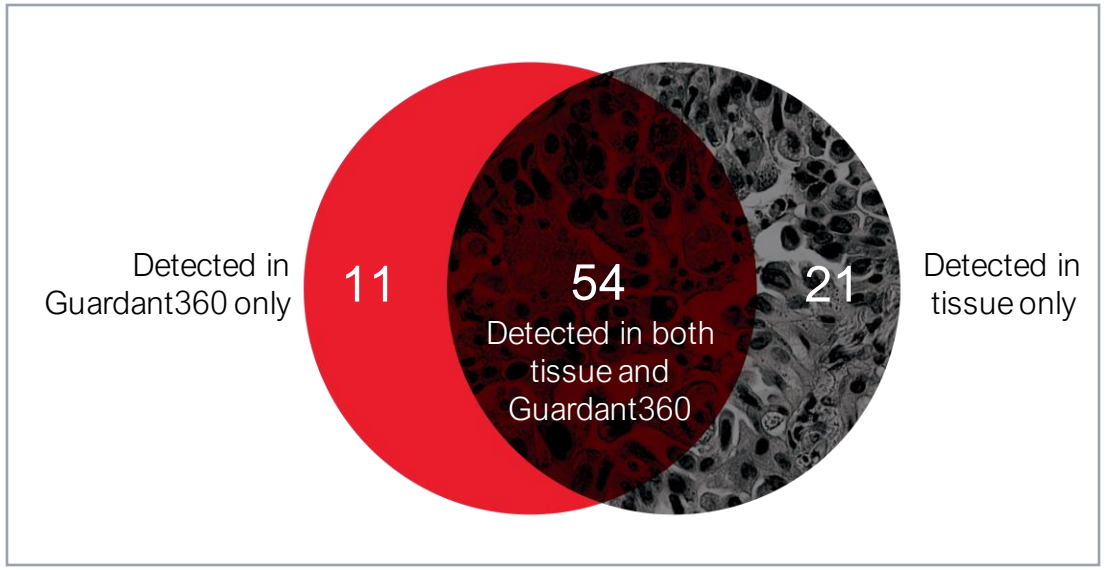


- Only 8% of patients were tested for all 7 guideline-recommended alterations (including *MET*, *RET*, and *ERBB2* [HER2])

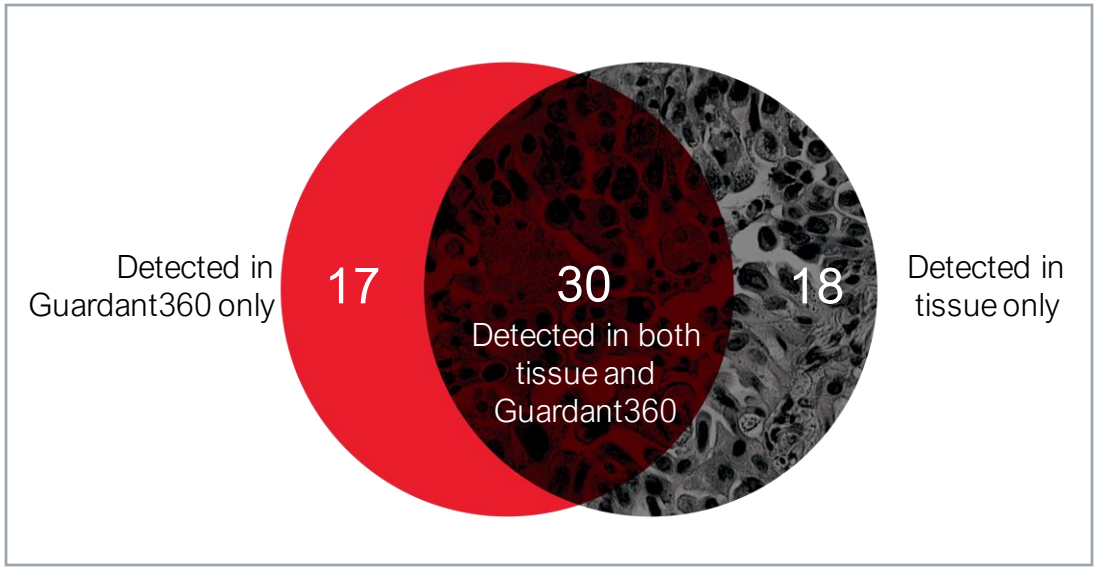
6. Gutierrez et al. 2017 *Clin Lung Cancer*

In prospective studies, Guardant360 had comparable detection of targetable biomarkers vs comprehensive tissue testing

Study 1 Results from 128 patients with advanced NSCLC prior to first-line therapy and at progression[15]

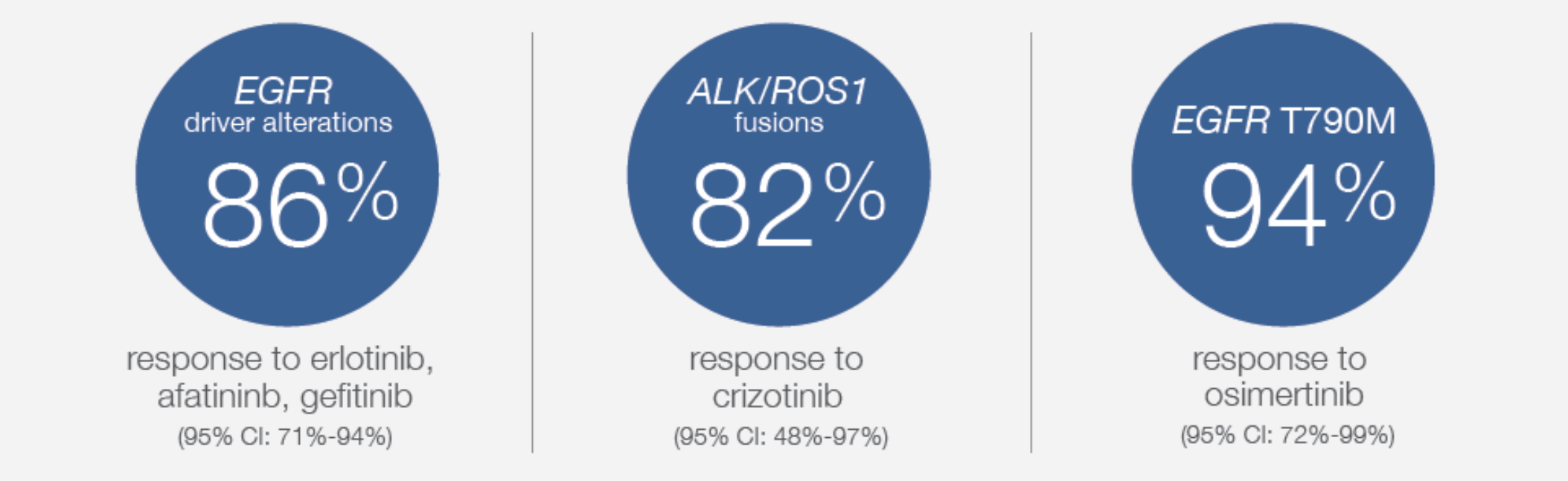


Study 2 Results from 185 patients with advanced NSCLC prior to first-line therapy[16]



15. Aggarwal et al. 2018 *JAMA Oncology* / 16. Palmero et al. 2018 IASLC 19th World Conference on Lung Cancer, Abstract #12561

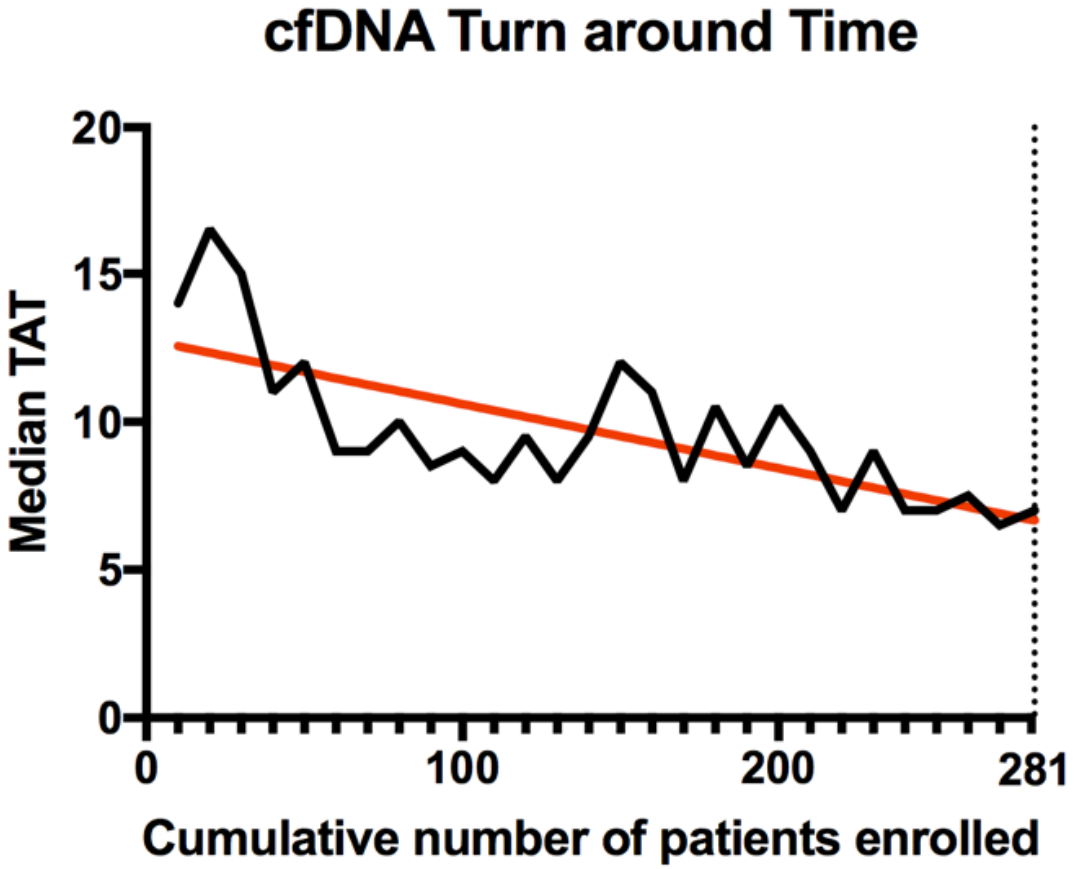
Treat what you find with Guardant360[17]



- Objective response was achieved regardless of variant allele fraction

17. Kim et al. 2017 *JCO Precision Onc*

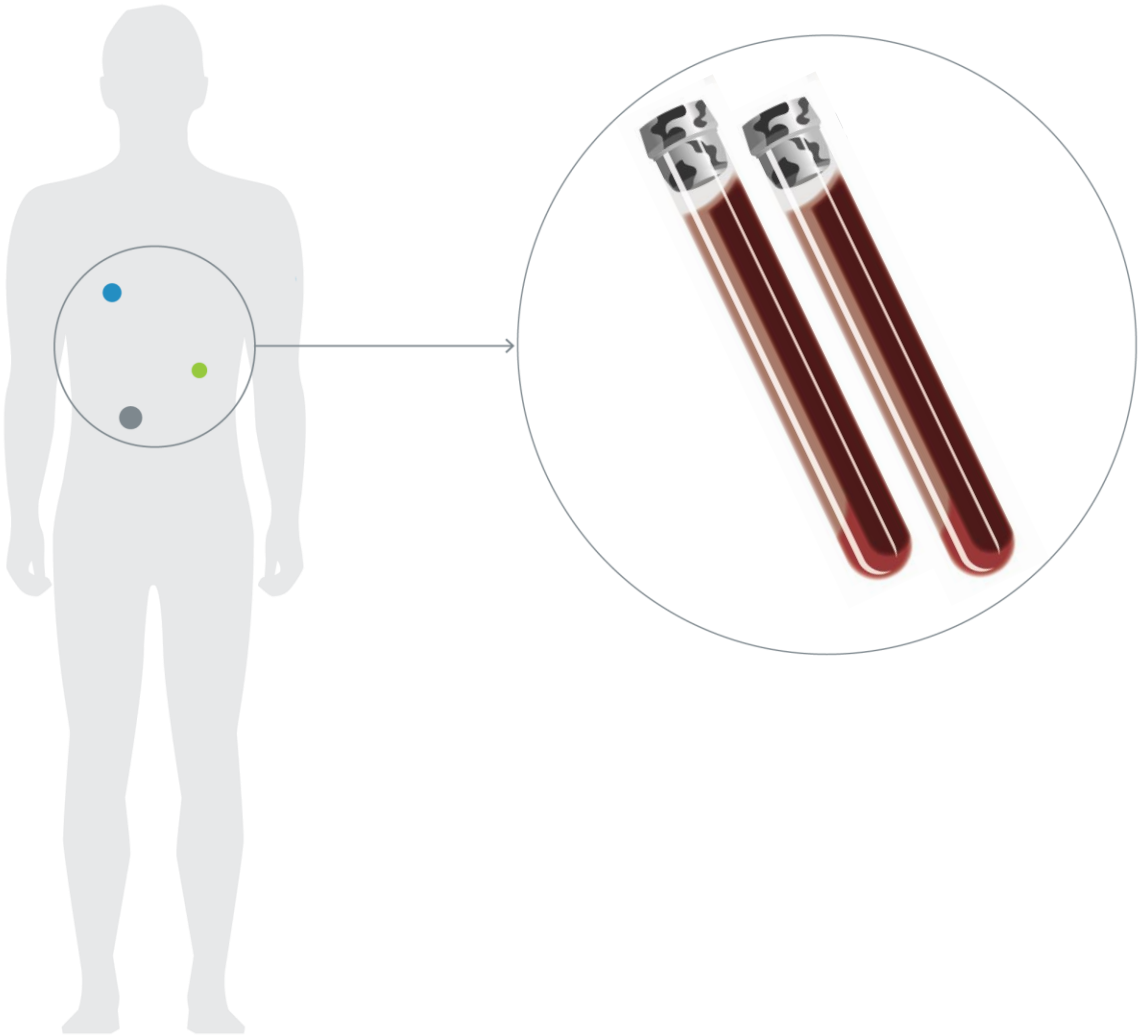
Results were reported significantly faster with Guardant360



- Median turn around time was significantly faster for Guardant360 as compared to tissue testing (9 vs 15 days; $p<0.0001$)

- Guardant360 median turn around time improved to 7 days over the course of the study

Benefits of Guardant360 at metastatic progression



Do not need to deal with the hassles of tracking down archival tissue

Get results in 7 days

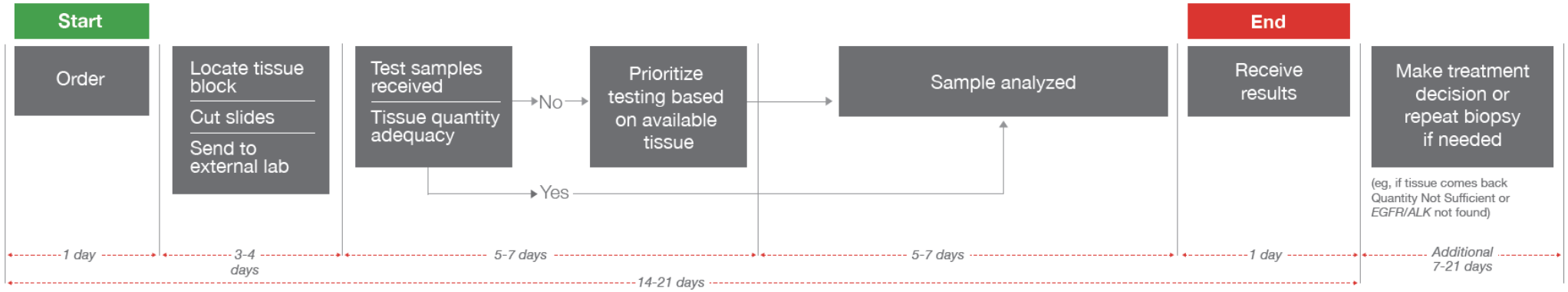
Patients are not subjected to the risks of complications of repeat biopsies

Find treatment or clinical trial recommendations by testing over 70 genes, including MSI-High and *NTRK1* fusions

Avoid the complexities and delays of tissue testing

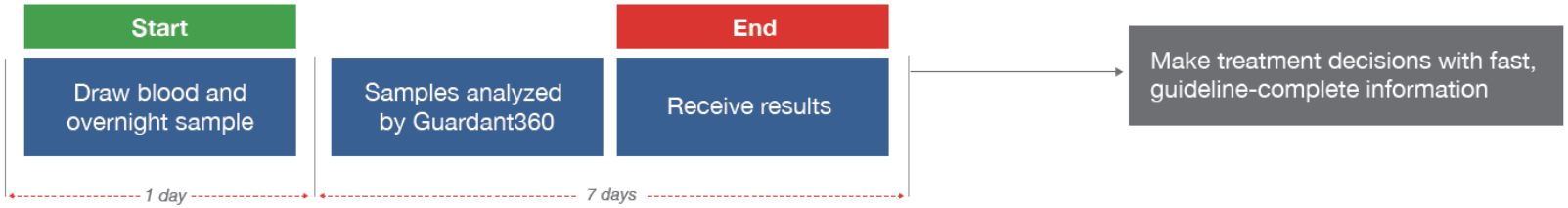
Tissue workflow for genomic profiling

An unpredictable and complex process that requires communication across multiple stakeholders and **can take >14 days** depending on tissue adequacy



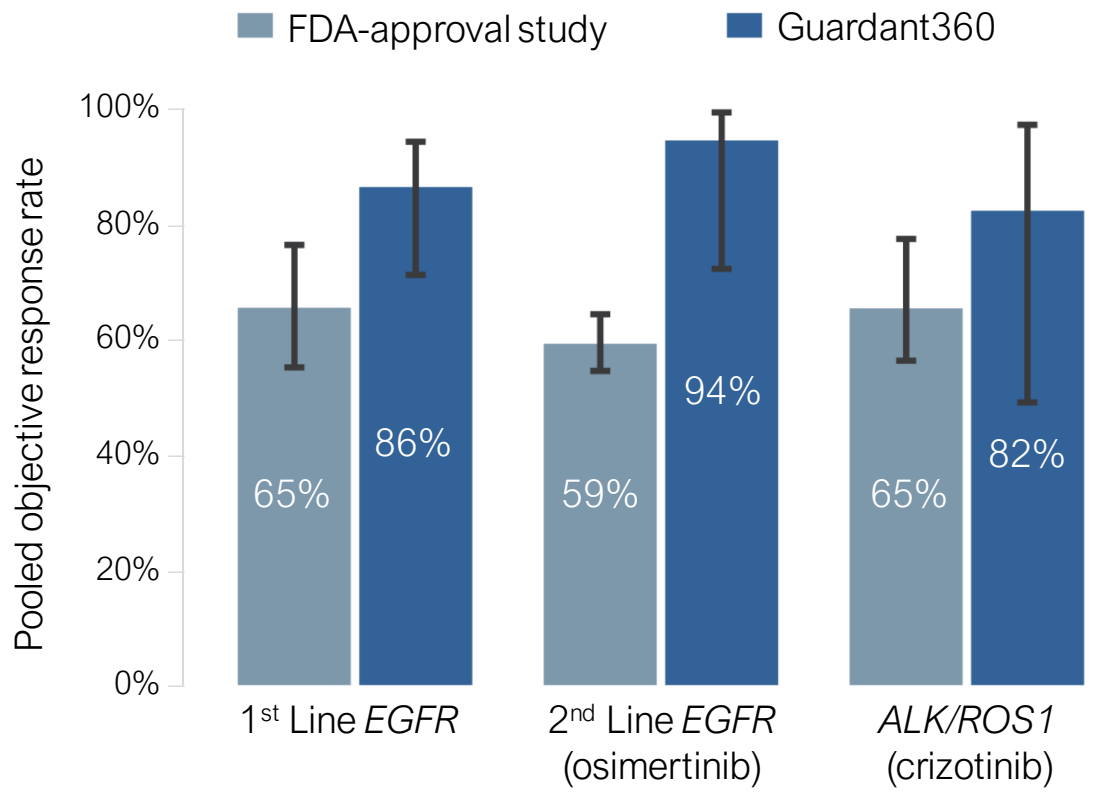
Genomic profiling with Guardant360

A fast and reliable process that delivers **guideline-complete results in 7 days**



Guardant360-detected alterations: patients respond

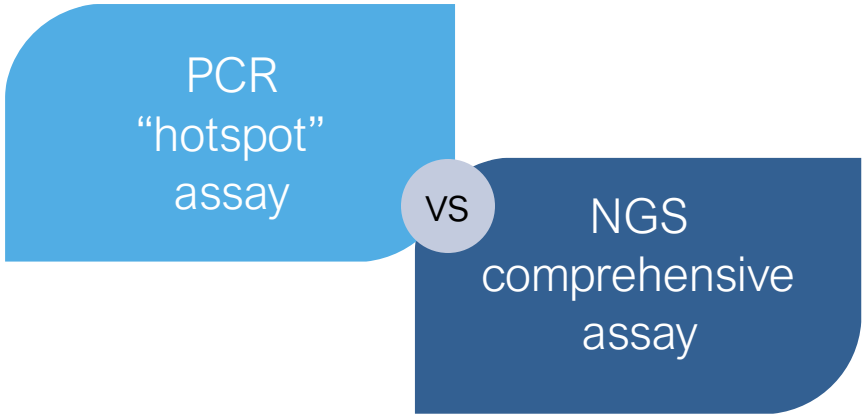
Pooled response rate of Guardant360 NSCLC studies compared to expected response rate



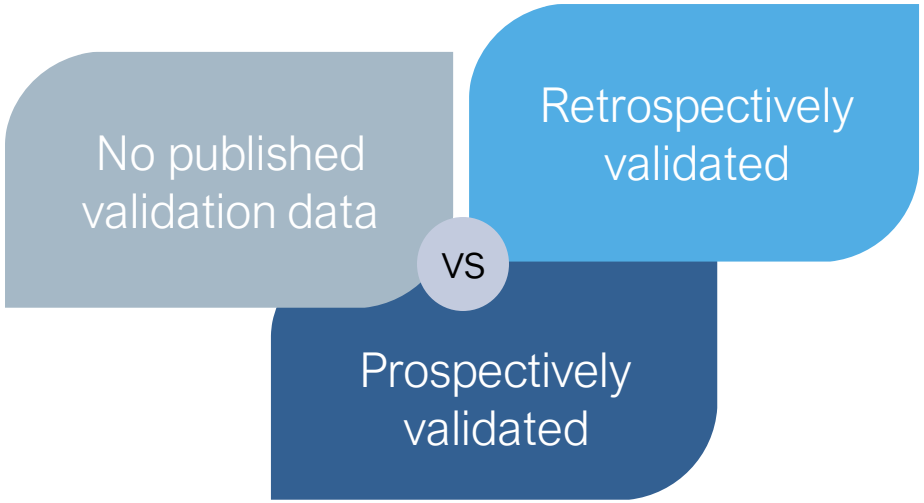
References in slide notes section

Considerations when choosing a liquid biopsy

Test Type



Validation Status

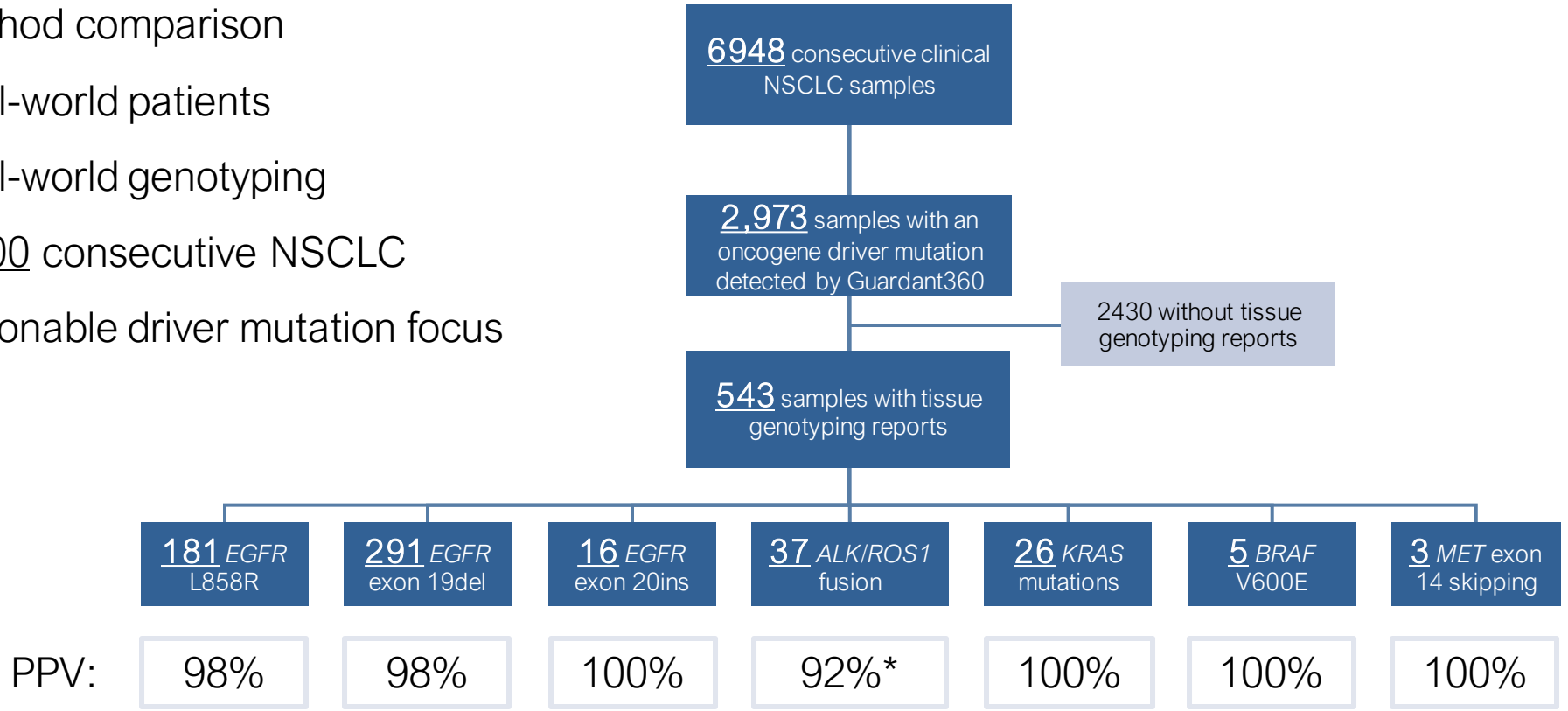


“The effective use of this promising new technology by clinicians hinges upon a shrewd understanding of the test characteristics and validation of a given assay . . .”

Sacher (Oxnard) et al. 2017 Application of plasma genotyping technologies in non-small cell lung cancer: A practical review, *JTO*

The COMPLETE study: Comparison Of Matched PLasma VErsus TissuE

- Method comparison
- Real-world patients
- Real-world genotyping
- 7,000 consecutive NSCLC
- Actionable driver mutation focus

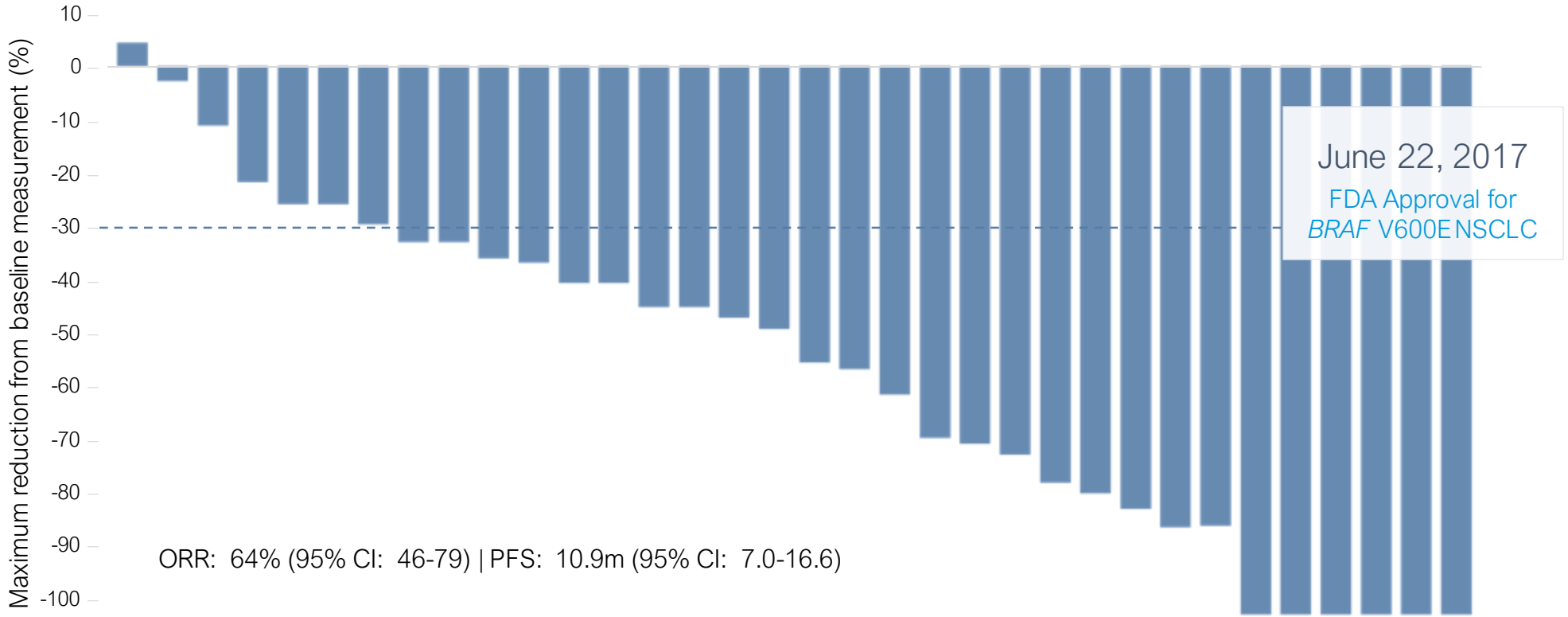


*3 of 3 Guardant360+ tissue- ALK fusions were treated with and responded to crizotinib

JCO 2016 34:18_suppl, ASCO Abstract LBA11501, updated JTO, 12(1):S263–S264 for World Congress Lung Cancer Abstract OA06.01 2016

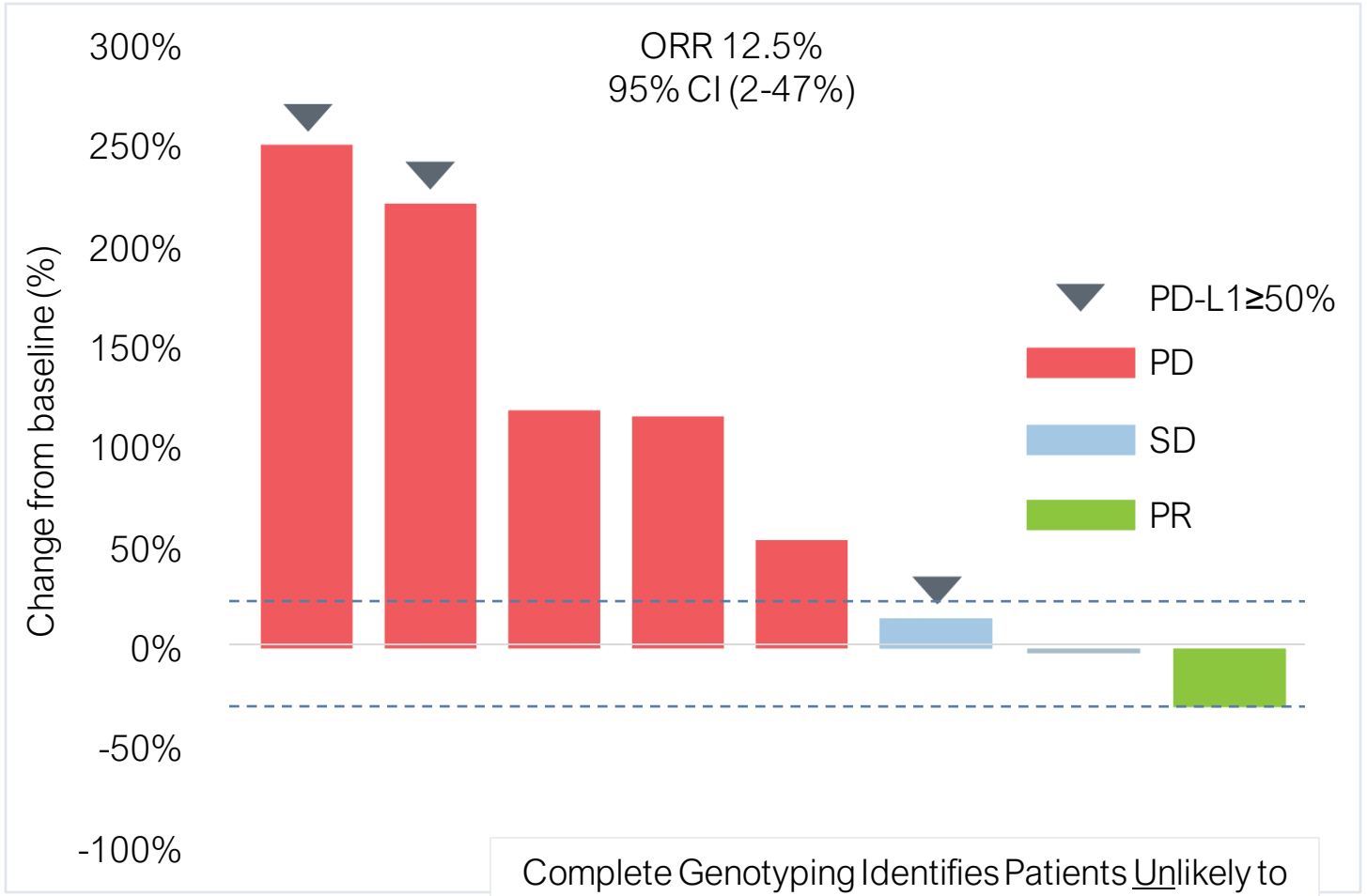
Targeting *BRAF* V600E in NSCLC

Tumor Responses to First-line Dabrafenib + Trametinib in *BRAF* V600E–mutant NSCLC



Planchard et al 2017 *Lancet Oncology*

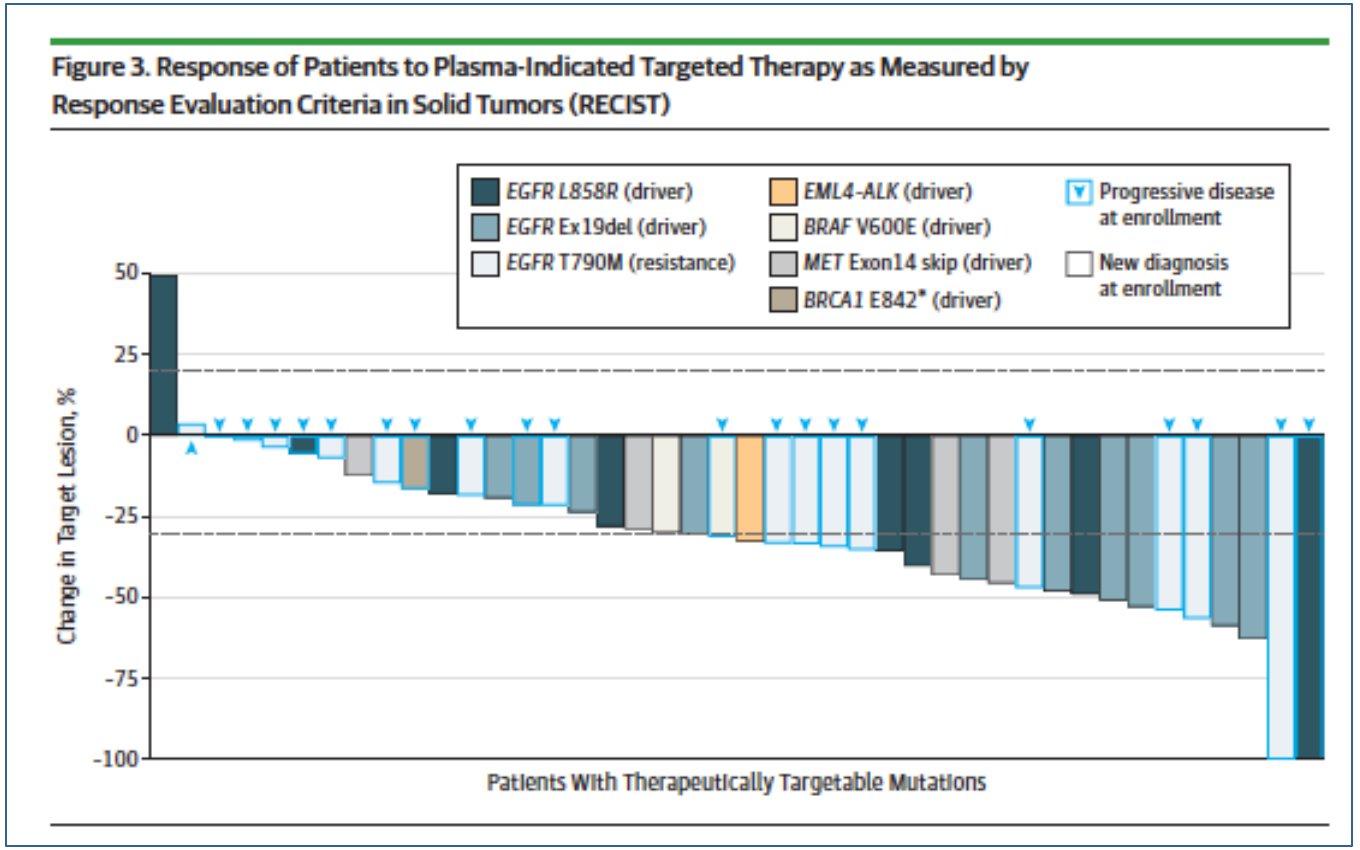
Poor response to IO in patients with *BRAF* V600E NSCLC



Complete Genotyping Identifies Patients Unlikely to Benefit from Immunotherapy

Dudnik et al. 2017 *JTO Suppl*

Patients treated based on Guardant360 results responded as expected to targeted therapies

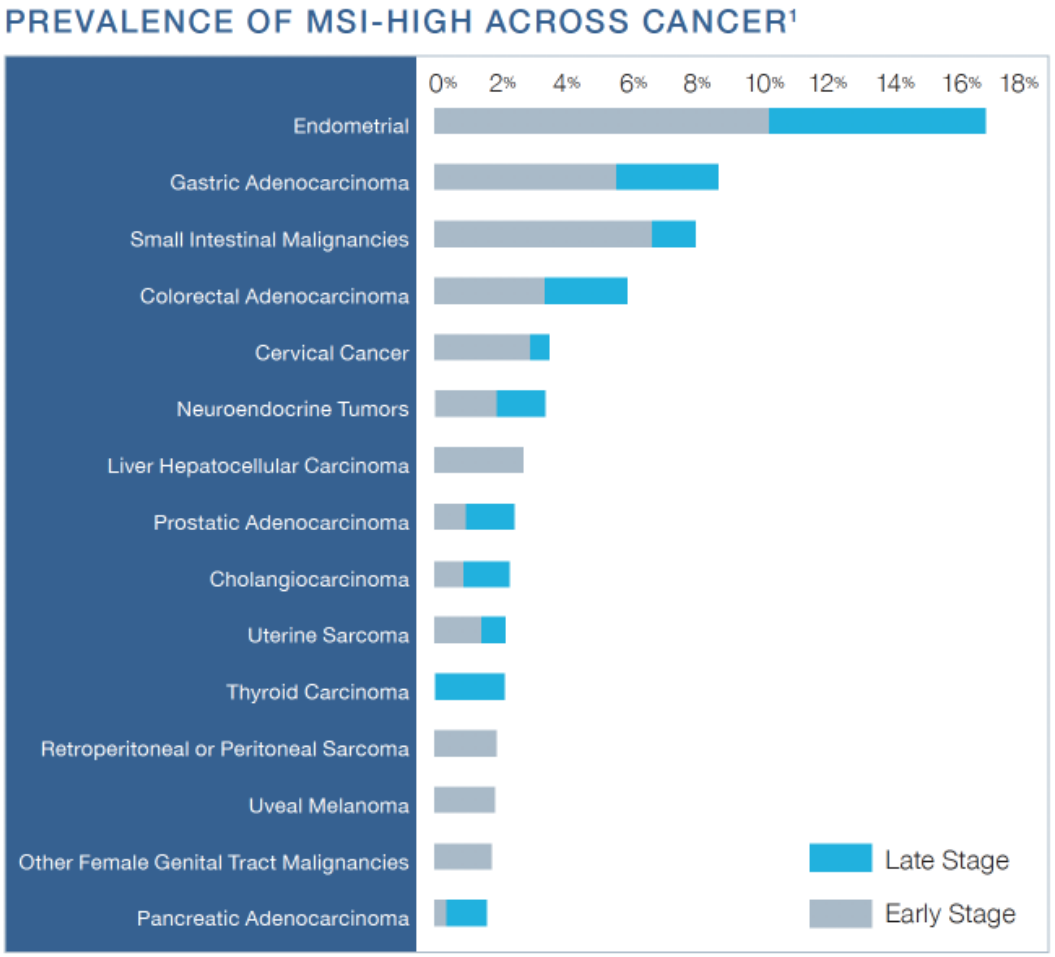


86% of NSCLC patients who were treated with targeted therapy based on Guardant360 results achieved a complete response, partial response, or stable disease

*Targetable mutations were in eight genes: *EGFR*, *ALK*, *MET*, *BRCA1*, *ROS1*, *RET*, *ERBB2*, and *BRAF*

18. Aggarwal et al. 2018 *JAMA Oncology*

Prevalence of MSI-High*



- Guidelines recommend MSI testing for patients with advanced cancers: colorectal, endometrial, gastric and gastroesophageal, pancreatic, and prostate

- Multiple checkpoint inhibitors have FDA approved indications of use across advanced cancers for MSI-High patients

*Please note: MSI status is not reported for specimens originating from New York State or for earlier panel versions.

Guardant360 technical specifications for MSI-High*

Technical Specifications[1]	Mutant Allele Frequency	Sensitivity	Specificity
	≥0.1%	95%	100%

Clinical Validation[2]: Mutant Allele Frequency: ≥0.4%; Sensitivity: 92%; Specificity: 99%;

Reportable Range: Detected/Not Detected

*Please note: MSI status is not reported for specimens originating from New York State or for earlier panel versions / 1. Analytical Validation / 2. Clinical Validation