THE ROLE OF BIOMARKER TESTING IN ADVANCED NSCLC

US-NONO-00378
03/23

EMD SERONO

Important Notices

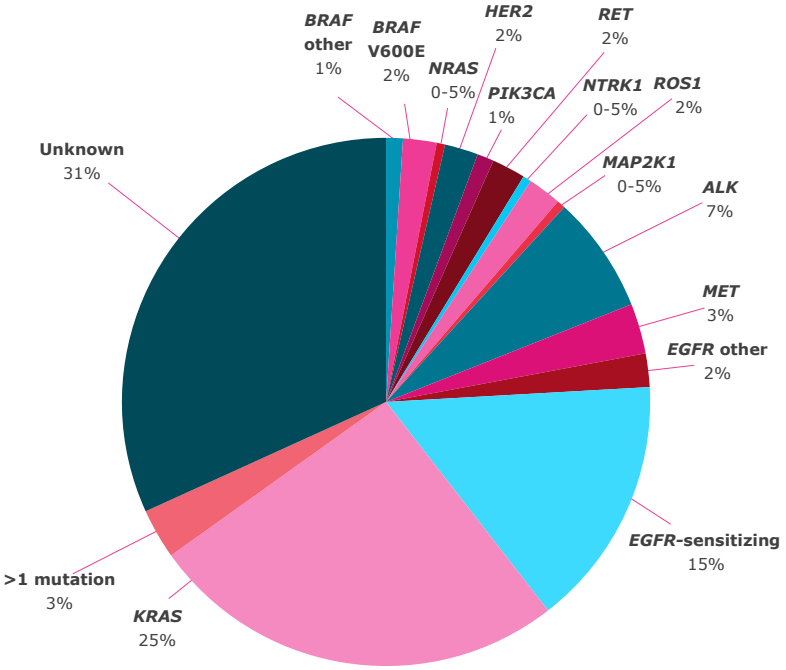
- Copyright in this document is owned by Merck KGaA, Darmstadt, Germany, or its affiliates (except for any third-party content that has been identified as such) unless otherwise specified and all rights are reserved
- All product names referred to in this document are trademarks of Merck KGaA, Darmstadt, Germany, or its affiliates except for those trademarks that are indicated as owned by other companies and all rights are reserved
- Unless otherwise stated, copyright clearance for the use of images and graphs has been sought throughout this presentation
- EMD Serono is the Healthcare business of Merck KGaA, Darmstadt, Germany, in the United States and Canada
- This presentation is for discussion purposes only, not for distribution, and is intended for US healthcare providers only

OVERVIEW OF BIOMARKERS IN NSCLC

Known biomarkers and use of biomarker testing for patient care

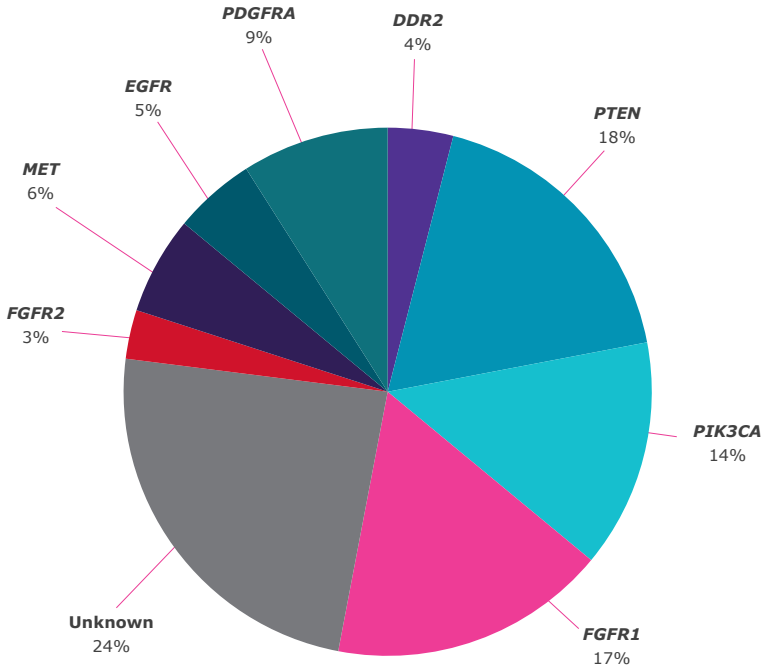
NSCLC is a heterogenous group of diseases with distinct histological subtypes and numerous oncogenic drivers

ONCOGENIC DRIVERS IN ADENOCARCINOMA[1]



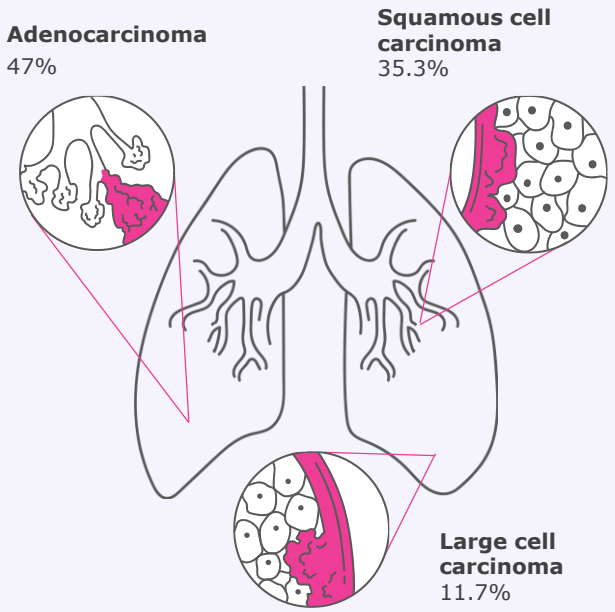
Up to 60% of patients with adenocarcinoma have a known oncogenic driver[2]

ONCOGENIC DRIVERS IN SQUAMOUS CELL CARCINOMA[1]



50% to 80% of patients with squamous cell carcinoma have a known oncogenic driver[2]

NSCLC INCLUDES 3 MAIN HISTOLOGICAL SUBTYPES[1]



Known oncogenic drivers differ in commonality between these subgroups[1]

Oncogenic drivers may serve as prognostic or predictive biomarkers to help guide patient management[3]

NSCLC, non-small cell lung cancer.

1. Lungevity. Types of Lung Cancer. https://www.lungevity.org/for-patients-caregivers/lung-cancer-101/types-of-lung-cancer. Accessed March 2, 2023. 2. Chan BA, et al. Transl Lung Cancer Res. 2015;4:36-54. 3. Ballman KV. J Clin Oncol. 2015;33:3968-3971.

Characteristics of patients with different driver mutations*

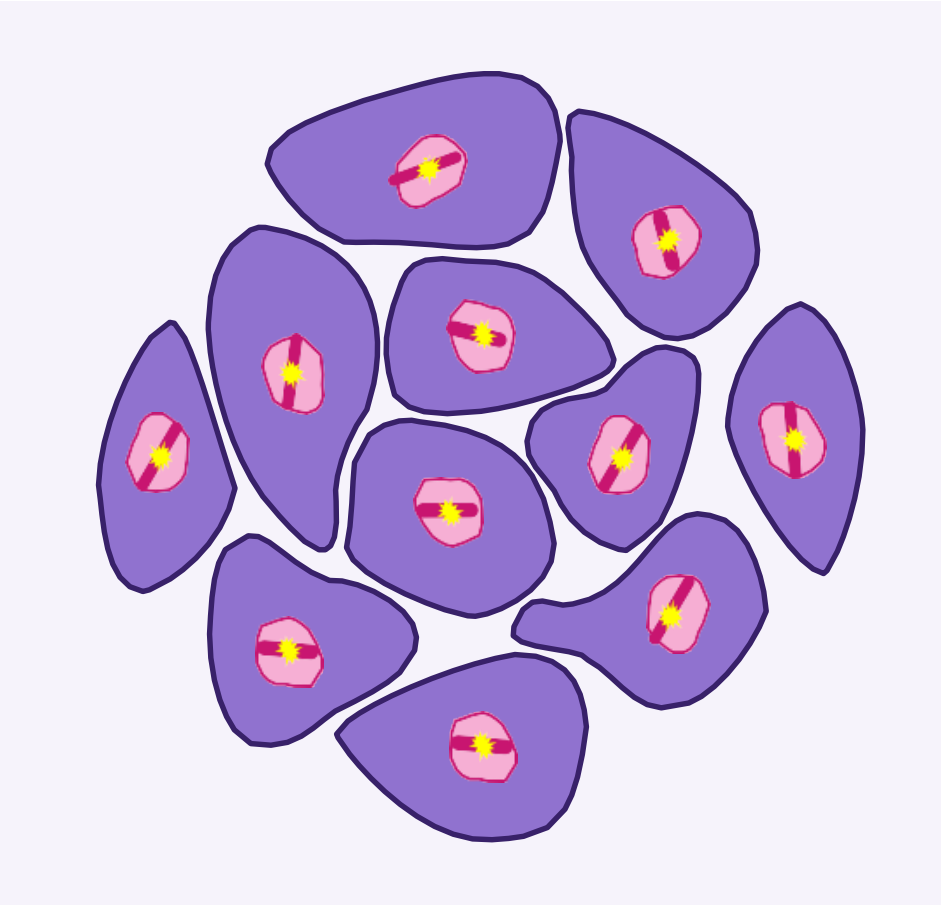
Mutation	Age (yrs) Mean ± SD	Smoking history (%)		Gender (%)		Stage (%)	
		Ever smoked	Never smoked	Female	Male	IA-IIIA	IIIB-IV
ALK positive	55.0 ± 13.7	41.7	58.3	48.1	51.9	70.4	39.6
EGFR positive	63.5 ± 10.9	32.1	67.9	54.4	45.6	84.2	15.8
KRAS positive	64.7 ± 9.1	79.6	20.4	18.0	82.0	86.4	13.6
METex14 skipping	73.7 ± 11.6	50.0	50.0	38.9	61.1	83.3	16.7
*MET*amp (high)	65.5 ± 11.7	100.0	0.0	12.5	87.5	57.1	42.9
ROS1 positive	53.9 ± 16.2	42.9	57.1	60.0	40.0	40.0	60.0

*Note that the data presented may have been calculated from small population sizes (range: 8-180).[1]

*MET*amp, *MET* amplification; *MET*ex14, *MET* exon 14; NSCLC, non-small cell lung cancer; SD, standard deviation.

1. Tong JH, et al. Clin Cancer Res. 2016 Jun 15;22(12):3048-3056.

Importance of biomarker testing in NSCLC[1-3]



- NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) recommend **biomarker testing in all appropriate patients with NSCLC** based on data showing clinical benefit for patients receiving appropriate targeted therapy or immunotherapy as opposed to chemotherapy options[1]
    - **Predictive biomarkers** are indicative of therapeutic efficacy because there is an interaction between the biomarker and therapy on patient outcome[1]
    - **Prognostic biomarkers** are indicative of patient survival independent of the treatment received[1]
- Molecular testing to detect actionable targets as part of a diagnostic work-up can help **personalize care**
- Longitudinal biomarker testing can provide **insights into tumor evolution, heterogeneity, and resistance**

NCCN, National Comprehensive Cancer Network; NSCLC, non-small cell lung cancer.
1. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-Small Cell Lung Cancer V.2.2023. © National Comprehensive Cancer Network, Inc. 2023. All rights reserved. Accessed March 2, 2023. To view the most recent and complete version of the guideline, go online to NCCN.org. 2. Kerr KM, et al. Lung Cancer. 2021;154:161-175. 3. Ahmadzada T, et al. J Clin Med. 2018;7(6):153.

Current actionable biomarkers in metastatic NSCLC according to NCCN Guidelines®[1]

Patients receiving appropriate targeted therapy or immunotherapy based on biomarker testing show greater clinical benefit as opposed to patients receiving chemotherapy*

PREDICTIVE BIOMARKERS ASSOCIATED WITH RESPONSIVENESS TO TARGETED THERAPY

- *EGFR*† mutations such as exon 19 indels, exon 20 mutations (eg, p.T790M), or exon 21 mutations (eg, p.L858R)
- *ALK*† rearrangements
- *ROS1*† gene fusions
- *BRAF* V600E point mutations
- *ERBB2* (*HER2*) mutations
- *KRAS* G12C point mutations
- *MET*ex14 skipping mutations
- *RET* gene rearrangements
- *NTRK1/2/3* gene fusions

PREDICTIVE BIOMARKERS ASSOCIATED WITH RESPONSIVENESS TO IMMUNOTHERAPY

- PD-L1 protein expression level

EMERGING BIOMARKERS

- High-level *MET* amplification‡

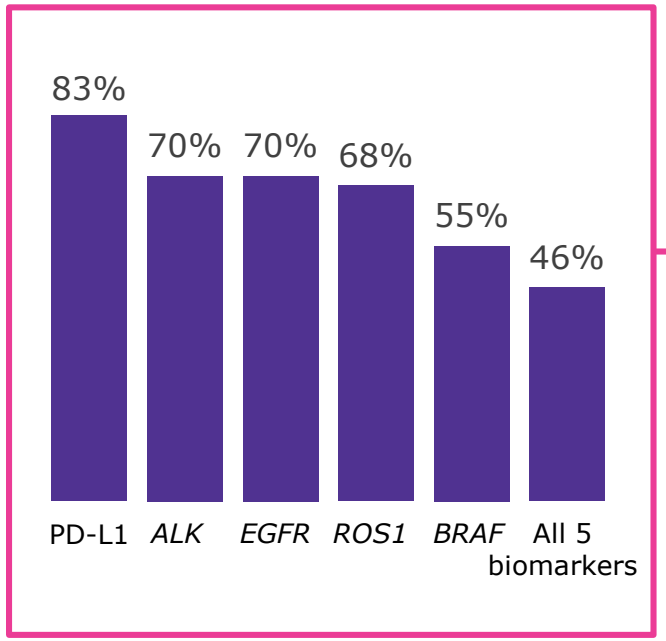
*The NCCN Guidelines® for NSCLC provide recommendations for individual biomarkers that should be tested and recommend testing techniques but do not endorse any specific commercially available biomarker assays or commercial laboratories.[1]
†Considered must test biomarkers by CAP-IASLC molecular testing guidelines.[2] ‡The definition of high-level *MET* amplification is evolving and may differ according to the assay used for testing. For NGS-based results, a copy number greater than 10 is consistent with high-level *MET* amplification.[1]
NCCN, National Comprehensive Cancer Network; *MET*ex14, *MET* exon 14; NSCLC, non-small cell lung cancer; PD-L1, programmed death ligand 1.
1. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-Small Cell Lung Cancer V.2.2023. Accessed March 2, 2023. To view the most recent and complete version of the guideline, go online to NCCN.org. 2. Lindeman NI et al. J Mol Diagn. 2018;20(2):129-159.

EMD SERONO

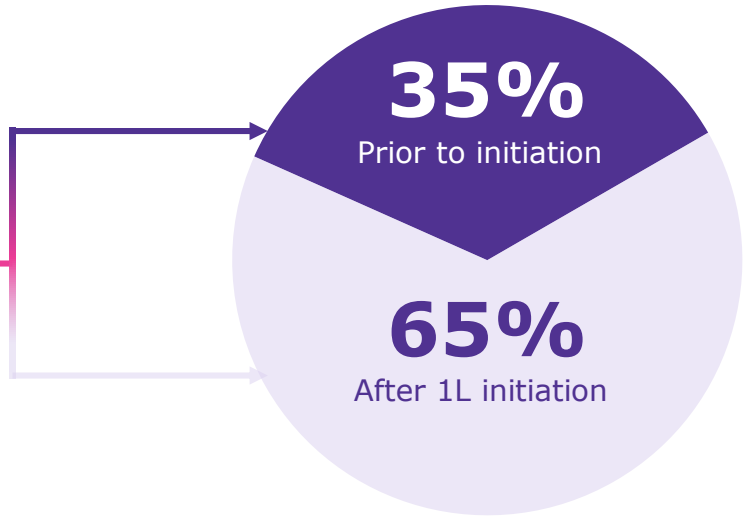
Despite the identification of actionable biomarkers and known patient benefit, biomarker testing may be limited

Although biomarker testing rates have increased in the last few years, challenges to biomarker testing in NSCLC remain[1-3]

BIOMARKER TESTING RATES[1,*] (% OF PATIENTS TESTED; N=3474)



BIOMARKER TESTING PRIOR TO 1L INITIATION[1,*]



Only about a third of patients with actionable mutations received biomarker testing results before the initiation of 1L therapy[1]

Current challenges to biomarker testing include[3,4]:

- Tissue sample adequacy
- Selecting the appropriate biomarker test
- Interpretation of biomarker test results
- Financial considerations
- Turnaround time for some results

*A retrospective study in patients with metastatic NSCLC who initiated 1L systemic therapy in The US Oncology Network between April 1, 2018, and March 31, 2020.
1L, first line; NSCLC, non-small cell lung cancer; PD-L1, programmed death ligand 1.
1. Robert NJ, et al. Lung Cancer. 2022;166:197-204. 2. Griesinger F, et al. Lung Cancer. 2021;152:174-184. 3. Kim ES, et al. J Thorac Oncol. 2019;14(3):338-342. 4. Kerr KM, et al. Lung Cancer. 2021;154:161-175.

NSCLC tissue biopsy size is often limited – NILE study[1]

A CORE LUNG BIOPSY* WILL GIVE 200 µm OF MATERIAL[1]

Block trimming waste 10 µm

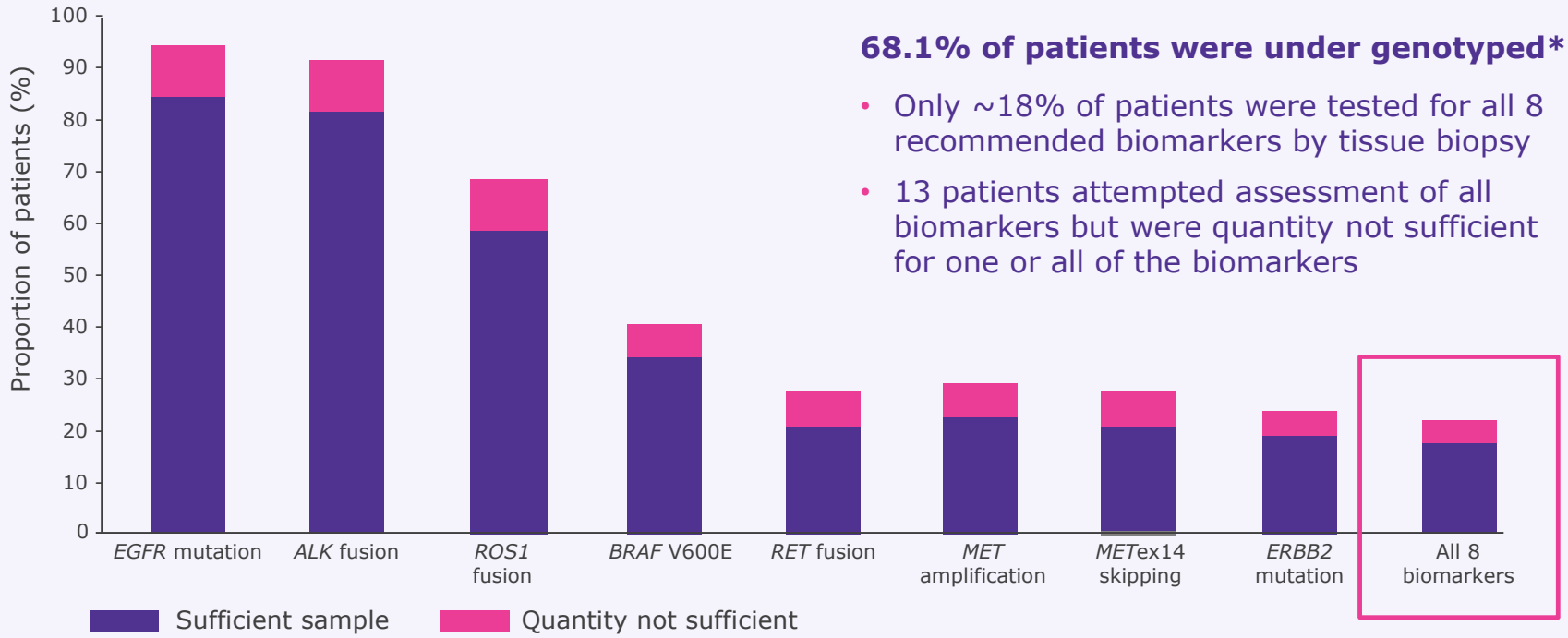
NGS 10 x 5 µm for NGS testing = 50 µm for tests + wastage	Total = **60 µm**
***ALK, ROS1,* PD-L1** 5 x 4 µm for *ALK* and *ROS1* FISH/IHC and PD-L1 IHC = 20 µm for tests + wastage	Total = **30 µm**
EGFR 6 x 10 µm for *EGFR* testing = 60 µm for tests + wastage	Total = **70 µm**
H&E, IHC 1 x 4 µm H&E 4 x 4 µm additional Ab 2 x 4 µm controls = 28 µm for tests + wastage	**Total = 38 µm**
	Total = 198 µm (leaving just 2 µm for additional testing)

- Tissue biopsy is often small and sample amount may not be sufficient for testing all actionable biomarkers[1]
- Use of multiplex arrays may increase efficiency with small tissue samples and allow simultaneous detection of multiple biomarkers[2]

*Core needle biopsies provide more intact material than fine needle aspiration.[1]
Ab, antibody; FISH, fluorescence in situ hybridization; H&E, hematoxylin and eosin; IHC, immunohistochemistry; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; PD-L1, programmed death ligand 1.
1. Data on file. 2. Engstrom PF, et al. JNCCN. 2011;9(6).

NSCLC tissue biopsy size is often limited – NILE study[1]

PROPORTION OF PATIENTS WITH SUFFICIENT TISSUE FOR BIOMARKER ASSESSMENT



68.1% of patients were under genotyped*

- Only ~18% of patients were tested for all 8 recommended biomarkers by tissue biopsy
- 13 patients attempted assessment of all biomarkers but were quantity not sufficient for one or all of the biomarkers

- Sequential biomarker testing using a tissue biopsy occurred in 84.8% of patients
- Of the patients with complete genotyping using a tissue sample:
  - 68.6% had comprehensive NGS genotyping
  - 31.3% had sequential testing of all 8 biomarkers

With cfDNA available, all 8 guideline-recommended biomarkers were **fully assessed in 95% of patients**

If all currently recommended tests are performed sequentially, there may not be sufficient sample to test all biomarkers

*Did not have a guideline-recommended biomarker identified and were not assessed for all guideline-recommended biomarkers.
cfDNA, cell-free DNA; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer.
1. Leighl NB, et al. Clin Cancer Res. 2019;25:4691-4700.

TESTING FOR BIOMARKERS IN NSCLC

Technical approaches, testing needs, and clinical guideline recommendations

EMD SERONO

Sample collection – tissue biopsy

Tissue biopsy is well established and sensitive, but has significant challenges

Tissue biopsy[1,2]

Isolation of tissue sample

FFPE samples, cytoblocks, or cytology smears

Pathology, IHC, and tumor genotyping

Biomarker identification and treatment decision

Advantages[1]

- **Highly specific and sensitive**
- **Allows assessment of both DNA and non-DNA biomarkers**
- **Provides pathology information**
- **Allows PD-L1 assessment**

Disadvantages[1]

- **May have longer turnaround time**
- **Limited tissue quantities**
- **Invasive**
- **Re-biopsy not always possible in case of progressive disease**
- **May not capture tumor heterogeneity**

FFPE, formalin-fixed, paraffin-embedded; IHC, immunohistochemistry; PD-L1, programmed death ligand 1.

1. Rolfo C, et al. J Thorac Oncol. 2021;16(10):1647-1662. 2. National Cancer Institute. Biomaker Testing for Cancer Treatment. https://www.cancer.gov/about-cancer/treatment/types/biomarker-testing-cancer-treatment. Accessed March 2, 2023.

Sample collection – liquid biopsy

Liquid biopsy makes repeat sampling and detecting tumor heterogeneity easier, but may have limited sensitivity

Liquid biopsy[1,2]

Plasma sampling

cfDNA isolation

Tumor genotyping

Biomarker identification and treatment decision

Advantages[1]

- **High concordance rate**
- **May have rapid turnaround time**
- **Minimally invasive**
- **Repeatable over time**
- **Captures tumor heterogeneity and clonal evolution**

Disadvantages[1]

- **Non-DNA biomarkers not evaluable**
- **Concurrent use with tissue testing can increase costs**
- **False negatives**
- **Low concentrations of ctDNA may be difficult to detect**

cfDNA, cell-free DNA; ctDNA, circulating tumor DNA.

1. Rolfo C, et al. J Thorac Oncol. 2021;16(10):1647-1662. 2. National Cancer Institute. Biomaker Testing for Cancer Treatment. https://www.cancer.gov/about-cancer/treatment/types/biomarker-testing-cancer-treatment. Accessed March 2, 2023.

Sample collection – National Comprehensive Cancer Network® (NCCN®) recommendations[1]

The use of plasma cfDNA/ctDNA testing (plasma testing) for metastatic NSCLC **can be considered in specific clinical circumstances**:

- If a patient is medically unfit for invasive tissue sampling
- In the initial diagnostic setting following pathologic confirmation of NSCLC if there is insufficient material for molecular analysis and if follow-up tissue-based analysis is planned for patients without oncogenic drivers identified

Plasma cfDNA/ctDNA testing:

Should not be used in lieu of a histologic tissue diagnosis

Has very high specificity, but significantly compromised sensitivity (up to 30% false-negative rate)

Does not have established standards/guidelines for analytical performance characteristics

Can identify alterations that are unrelated to a lesion of interest

cfDNA, cell-free DNA; ctDNA, circulating tumor DNA; NCCN, National Comprehensive Cancer Network; NSCLC, non-small cell lung cancer.

Overview of assessment techniques

Method	Used to assess/detect:	Sensitivity (%)	Turnaround time	Biopsy method[3]	Point mutations	Small indels	CNAs	Rearrangements
PCR and Sanger sequencing[1,2]	DNA changes, including point mutations, insertions, or deletions	20 to 50	3 to 4 days	• Liquid • Tissue	✓	✓		
RT-PCR[1,2,4]	RNA expression, including fusion transcripts	0.00001	2 to 3 days	• Liquid • Tissue	✓	✓		✓
FISH[1,2]	Gene rearrangements including deletions, amplifications, translocations, and fusions	<1	2 to 3 days	• Tissue			✓	✓
NGS: targeted approach[1,2,5]	Genetic changes in multiple genes simultaneously	1 to 10	7 to 20 days	• Liquid • Tissue	✓	✓	✓	May not reliably detect fusions
NGS: WES/ WGS[1,2,5]		Variable	Weeks	• Liquid • Tissue	✓	✓	✓	✓ (As long as in design)
IHC[2,5,6]	Protein expression, localization or specific alterations, including fusions	Variable	1 to 2 days	• Tissue				✓

It is important to choose the technique that ensures accurate and reliable detection of the selected biomarkers within a reasonable turnaround time

CNA, copy number alteration; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NGS, next-generation sequencing; PCR, polymerase chain reaction; RT-PCR, reverse-transcription PCR; WES, whole-exome sequencing; WGS, whole-genome sequencing.

1. Pennell NA, et al. Am Soc Clin Oncol Educ Book. 2019;39:531-542. 2. El-Deiry WS, et al. CA Cancer J Clin. 2019;69(4):305-343. 3. Rolfo C, et al. J Thorac Oncol. 2021;16(10):1647-1662. 4. Tests used on biopsy and cytology specimens to diagnose cancer. American Cancer Society. https://www.cancer.org/treatment/understanding-your-diagnosis/tests/testing-biopsy-and-cytology-specimens-for-cancer/special-tests.html. Accessed March 2, 2023). 5. Dong J, et al. Front Pharmacol. 2019;10:230. 6. Doshi S, et al. Diagnostics (Basel). 2016;6(1):4.

Advantages and disadvantages of assessment techniques

	DNA and RNA				Protein
	NGS[1-3]	RT-PCR[3-5]	Sanger sequencing[6]	FISH[3,4]	IHC[4,7]
	• High sensitivity • Broad-panel testing • Detects gene rearrangements • Detects gene amplifications	• Highly sensitive • Detects fusion transcripts at the RNA level • Detects gene rearrangements • Turnaround time	• Ability to identify all possible mutations in the analyzed fragment	• Knowledge of fusion partner not required • Rearrangements can be discriminated from polysomy/ amplifications	• Sensitive • Familiar • Time saving and easily automatable • Cost-friendly • Many validated antibodies available
	• Turnaround time • Sophisticated bioinformatics needs • Large data storage capabilities • Reports can be hard to interpret • Cost	• Poor quality of FFPE RNA samples • Limited number of variants tested at once	• Low sensitivity assay requiring tumor enrichment	• Not all rearrangements produce an expressed fusion transcript • May miss unknown variants	• May require confirmatory test • Accuracy can vary by fixative and background • Insufficient tumor content of tissue • Skilled pathologist required

FFPE, formalin-fixed paraffin embedded; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NGS, next-generation sequencing; RT-PCR, reverse-transcription polymerase chain reaction.

1. Rolfo C, et al. J Thorac Oncol. 2021;16(10):1647-1662. 2. Whole-genome sequencing. Healio Learn Genomics. https://www.healio.com/hematology-oncology/learn-genomics/whole-genome-sequencing/strengths-and-limitations-of-next-generation-sequencing. Accessed March 2, 2023. 3. Pennell NA, et al. Am Soc Clin Oncol Educ Book. 2019;39:531-542. 4. Bruno R, Fontanini G. Diagnostics. 2020;10:521. 5. El-Deiry WS, et al. CA Cancer J Clin. 2019;69(4):305-343. 6. Jain D, and Roy-Chowdhuri, S. Arch Pathol Lab Med. 2018;142(9):1127-1133. 7. Jain D, et al. Cancer Cytopathol. 2019;127:325-339.

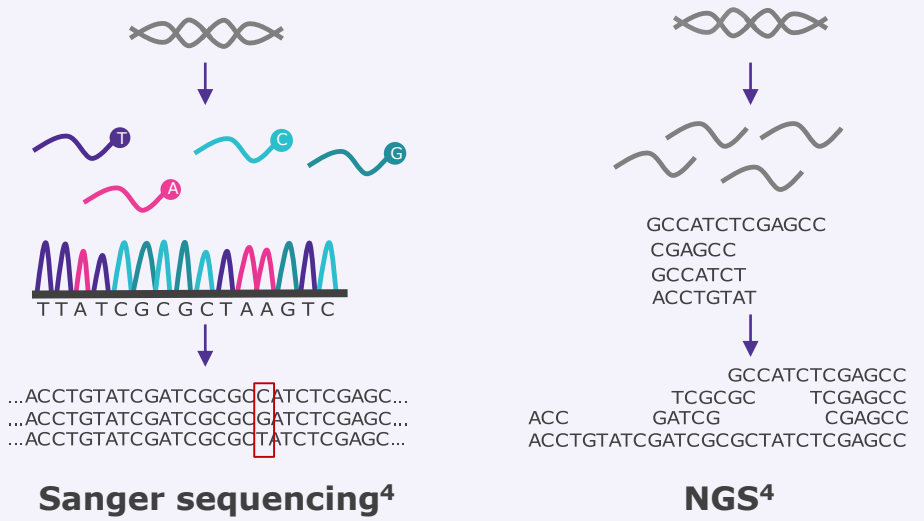
Recommended assays to assess for actionable biomarkers according to NCCN Guidelines[1,*]

Biomarker	DNA					PROTEIN
	NGS	Sanger[†]	RT-PCR	PCR	FISH	IHC
EGFR	✓	✓	✓			
ALK	✓		✓ (Unlikely to detect fusions with novel partners)		✓	✓
ROS1	✓ (DNA-based NGS may under detect)		✓ (Unlikely to detect fusions with novel partners)		✓ (May under-detect *FIG-ROS1* variant)	✓ (Low specificity)
BRAF	✓	✓	✓			
KRAS	✓	✓	✓			
***MET*ex14 skipping**	✓ (RNA-based NGS may have improved detection)					
RET	✓ (RNA-based NGS preferred)		✓ (Unlikely to detect fusions with novel partners)		✓ (May under-detect some variants)	
NTRK1/2/3	✓ (DNA-based NGS may under-detect *NRTK1/3* fusions)			✓	✓ (May require ≥3 probe sets for full analysis)	✓ (May be complicated by baseline expression)
ERBB2	✓	✓		✓		
PD-L1						✓ (Definition of positive or negative depends on assay)

*The NCCN Guidelines® for NSCLC provide recommendations for individual biomarkers that should be tested and recommend testing techniques but do not endorse any specific commercially available biomarker assays or commercial laboratories.[1]
[†]Ideally paired with tumor enrichment.[1]
FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; *MET*ex14, *MET* exon 14; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; PCR, polymerase chain reaction; PD-L1, programmed death ligand 1; RT-PCR, reverse-transcription PCR.
1. Adapted with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-Small Cell Lung Cancer V.2.2023.

NCCN Guidelines recommend a broad, panel-based approach to test for biomarkers prior to initiating treatment in eligible patients with metastatic NSCLC[1]

NGS can provide a large profile of oncogenic alterations at a point in the patient's journey without sequential testing, with limited tissue sample and through either tissue or plasma testing (also known as liquid biopsy)[2,3]



Sanger sequencing[4] **NGS[4]**

Adapted from Parikh et al. 2017.

Additional benefits of NGS[5]:

- More cost effective than single gene testing
- May facilitate an increase in life-years gained in advanced NSCLC, a 10% increase in NGS use compared to single-gene testing resulted in 2630 life-years gained
- Easier to add new biomarker genes in patient assessment
- Can provide value for low frequency biomarkers

Testing tissue samples with NGS following a negative result with non-NGS methods revealed genomic alterations with a corresponding targeted therapy in 26% of retested samples, and a targeted agent in a clinical trial was available for 39% of retested samples[6]

NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer.

1. Adapted with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-Small Cell Lung Cancer V.2.2023. 2. Lindeman NI, et al. J Thorac Oncol. 2018;13(3):323-358. 3. Rolfo C, et al. J Thorac Oncol. 2019;13(9):1248-1268. 4. Parikh VN, Ashley EA. Circulation. 2017;135(5):406-409. 5. Kerr KM, et al. Lung Cancer. 2021;154:161-175. 6. Drilon A, et al. Clin Cancer Res. 2015;21(16):3831-3639.

DNA-based versus RNA-based NGS assays

NGS assays vary widely in the information they provide in terms of sensitivity, specificity, comprehensiveness, tissue requirements, and turnaround times

DNA-based NGS assays[1,2]

- Allows the characterization of the exact gene fusion breakpoints and other genetic alterations
- Can detect genetic alterations that lead to aberrant isoforms
- Does not require an additional RNA purification step

- Does not indicate expression of the rearranged locus of some fusion events
- Involves intronic regions

Identification of key biomarkers

Tumor biopsy and sample preparation for NGS

NGS assay

Interpretation of results and treatment decision

RNA-based NGS assays[1,2]

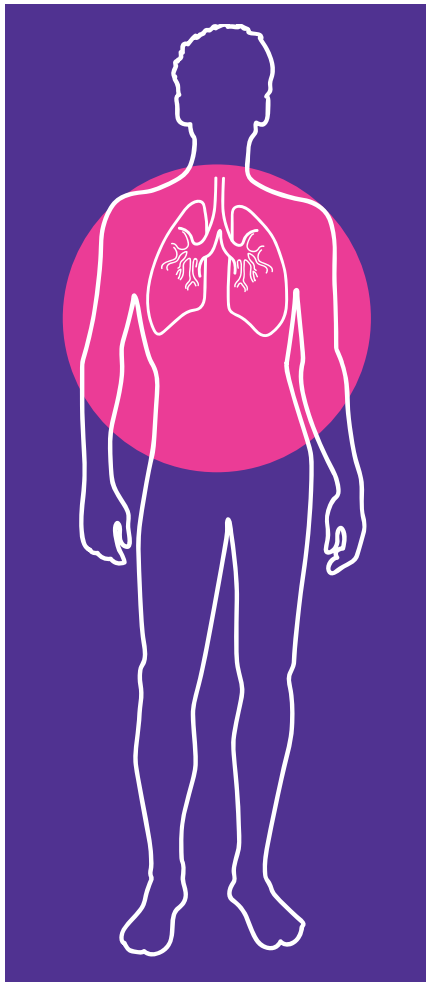
- Can be more sensitive, efficient, and functionally definitive
- Can discriminate splicing isoforms and quantify fusion transcripts
- Not impacted by intronic regions

- RNA is more complicated to handle
- RNA can be highly degraded in FFPE specimens
- Fusion gene detection limited to those functionally expressed

FFPE, formalin-fixed paraffin embedded; NGS, next-generation sequencing.

1. Haynes BC, et al. Transl Oncol. 2019;12(6):836-845. 2. Bruno R, Fontanini G. Diagnostics (Basel). 2020;10(8):521.

Biomarker testing to guide care of treatment-naive NSCLC[1]



PATIENT WITH ADVANCED TREATMENT-NAIVE NSCLC

MOLECULAR PROFILING
(IDEALLY SAMPLING THE NON-SQUAMOUS COMPONENT OF TUMOR)

Surgical specimen is available

Yes

- Molecular analysis* on surgical specimen†
- NGS is preferred‡
- Treat with SOC based on presence or absence of oncogenic driver
- PD-L1 IHC as needed

No

Tissue biopsy specimen sufficient for molecular testing

Yes

- Perform molecular analysis* on tissue biopsy specimen†
- NGS is preferred‡
- Treat with SOC based on presence or absence of oncogenic driver
- PD-L1 IHC as needed

No

Perform molecular analysis* on liquid biopsy (ctDNA); NGS is preferred‡

Therapeutic target negative

Therapeutic target positive

Tissue re-biopsy

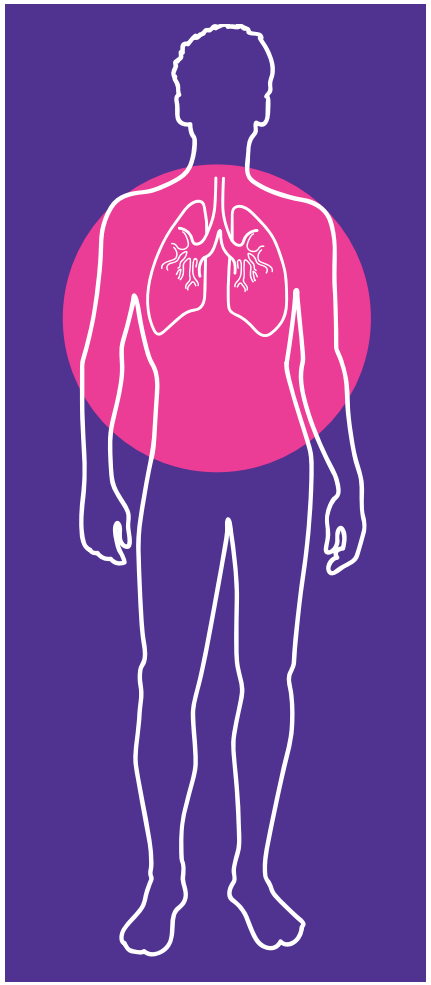
Treat with SOC based on presence of oncogenic driver

*_EGFR, ALK, ROSI,_ and _BRAF_ at minimum, but a panel if available. †Strongly suggest tissue sparing to facilitate participation in clinical trials. ‡While NGS is preferred, based on availability, other validated assays are acceptable.
ctDNA, circulating tumor DNA; IHC, immunohistochemistry; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; PD-L1, programmed death ligand 1; SOC, standard of care.

1. Pennell NA et al. Am Soc Clin Oncol Educ Book. 2019;39:531–542.

EMD SERONO

Biomarker testing to guide care of progressive or recurrent NSCLC[1]



PATIENT WITH NSCLC PROGRESSIVE OR RECURRENT DISEASE DURING TREATMENT WITH TKI

PERFORM MOLECULAR ANALYSIS* ON LIQUID BIOPSY (ctDNA)

Targetable resistance mutation absent

Tissue re-biopsy

Feasible

- Perform molecular analysis* on tissue biopsy specimen†
- NGS is preferred‡
- Treat with recommended therapy based on presence or absence of oncogenic driver
- PD-L1 IHC as needed

Not feasible

Evaluate the potential benefit of other therapy for marker unknown or BSC

Targetable resistance mutation present

Treat with recommended therapy based on presence of oncogenic driver

Retesting a tumor after progression on targeted therapy can support the appropriate next therapeutic steps[2]

*PCR 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.
BSC, best supportive care; ctDNA, circulating tumor DNA; IHC, immunohistochemistry; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; PCR, polymerase chain reaction; PD-L1, programmed death ligand 1; TKI, tyrosine kinase inhibitor.

1. Pennell NA et al. Am Soc Clin Oncol Educ Book. 2019;39:531–542. 2. Adapted with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-Small Cell Lung Cancer V.2.2023.

EMD SERONO

Interpreting biomarker test results

Depending on the testing approach and the facility, testing results may be reported differently, and results may include genes tested, probes used, qualitative data, and quantitative data.[1]

However, there have been efforts to standardize reports through templates.[1]

NGS reports may include[2]:

- A top-line summary of the key findings
- Clinically relevant biomarkers with an associated FDA-approved therapy
- Biomarkers that are potentially relevant but without a clear consensus
- Negative results that are clinically relevant but have not been identified
- A list of clinical trials for which a patient may be eligible based on the presence of an identified biomarker

FDA, US Food and Drug Association.
1. Jones S. Presented at 2014 NAACCR Annual Conference. naaccr.org/wp-content/uploads/2016/11/022-NAACCR-2014-Biomarkers-Jones-20140624.pdf. Accessed March 2, 2023. 2. Pennell NA. et al. Am Soc Clin Oncol Educ Book. 2019;39:531–542.

NCCN Guidelines overview for advanced or metastatic NSCLC[1,*]

VALIDATED TESTING SHOULD ASSESS A MINIMUM OF:

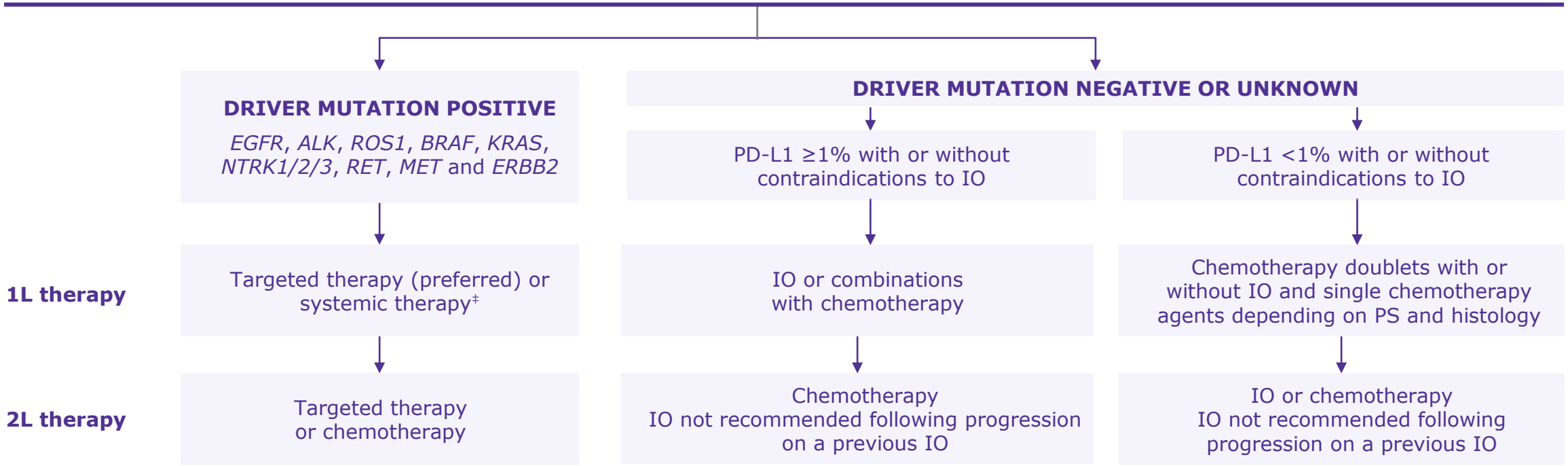
***EGFR*[†] mutations**
***BRAF* mutations**

***MET*ex14 skipping**
***RET* rearrangements**

***ALK*[†] fusions**
***ROS1*[†] fusions**

***KRAS* mutations**
NTRK1/2/3

ERBB2
PD-L1



When patients do not have an identifiable driver oncogene, broad panel testing with RNA-based NGS should be considered to maximize detection of fusion events[1]

*See the NCCN Guidelines® for detailed recommendations, including treatment regimens.[1] †Considered must test biomarkers by CAP-IASLC molecular testing guidelines.[2] ‡Chemotherapy ± immunotherapy regimens are recommended as first-line therapy for patients with *KRAS* mutations or *ERBB2* mutations.

1L, first line; 2L, second line; IO, immuno-oncology; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; PD-L1, programmed death ligand 1; PS, performance status.

1. Adapted with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-Small Cell Lung Cancer V.2.2023. 2. Lindeman NI et al. J Mol Diagn. 2018;20(2):129-159.

SUMMARY

Summary

NSCLC is both **histologically and genetically diverse**[1]

Current actionable biomarkers for eligible patients with metastatic NSCLC according to the **NCCN include *EGFR*, *ALK*, *ROS1*, *BRAF*, *MET*ex14 skipping mutations, *RET*, *KRAS*, *ERBB2*, *NTRK1/2/3*, and PD-L1**; the NCCN recommends that when feasible, molecular testing be performed via a broad, panel-based approach[2]

If tissue quantity and testing methods limit testing for all recommended biomarkers during initial diagnosis of metastatic NSCLC, **repeat biopsy or cfDNA/ctDNA testing should be done**[2]

Biomarkers can be assessed via well-characterized techniques such as NGS, RT-PCR, PCR, FISH, and IHC, with assay selection depending on biomarker[2,3]

Biomarker testing can help **guide patient management and treatment** for eligible patients with metastatic NSCLC[2]

Broad, panel-based testing can provide a view of the patient's biomarker profile without high tissue demands of sequential testing[2,3]

cfDNA, cell-free DNA; ctDNA, circulating tumor DNA; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; *MET*ex14, *MET* exon 14; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; PCR, polymerase chain reaction; PD-L1, programmed-death ligand 1; RT-PCR, reverse transcription PCR.

1. Lungevity. Types of Lung Cancer. https://www.lungevity.org/for-patients-caregivers/lung-cancer-101/types-of-lung-cancer. Accessed March 2, 2023. 2. Adapted with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-Small Cell Lung Cancer V.2.2023. © 2023 National Comprehensive Cancer Network, Inc. All rights reserved. The NCCN Guidelines® and illustrations herein may not be reproduced in any form for any purpose without the express written permission of NCCN. To view the most recent and complete version of the NCCN Guidelines, go online to NCCN.org. The NCCN Guidelines are a work in progress that may be refined as often as new significant data becomes available. 3. Lindeman NI, et al. J Thorac Oncol. 2018;13(3):323-358.

EMD SERONO