Biomarker Testing in NSCLC

NSCLC IS BOTH HISTOLOGICALLY AND GENETICALLY DIVERSE

Types of Non-Small Cell Lung Cancer

In **adenocarcinoma (47.0%),** up to **60%** of patients have 1 known oncogenic driver

Large cell carcinoma (11.7%)

In **squamous cell carcinoma (35.3%),** **50% to 80%** of patients have 1 known oncogenic driver

CHALLENGES IN BIOMARKER TESTING

Insufficient biopsy tissue sample

Appropriate assessment technique selection, sensitivity, and turnaround time

NCCN Guidelines®–recommended assessment techniques

SAMPLE COLLECTION TECHNIQUES: ADVANTAGES AND CHALLENGES

Tissue biopsy

Liquid biopsy

GUIDANCE FOR BIOMARKER TESTING IN PATIENTS WITH NSCLC

Treatment-naive NSCLC

Progressive or recurrent NSCLC

NCCN Guidelines overview for advanced or metastatic NSCLC

NCCN®-recommended biomarkers to guide NSCLC treatment

EMD Serono

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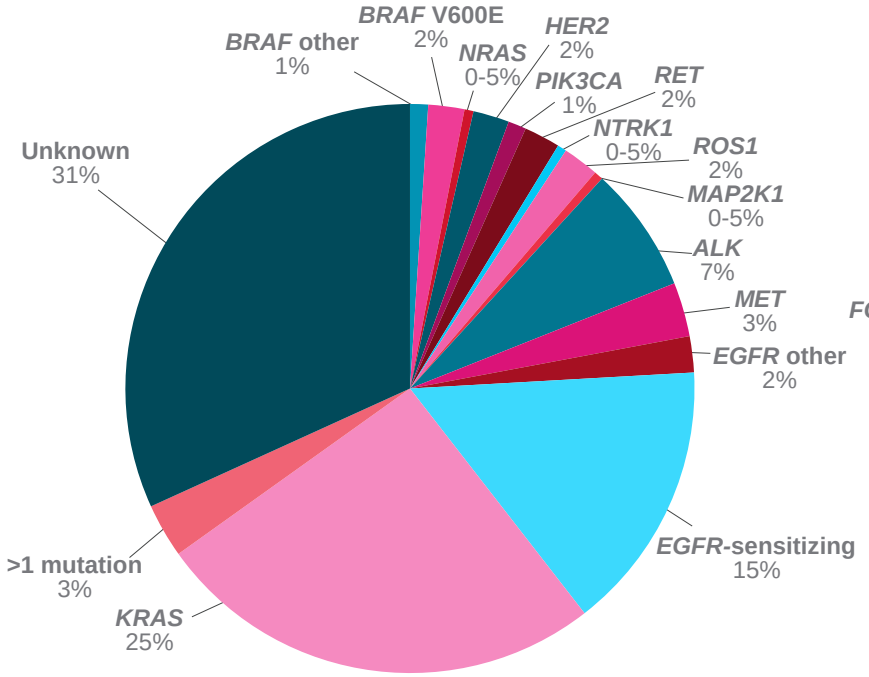
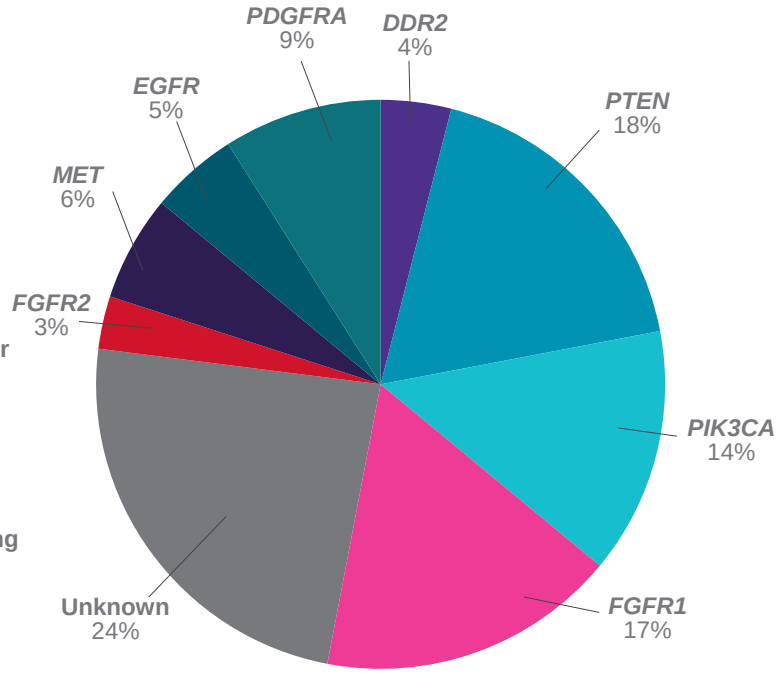
NSCLC: Histological subtypes defined by distinct oncogenic drivers

ONCOGENIC DRIVERS IN ADENOCARCINOMA[1]



Up to 60% of patients with adenocarcinoma have 1 known oncogenic driver[2]

ONCOGENIC DRIVERS IN SQUAMOUS CELL CARCINOMA[1]



50% to 80% of patients with squamous cell carcinoma have 1 known oncogenic driver[2]

CHARACTERISTICS OF PATIENTS WITH DIFFERENT DRIVER MUTATIONS[4,*]

Mutation	Age (yrs) Mean ± SD	Ever smoked (%)	Female (%)
ALK positive	55.0 ± 13.7	41.7	48.1
EGFR positive	63.5 ± 10.9	32.1	54.4
KRAS positive	64.7 ± 9.1	79.6	18.0
*MET*ex14 skipping	73.7 ± 11.6	50.0	38.9
*MET*amp (high)	65.5 ± 11.7	100.0	12.5
ROS1 positive	53.9 ± 16.2	42.9	60.0

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

*Note that the data presented may have been calculated from small population sizes (range: 8-180)[4]
*MET*amp, *MET* amplification; *MET*ex14, *MET* exon 14; NSCLC, non-small cell lung cancer; SD, standard deviation.
1. Types of Lung Cancer. Lungevity. https://www.lungevity.org/for-patients-caregivers/lung-cancer-101/types-of-lung-cancer (accessed April 2022). 2. Chan BA et al. Transl Lung Cancer Res. 2015;4:36–54.
3. Ballman KV. J Clin Oncol. 2015;33:3968–3971. 4. Tong JH, et al. Clin Cancer Res. 2016;22(12):3048-56.

Challenges in biomarker testing: Insufficient biopsy tissue sample

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]

Part 1 | **Part 2**

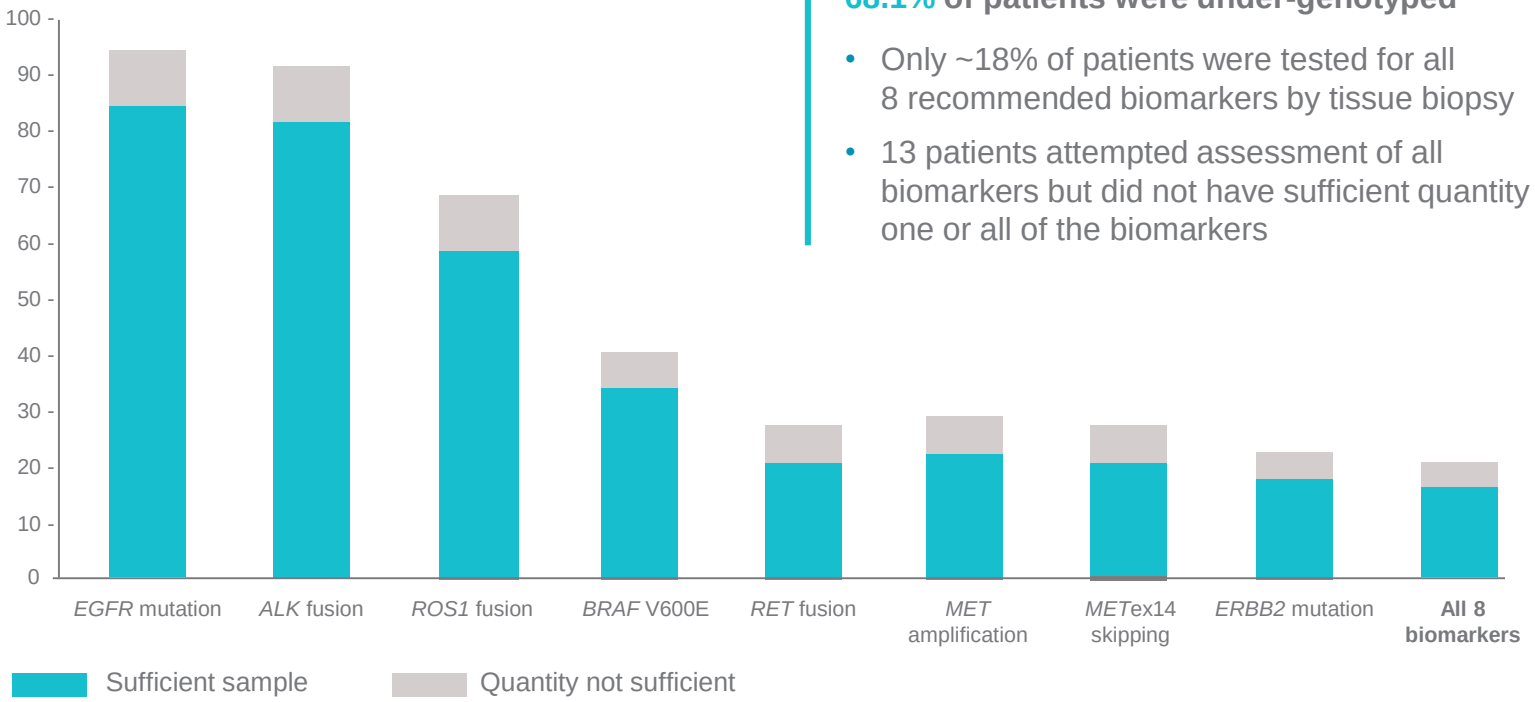
*Core needle biopsies provide more intact material than fine needle aspiration.[1]

Ab, antibody; FISH, fluorescence in situ hybridization; H&E, hematoxylin and eosin; IHC, immunohistochemistry; NGS, next-generation sequencing; PD-L1, programmed death ligand 1.

1. Data on file. 2. Engstrom PF et al. JNCCN. 2011;9(6):S1–S16.

Challenges in biomarker testing: Insufficient biopsy tissue sample (NILE study)

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 did not have sufficient quantity 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

Part 1 **Part 2**

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

Challenges in biomarker testing: Assessment technique selection, sensitivity, and turnaround time

CHOOSING A TECHNIQUE THAT ENSURES ACCURATE AND RELIABLE DETECTION OF SELECTED BIOMARKERS WITHIN A REASONABLE TURNAROUND TIME IS IMPORTANT

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	20–50	3–4 days	• Liquid • Tissue	✓	✓		
RT-PCR[1,2,4]	RNA expression, including fusion transcripts	0.00001	2–3 days	• Liquid • Tissue	✓	✓		✓
FISH[1,2]	Gene rearrangements including deletions, amplifications, translocations, and fusions	<1	2–3 days	• Tissue			✓	✓
NGS: targeted approach[1,2,5]	Genetic changes in multiple genes simultaneously	1–10	7–20 days	• Liquid • Tissue	✓	✓	✓	May not reliably detect fusions
NGS: WES/ WGS[1,2,5]	Genetic changes in multiple genes simultaneously	Variable	Weeks	• Liquid • Tissue	✓	✓	✓	✓ (As long as in design)
IHC[2,5,6]	Protein expression, localization, or specific alterations, including fusions	Variable	1–2 days	• Tissue				✓

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 April 2022). 5. Dong J et al. Front Pharmacol. 2019;10:230. 6. Doshi S et al. Diagnostics (Basel). 2016;6(1):4.

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* exon 14 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)	
NTRK 1/2/3	✓ (DNA-based NGS may under-detect *NRTK1/3* fusions)			✓	✓ (May require ≥3 probe sets for full analysis)	✓ (May be complicated by baseline expression)
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; 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.

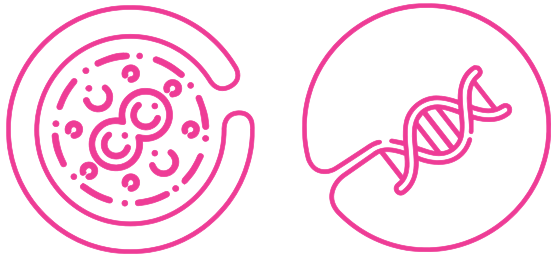
Advantages and challenges associated with sample collection

A direct sample of the tumor tissue[1]

TUMOR BIOPSY[2]

- Highly sensitive
- Assessment of DNA and non-DNA biomarkers
- Provides pathology information
- Allows PD-L1 assessment

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



A sample of the CTCs and cfDNA shedding from the tumor to the blood[3]

LIQUID BIOPSY[2]

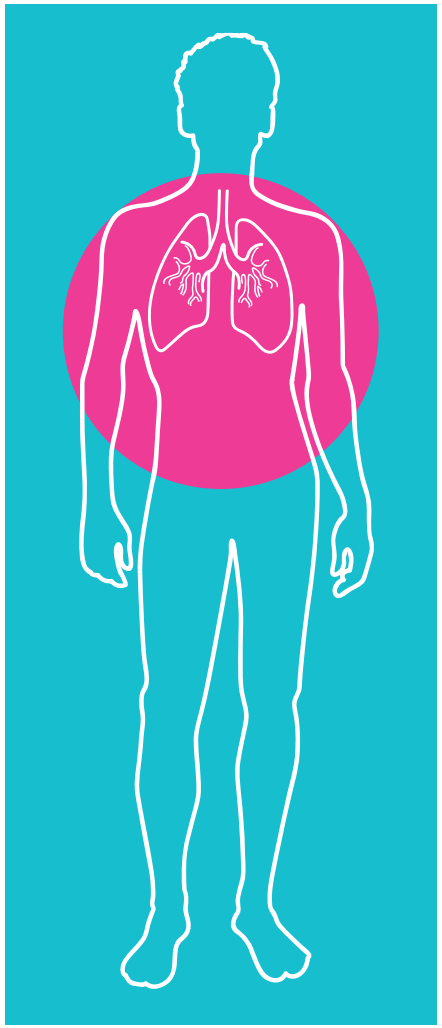
- High concordance rate
- May have rapid turnaround time
- Minimally invasive
- Repeatable over time
- Captures tumor heterogeneity and clonal evolution

- Non-DNA biomarkers not evaluable
- Concurrent use with tissue testing can increase costs
- False negatives
- Low concentrations of cfDNA may be difficult to detect[4]

cfDNA, circulating free DNA; CTC, circulating tumor cell; PD-L1, programmed death ligand 1.

1. How is Cancer Diagnosed? American Cancer Society. https://www.cancer.org/treatment/understanding-your-diagnosis/tests/testing-biopsy-and-cytology-specimens-for-cancer/how-is-cancer-diagnosed.html (accessed April 2022). 2. Rolfo C et al. J Thorac Oncol. 2021;16(10):1647–1662. 3. Saarenheimo J et al. Front Oncol. 2019;9:129. 4. Crowley E et al. Nat Rev Clin Oncol. 2013;10:472–484.

Biomarker testing to guide care of treatment-naive NSCLC



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

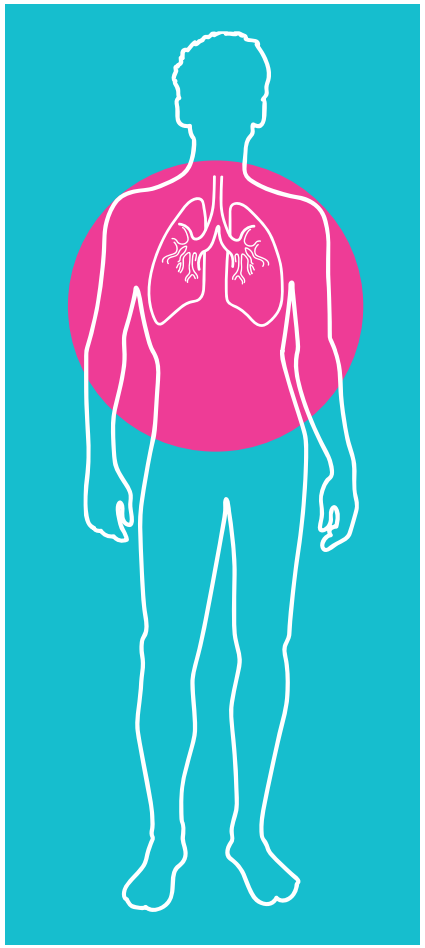
Treat with SOC based on presence of oncogenic driver

**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.

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‡
      - Treatment options 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
  - Treatment options 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. §See the NCCN Guidelines for detailed recommendations, including specific treatment regimens.[2]
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; SOC, standard of care; 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.3.2022.

NCCN Guidelines: Overview for advanced or metastatic NSCLC*[1]

VALIDATED TESTING SHOULD ASSESS A MINIMUM OF:

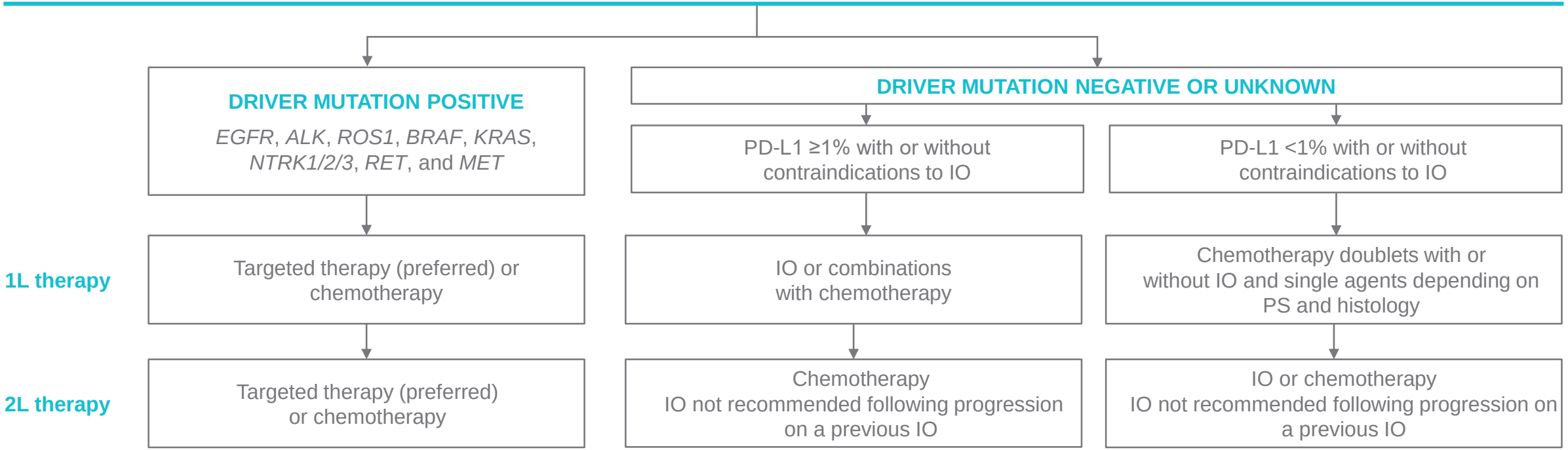
***EGFR*† mutations**
***BRAF* mutations**

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

***ALK*† fusions**
***ROS1*† fusions**

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

PD-L1



When patients do not have an identifiable driver oncogene, broad panel testing RNA-based NGS should be considered[1]

*See the NCCN Guidelines® for detailed recommendations, including treatment regimens.[1] †Considered must test biomarkers by CAP-IASLC molecular testing guidelines.[2]
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.

 2. Lindeman NI et al. J Mol Diagn. 2018;20(2):129-159.

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

Patients receiving appropriate targeted therapy or immunotherapy based on biomarker testing show 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)
- Fusion between *ALK*† and other genes
- *ROS1*† gene fusions
- *BRAF* V600E point mutations
- *KRAS* G12C point mutations
- *MET* exon 14 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‡
- *ERBB2* (*HER2*) mutations

*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; NSCLC, non-small cell lung cancer; PD-L1, programmed death ligand 1.

1. Adapted with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-Small Cell Lung Cancer V.3.2022. © 2022 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. 2. Lindeman NI et al. J Mol Diagn. 2018;20(2):129-159.