THE ROLE OF BIOMARKER TESTING IN ADVANCED NSCLC

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EMD SERONO

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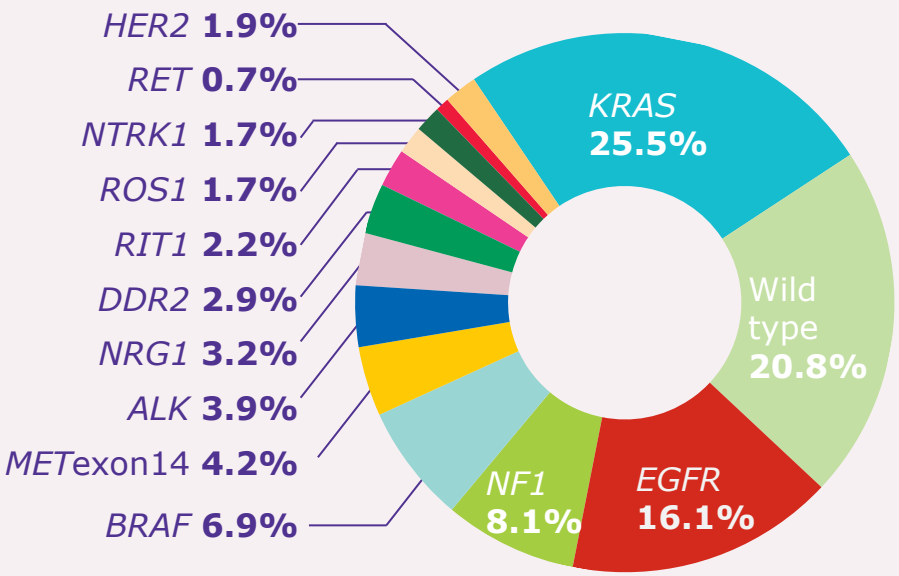
OVERVIEW OF BIOMARKERS IN NSCLC

Known biomarkers and use of biomarker testing for patient care

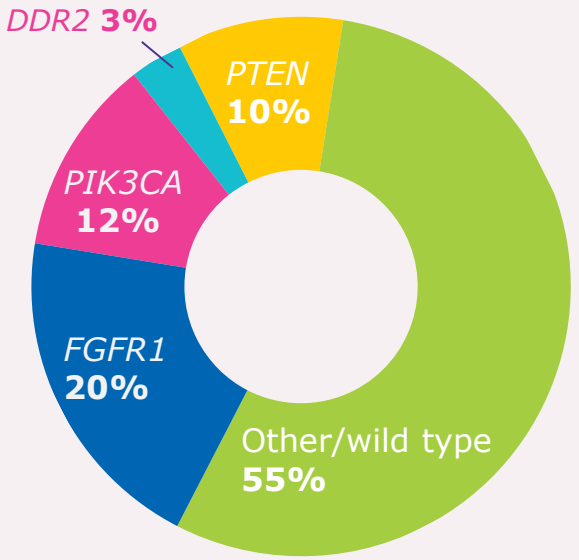
NSCLC is a heterogenous group of diseases with distinct histological subtypes and numerous oncogenic drivers

Oncogenic drivers in adenocarcinoma[1]



Up to 60% of patients with adenocarcinoma have ≥1 known oncogenic driver[2,3]

Oncogenic drivers in squamous cell carcinoma[1]



50% to 80% of patients with squamous cell carcinoma have ≥1 known oncogenic driver[2,3]

NSCLC includes 3 main histological subtypes[4]:

- Adenocarcinoma (49.7%)
- Squamous cell carcinoma(22.7%)
- Large cell carcinoma (1.4%)

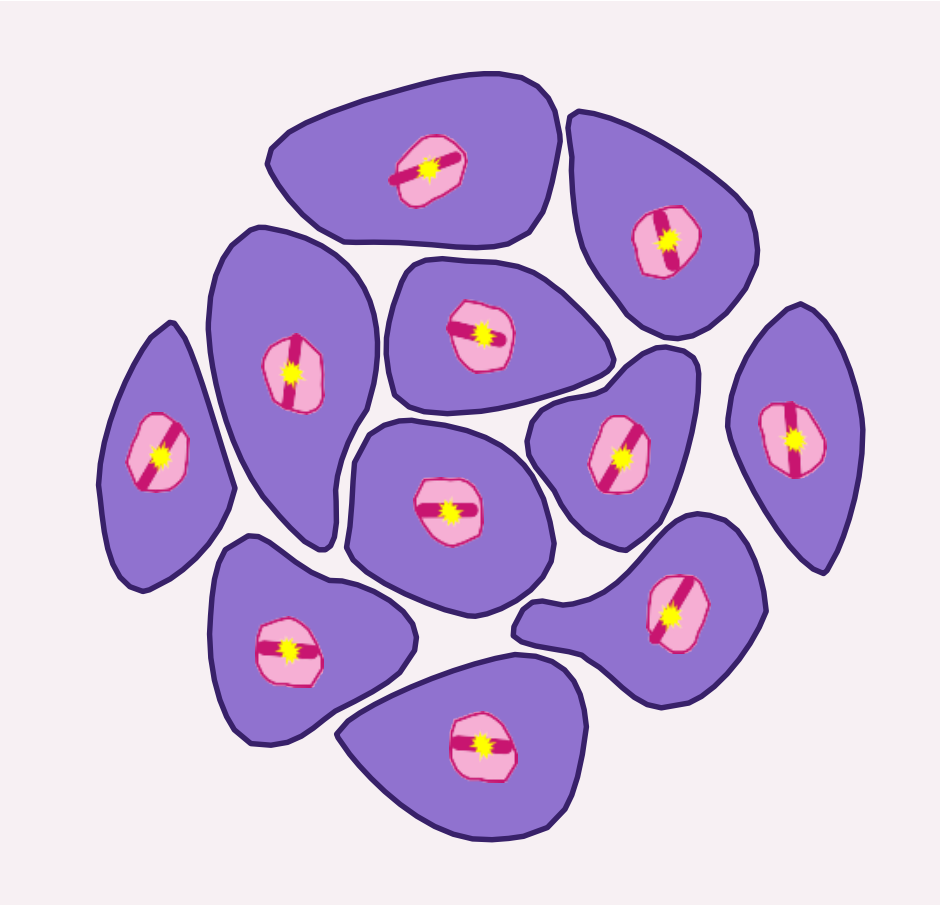
Known oncogenic drivers differ in commonality between these subgroups[1]

- Actionable oncogenic drivers that occur in adenocarcinoma also occur in squamous-cell carcinoma, but at lower frequencies.[6]

Oncogenic drivers may serve as prognostic or predictive biomarkers to help guide patient management.[5]

1. Rosell R, Karachaliou N. Lancet. 2016;387(10026):1354–1356. 2. Chan BA, et al. Transl Lung Cancer Res. 2015;4:36-54. 3. Dearden S, et al. Ann Oncol. 2013; 24:2371–2376. 4. Lung and Bronchus CSR. SEER. https://seer.cancer.gov/archive/csr/1975_2016/results_merged/sect_15_lung_bronchus.pdf (accessed 01/2021). 5. Ballman KV. J Clin Oncol. 2015;33:3968–3971. 6. Griffin R, Ramirez R. 2017; Ochsner Journal 17:388–392.

Importance of biomarker testing in NSCLC[1-3]



- NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) recommend **biomarker testing in all appropriate patients** with metastatic 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
  - **Prognostic biomarkers** are indicative of patient survival independent of the treatment received
- 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**

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

- Numerous gene alterations have been identified that impact therapy selection in NSCLC
- Testing for these alterations not only helps identify potentially efficacious targeted therapies, but also those therapies unlikely to provide clinical benefit+

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
KRAS mutations±[2]
BRAF V600E 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

- Numerous other mutations are under investigation for biomarker use[2]

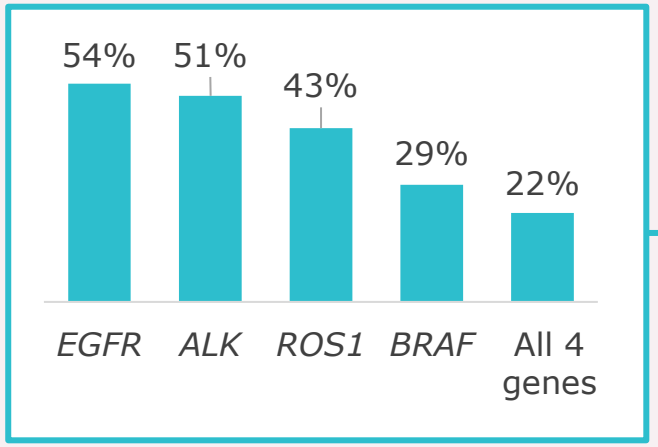
+The NCCN Guidelines for NSCLC provide recommendations for certain individual biomarkers that should be tested and recommend testing techniques but do not endorse any specific commercially available biomarker assays or commercial laboratories.[1] *Considered must test biomarkers by CAP-IASLC molecular testing guidelines. ±KRAS mutations are a prognostic biomarker in the NCCN Guidelines[1]

1. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for NSCLC V.5.2021.© National Comprehensive Cancer Network, Inc. 2021. All rights reserved. Accessed June 15, 2021. To view the most recent and complete version of the guidelines, go to NCCN.org. 2. Bernicker E, et al. J Thorac Dis. 2019;11(Suppl 1): S81–S88.

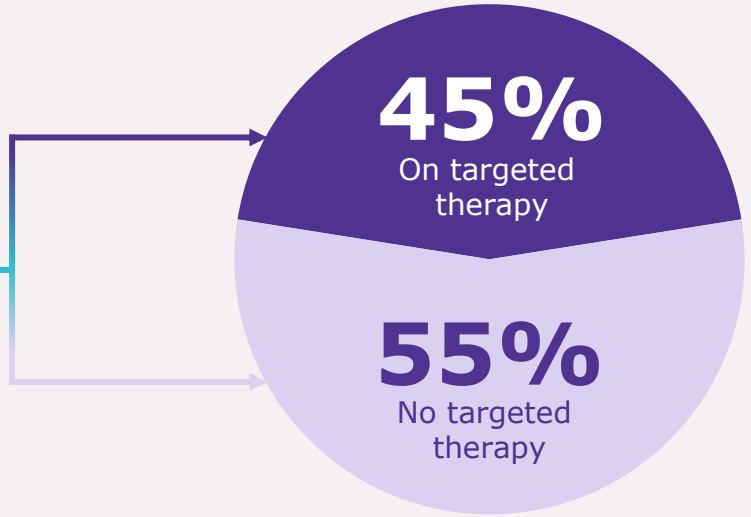
Despite the identification of actionable biomarkers and known patient benefit, biomarker testing may be limited

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

Biomarker testing rates, 2017-2019 (% of patients tested; N=1203)



Treatment of biomarker-positive patients



Less than half of patients in community practices with actionable mutations received targeted therapy despite being biomarker positive[2]

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

ALK, anaplastic lymphoma kinase gene; BRAF, B-Raf proto-oncogene; EGFR, epidermal growth factor receptor gene; NSCLC, non-small cell lung cancer; ROS1, ROS proto-oncogene 1.

1. Shan-Manek B, et al. J Clin Oncol. 2018;36 (no 15_suppl). 2. Gierman, HJ, et al. J Clin Oncol. 2019;37(15_suppl):1585-1585. 3. Kim ES, et al. J Thorac Oncol. 2019;14(3):338-342. 4. Kerr KM, et al. Lung Cancer. 2021.

RECENT DATA ON TESTING RATE

Real world biomarker testing rates in US oncology network community practices

- Retrospective observational chart review of mNSCLC patients initiating 1L therapy between (4/2018-3/2020): N=3,474[1]
- Assessed testing rates for *ALK, BRAF, EGFR*, PD-L1, *ROS1*:
  - 90% of patients received ≥1 biomarker test
  - 46% received all 5 biomarker tests
  - NGS testing increased from 33% to 44% (p<0.0001)
- Median (IQR) time from dx to 1L therapy: 35 (22, 55) days
  - Median (IQR) turn around time (TAT) from biomarker testing orders to results: 10 (6, 17) - 15 (10, 22) days
  - Median (IQR) time from mNSCLC dx to biomarker results: 14 (7, 26) to 21 (12, 36) days

Biomarker testing rates, 2018-2020

	Total Patients	Cohort 1 biomarker test result received prior to 1L	Cohort 2 biomarker test result received during/after 1L	Cohort 3 no biomarker test
Overall n (%)[a]	3474	2752 (79)	371 (11)	351 (10)
Any biomarker test[b]	3123	2752 (88)	371 (12)	NA
All 5 biomarker tests[b]	1602	1230 (77)	372 (23)	NA
Biomarker testing, n (%)[a]				
ALK	2446	1986 (57)	460 (13)	1028 (30)
BRAF	1912	1489 (43)	423 (12)	1562 (45)
EGFR	2443	1979 (57)	464 (13)	1031 (30)
PD-L1	2882	2526 (73)	356 (10)	592 (17)
ROS1	2348	1897 (55)	451 (13)	1126 (32)

[a]Row percentage denominator: 3474 [b]Row percentage denominator: total patients with test.

1. Robert NJ, et al. Biomarker tissue journey among patients (pts) with untreated metastatic non-small cell lung cancer (mNSCLC) in the U.S. Oncology Network community practices. DOI: 10.1200/JCO.2021.39.15_suppl.9004 Journal of Clinical Oncology 39, no. 15_suppl (May 20, 2021) 9004-9004.

NSCLC tissue biopsy size is often small and may not be sufficient to test the increasing number of actionable biomarkers

A core lung biopsy* will give 200µm of material[1]

Block trimming waste 10µm

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

*Core needle biopsies provide more intact material than fine needle aspiration[2]

Efficient use of tissue is important so that critical molecular testing can be performed[3]:

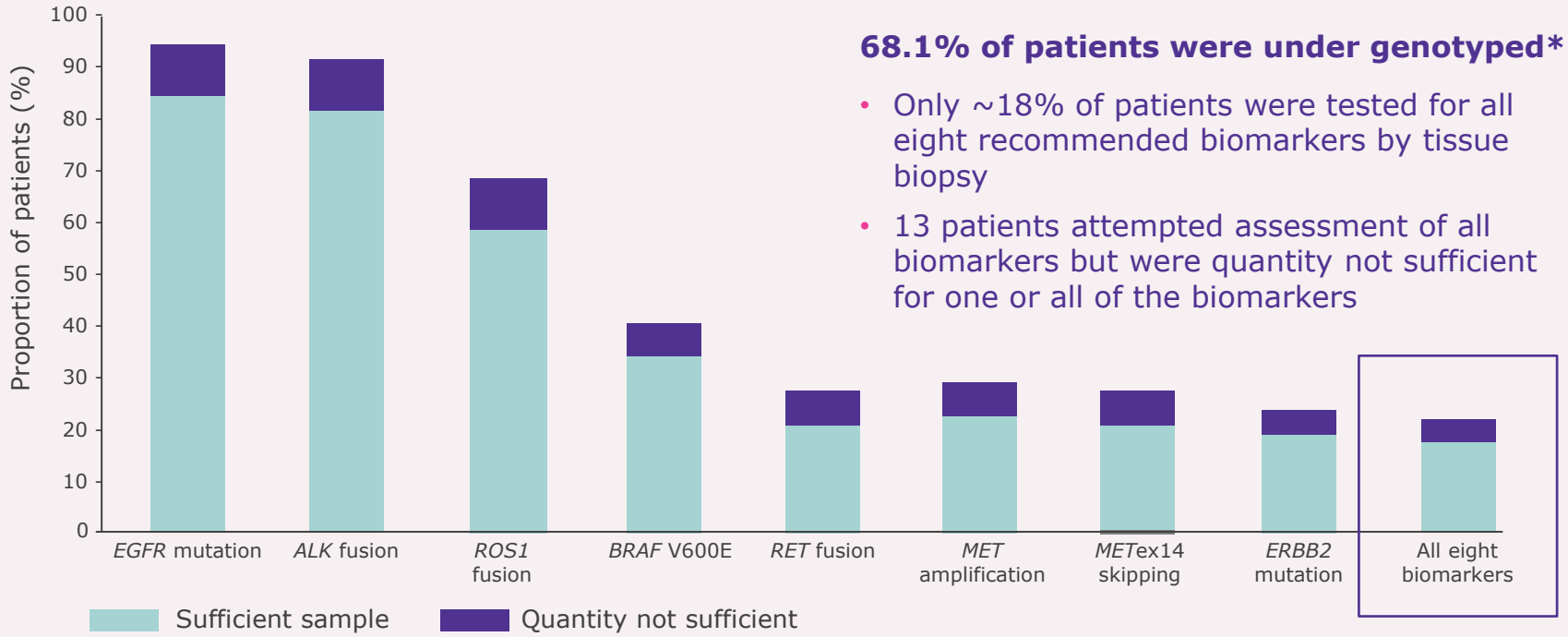
- On adequate tissue
- In a timely fashion

Simultaneous detection of multiple biomarkers (eg, through multiplex arrays) may allow for increased efficiency with small tissue samples[3]

1. Internal Data on File. 2. VanderLaan PA. Cancer Cytopathol. 2016;124(12):862-870. 3. 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 eight recommended biomarkers by tissue biopsy
- 13 patients attempted assessment of all biomarkers but were quantity not sufficient for one or all of the biomarkers

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

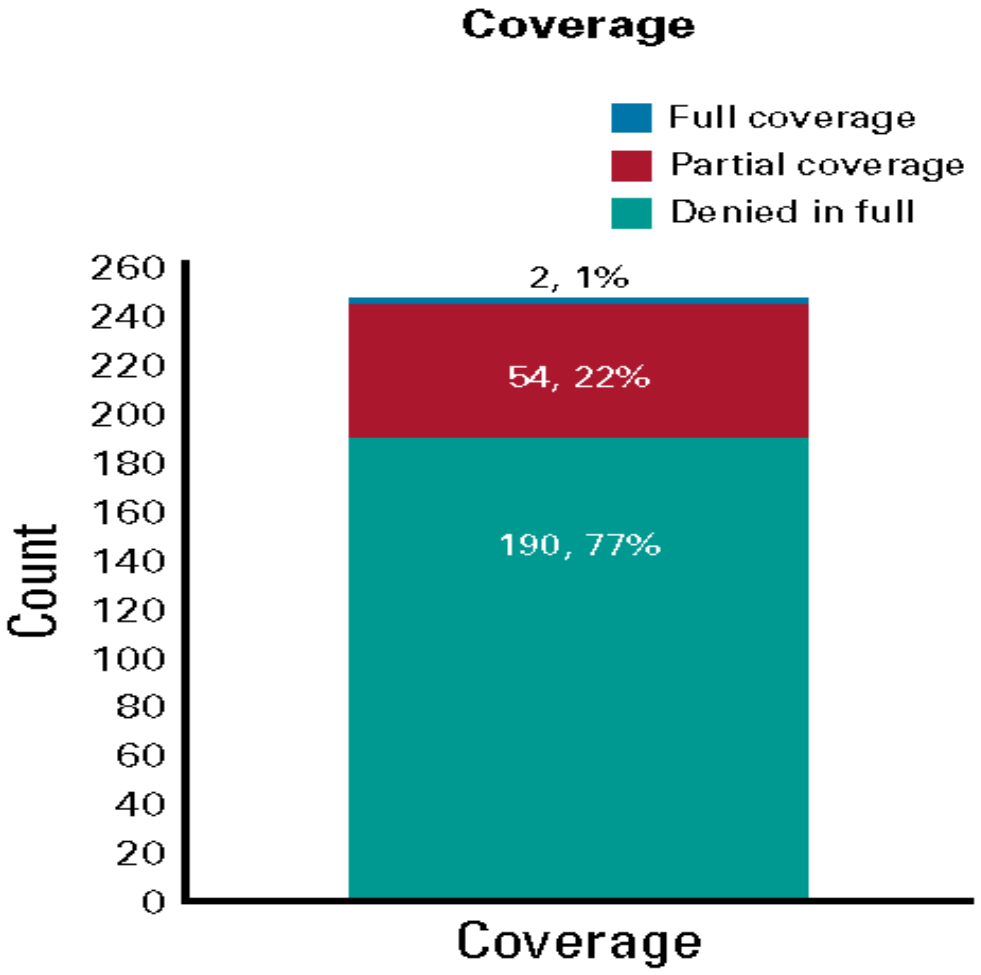
With cfDNA available, all eight guideline-recommended biomarkers were **fully assessed in 95% of patients**

* Did not have a guideline-recommended biomarker identified and were not assessed for all guideline-recommended biomarkers

1. Leighl NB, et al. Clin Cancer Res. 2019;25:4691-4700.

Payer coverage is one of the barriers to access to molecular testing in NSCLC[1]

- In 2020, a study among 246 cases (01/2017-04/2018) in New York showed **majority of tests denied payer coverage (77%, n = 190) and only 10.75% of the total NGS service charge was reimbursed[2]**
- In 2018, centers for Medicare & Medicaid Services (CMS) released a National Coverage Determination (NCD) for NGS testing for Medicare beneficiaries with advanced cancer[3]
  - Limited to patients who have not been previously tested using the same NGS test for the same primary cancer diagnosis
- Coverage through private payers may be variable



1. https://www.ncoda.org/wp-content/uploads/bp-attachments/11385/PTCE_NCODA_NSCLC-Biomarkers_Final-for-Handout_04.29.2021-1.pdf
2. Hsiao S, et al. JCO Precis Oncol 2020;4:1038-1048; Pennell NA, et al. Am Soc Clin Oncol Educ Book. 2019;39:531-542.
3. https://www.cms.gov/medicare-coverage-database/details/nca-decision-memo.aspx?NCAId=290

TESTING FOR BIOMARKERS IN NSCLC

Technical approaches, testing needs, and clinical guideline recommendations

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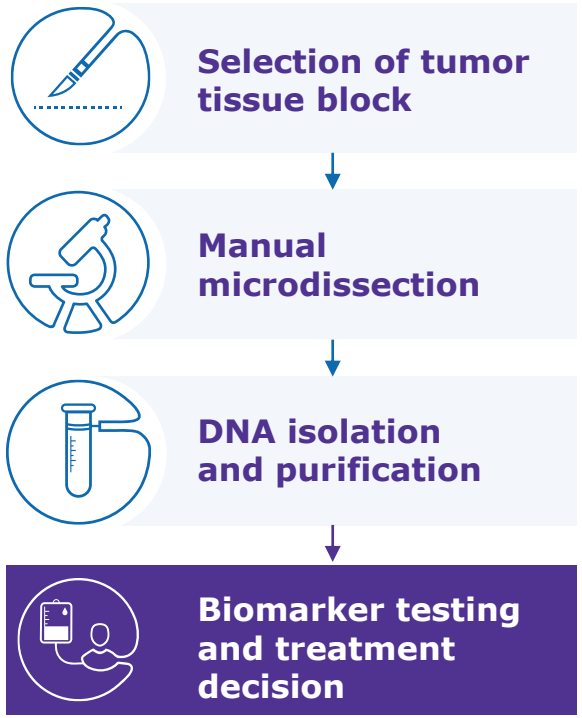
Sample collection – tissue biopsy

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

Tissue biopsy[1,2]



Strengths and Limitations

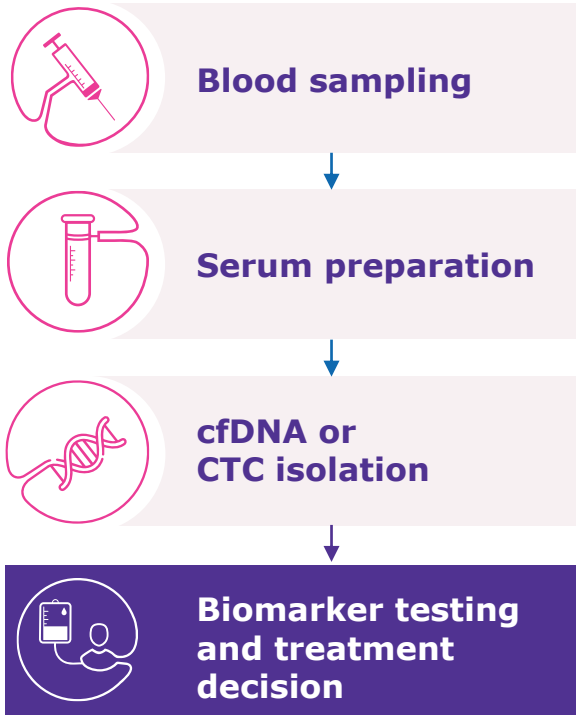
- Invasive and repeat samples may be needed to capture progression or treatment response, resistance, etc. **Tissue may not be accessible**[1]
- May not capture **tumor heterogeneity**[3]
- Samples often isolated from archival tissue; **may not represent the tumor** in its current form[2,3]
- Highly **sensitive**, requires a low number of tumor cells[4]
  Tissue biopsy is the **gold standard for molecular analysis**[3,5]

1. Aisner DL et al. Arch Pathol Lab Med. 2016;140:1206-1220. 2. Crowley E et al. Nat Rev Clin Oncol. 2013;10:472-484. 3. Garcia-Foncillas J et al. Ann Oncol. 2017;28:2943-2949.
4. Pennell NA et al. Am Soc Clin Oncol Educ Book. 2019;39:531-542. 5. Saarenheimo J et al. Front Oncol. 2019;9:129.

Sample collection – liquid biopsy

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

Liquid biopsy[1]



Strengths and Limitations

- ✓ **Minimally invasive**, repeat sampling to monitor acquired resistance mutations is easier[2]
  Captures tumor heterogeneity[1,2]
- ✓ **Faster preparation time** than tissue biopsy, more likely to represent current tumor environment[1,3]
- ✓ cfDNA breaks down rapidly, and therefore can be a real-time biomarker of tumor stage and other biological features[1]
- ⚠ cfDNA and CTC shedding varies by tumor type and stage; **low concentrations** of cfDNA and CTCs may be **difficult to detect**[1-3]
- ✓ Can detect cancers **earlier**, before disease progression[1,4]
- ⚠ **Clinical significance of early mutations** and the percentage of mutations detected are **not yet clear**[2]
  Not all techniques available; cost and limited availability[1,2]

1. Saarenheimo J et al. Front Oncol. 2019;9:129; doi:10.3389/fonc.2019.00129. 2. Crowley E et al. Nat Rev Clin Oncol. 2013;10:472-484. 3. Pennell NA et al. Am Soc Clin Oncol Educ Book. 2019;39:531-542. 4. Siravegna G et al. Nat Med. 2015;21:795-801.

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

The use of plasma cfDNA/ctDNA testing (plasma testing) **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

Cell free tumor DNA 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

1. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for NSCLC V.5.2021.© National Comprehensive Cancer Network, Inc. 2021. All rights reserved. Accessed June 15, 2021. To view the most recent and complete version of the guidelines, go to NCCN.org.

Overview of assessment techniques

Method	Used to assess/detect:	Sensitivity (%)	Turnaround time	Biopsy method	Point mutations	Small indels	CNAs	Rearrangements
PCR and Sanger Sequencing[1,2]	DNA changes, including point mutations, insertions, or deletions	20–50	3 to 4 days	• Liquid • Tissue	✓	✓		
RT-PCR[1-3]	RNA expression, including fusion transcripts	0.00001	2 to 3 days	• Liquid • Tissue	✓	✓		✓
FISH[2-6]	Gene rearrangements including deletions, amplifications, translocations, and fusions	<1	2 to 3 days	• Tissue			✓	✓
NGS: targeted approach[1,4]	Genetic changes in multiple genes simultaneously	1–10	7–20 days	• Liquid • Tissue	✓	✓	✓	May not reliably detect fusions
NGS: WES/ WGS[1,4]		Variable	Weeks	• Liquid • Tissue	✓	✓	✓	✓ (As long as in design)
IHC[4,5,7,8]	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 biomarker(s); some techniques require the knowledge of a specific change in DNA/RNA or protein for biomarker detection[1,9]

1. Dong J, et al. Front Pharmacol. 2019;10:230. 2. FISH. NIH Genome Research Institute. https://www.genome.gov/genetics-glossary/Fluorescence-In-Situ-Hybridization (accessed 02/2021). 3. El-Deiry WS, et al. CA Cancer J Clin. 2019;69(4):305-343. 4. Pennell NA. et al. Am Soc Clin Oncol Educ Book. 2019;39:531–542. 5. Bruno R, Fontanini G. Diagnostics. 2020;10:521;doi:10.3390/diagnostics10080521. 6. Wadowska K, et al. Int J Mol Sci. 2020;21(13):4569. 7. Torlakovic E, et al. Mod Pathol. 2020;33(1):4-17. 8. Doshi S, et al. Diagnostics (Basel). 2016;6(1):4. 9. Chen M, Zhao H. Human Genomics. 2019;13:34.

Advantages and disadvantages of assessment techniques

	DNA and RNA				Protein
	NGS[1]	RT-PCR[2]	PCR [2,3]	FISH[2,3]	IHC[2,4]
✓	• Large throughput • High accuracy • Rich content information • Multiple types of genetic alterations	• Highly sensitive • Detects fusion transcripts at the RNA level	• Allows for rapid testing	• 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 • Tissue sample needs • Reports can be hard to interpret • Wide variety of NGS assay platforms	• Poor quality of FFPE RNA samples • Limited number of variants tested at once	• Only test 1 gene at a time • Requires high 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

1. Dong J, et al. Front Pharmacol. 2019;10:230. doi: 10.3389/fphar.2019.00230. 2. Bruno R, Fontanini G. Diagnostics. 2020;10:521;doi:10.3390/diagnostics10080521. 3. Pennell NA. et al. Am Soc Clin Oncol Educ Book. 2019;39:531–542. 4. Jain D, et al. Cancer Cytopathol. 2019;127:325–339.

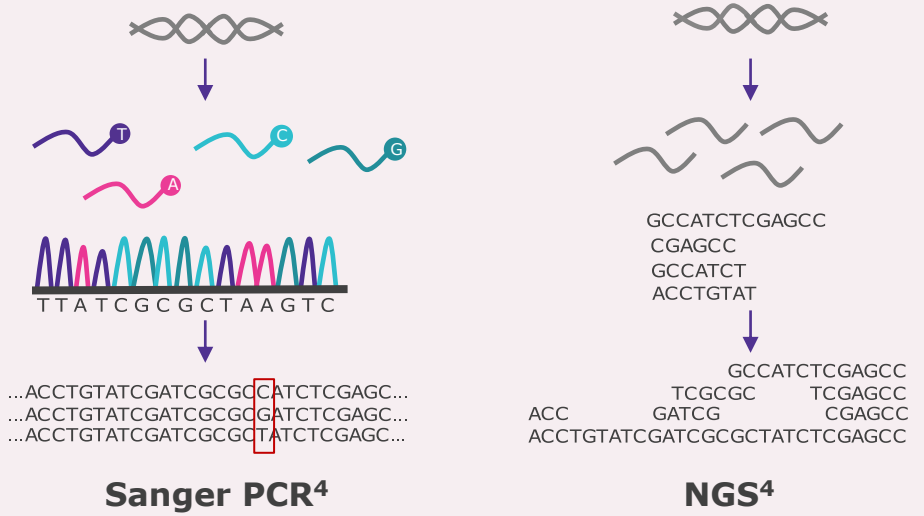
NCCN recommended use of assessment techniques*[1]

	DNA & RNA				Protein
	NGS	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	✓	✓	✓		
***MET* exon 14 skipping**	✓				
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)		✓	✓ (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 certain individual biomarkers that should be tested and recommend testing techniques but do not endorse any specific commercially available biomarker assays or commercial laboratories.

NCCN Guidelines recommend a broad, panel-based approach (most typically performed by NGS) to test for biomarkers prior to initiating treatment in eligible patient 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]



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]

Multiplex gene testing, including NGS, provides an efficient method for identifying predictive biomarkers in patients with NSCLC

Liquid biopsy was found to cost less and cause fewer complications than tissue biopsy

Study	Descriptions	Findings
Yu, 2018[1]	Budget impact from a **US health care payer** perspective was modeled in 2018 to compare NGS to single gene testing.	The overall impact of **NGS was expected to be minimally cost additive**, this was due to more accurate identification of mutations and more use of target therapy in first line. First-line and maintenance treatment costs increased but were offset by a decrease in second-line and palliative care costs. Over 5 years, the total budget impact was $432,554 ($0.0072 PMPM).
Steuten, 2019[2]	Estimated the clinical and cost-effectiveness of multigene panel sequencing (MGPS) relative to single-marker genetic testing (SMGT) in patients diagnosed with aNSCLC using the Flatiron Health database.	The incremental cost-effectiveness ratio of MGPS versus SMGT was $148,478 per LY gained, demonstrating **moderate cost effectiveness of MGPS compared with SMGT** in patients with NSCLC.
Pennel, 2019[3]	Assessed the economic impact of NGS versus single gene testing from perspective of ***the CMS*** and ***US commercial*** payers.	**Upfront NGS testing** in patients with metastatic NSCLC was associated with **substantial cost savings** and shorter time-to-test results for both CMS and commercial payers than alterative testing approaches.
Armaud, 2016[4]	Compared the clinical costs and complications of solid biopsies with blood-based biopsies for biomarker testing in NSCLC from a ***Medicare*** reimbursement perspective.	The biomarker testing via the **blood-based test was both significantly cheaper and patients had fewer complications than both computerized tomography (CT)-guided biopsy and navigational bronchoscopy**.

1. Yu, TM, et al. Budget Impact of Next-Generation Sequencing for Molecular Assessment of Advanced Non-Small Cell Lung Cancer. Value Health, 2018. 21(11): p. 1278-1285.
2. Steuten, L, et al. Cost Effectiveness of Multigene Panel Sequencing for Patients With Advanced Non-Small-Cell Lung Cancer. JCO Clin Cancer Inform, 2019. 3: p. 1-10.
3. Pennell, NA, et al. Economic Impact of Next-Generation Sequencing Versus Single-Gene Testing to Detect Genomic Alterations in Metastatic Non–Small-Cell Lung Cancer Using a Decision Analytic Model. JCO Precision Oncology, 2019(3): p. 1-9.
4. Arnaud, A, Costs and outcomes comparison of tissue and blood-based biopsies for the purpose of biomarker testing. Value in Health, 2016. 19(3): p. A143-A144.

MGPS: multigene panel sequencing; SMGT: single-marker genetic testing; CMS: the Centers for Medicare & Medicaid Services

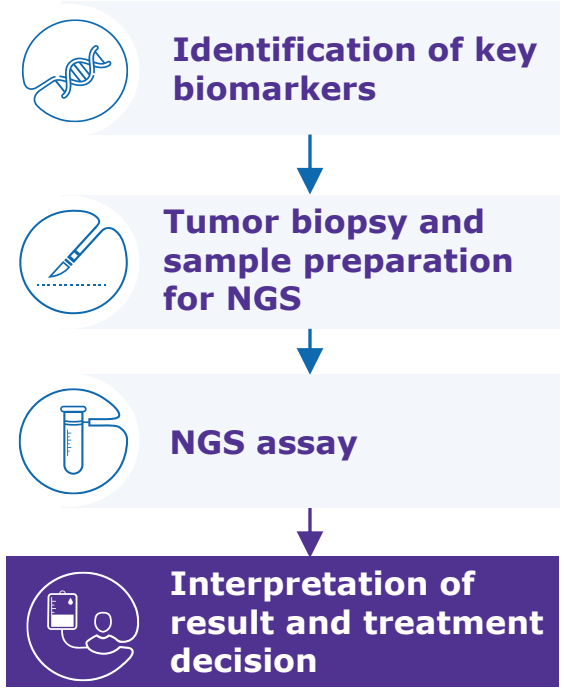
DNA-based versus RNA-based NGS assays

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



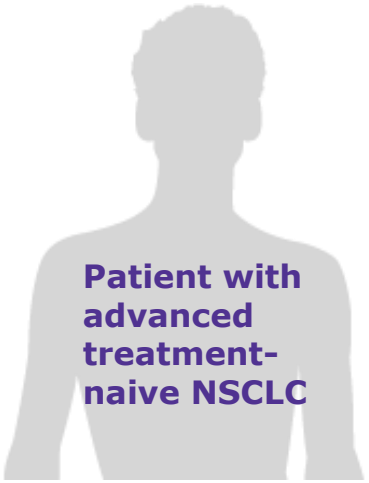
RNA-based NGS assays[1,2]

✓
- Can be more sensitive, efficient, and functionally definitive
- Fusion gene detection limited to those functionally expressed
- 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

1. Haynes BC, et al. Transl Oncol. 2019;12(6):836-845. 2. Bruno R, Fontanini G. Diagnostics. 2020;10:521; doi:10.3390/diagnostics10080521.

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



Molecular profiling on all with non squamous component, or if clinical features may suggest a molecular driver

Surgical specimen is available

No

Tissue biopsy specimen sufficient for molecular testing

Yes

No

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

- 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

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

Therapeutic target negative

Therapeutic target positive

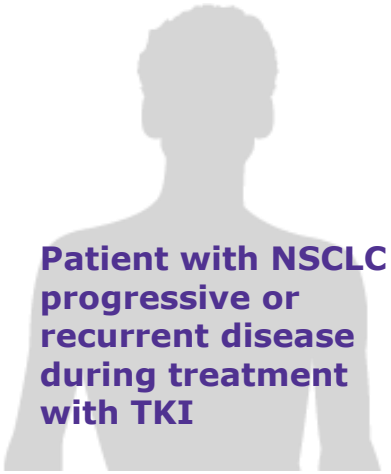
Tissue re-biopsy

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.

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†
- Treat with SOC 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 SOC based on presence of oncogenic driver

*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.

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

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

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]:

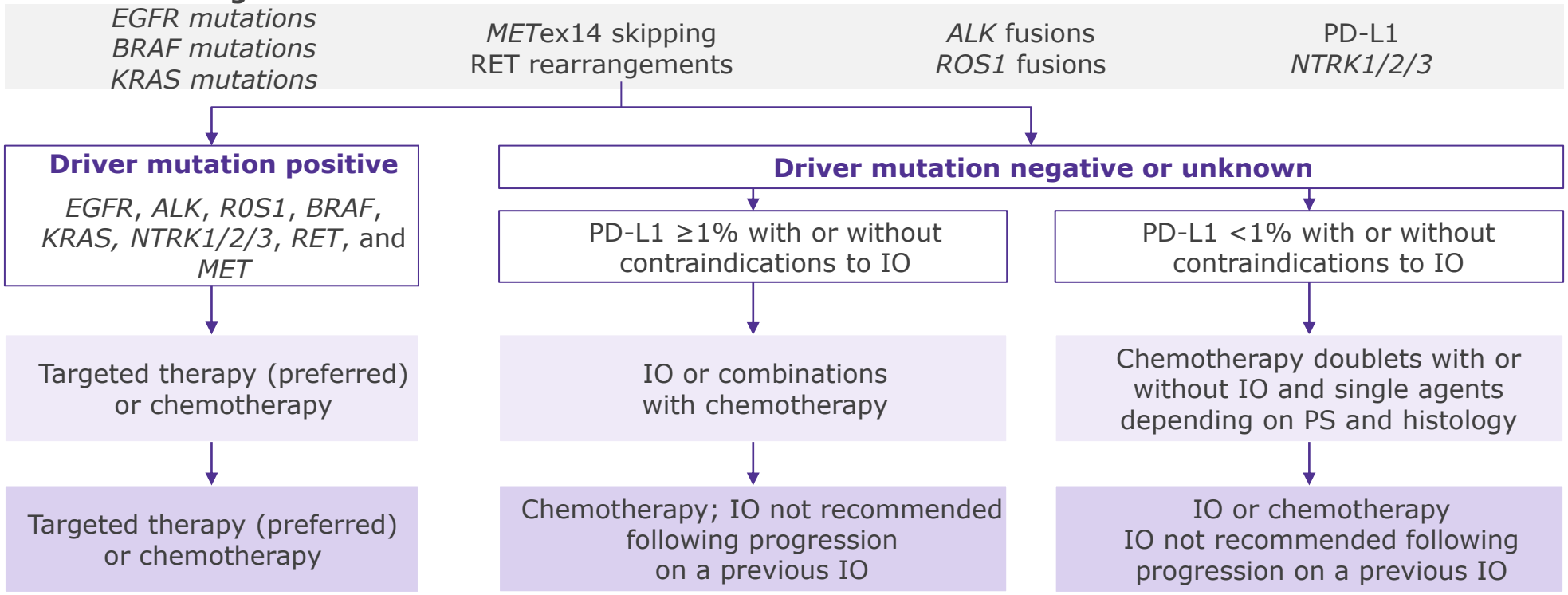
- A top-line summary of the key findings
- Clinically relevant biomarkers with an associated FDA-approved therapy
- Biomarkers that are potentially relevant but without a clear consensus
- Negative results that are clinically relevant but have not been identified
- A list of clinical trials for which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

1. Jones S. Presented at 2014 NAACCR Annual Conference. naaccr.org/wp-content/uploads/2016/11/022-NAACCR-2014-Biomarkers-Jones-20140624.pdf (accessed 03/2021). 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
KRAS mutations
*MET*ex14 skipping
RET rearrangements
ALK fusions
ROS1 fusions
PD-L1
NTRK1/2/3



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

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

SUMMARY

Summary

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

Current actionable biomarkers according to the **NCCN include *EGFR*, *ALK*, *ROS1*, *BRAF*, *KRAS*, *MET* exon 14 skipping mutations, *RET*, *NTRK1/2/3* and PD-L1**; NCCN recommends that when feasible, molecular testing be performed via a broad, panel-based approach[3]

Patients should be assessed for biomarker expression **at multiple points in the treatment pathway**, including at diagnosis and when starting a new therapy[2]

Biomarkers can be assessed via well-characterized techniques such as NGS, RT-PCR, PCR, FISH, and IHC, with assay selection depending on biopsy type[3,4]

Biomarker testing can help **guide patient management and treatment**[3]

Broad, panel-based testing can provide a view of the patient's genetic profile without high tissue demands of sequential testing[3,4]

1. Lung and Bronchus CSR. SEER. https://seer.cancer.gov/archive/csr/1975_2016/results_merged/sect_15_lung_bronchus.pdf (accessed 01/2021). 2. Pennell NA. et al. Am Soc Clin Oncol Educ Book. 2019;39:531–542. 3. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for NSCLC V.5.2021.© National Comprehensive Cancer Network, Inc. 2021. All rights reserved. Accessed June 15, 2021. To view the most recent and complete version of the guidelines, go to NCCN.org. 4. Lindeman NI, et al. J Thorac Oncol. 2018;13(3):323–358.

ADDITIONAL SLIDES

Glossary

Ab = antibody
ALK = anaplastic lymphoma kinase
BSC = best supportive care
BRAF = B-Raf proto-oncogene
cfDNA = circulating free DNA
ctDNA = circulating tumor DNA
CTC = circulating tumor cell
CGP = cancer gene panel
CNA = copy number alterations
DDR2 = discoidin domain receptor tyrosine kinase 2 gene
EGFR = epidermal growth factor receptor gene
ERBB2 = erb-b2 receptor tyrosine kinase 2 gene
FDA = Food and Drug Administration
FFPE = formalin-fixed paraffin embedded
FGFR1 = fibroblast growth factor receptor 1 gene
FISH = fluorescence in situ hybridization
H&E = hematoxylin and eosin
HER2 = human epidermal receptor 2 gene
ICI = immune checkpoint inhibitor
IHC = immunohistochemistry
IO = immunotherapy
KRAS = kirsten rat sarcoma viral oncogene homolog
L = leucine
M = methionine
MET = mesenchymal-epithelial transition proto-oncogene
MET = MET receptor tyrosine kinase
***MET*ex14** = *MET* exon 14
NCCN = National Comprehensive Cancer Network
NF1 = neurofibromin 1 gene
NGS = next generation sequencing
NRG1 = neuregulin 1 gene
NSCLC = non-small cell lung cancer
NTRK = neurotrophic receptor tyrosine kinase gene
PCR = polymerase chain reaction
PD-L1 = programmed-death ligand 1
PIK3CA = phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha gene
PS = performance score
PTEN = phosphatase and tensin homolog gene
R = arginine
RET = RET proto-oncogene
RIT1 = Ras like without CAAX 1 gene
ROS1 = ROS proto-oncogene 1
RT-PCR = reverse transcription PCR
SOC = standard of care
T = threonine
Trk = tropomyosin receptor kinase
TRS = targeted region sequencing
WES = whole exome sequencing
WGS = whole genome sequencing

Overview of assessment techniques

	DNA and RNA				Protein
	NGS[1,2]	RT-PCR[1-3]	PCR (Sanger)[1-4]	FISH[2-5]	IHC[2,5]
Overview	NGS is a high throughput sequencing technique performed on DNA or RNA, and includes targeted (TRS, CGP) and broad approaches (WES, WGS) which do not need a specific target • **Used to assess genetic changes in multiple genes simultaneously**	RT-PCR converts RNA to DNA for amplification and analysis • **Used to assess RNA expression, including fusion transcripts**	PCR allows for the amplification of a specific piece of DNA • **Used to assess DNA changes, including point mutations, insertions, or deletions**	FISH uses fluorescent probes to detect specific gene changes at the DNA level, where the probe binds to a specific sequence • **Used to detect gene rearrangements including deletions, amplifications, translocations, and fusions**	IHC uses commercially available antibodies to assess specific proteins • **Used to detect change in protein expression, localization or specific alterations, including fusion proteins**
Biopsy method[6]	Liquid and tissue biopsy	Liquid and tissue biopsy	Liquid and tissue biopsy	Tissue biopsy only	Tissue biopsy only
Sensitivity[2]	Variable with broader approaches; 1-10% with targeted approaches	0.0001%	20-50%	<1%	
Turnaround time[2]	Days to weeks depending on NGS approach	2-3 days	3-4 days	2-3 days	
Variants detected[2]	Point mutations Small indels CNA* Rearrangements*	Point mutations Small indels Rearrangements	Point mutations Small indels	Point mutations CNA Rearrangements	Rearrangements Protein expression

It is important to choose the technique that ensures accurate and reliable detection of the selected biomarker(s); some techniques require the knowledge of a specific change in DNA/RNA or protein for biomarker detection[1,6]

*Excluding amplicon capture

1. Dong J, et al. Front Pharmacol. 2019;10:230. doi: 10.3389/fphar.2019.00230. 2. Pennell NA. et al. Am Soc Clin Oncol Educ Book. 2019;39:531–542. 3. El-Deiry WS, et al. CA Cancer J Clin. 2019;69(4):305-343. 4. FISH. NIH Genome Research Institute. https://www.genome.gov/genetics-glossary/Fluorescence-In-Situ-Hybridization (accessed 02/2021). 5. Bruno R, Fontanini G. Diagnostics. 2020;10:521;doi:10.3390/diagnostics10080521. 6. Chen M, Zhao H. Human Genomics. 2019;13:34.