See the complete picture of NSCLC

Next-Generation Sequencing (NGS)
A clinician's guide to report interpretation and consultation

PP-US-8201a-1585. 11/22.

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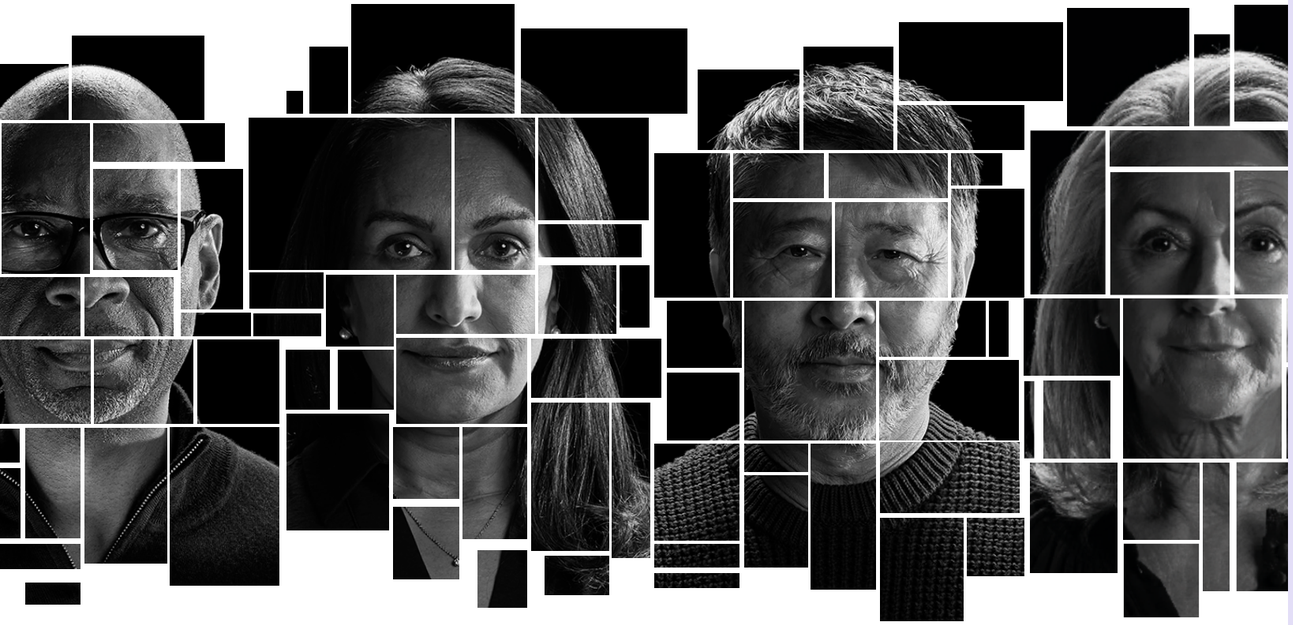
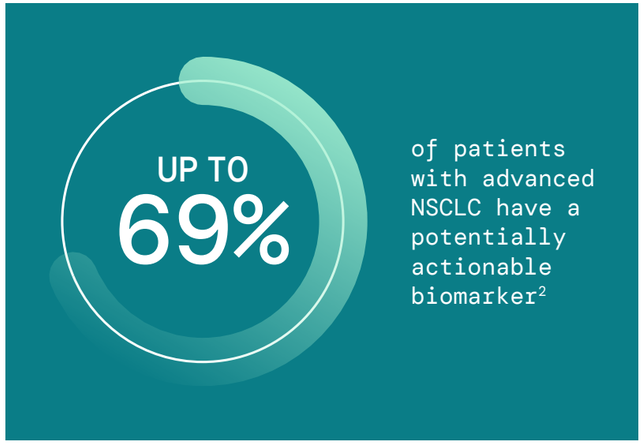


Lung cancer is not just one disease

Individualized approaches to lung cancer treatment are possible with targeted therapies and immunotherapies, which are available for different molecular subtypes of metastatic NSCLC. These therapies offer personalized treatment options for eligible patients with the potential for better clinical outcomes.[1]



of patients with advanced NSCLC have a potentially actionable biomarker[2]

Biomarker testing in patients with metastatic NSCLC is critical for determining eligibility for biomarker-driven therapies[1,2,3]

NSCLC, non-small cell lung cancer.

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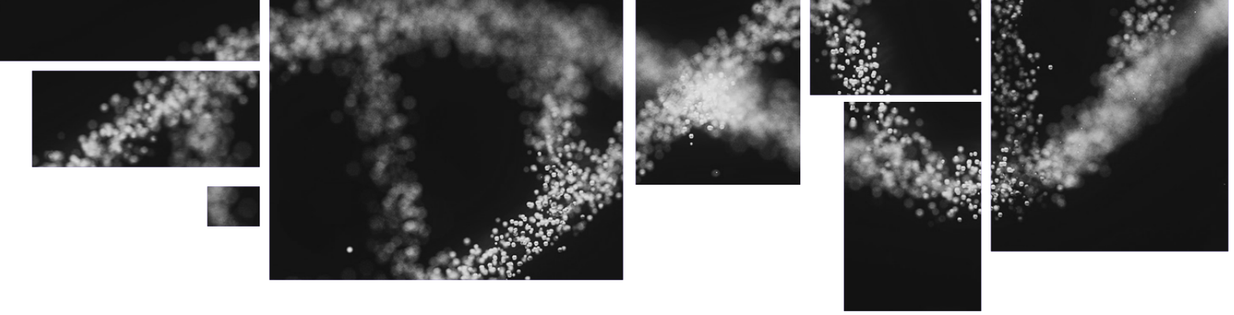
Overview of key biomarkers in NSCLC

An increasing number of key biomarkers that inform treatment decisions are being identified in NSCLC. In addition, there are now more than 25 biomarker-driven therapies FDA-approved for patients with NSCLC.[1]

Key biomarkers in NSCLC[2]	Prevalence in NSCLC
Actionable biomarkers*	
ALK gene rearrangements (fusions)	~7%[3]
BRAF V600E mutation	~1–2%[4,5]
EGFR mutations	~21%[3]
HER2 (ERBB2) mutations	~2–4%[6]
KRAS G12C mutation	~13%[7]
MET exon 14 skipping mutations	~3–4%[8]
NTRK1/2/3 fusions	<1%[9]
PD-L1 expression ≥1%†	~47–67%[10,11]‡
PD-L1 expression ≥50%†	~21–24%[10,12]‡
RET gene rearrangements (fusions)	~2%[3]
ROS1 gene rearrangements (fusions)	~2%[3]
Emerging biomarkers**	
High-level MET amplification	~2–4%[13,14]

*Biomarker in NSCLC with an associated FDA-approved biomarker-driven therapy. Please refer to the appropriate clinical guidelines or institutional treatment protocol to determine the appropriate therapy.
**Biomarker that does not have an associated FDA-approved therapy within NSCLC.
†PD-L1 expression level is determined by immunohistochemistry, not molecular testing.[1,15]
‡Prevalence is specific to advanced and metastatic disease.
ALK, anaplastic lymphoma kinase; BRAF, B-Raf proto-oncogene; EGFR, epidermal growth factor receptor; ERBB2, Erb-B2 receptor tyrosine kinase 2 HER2, human epidermal growth factor receptor 2; KRAS, Kirsten rat sarcoma virus; MET, mesenchymal epithelial transition factor receptor; NCCN, National Comprehensive Cancer Network® (NCCN®); NSCLC, non-small cell lung cancer; NTRK, neurotrophic tyrosine receptor kinase; PD-L1, programmed death-ligand 1; RET, rearranged during transfection; ROS1, ROS proto-oncogene 1; TMB, tumor mutational burden.

1. National Cancer Institute. Drugs Approved for Lung Cancer. Available at: https://www.cancer.gov/about-cancer/treatment/drugs/lung. Accessed May 2022. 2. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-Small Cell Lung Cancer V.5.2022. © National Comprehensive Cancer Network, Inc. 2022. All rights reserved. Accessed September 26th, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way. 3. Tsao A et al. J Thorac Oncol 2016; 11(5): 613–638. 4. Baik CS. PER® Winter Lung Cancer Conference. Available at: https://www.onclive.com/view/braf-v600e-testing-necessary-in-lung-cancer-but-physicians-unsure-of-optimal-setting-with-brafmek-combo. Accessed: April 2022. 5. Alvarez J and Otterson G. Drugs Context 2019; 13(8): 212566. 6. Zhao J and Xia Y. JCO Precis Oncol 2020; 4: 411–425. 7. Palma G et al. NPJ Precis Oncol 2021; 5(1): 98. 8. Hong L et al. Ther Adv Med Oncol 2021; 13:175883592199276. 9. Farago A et al. JCO Precis Oncol 2018; PO.18.00037. 10. Dietel M et al. Lung Cancer 2019; 134: 174-179. 11. Aggarwal C et al. Ann Oncol 2016; 27(Supplement 6): vi363. 12. Garon E et al. N Engl J Med 2015; 372(21): 2018-28. 13. Skoulidis F and Heymach JV. Nat Rev Cancer 2019; 19(9): 495–509. 14. Schubart C et al. Cancers 2021; 13: 5023. 15. Riely GL. J Natl Compr Canc Netw 2017; 15(5S): 686-688.



Broad molecular profiling using Next-Generation Sequencing

Broad molecular profiling using Next-Generation Sequencing (NGS) can simultaneously detect all four classes of genomic alterations: point mutations, small insertions and deletions, gene amplification, and gene rearrangements (fusions).[1]

Additionally, broad molecular profiling with NGS can detect molecular signatures (e.g. TMB, MSI), which is not possible with single-gene testing (PCR/IHC/FISH), or multigene hotspot NGS.[2,3]

Several NGS-based assays are validated to detect key biomarkers in NSCLC.[5–7]

Broad molecular profiling using NGS is an efficient method for identifying key biomarkers in NSCLC.[8]

Note: PD-L1 expression level is determined by immunohistochemistry, not NGS.

Clinical guidelines endorse NGS testing[4]

– The NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Panel for NSCLC strongly advises broad molecular profiling for eligible patients with metastatic NSCLC, typically with NGS, with the goal of identifying rare driver mutations for which effective drugs may already be available, or to appropriately counsel patients regarding the availability of clinical trials[4*]

*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.

FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; MSI, microsatellite instability; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; PCR, polymerase chain reaction; PD-L1, programmed death-ligand 1; TMB, tumor mutational burden.

1. Types of Molecular Tumor Testing. My Cancer Genome. Available at: https://www.mycancergenome.org/content/molecular-medicine/types-of-molecular-tumor-testing/. Accessed: April 2022; 2. Colomer R et al. EClinicalMedicine 2020; 25: 100487. 3. Chakravarty D et al. J Clin Oncol 2022. https://doi.org/10.1200/JCO.21.02767. [Epub ahead of print]. 4. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-Small Cell Lung Cancer V.5.2022. © National Comprehensive Cancer Network, Inc. 2022. All rights reserved. Accessed September 26th, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way. 5. D'Haene N et al. PLoS One 2015; 10(9): e0138245; 6. Martínez-Fernández P et al. J Pers Med 2021; 11(5): 360; 7. Poh J et al. PLoS One 2022; 17(4): e0267389; 8. Pennell NA et al. JCO Precis Oncol 2019; 3: 1–9.

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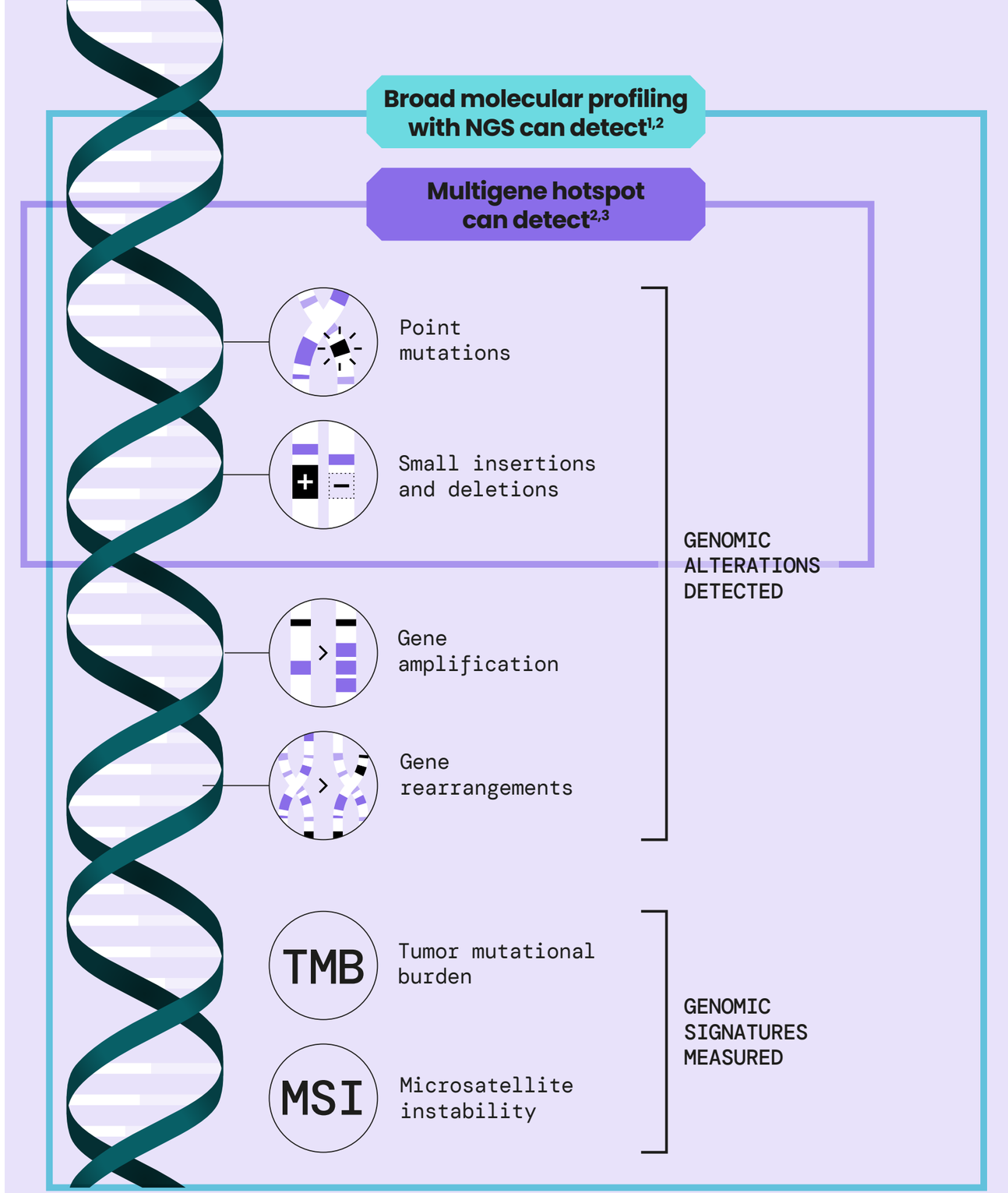

1. Vnencak-Jones, C., et al. 2016. Types of Molecular Tumor Testing. Available at: mycancergenome.org/content/molecular-medicine/types-of-molecular-tumor-testing/ Accessed May 2022. 2. Colomer R et al. EClinicalMedicine 2020; 25: 100487. 3. Chakravarty D et al. J Clin Oncol 2022. https://doi.org/10.1200/JCO.21.02767. [Epub ahead of print].

Benefits and potential limitations of tissue and liquid biopsy

In view of the recent technological advances and growing clinical application of plasma-based NGS, the IASLC revisited the role of liquid biopsy in therapeutic decision-making and the question of "liquid first" versus "tissue first" approaches for molecular testing.

Liquid biopsy is complementary to tumor tissue testing, but its use is expected to increase.[1]

Tissue biopsy

Considered the 'gold standard' for diagnostic and biomarker testing.[1]

14–17 days TAT[2] (NGS)

Benefits[5-9]

- Gold standard for biomarker testing
- Direct assessment of tumor
- Assessment of molecular biomarkers and PD-L1 (IHC)
- Provides histologic diagnosis

Potential Limitations[2,5-6]

- Requires sufficient quality and quantity of tumor tissue
- Acquisition of tissue biopsy is more time-consuming than a blood draw
- Analysis of a single tumor sample may not capture heterogeneity

Liquid biopsy
ctDNA analysis of blood

Useful in cases of tissue insufficiency or contraindication for biopsy (e.g. bleeding risk).[1]

8-12 days TAT[3] (NGS)

Benefits[4,5,10]

- Minimally invasive blood draw is conducive for repeat sampling
- Has the potential to capture tumor heterogeneity and clonal evolution
- Typically shorter turnaround time

Potential Limitations[5,11]

- May not detect biomarkers due to variability in tumor ctDNA shedding
- Negative results are not informative and require reflex testing of tumor tissue

ctDNA, circulating tumor DNA, DNA, deoxyribonucleic acid; IASLC, International Association for the Study of Lung Cancer; IHC, immunohistochemistry; NGS, next-generation sequencing; PD, progressive disease; PD-L1, programmed death-ligand 1. TAT, turnaround time.

1. Kerr KM et al. Lung Cancer 2021; 154: 161–175. 2. Zheng Y et al. Future Oncol 2022; 18(4): 505–518. 3. Lee Y et al. JCO Precis Oncol 2020; 4: 1098–1108. 4. Robert NJ, et al. J Clin Oncol. 2021; 39 (suppl 15; abstr 9004). 5. Rolfo C et al. J Thorac Oncol 2019; 13(9): 1248–1268. 6. Duffy MJ and Crown J. J Pers Med 2022; 12(1): 99. 7. National Cancer Institute. Pathology Reports. Available at: https://www.cancer.gov/about-cancer/diagnosis-staging/diagnosis/pathology-reports-fact-sheet#what-information-does-a-pathology-report-usually-include. Accessed June 2022. 8. Wistuba II. Am Soc Clin Oncol Educ Book 2012: 459-64. 9. Schehr J et al. Biomark Res 2022; 10(1): 26. 10. Marrugo-Ramírez J et al. Int J Mol Sci 2018; 19(10): 2877. 11. Schwartzberg L et al. NPJ Precis Oncol 2020; 4: 15.

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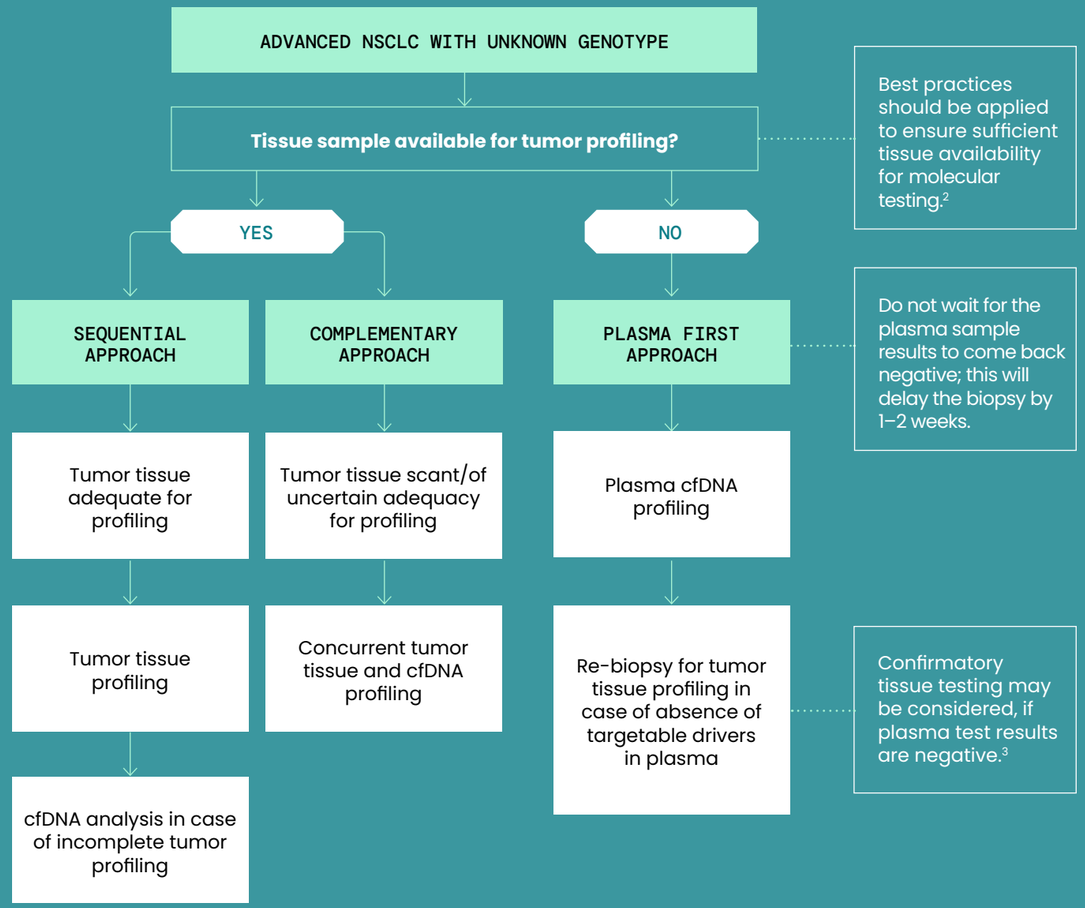
Diagnostic algorithm for liquid biopsy use in treatment-naive advanced/metastatic NSCLC[1-3,5]



Adapted from Rolfo, et al. J Thorac Oncol 2021; 16(10): 1647–1662 and Aggarwal C et al. Nat Rev Clin Oncol. 2021 Jan; 18(1): 56-62.

Optimal biomarker testing strategies, including reflex testing protocols, may reduce overall testing TAT and improve the identification of biomarkers.[4]

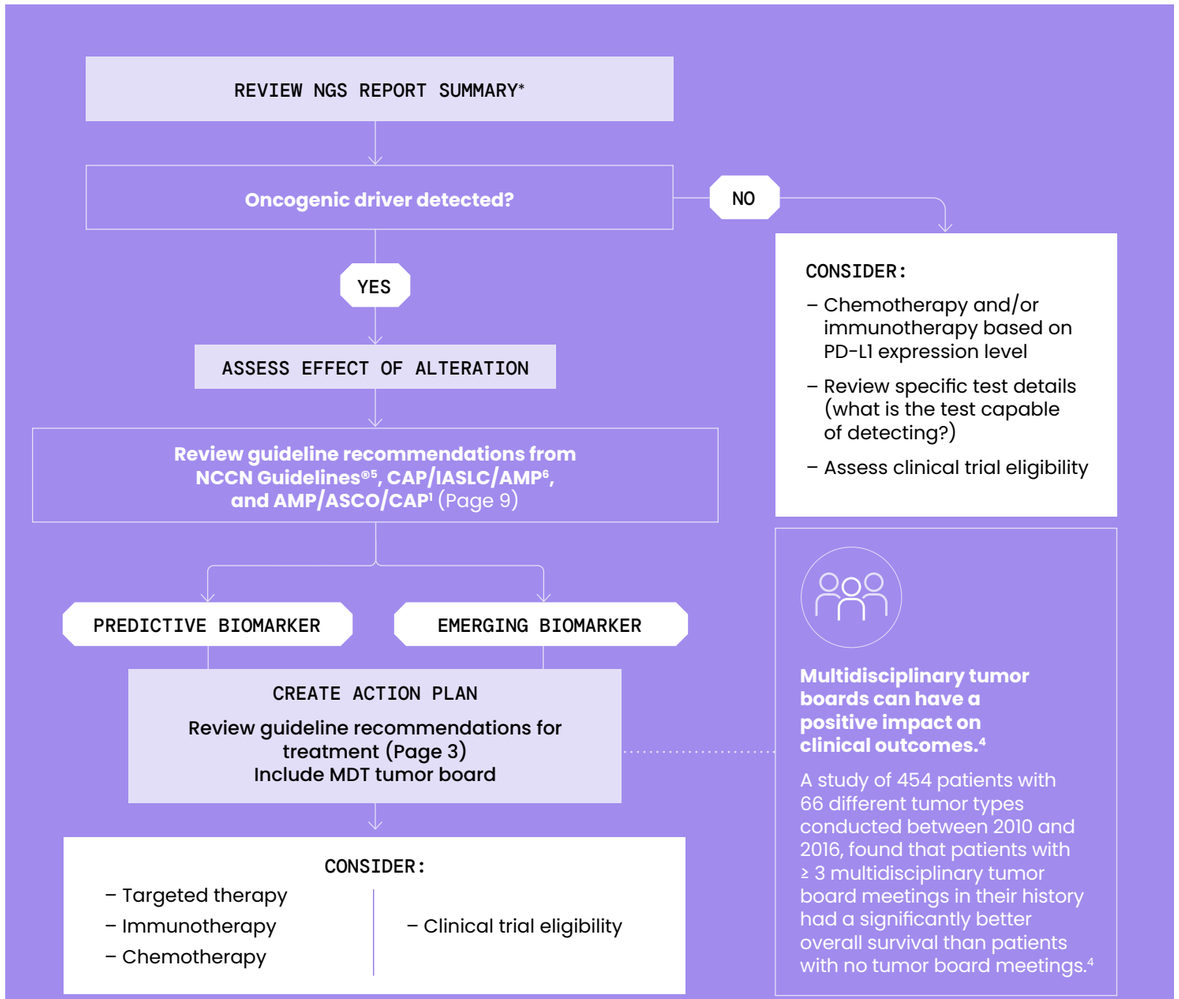
cfDNA, cell-free DNA; NSCLC, non-small cell lung cancer. TAT, turnaround time.

1. Rolfo et al. J Thorac Oncol 2021; 16(10): 1647–1662. 2. Gregg JP et al. Transl Lung Cancer Res. 2019; 8(3): 286–301 . 3. Guibert N et al. Eur Respir Rev 2020; 29: 190052. 4. Anand K et al. Clin Lung Cancer 2020; 21(5): 437–442. 5. Aggarwal C et al. Nat Rev Clin Oncol. 2021 Jan; 18(1): 56-62.

Decoding an NGS report

The results of NGS testing are reported in a variety of templates, with large amounts of data that can be difficult to interpret.[1]

The approach presented in the flow chart below, provides simple guidance and a framework to help navigate the results of a typical NGS report.[1-5]



Adapted from Brown NA et al. Am Soc Clin Oncol Educ Book 2018; 38: 708–715; Dienstmann R Mol Oncol. 2014; 8(5): 859–873.

AMP, Association for Molecular Pathology; CAP, College of American Pathologists; IASLC, International Association for the Study of Lung Cancer; MDT, multi-disciplinary team; NGS, next-generation sequencing; PD-L1, programmed death-ligand 1.

*The NGS report summary may extend beyond one page and it is important to be read in its entirety.

1. Li MM et al. J Mol Diagn 2017;19:4–23. 2. Brown NA et al. Am Soc Clin Oncol Educ Book 2018; 38: 708–715; 3. Dienstmann R Mol Oncol. 2014; 8(5): 859–873. 4. Freytag M et al. BMC Cancer 2020; 20: 355. 5. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-Small Cell Lung Cancer V.5.2022. © National Comprehensive Cancer Network, Inc. 2022. All rights reserved. Accessed September 26th, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way. 6. Lindeman N et al. Arch Pathol Lab Med 2018; 142(3): 321-346.

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Clinical NGS reports share common features and basic information about the test:[1]

- A high-level summary of the key findings
- Potentially clinically relevant biomarkers, with any matched clinical trials
- Identified biomarkers with established utility
- Pertinent negative results considered to be clinically relevant

Variant classification[2]

Guideline recommendations for biomarker test reporting use evidence-based variant categorization – tier I to IV – to rate biomarkers by clinical significance and level of confidence in clinical effect.[2]

An NGS report summary contains the most important and actionable information for establishing a treatment plan.

Tier I Variants with Strong Clinical Significance[2]	Therapeutic, prognostic, and diagnostic with **Level A and Level B evidence**
Tier II Variants with Potential Clinical Significance[2]	Therapeutic, prognostic, and diagnostic with **Level C and Level D evidence**
Tier III Variants of Unknown Clinical Significance[2]	Appropriate tracking of VUS is key, for future reclassification as could affect patient management
Tier IV Benign or Likely Benign[2]	Mostly rare germline variants and not recommended for inclusion in the NGS report

Evidence level[2]

- **A** FDA-approved therapies, included in professional guidelines
- **B** Well-powered studies with consensus from experts in the field
- **C** FDA-approved therapies for different tumor types or investigational therapies. Multiple small published studies with some consensus
- **D** Preclinical trials or a few case reports without consensus

FDA, Food and Drug Administration; VUS, variants of uncertain (or unknown) significance; NGS, next-generation sequencing.

1. Brown NA et al. Am Soc Clin Oncol Educ Book 2018; 38: 708–715. 2. Li MM et al. J Mol Diagn 2017;19:4–23.

A prep tool for discussing biomarker results

As you prepare to communicate biomarker results with your patients, use this template as a preparation tool to help you summarize key information from the comprehensive report to discuss with your patients, as part of a shared decision making process.

KEY RESULTS FROM NGS REPORT			
BIOMARKER	✓	SPECIFIC ALTERATION	ASSOCIATED THERAPY (EVIDENCE LEVEL A-D)
ALK rearrangements (fusions)*			
BRAF V600E mutation*			
EGFR sensitizing mutations*†			
EGFR exon 20 insertion mutations*			
HER2 (ERBB2) mutations*			
KRAS G12C mutation*			
MET exon14 skipping mutations*			
NTRK1/2/3 fusions*			
PD-L1 expression ≥1%*‡			
PD-L1 expression ≥50%*‡			
RET gene rearrangements (fusions)*			
ROS1 gene rearrangements (fusions)*			
High-level MET amplification**			
PLAN OF ACTION Consider recommendations on FDA-approved treatment or clinical trial eligibility			

*Biomarkers in NSCLC with an associated FDA-approved biomarker-driven therapy. Please refer to the appropriate clinical guidelines or institutional treatment protocol to determine the appropriate therapy.

**Biomarker that does not have an associated FDA-approved therapy within NSCLC.

†Including exon 19 deletions, L858R mutations, S768I mutations, L861Q mutations, or G719X mutations.

‡PD-L1 expression level is determined by immunohistochemistry, not NGS.[1] It can be targeted by FDA-approved therapies.[1]

ALK, anaplastic lymphoma kinase; BRAF, B-Raf proto-oncogene; EGFR, epidermal growth factor receptor; ERBB2, Erb-B2 receptor tyrosine kinase 2; FDA, Food and Drug Administration HER2, human epidermal growth factor receptor 2; KRAS, Kirsten rat sarcoma virus; MET, mesenchymal epithelial transition factor receptor; NTRK, neurotrophic tyrosine receptor kinase; PD-L1, programmed death-ligand 1; RET, rearranged during transfection; ROS1, ROS proto-oncogene 1.

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Begin the biomarker conversation

It is important to discuss biomarker testing and its treatment implications with your patients. Below are some questions you should be prepared to answer.

- **Who will order my test, and how long will it take to get the results?**
- **Why is biomarker testing important?**
- **Which biomarkers will be tested?**
- **What does 'broad molecular profiling' mean?**
- **What will it tell me about my cancer and treatment options?**
- **What if an actionable biomarker is not detected?**
- **What sample(s) will be tested?**
- **Is there enough tumor tissue to obtain results from the biomarker testing?**

When discussing testing, patients may be more familiar with the terms 'biomarker testing' or 'comprehensive biomarker testing.'

Refer to the Biomarker Information Sheet in the accompanying Patient NGS Booklet to help facilitate discussions regarding biomarker test results during consultations with your patients.

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