Sample Requirements and Testing Approaches in mNSCLC

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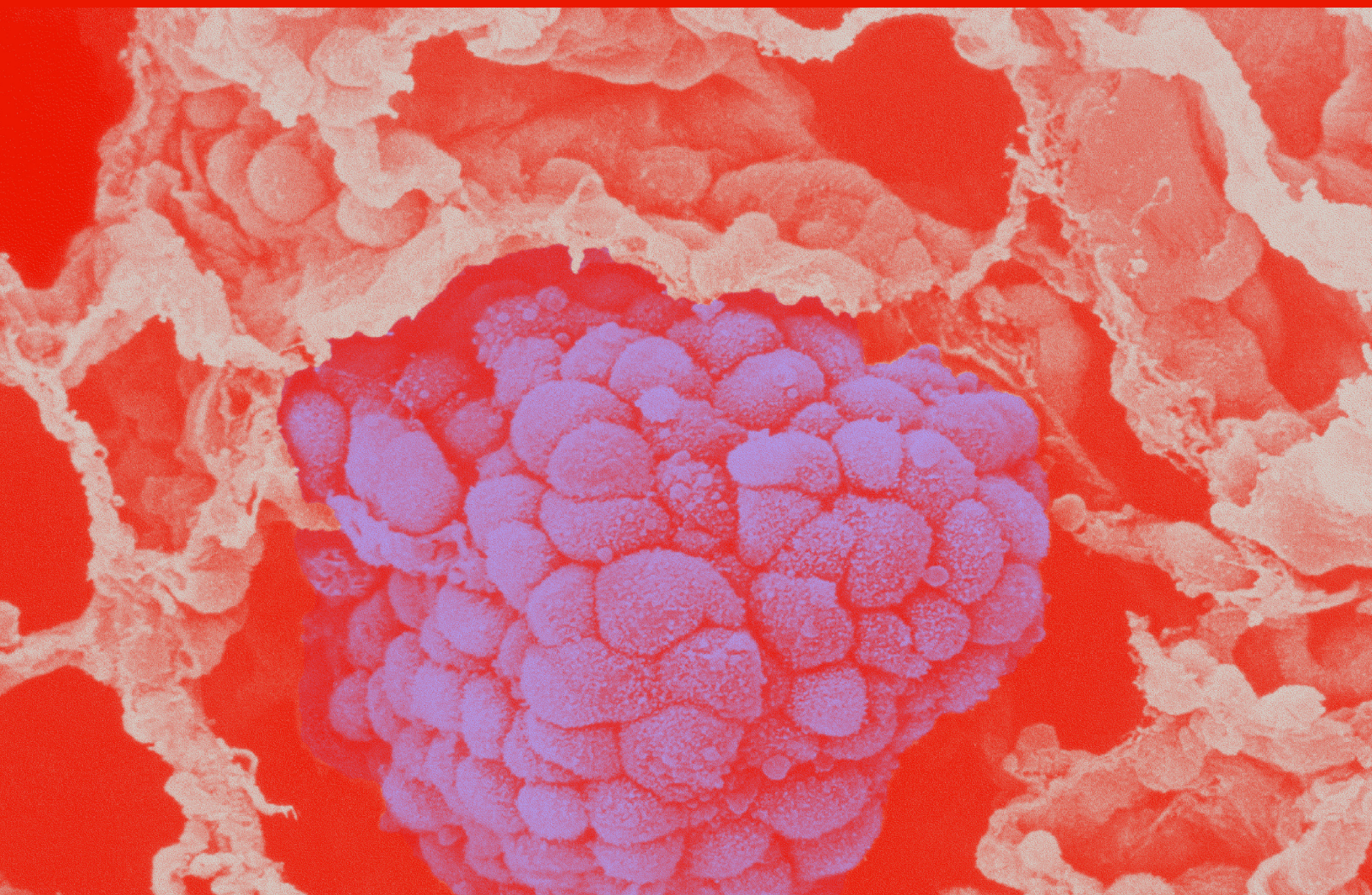

Key factors for successful biomarker-informed care include sample acquisition, testing method, and test interpretation[1–14]*

Sample acquisition

- Amount and quality of tissue collected impacts the feasibility of biomarker testing[1–7,9–11,13]*
- The type of sample may also impact biomarker testing options[1–7,11–13]*
- Consider alternate sample types, ie, liquid biopsy[3]*

Testing method

Selecting the appropriate test depends on:

- Actionable biomarkers to be detected[1,2,5–7,9]
- Choice of testing methodologies[3,4,7,12]*
- Test specificity and sensitivity[3,4,7,9,10,12]*
- Turnaround time (TAT)[4,7,9]

Test interpretation

- Understanding the spectrum of alterations being tested and not tested when interpreting the report[3,5,14]*
- Ensuring patients are well-informed[14]
- Matching testing results to available targeted therapies[3,5]*

When preparing for biopsy, the sample method could impact quality and testing options[1–14]*

Lung tumor tissue and DNA can be accessed through minimally invasive techniques[1,15,16]

	Transthoracic procedure	Endobronchial ultrasound-guided transbronchial procedure	Navigational bronchoscopy	Liquid biopsy
Imaging method	Computed tomography[1,15]	Ultrasound[1,15]	Computer-generated mapping software guided by a robotic bronchoscope[15]	Molecular testing complementing imaging tests[16]
Sample location	Peripheral pulmonary lesions[1,15]	Mediastinal and hilar lymph nodes and centrally located parenchymal lesions[1,15]	Central and peripheral lesions[15]	Plasma, serum, sputum, bronchoalveolar lavage fluid[16]
Small biopsy technique	FNA or CNB may be used for tissue biopsy[1,15]			N/A

In mNSCLC, tissue biopsy method may impact biomarker testing options[1,2,17]

	Fine-needle aspiration (FNA) accounts for ~80% of biopsies in advanced NSCLC[17]	**Core needle biopsy (CNB)** accounts for an additional 10% of biopsies in advanced NSCLC[17]
Needle[1,2]	• Fine needle (20–25 gauge, ≤0.72 mm thick)	• Hollow-core needle (14–20 gauge, 0.91–2.1 mm thick)
Use[1,2]	• Can be used to prepare direct smears • Excellent for FISH, may be used for other biomarker testing	• Create tissue block for histologic assessment • Validated for ancillary studies/IHC • **Better molecular testing success due to less tissue lost**
Limitations[2]	• Limited tissue architecture hinders diagnosis of in situ versus invasive cancer • Cytologic specimen processing may pose validation challenges for IHC or molecular testing • Lower diagnostic rates due to lack of technical expertise	• More expensive • Longer tissue fixation and processing time

Tissue biopsy remains the primary sample type for biomarker testing in NSCLC[3,18]*

Strengths

- Can be used for all testing technologies and methods[11,18]
- Provides a snapshot of the histology and molecular makeup of the tumor[18]
- Highly sensitive[18]

Rapid on-site evaluation (ROSE) involves an **on-site cytopathologist or cytotechnologist performing a rapid stain** in the suite or operating room to confirm the **presence of tumor cells** and estimate **neoplastic cell content** in the biopsy[11]

Challenges

- Invasive[16,18]
- Does not assess tumor heterogeneity within the primary tumor and across tumors in metastatic sites[16,18]
- Cannot be used on inaccessible tumors or in patients who are not stable enough for biopsy[18]
- Serial tissue biopsies are not recommended[11]
  - In a systematic review and meta-analysis, testing with **ROSE improved diagnostic yield** by 14% and **decreased the amount of needle passes** (MD -0.99, 95% CI: -1.89 to -0.09)[19]

Opportunity to optimize

ROSE may enhance tissue biopsies by optimizing sample quality and diagnostic/molecular yield[3,11]*

Liquid biopsy samples are approved for biomarker testing in NSCLC[20–22]

Liquid biopsy biomarker testing utilizes bodily fluids, typically blood,† to detect ctDNA, ctRNA, CTC, and exosomes for genomic testing[20–22]

Strengths

- Minimally invasive[20–22]
- Can provide an overview of molecular heterogeneity from all sites (primary and metastatic)[20,22]
- Serial biopsies can monitor sub-clonal evolution/ acquired resistance[21]
- May have more rapid overall TAT compared with tissue-based NGS[16,23]
- Positive test result may direct treatment[16,21,22]

Challenges

- Low specificity and sensitivity – sample content may be below the limit of detection, depending on sample type, concentration, and variant being detected[20,24]
- May be associated with false negatives (up to 30%) or may identify variants of unknown significance (VUS)[3,22]*
- Negative test result requires reflex testing of tissue sample[25]

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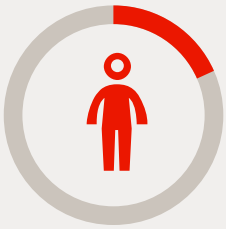
†Urine, saliva, CSF, and other bodily fluids may also be used for detection, disease monitoring, or assessing acquired resistance.[20,22]

Liquid biopsy complements tissue biopsy biomarker testing[20,26]

Both tissue and liquid biopsy testing can miss actionable biomarkers.[20,24] For example:

- Tissue samples may not capture a mutation in a part of a tumor that is not sampled[20,27]
- Liquid samples may give a false negative if the alteration is below the limit of detection[3,24]*

In a prospective observational study, NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) recommended biomarkers were identified in[26]:



21.3% of patients who underwent **tissue biopsy testing**

27.3% of patients who underwent **tissue and/or liquid biopsy testing**

10.3% of patients who underwent **liquid biopsy testing** whose biomarkers were **not identified in tissue**

In another study, in patients who received BOTH liquid and tissue-based NGS testing[28]:

- **99.5%** received complete molecular testing†
- **100%** had testing results available prior to first-line therapy

Opportunity to optimize

Consider liquid biopsy biomarker testing in NSCLC as a complement to tissue testing. Liquid biopsy stand-alone testing is an option when tissue can not be obtained, or when a tissue sample may be insufficient[3]*

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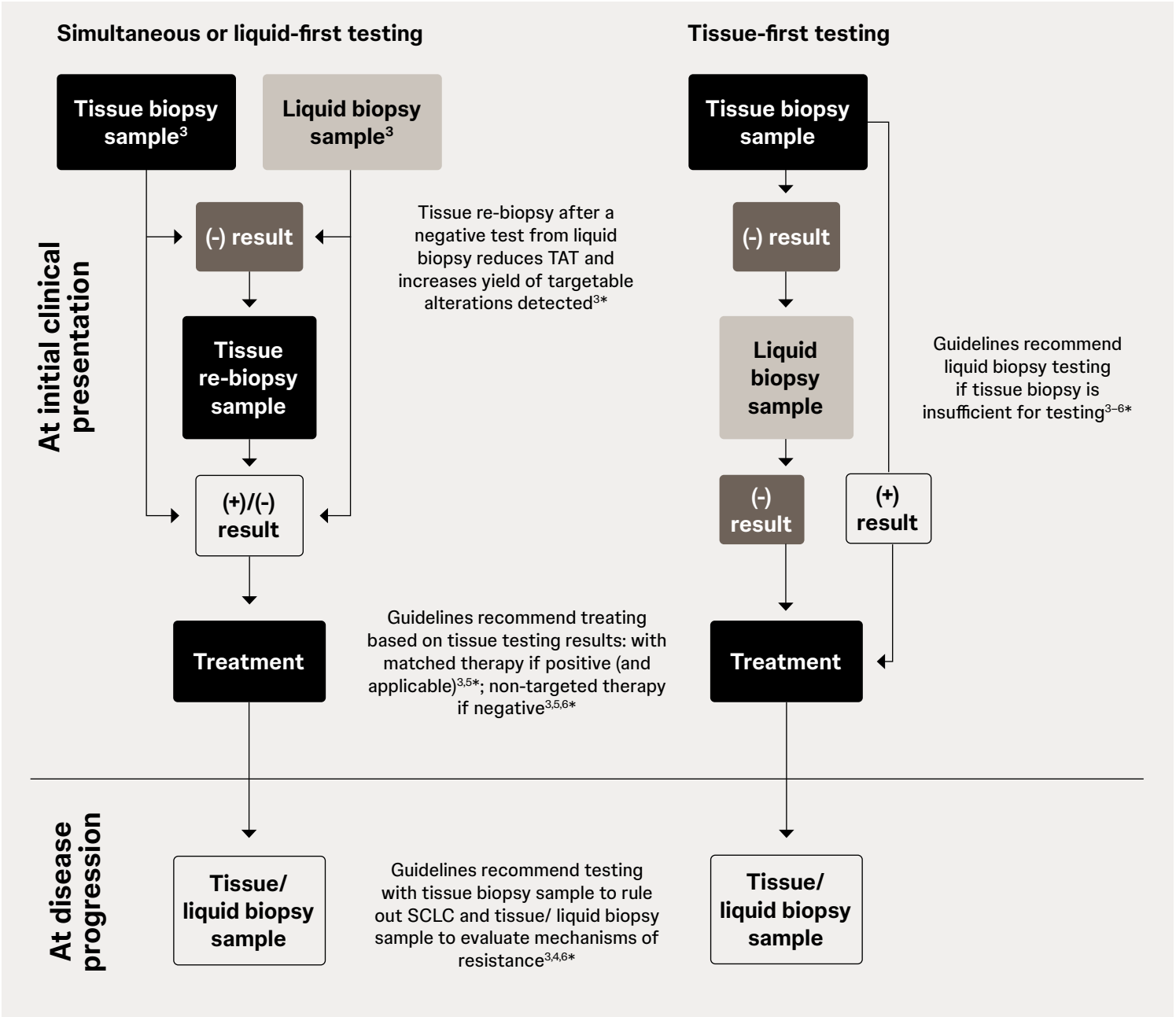
†Complete based on guideline recommendations at the time of the study. *EGFR, ALK, BRAF, ROS1, MET, RET,* and *NTRK* were tested.[28]

Depending on different conditions, guidelines recommend using tissue and liquid biopsies simultaneously or sequentially for biomarker testing to aid in clinical decision-making[3,4,18,29]*



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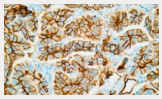
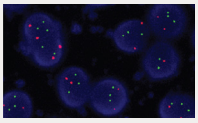
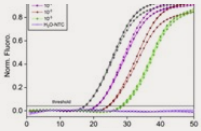
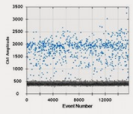
Different methods of biomarker assays have different capabilities[3,7]*

	Broad-based panel	Single-biomarker test			
Test type	**NGS**[3,7,9,10]* 	**IHC**[3,10]* 	**FISH**[3,9,10,12]* 	**RT-PCR**[3,9,10]* 	**ddPCR**[10] 
Actionable biomarkers detected	*ALK, BRAF, EGFR,* HER2*, KRAS, MET, NTRK, RET, ROS1*	ALK, HER2, NTRK, ROS1, PD-L1 **Notes:** • IHC is **not** a method for detecting *METex14* skipping or *EGFR* mutations[3]* • Presumptive *ROS1* fusions must be confirmed by FISH[3]*	*ALK, NTRK, RET, ROS1*	*ALK, BRAF, EGFR,* HER2*, KRAS, NTRK, RET, ROS1*	*ALK, EGFR, KRAS*

NGS detects biomarkers that may be missed by single-gene tests[3,7]*

- ✓ Simultaneously screens hundreds of genes[7,23]
- ✓ Generates more data from smaller amounts of DNA due to greater resolution of genomic variants[7,23]
- ✓ Discovers actionable and emerging biomarkers through comprehensive genomic coverage[3,7]*

Images adapted from Cao P, Yu Y, Wang W, et al. Fluorescence in situ hybridization comparison of the prognostic factors in adult and pediatric acute lymphoblastic leukemia: a retrospective analysis of 282 cases. *Exp Ther Med*. 2018;16(6):4674-4684; Kipf E, Schlenker F, Borst N, et al. Advanced minimal residual disease monitoring for acute lymphoblastic leukemia with multiplex mediator probe PCR. *J Mol Diagn*. 2022;24(1):57–68; Isaka T, Yokose T, Ito H, et al. Detection of EGFR mutation of pulmonary adenocarcinoma in sputum using droplet digital PCR. *BCM Pulm Med*. 2021;21(1):100. doi:10.1186/s12890-021-01468-9; Watson CM, Nadat F, Ahmed S, et al. Identification of a novel MAGT1 mutation supports a diagnosis of XMEN disease. *Genes Immun*. 2022;23(2):66–72.

NGS panels detect different alterations and biomarkers[30]

NGS panels may:

Use RNA and DNA[30]

DNA-based NGS

- Can **detect indels, SNVs, and CNVs,** as well as some **gene fusions or rearrangements**[30]
- **May miss** certain rearrangements or gene fusions, particularly **novel or unusual gene fusions**[30]

RNA-based NGS

- Can reliably detect **gene fusions or rearrangements** that are missed by DNA sequencing[30,31]
- **Rarely used for somatic SNV or indel testing** due to instability, variable expression, and lack of double-stranded context[30]

Be small or large[32,33]

Small panels (typically, around 50 genes or fewer)

✓ May have **faster TAT** and may be more likely to be **covered by insurance**[32,33]

⊗ May not reliably detect fusions or CNVs[32]

Large panels (larger than 50 genes)

✓ May **increase the probability of detecting an actionable biomarker** for currently available treatment or for clinical trial[32,33]

RNA sequencing can identify 26.5%–31.9% of DNA sequencing undetectable gene alterations in lung adenocarcinomas[31]

Opportunity to optimize

An NGS-first approach to testing may help optimize tissue efficiency[7,34]

NGS testing optimizes tissue and time efficiency compared with single-gene testing[7,34]

A recent study examined the tissue consumption and success rates of NGS after single-gene testing across >80 community practices[34]

Single-gene testing[34]

10 slides for diagnosis (H&E + immunostains)

↓

35 slides for single-gene testing
Block is refaced each time if results are negative

↓

20 slides for CGP

NGS success rate: 66%[34]

Single-gene testing often leads to re-biopsy, delaying test results and increasing testing cost[7,34]

CGP[34]

10 slides for diagnosis (H&E + immunostains)

↓

20 slides for CGP
Block is refaced once, leading to less risk of insufficient tissue

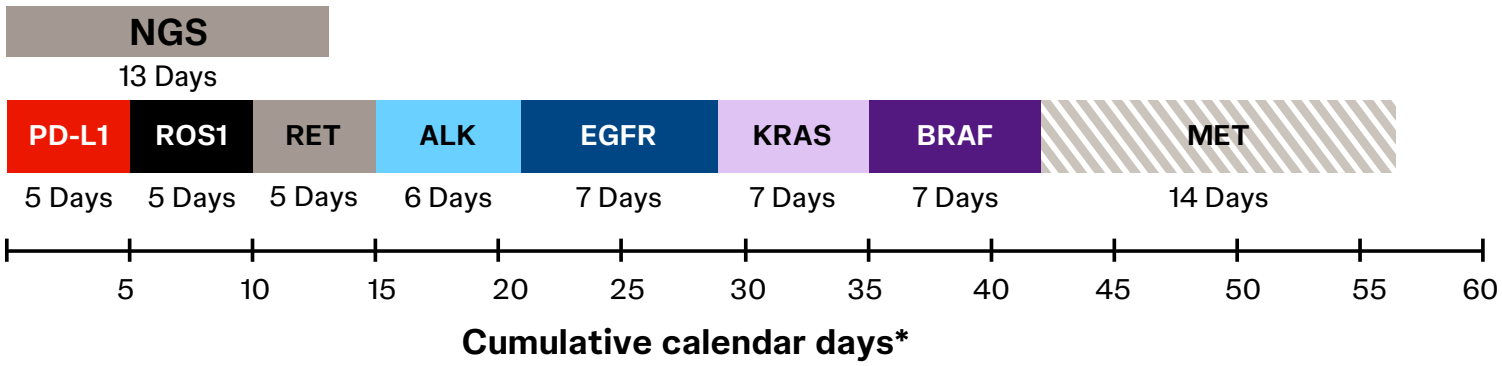
NGS-first success rate: 82%[34]

~Twice the number of CGP orders were **cancelled** due to tissue insufficiency from a single-gene testing-first strategy[34]

This study found median TAT for testing to be shorter for NGS compared with sequential single-gene tests[34]

Only 3% of patients with NSCLC had all available single-gene tests performed for all guideline-indicated biomarkers[34]

Median TAT[34]



Upfront single-gene testing may put patients with NSCLC at risk for missed targeted therapy due to incomplete testing[34]

Opportunity to optimize

Reflex testing can reduce time to treatment initiation versus physician-ordered, on-demand testing and facilitate optimal use of tissue and increase overall testing rates[7,35]

*Cumulative calendar days is theoretical summation of median TAT for each single-gene test.

NGS optimizes cost-efficiency compared with PCR[36]

NGS was associated with a cost savings of $7,386 for the health system over the first year per patient, compared with PCR[36]

In addition to costs associated with testing being lower, the **total cost of treatment was found to be lower with NGS** when taking into account[36]:

- Wasted healthcare expenditures associated with initiating suboptimal treatment[36]
- Cost associated with initiating treatment prior to receiving results[36]
- Costs associated with delaying care[36]

NGS is an efficient option for biomarker testing in NSCLC[3,7,34,36,37*]

Reduces need for re-biopsy[37]	NGS efficiently uses limited tissue while maximizing diagnostic yield[7,34]
Decreases time to obtain test results[37]	NGS has been associated with a faster time to appropriate therapy than PCR or single-gene testing[34,37]
Offers comprehensive testing[37]	NGS can detect actionable biomarkers, as well as emerging and those associated with clinical trials[3,7,9,10*]
Provides cost savings[37]	NGS was shown to result in higher projected savings than PCR or single-gene testing[36]
Negates need for sequential single-gene testing[37]	NCCN recommends, when feasible, testing be performed via a broad, panel-based approach, most typically performed by NGS[3*]

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ALK, anaplastic lymphoma kinase; BRAF, B-Raf proto-oncogene; CGP, comprehensive genomic profiling; CI, confidence interval; CNB, core needle biopsy; CNV, copy number variation; CSF, cerebrospinal fluid; CTC, circulating tumor cell; ctDNA, circulating tumor deoxyribonucleic acid; ctRNA, circulating tumor ribonucleic acid; ddPCR, digital droplet polymerase chain reaction; DNA, deoxyribonucleic acid; EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; FNA, fine-needle aspiration; H&E, hematoxylin and eosin; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; KRAS, Kirsten rat sarcoma virus; MD, median deviation; mNSCLC, metastatic non-small cell lung cancer; MET, MET proto-oncogene 1, receptor tyrosine kinase; N/A, not applicable; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; NTRK, neurotrophic tyrosine receptor kinase; PCR, polymerase chain reaction; PD-L1, programmed death-ligand 1; RET, receptor tyrosine kinase; RNA, ribonucleic acid; ROS1, ROS proto-oncogene 1, receptor tyrosine kinase; ROSE, rapid on-site evaluation; RT-PCR, reverse transcription polymerase chain reaction; SCLC, small cell lung cancer; SNV, single nucleotide variant; TAT, turnaround time; VUS, variants of unknown significance.

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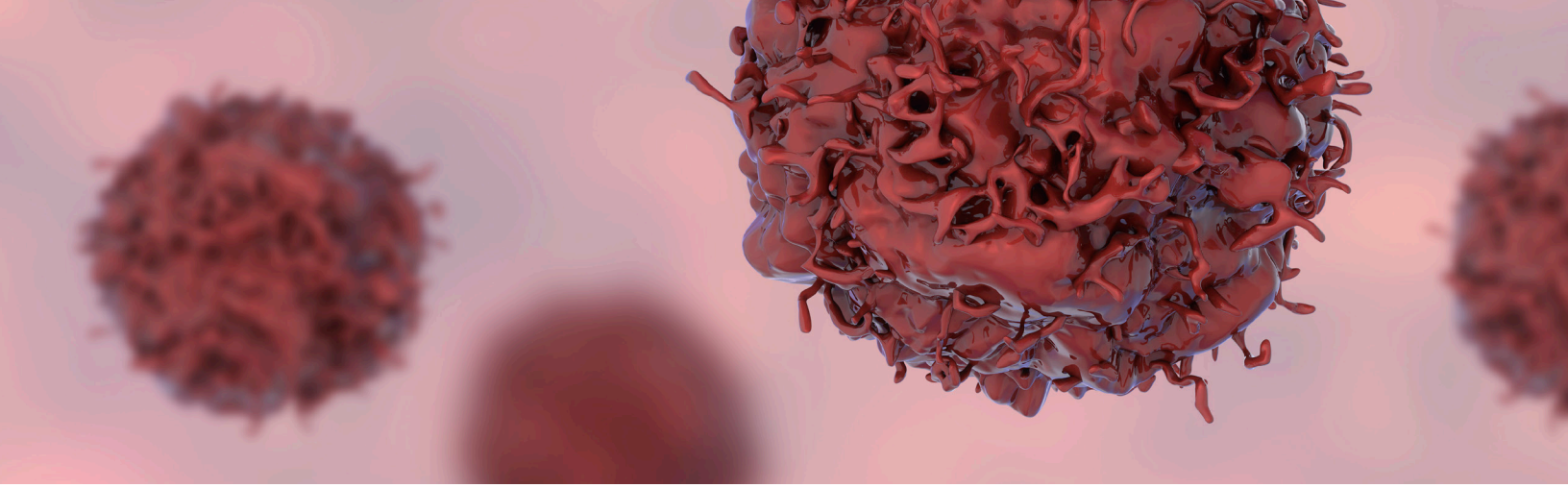

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