Liquid Biopsy for Guiding Treatment Decisions in Advanced Non–Small Cell Lung Cancer

GRETCHEN SUK, DMSc, PA-C

From Guardant Health, Inc., Palo Alto, California, and University of Lynchburg, Lynchburg, Virginia

Author's disclosure of conflict of interest is found at the end of this article.

Correspondence to: Gretchen D. Suk, DMSc, PA-C, 3100 Hanover Street, Palo Alto, CA 94304. E-mail: gsuk14@gmail.com

https://doi.org/10.6004/jadpro.2022.13.8.5

Abstract

Lung cancer is the leading cause of cancer-related deaths in the United States. The 5-year survival rates are poor with traditional therapy alone. New scientific advances in technology involving the human genome, including diagnostic tools to inform on tumor-derived acquired (somatic) mutations that drive cancer formation, are essential to utilize. Targeting cancer cells paired with actionable drugs to shut off growth pathways has significantly improved patient survival. Obtaining mutational analysis can be performed via traditional methods such as tissue; new advances allow comparable information obtained through liquid biopsy to inform targeted treatment decision-making. Getting tissue for additional molecular analysis can pose several challenges for patients. Liquid biopsy is a minimally invasive test (typically blood) analyzed by next-generation sequencing for tumor shed to obtain actionable information for treatment decisions. Analyses between blood and tissue consistently yield high concordance, with liquid biopsy providing faster turnaround time for results than tissue. The utility of liquid biopsy is well proven but not standardized and cannot diagnose lung cancer histopathology, which requires a tissue diagnosis.

The National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) data estimate 130,180 lung cancer deaths in the United States in 2022. The American Cancer Society estimates approximately 236,740 new lung cancer cases in 2022. Approximately 85% of these cases will be non–small cell lung cancer (NSCLC). The current 5-year survival rate of advanced NSCLC is about 22% (ASCO, 2022). Several factors that can affect the survival rate include tumor pathology type, comorbid medical conditions, and smoking history.

Historically, to diagnose cancer, a tissue biopsy sample must be obtained and confirmed by a pathologist for tumor primary and histologic type. It is also now standard to test for molecular biomarkers. Using this same tissue, molecular genomic

J Adv Pract Oncol 2022;13(8):790–795

analysis is performed separately. Recently, liquid biopsies have become commercially available to obtain similar genotyping data as the tissue-based approach for molecular analysis. Liquid biopsy is the fluid extraction (typically blood) sent for diagnostic testing to obtain genomic alterations from the tumor shed. Since the Human Genome Project was completed in 2003, molecular genetic research companies have been able to identify and interrogate targetable mutations on tumor cells that elicit molecular abnormalities resulting from cancer cell formation. Currently, in academic and commercial settings, methods for evaluating cancer cells are still not standardized. However, a personalized medicine approach to target a drug to a somatic (acquired) mutation is now the standard of care.

CLINICAL SIGNIFICANCE

Many patients with newly diagnosed or progressive lung cancers are advanced in age, have comorbid medical conditions (such as chronic obstructive pulmonary disease or emphysema), are immune compromised, or have surgical access issues secondary to COVID-19. The ability to obtain and analyze tissue specimens to accurately diagnose and treat patients with a newly diagnosed NSCLC can be complicated and time-consuming, requiring input from multiple medical subspecialties. Timely and accurate diagnosis and treatment can improve outcomes in patients with advanced cancers. The standard of care for the diagnosis of NSCLC has been through surgical methods to obtain tissue for histopathologic diagnosis. Several barriers to tissue testing include the procedure's invasiveness with related complications, insufficient DNA or tissue volume, deteriorated DNA quality (Sone, 2020), and time-consuming pathological analysis of tissue specimens (Hyun et al., 2016). Obtaining a sample through this method represents a snapshot of the tumor cells at one point in time.

A liquid biopsy analyzes cancer-derived materials from various body fluids, is minimally invasive, can pick up low levels of tumor shed, has FDA approval to inform treatment decisions in the newly diagnosed setting, and can inform the treatment of new resistance mutations upon progression without the need for additional biopsies. Liquid biopsy can now be used to provide prognostication and inform treatment decisions from genomic alterations in the bloodstream (Siravegna et al., 2017). We know that there is a clonal evolution to cancer cells over the lifetime of the disease; therefore, liquid biopsy is more practical for real-time monitoring of disease progression than tissue biopsy (Hyun et al., 2016). This minimally invasive test, typically a blood draw in an office, can be performed serially to monitor the disease process's clonal evolution, which guides treatment decisions. Despite the lack of standardization methods in academic and commercial settings, there are several accurate ways to extract the same information obtained by tissue.

DISCUSSION

Diagnosis

The three main types of non–small cell histopathology include squamous cell carcinoma, adenocarcinoma, and bronchoalveolar carcinoma, with the first two making up the majority of lung cancer types. Tissue biopsy is sent to the pathologist, who evaluates the tissue through a method called immunohistochemical staining (Yatabe et al., 2019). Immunohistochemical staining can determine the histopathologic type. The tissue is then sent for molecular diagnostic testing, called genotyping, through techniques like NGS or PCR hotspot testing.

The College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP) regulate the industry's pathology and molecular testing guidelines. The American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network (NCCN) endorse the CAP/IASLC/AMP guidelines. In 2018, the NCCN Guidelines were updated to include liquid biopsy testing, preferably by NGS methodology, where tissue testing is unavailable.

Tissue Insufficiency

There may be some downsides to tissue biopsies for diagnostic workup. Hagemann and colleagues (2015) revealed that of 209 samples obtained, only 55% could be genotyped after histopathologic diagnosis was made. An excisional biopsy had the highest yield of 95%, an endoscopic biopsy rendered 66% evaluation, and a core biopsy

only 40%. Schneider and colleagues (2015) compiled 52 core needle biopsies (CNB) and 120 fine needle aspirate (FNA) specimens. They revealed that the CNB specimens yielded a significantly higher number of samples sufficient for molecular testing than FNA specimens, 67% vs. 46%, respectively. Various tumors express intratumor heterogeneity; if different parts of the tumor show variations in genetic expressions, a single tissue biopsy may not obtain all alterations within a tumor (Burrell et al., 2013). In their database search of biopsy procedures, Heerink and colleagues (2017) found that the rate of minor complications for CNB and FNA biopsies was 38.8% and 24.0%, respectively. A disadvantage to these diagnostic yields is that an additional biopsy is warranted if a lack of tissue or non-diagnostic tissue is obtained.

Unmet Need

Given the factors that affect lung cancer patients and the challenges facing them, a one-size-fits-all diagnostic algorithm is untenable. The NCCN supports the diagnostic workup for advanced NSCLC to include testing for molecular biomarkers. The NCCN is careful to remind clinicians that biomarker testing cannot be used as a surrogate for obtaining a histopathologic diagnosis (NCCN, 2021). The NCCN Guidelines recognize plasma cell-free DNA (cfDNA) testing in patients unfit for additional tissue testing in newly diagnosed patients when insufficient material for molecular analysis is available. The NCCN also recognizes the need for testing upon progression, where the original biopsy did not derive a molecular biomarker.

Treatment

Two separate specimens are required for adequate diagnostic workup of advanced NSCLC, the first looking for the histopathologic type and the latter looking for molecular and immune biomarkers, such as *EGFR, ALK, RET, MET, BRAF, NTRK, ROS1, ERBB2, KRAS,* and PD-L1. These specific biomarkers are NCCN endorsed and have single actionable drugs or dual-drug combinations that can target the abnormal gene mutation. Currently, there are 23 oral tyrosine kinase inhibitors (TKIs) and two intravenous antibody-related therapies that have FDA approvals in either the front-line or second-line treatment setting for advanced NSCLC. Over 50% of NSCLC patients have identifiable mutations (Vu & Patel, 2019). If an actionable target is not identified, patients are candidates for standard-of-care therapy, including chemotherapy with or without an immune checkpoint inhibitor (which requires PD-L1 analysis) or anti–VEGF treatment. If a driver mutation is found, median overall survival response rates for targeted therapy range from 38 to 62 months (Ramalingam et al., 2020; Gettinger et al., 2016). Otherwise, without a mutation, the response to traditional chemotherapy for advanced disease is a median of 11.1 months with first-line treatment (Simeone et al., 2019).

The role of targeted therapy is continuing to expand outside the metastatic setting. Osimertinib (Tagrisso), initially approved for *EGFR*-mutant metastatic NSCLC, is now the first oral biomarker therapy used in the adjuvant setting based on results from the ADAURA trial. The trial results showed that osimertinib significantly increased disease-free survival in stage II to IIIA NSCLC patients treated with this drug compared with placebo (Wu et al., 2020).

The newest advanced-stage FDA approvals include therapies that target *KRAS*, *EGFR* exon 20 insertion, and *ERBB2* mutations. *KRAS* is seen in up to approximately 33% of all NSCLC cases and was recently approved by the FDA in patients with a *KRAS* G12C-specific mutation (Biernacka et al., 2016). Amivantamab-vmjw (Rybrevant) and mobocertinib (Exkivity) were approved in 2021 for patients who harbor an *EGFR* exon 20 insertion mutation and have failed prior therapy. In August 2022, the antibody-drug conjugate, fam-trastuzumab deruxtecan-nxki (Enhertu), which targets *ERBB2* mutations, was given accelerated approval based on the DESTINY-Lung02 trial. Based on the phase II clinical trial, they showed an objective response rate of 55% and a median overall survival of 17.8 months in previously treated patients (Li et al., 2022). The recent approvals highlight how quickly the biomarker landscape continues to evolve.

Lastly, microsatellite instability and tumor mutational burden inform treatment decisions for immune checkpoint inhibitor therapy; however, neither have recommendations from the NCCN, and research continues. These targeted drugs are

the newest form of treatment for lung cancer, but to have this option, testing must be performed either through tissue or liquid biopsy.

Progression

Metastatic NSCLC patients can progress despite treatment. Upon progression, liquid biopsy can help inform additional treatment options. For example, patients with an actionable *EGFR* exon 19 deletion or exon L858R mutation upon diagnosis are treated with an FDA-approved oral TKI therapy. Over time, patients can develop acquired resistance to the TKI treatment. The most well-studied mutation that can readily be assessed through liquid biopsy techniques is *EGFR* T790M. This mutation develops after treatment on first- and second-generation *EGFR* TKI therapy due to cancer-adaptive biology. Osimertinib, an irreversible third-generation TKI, has been approved by the FDA for *EGFR* T790M mutations after the progression of a first or second-generation TKI. Leonetti and colleagues (2019) found that tumor heterogeneity can have various evolution patterns when treating with different TKI therapies, causing the tumor's clonal evolution to develop further genomic alterations.

Given the persistent clonal expansion of cancer, serial liquid biopsy testing provides ease of testing due to the minimal invasiveness compared with an invasive rebiopsy. Liquid biopsy can attain results within a 1- to 2-week turnaround time, which can expedite discussions with the patient for new treatment options and therefore help minimize patient anxiety.

Technical

Liquid biopsy is a relatively new diagnostic method, with research on many solid tumor types, although data on NSCLC is the most robust. Currently, two primary techniques are used to extract DNA from plasma performed by a process called NGS, as mentioned previously. Next-generation sequencing platforms perform sequencing of millions of small DNA fragments in parallel with over three billion bases of the human genome sequenced multiple times so DNA variation can be mapped (Behjati & Tarpey, 2013). In the first technique, circulating tumor cell (CTC) tests are liquid biopsy tests that look for whole tumor cells found in the bloodstream. The second technique uses ctDNA or cfDNA tests that analyze DNA from tumor cells circulating in the bloodstream. Each technique strives to obtain diagnostic information on relevant biomarkers to drive patient management, measure the cancer's response to treatment, and monitor for relapse after treatment (Yang, 2018).

When tumor cells are shed from either the primary tumor or its metastatic sites, tumor-derived DNA enters the bloodstream, allowing modern technology to evaluate for somatic alterations through the techniques discussed previously. Recent landmark publications have identified the clinical utility of liquid assays while measuring the concordance rates comparing blood and tissue. Mack and colleagues (2020) analyzed the DNA of over 8,000 cases of advanced NSCLC. They were able to detect somatic alterations in 86% of advanced lung cancer samples by ctDNA methods, 46% of which were actionable. They were also able to increase biomarker detection by 65% of samples that could not be evaluated by tissue. Additionally, Aggarwal and colleagues (2019) determined that of 323 advanced NSCLC patients evaluated at diagnosis with metastatic disease, the concordance between ctDNA and tissue testing of alterations was 90%. Another study reported concordance findings between ctDNA and tissue to be between 92% and 100% depending on the gene identified (Odegaard et al., 2018). Leighl and colleagues (2019) showed 90% concordance between blood and tissue results, and the turnaround time to obtain results from the blood-based analysis was 1 week faster than that of tissue testing.

Similarly, analytic validation studies have been performed to discern how well NGS techniques can pick up actual alterations. Odegaard and colleagues (2018) were able to pick up mutations as small as 0.02% to 0.04% of the sample obtained using cfDNA methods. This percentage is known as the variant allele fraction (VAF), the portion of DNA molecules in the specimen that carries the variant (Strom, 2016).

CONCLUSION

New diagnostic modalities have been developed to improve treatment algorithms for advanced NSCLC. It is critical to know not only the histopathologic type of lung cancer, but also the molec-

ular biomarkers of cancer cells to drive treatment decisions, response, and survival discussions. A systematic review extracted data from 38 studies and found that liquid biopsy's clinical usefulness has several advantages over tissue biopsy. More scientific data and technology are needed to make it the standard of care (Esagian et al., 2020). For newly diagnosed and advanced NSCLC cancer patients with various needs, clinicians have expanded options to help aid in treatment planning for patients. More research is needed. For now, liquid biopsy will remain as a companion diagnostic tool in addition to tissue biopsy. While the advantages of this minimally invasive test can outweigh a biopsy's invasiveness, an initial histologic diagnosis obtained by tissue biopsy remains the mainstay.

Acknowledgment

The author would like to thank Leylah Drusbosky, PhD.

Disclosure

Dr. Suk is an employee of Guardant Health, Inc.

References

Aggarwal, C., Thompson, J. C., Black, T. A., Katz, S. I., Fan, R., Yee, S. S.,...Carpenter, E. L. (2019). Clinical implications of plasma-based genotyping with the delivery of personalized therapy in metastatic non-small cell lung cancer. JAMA Oncology, 5(2), 173–180. https://doi.org/10.1001/jamaoncol.2018.4305

American Cancer Society. (2022). Key statistics for lung cancer. https://www.cancer.org/cancer/lung-cancer/about/key-statistics.html

American Society of Clinical Oncology. (2022). Lung cancer – non-small cell: Statistics. https://www.cancer.net/cancer-types/lung-cancer-non-small-cell/statistics

Behjati, S., & Tarpey, P. S. (2013). What is next generation sequencing? *Archives of Disease in Childhood. Education and Practice Edition, 98*(6), 236–238. https://doi.org/10.1136/archdischild-2013-304340

Biernacka, A., Tsongalis, P. D., Peterson, J. D., de Abreu, F. B., Black, C. C., Gutmann, E. J.,...Tsongalis, G. J. (2016). The potential utility of re-mining results of somatic mutation testing: *KRAS* status in lung adenocarcinoma. *Cancer Genetics, 209*(5), 195–198. https://doi.org/10.1016/j.cancergen.2016.03.001

Burrell, R. A., McGranahan, N., Bartek, J., & Swanton, C. (2013). The causes and consequences of genetic heterogeneity in cancer evolution. *Nature, 501*(7467), 338–345. https://doi.org/10.1038/nature12625

Esagian, S. M., Grigoriadou, G. I, Nikas, I. P., Boikou, V., Sadow, P. M., Won, J., & Economopoulos, K. P. (2020). Comparison of liquid-based to tissue-based biopsy analysis by targeted next generation sequencing in advanced non-small cell lung cancer: A comprehensive systematic review. *Journal of Cancer Research and Clinical Oncology, 146*(8), 2051–2066. https://doi.org/10.1007/s00432-020-03267-x

Gettinger, S., Rizvi, N. A., Chow, L. Q., Borghaei, H., Brahmer, J., Ready, N.,...Hellmann, M. D. (2016). Nivolumab monotherapy for first-line treatment of advanced non-small-cell lung cancer. *Journal of Clinical Oncology, 34*(25), 2980–2987. https://doi.org/10.1200/JCO.2016.66.9929

Hagemann, I. S., Devarakonda, S., Lockwood, C. M., Spencer, D. H., Guebert, K., Bredemeyer, A. J.,...Govindan, R. (2015). Clinical next-generation sequencing in patients with non–small cell lung cancer. *Cancer, 121*(4), 631–639. https://doi.org/10.1002/cncr.29089

Heerink, W. J., de Bock, G. H., de Jonge, G. J., Groen, H. J., Vliegenthart, R., & Oudkerk, M. (2017). Complication rates of CT-guided transthoracic lung biopsy: Meta-analysis. European *Radiology, 27*(1), 138–148. https://doi.org/10.1007/s00330-016-4357-8

Hyun, K., Kim, J., Gwak, H., & Jung, H. (2016). Isolation and enrichment of circulating biomarkers for cancer screening, detection, and diagnostics. *The Analyst, 141*(2), 382–392. https://doi.org/10.1039/c5an01762a

Leighl, N. B., Page, R. D., Raymond, V. M., Daniel, D. B., Divers, S. G., Reckamp, K. L.,...Papadimitrakopoulou, V. A. (2019). Clinical utility of comprehensive cell-free DNA analysis to identify genomic biomarkers in patients with newly diagnosed metastatic non-small cell lung cancer. *Clinical Cancer Research, 25*(15), 4691–4700. https://doi.org/10.1158/1078-0432.CCR-19-0624

Leonetti, A., Sharma, S., Minari, R., Perego, P., Giovannetti, E., & Tiseo, M. (2019). Resistance mechanisms to osimertinib in EGFR-mutated non-small cell lung cancer. *British Journal of Cancer, 121*(9), 725–737. https://doi.org/10.1038/s41416-019-0573-8 https://doi.org/

Li, B. T., Smit, E. F., Goto, Y., Nakagawa, K., Udagawa, H., Mazières, J.,...Planchard, D. (2022). Trastuzumab deruxtecan in HER2-mutant non–small-cell lung cancer. *New England Journal of Medicine, 386*(3), 241–251. https://doi.org/10.1056/nejmoa2112431

Mack, P. C., Banks, K. C., Espenschied, C. R., Burich, R. A., Zill, O. A., Lee, C. E.,...Gandara, D. R. (2020). Spectrum of driver mutations and clinical impact of circulating tumor DNA analysis in non-small cell lung cancer: Analysis of over 8000 cases. *Cancer, 126*(14), 3219–3228. https://doi.org/10.1002/cncr.32876

National Cancer Institute. (2022). Surveillance, Epidemiology and End Results Program. Cancer Stat Facts: Lung and Bronchus Cancer. https://seer.cancer.gov/statfacts/html/lungb.html

National Comprehensive Cancer Network. (2021). NCCN Clinical Practice Guidelines in Oncology: Non–Small Cell Lung Cancer. V1.2021. https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf

Odegaard, J. I., Vincent, J. J., Mortimer, S., Vowles, J. V., Ulrich, B. C., Banks, K. C.,...Talasaz, A. (2018). Validation of a plasma-based comprehensive cancer genotyping assay utilizing orthogonal tissue- and plasma-based methodologies. *Clinical Cancer Research, 24*(15), 3539–3549. https://doi.org/10.1158/1078-0432.CCR-17-3831

Ramalingam, S. S., Vansteenkiste, J., Planchard, D., Cho, B. C., Gray, J. E., Ohe, Y.,...FLAURA Investigators. (2020). Overall survival with osimertinib in untreated, EGFR-mutated advanced NSCLC. *New England Journal of*

Medicine, 382(1), 41–50. https://doi.org/10.1056/NEJMoa1913662

Schneider, F., Smith, M. A., Lane, M. C., Pantanowitz, L., Dacic, S., & Ohori, N. P. (2015). Adequacy of core needle biopsy specimens and fine-needle aspirates for molecular testing of lung adenocarcinomas. *American Journal of Clinical Pathology, 143*(2), 193–200. https://doi.org/10.1309/AJCPMY8UI7WSFSYY

Simeone, J. C., Nordstrom, B. L., Patel, K., & Klein, A. B. (2019). Treatment patterns and overall survival in metastatic non-small-cell lung cancer in a real-world, US setting. *Future Oncology, 15*(30), 3491–3502. https://doi.org/10.2217/fon-2019-0348

Siravegna, G., Marsoni, S., Siena, S., & Bardelli, A. (2017). Integrating liquid biopsies into the management of cancer. *Nature Reviews Clinical Oncology*, *14*(9), 531–548. https://doi.org/10.1038/nrclinonc.2017.14

Sone, M., Arai, Y., Sugawara, S., Kubo, T., Itou, C., Hasegawa, T.,...Kubo, T. (2019). Feasibility of genomic profiling with next-generation sequencing using specimens obtained by image-guided percutaneous needle biopsy. *Upsala Journal of Medical Sciences, 124*(2), 119–124. https://doi.org/10.1080/03009734.2019.1607635

Strom, S. P. (2016). Current practices and guidelines for clinical next-generation sequencing oncology testing. *Cancer Biology & Medicine, 13*(1), 3–11. https://doi.org/10.28092/j.issn.2095-3941.2016.0004

Vu, P., & Patel, S. (2019). Non-small cell lung cancer targetable mutations: Present and future. *Precision Cancer Medicine, 3*(March 2020). https://pcm.amegroups.com/article/view/5233

Wu, Y., Tsuboi, M., He, J., John, T., Grohe, C., Majem, M.,... Herbst, R. S. (2020). Osimertinib in resected EGFR-mutated non–Small-cell lung cancer. *New England Journal of Medicine, 383*(18), 1711–1723. https://doi.org/10.1056/NEJMoa2027071

Yang, M., Forbes, M. E., Bitting, R. L., O'Neill, S. S., Chou, P., Topaloglu, U.,...Zhang, W. (2018). Incorporating blood-based liquid biopsy information into cancer staging: Time for a TNMB system? *Annals of Oncology, 29*(2), 311–323. https://doi.org/10.1093/annonc/mdx766

Yatabe, Y., Dacic, S., Borczuk, A. C., Warth, A., Russell, P. A., Lantuejoul, S.,...Chen, G. (2019). Best practices recommendations for diagnostic immunohistochemistry in lung cancer. *Journal of Thoracic Oncology*, *14*(3), 377–407. https://doi.org/10.1016/j.jtho.2018.12.005