Review Article

Improving diagnostic capabilities in lung cancer through next-generation sequencing: a narrative review

Andrew Figueroa[1], Shadi Safar Gholi[2]^, Girish Jayant[3]^, Raj Wadgaonkar[4]^, Ahmed Gubran[2], Stephen J. Kuperberg[2,5]^

[1]Department of Pulmonary Medicine, Doylestown Hospital, Ambler, PA, USA; [2]Department of Internal Medicine, New York City Health and Hospitals, New York, NY, USA; [3]Department of Internal Medicine, Montefiore Medicine Center, The Bronx, NY, USA; [4]College of Medicine, SUNY Downstate Medical Center, Brooklyn, NY, USA; [5]New York University Grossman School of Medicine, New York, NY, USA

Contributions: (I) Conception and design: A Figueroa, SJ Kuperberg; (II) Administrative support: None; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: None; (V) Data analysis and interpretation: None; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Stephen J. Kuperberg, MD, MPH. Department of Internal Medicine, New York City Health and Hospitals, Woodhull, New York, NY, USA; New York University Grossman School of Medicine, 760 Broadway 8th Floor, Brooklyn, New York, NY 11206, USA. Email: stephenkuperbergmd@gmail.com.

Background and Objective: Lung neoplasia is the leading cause of cancer death worldwide, thus, early detection and accuracy in establishing a diagnosis is paramount. As a consequence of decades of basic and translational studies revealing the genetic basis of lung cancer, a paradigm shift has occurred toward a personalized approach to medicine whereby mutational analysis confers an opportunity for safer, and expedient treatment options. In this context, next-generation sequencing (NGS) has emerged as a vital technological advance, and has become increasingly established as a core method for rapidly and effectively identifying actionable mutations in lung cancer. For these reasons, an updated review of the literature across invasive and non-invasive diagnostic modalities in lung cancer is warranted to inform diagnostic approaches and prompt new investigations. The objective of the present review is to provide a focused update on applications of NGS in lung cancer diagnostics, with a special focus on tissue acquisition methodologies and mutational analysis.

Methods: The search strategy included a survey of the current literature from 2005 to 2024 in PubMed, Medline, Scopus, and Google Scholar. Eligible study types included original research, literature reviews (narrative and systematic), and observational studies. which encompassed findings pertinent to the lung cancer diagnostics, mutational analysis and lung cancer treatment overlapping with applications and use of NGS technologies.

Key Content and Findings: There are extensive and diverse advantages to the use of NGS in lung cancer diagnostics, especially when compared to traditional sequencing techniques including, speed, effectiveness, easy adoption in the context of analysis of samples prepared for lung cancer diagnosis. Advances in cell-free DNA reinforce the firm role of NGS in novel approaches.

Conclusions: NGS implementation is a crucial and beneficial technological leap in lung cancer diagnosis, especially given the environment of novel and established targeted and immune based therapies which require mutational testing. Its numerous benefits such as expedient results and reduced sample requirements will continue to ability optimize lung cancer outcomes by virtue of improved patient safety, reduction of unnecessary procedures, and provision of accurate results.

Keywords: Lung cancer; bronchoscopy; lung tissue acquisition; image guided needle aspiration; genomic profiling

Submitted Apr 20, 2024. Accepted for publication Nov 29, 2024. Published online Jan 22, 2025.
doi: 10.21037/jtd-24-488
View this article at: https://dx.doi.org/10.21037/jtd-24-488

^ ORCID: Shadi Safar Gholi, 0009-0005-4003-7101; Girish Jayant, 0000-0001-6037-7852; Raj Wadgaonkar, 0009-0008-5121-6414; Stephen J. Kuperberg, 0000-0002-6819-4425.

Introduction

As the most common cause of cancer-related death worldwide, lung neoplasia represents an important target of basic and translational and clinical effort in disease understanding (1). Over the past two decades, new and exciting methods for understanding its genetic basis have been developed (2), however the foundation of current understanding is the historic sequencing of the human genome by the National Institutes of Health (NIH) in 2001 in the Human Genome Project (3).

The initial repository, followed by subsequent landmark projects—such as the Human Genome Atlas consortium (4) have together enabled the scientific community to sustain unimaginable achievements in genetics, exome analysis, and propelled it towards important goals in precision and personalized medicine and cancer diagnosis in particular (5).

The Cancer Moonshot Initiative was launched in 2016 by the National Cancer Institute in an effort to reduce cancer mortality (6). Through a diverse set of federally funded programs, the initiative fosters data-driven efforts across multiple fields, in order to bolster novel mortality-reducing cancer treatments (7). This enables the investigation of molecular targets such as driver mutations, ultimately improving discovery of targeted therapies and immune-checkpoint inhibitors. In turn, these approaches have become essential in cancer treatment due to their effectiveness, favorable side effect profiles, and ease of administration (8,9). The rise of immuno-diagnostics and immunotherapies has significantly increased the demand for rapid gene sequencing from tissue samples, including wild-type sequences and their variations. This process necessitates effective technology capable of quickly sequencing mutations and allowing clinicians to assess whether they will respond to available immunotherapy, particularly in the evaluation of lung cancer. NGS can and does fill this role in modern clinical practice. This review will explore the role of NGS in enhancing lung cancer diagnosis and its integration into clinical practice. Until the early 2000s, first generation or "Sanger" sequencing was the only method for determining nucleotide sequence in DNA. This process utilized a chain termination method for sequencing single or low-throughput DNA (10). The turnaround time and cost-efficiency of traditional sequencing methods were hampered at least in part, by the use of gel electrophoresis, which is slower and has limited read length, and involves a labor-intensive process of meticulous handling. In contrast, next-generation sequencing (NGS) offers a rapid and efficient alternative, significantly enhancing diagnostic capabilities in oncology, with a particularly strong application in lung cancer (11). Indeed, sample size and quality can pose challenges for establishing a cancer diagnosis with polymerase chain reaction (PCR) testing, especially when samples are insufficient or degraded. In contrast, with even smaller sample sizes, NGS can systematically examine the entire genome for actionable mutation targets, enabling clinicians to offer earlier therapeutic interventions with lower rate of false negative results (12). This in turn allows for greater options for tissue acquisition; traditional surgical methods can now be supplemented or even substituted by transthoracic needle aspiration (TTNA) or endobronchial methods such as transbronchial needle aspiration (TBNA). Once tissue samples are acquired, NGS enables the sequencing of vast numbers of genetic variants in a single, parallel diagnostic test to identify important driver mutations (3). The National Comprehensive Cancer Network (NCCN) has issued that guidelines recommend screening for mutations in these major biomarkers: anaplastic lymphoma kinase (*ALK*), epidermal growth factor receptor (*EGFR*), receptor tyrosine kinase (*ROS1*), Kirsten rat sarcoma oncogene (*KRAS*), serine/threonine protein kinase (*BRAF*), rearranged during transfection (*RET*), neurotrophic tyrosine receptor kinase (*NTRK*) 1/2/3, mesenchymal-epithelial transition (*MET*) exon 14 skipping (METex14), and v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2 (ERBB2) (13-22). Of note, NGS panels vary in ways they detect mutations, and in copy number variations, thus clinicians must ensure that whichever panel they use covers these biomarkers (23). Analysis of existing NGS screening data shows wide variation in the types of mutations seen amongst different patient cohorts (24-31) (*Table 1*). We present this article in accordance with the Narrative Review reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-488/rc).

Methods

The research design for this study is a narrative literature review. The strategy used for this review was defined to encompass a broad spectrum of scientific literature pertinent to NGS in the context of lung cancer diagnosis (*Figure 1*).

Inclusion criteria

Eligible studies included literature reviews, observational

Table 1 Comparison of prevalence of common mutations and mutation variants of NSCLC found in the literature

Study	Country/ region	Age range (years old)	*KRAS* mutations	*EGFR* mutations	*MET* mutations	*BRAF* mutations	*ALK* mutations	*RET* mutations	*ROS1* mutations	*HER2* mutations
Passaro *et al.* (24)	Europe	Unspecified	43.7% total. G12C: 36.3%. Other: 63.7%	20% total. Ex19Del/ L858R: 80%. Other: 20%	9.4% total. Ex14 skip: 20.7%. Amp: 79.3%	3.2% total. V600: 30%. Other: 70%	13.3% total	5.2% total	3.9% total	1.3% total
Tanaka *et al.* (25)	Japan	≤40	2% total	30% total. Ex19del: 75%. L858R: 17%. Ex20ins: 8%	n/a	n/a	41% total	n/a	n/a	n/a
		>40	10% total	45% total. Ex19del: 43%. L858R: 48%. Ex20Ins: 1%. Others: 8%	n/a	n/a	n/a	n/a	n/a	n/a
Hou *et al.* (26)	China	≤45. Median: 36	3.4%	56.3%	n/a	3.4%	16.1%	1.1%	n/a	n/a
		>45	18.9%	52.2%	2.2%	1.1%	1.1%	1.1%	3.3%	2.2%
Kuang *et al.* (27)	Canada	19–97. Median: 68.6	32.2%. G12C: 35.8%. Non G12C: 66.8%. 2.6% >2 mutations	24.2%. Ex19Del: 43.6%. L858R: 40.6%. Ex20ins: 5.3%. T790M: 4.7%. Other: 16.6%. 29.1% >2 mutations	0.3%	2.1%. V600: 62%. Other: 38%	n/a	0.1%	n/a	n/a
Zheng *et al.* (28)	Puerto Rico	35–95. Median: 70	18.7%	24.0%. Ex19del: 55.1%. L858R: 31%. Ex20ins: 1.6%. T790M: 0.5%	10.2%	4.3%	3.9%	2.1%	2.2%	n/a
Cho *et al.* (8)	Taiwan, Korea, Japan	28–94. Median: 64	7.9%	48.6%	1.5%. Ex14 skip: 47%. Amp: 53%	1.3%	2.5%	1.5%	0.5%	n/a
Hondelink *et al.* (29)	The Netherlands	Unspecified	n/a	11%. Ex19Del: 42%. L858R: 30%. Ex20Ins: 15.5%. Other: 12%	n/a	n/a	n/a	n/a	n/a	n/a
Judd *et al.* (30)	USA	20–97. Median: 68	27.5%. G12C: 40%. Others: 60%	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Arbour *et al.* (31)	USA	27–93. Median: 69	30%. G12C: 46%. Others: 60%	n/a	n/a	n/a	n/a	n/a	n/a	n/a

NSCLC, non-small cell lung cancer; *KRAS*, Kirsten rat sarcoma oncogene; *EGFR*, epidermal growth factor receptor; *MET*, mesenchymal-epithelial transition; *BRAF*, rapidly accelerated fibrosarcoma, serine-threonine kinase; *ALK*, anaplastic lymphoma kinase; *RET*, rearranged during transfection; *ROS1*, receptor tyrosine kinase; *HER2*, human epidermal growth factor receptor 2; n/a, not applicable.

studies, original research, and meta-analyses found in peer-reviewed English-language journals from 2005 to 2024. The search for pertinent publications was conducted across databases PubMed, Medline, Scopus, and Google Scholar (*Table 2*).

Included studies were restricted to those focusing on NGS technologies in the context of lung cancer diagnosis, for either diagnostic or therapeutic purposes. Included

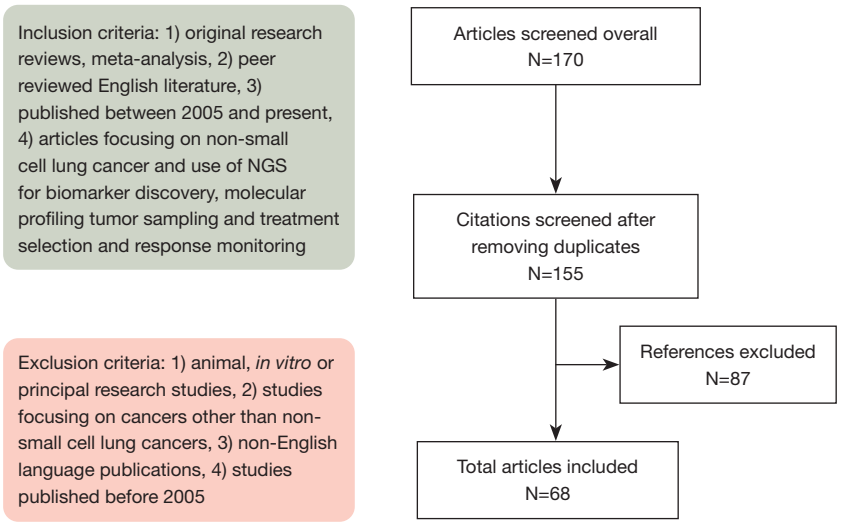

Figure 1 Visual summary of article search, selection, and inclusion. NGS, next-generation sequencing.

Table 2 The search strategy summary

Items	Specification
Date of search	May 5th, 2022 through October 3rd, 2024
Databases and other sources searched	PubMed, Medline, Scopus, and Google Scholar
Search terms used	Next-Generation Sequencing, Non-Small Cell Lung Cancer, NGS in Lung cancer
Timeframe	2005–2024
Inclusion and exclusion criteria	Inclusion criteria: (I) original research reviews, meta-analysis; (II) peer reviewed English literature; (III) published between 2005 and present; (IV) articles focusing on non-small cell lung cancer and use of NGS for biomarker discovery, molecular profiling tumor sampling and treatment selection and response monitoring. Exclusion criteria: (I) animal, *in vitro* or principal research studies; (II) studies focusing on cancers other than non-small cell lung cancers; (III) non-English language publications; (IV) studies published before 2005
Selection process	The selection process was conducted by the S.S.G., G.J., and A.G., who independently reviewed potential studies to ensure alignment with our objectives and focused on relevance to the research question. To ensure validity and consensus, selected studies were reviewed by S.J.K. and R.W.

NGS, next-generation sequencing.

studies were also required to report outcomes related to NGS-based biomarker discovery, molecular profiling, treatment selection, or monitoring treatment response. This criterion ensured that the selected literature contributes directly to understanding the clinical implications of NGS in lung cancer management.

Furthermore, we maintained a requirement that studies be published since the advent of NGS in order to maintain relevance to current clinical practice. This timeframe ensured that findings and methodologies discussed in the selected studies align with the latest advancements and standards in NGS technology application within the fields of pulmonary medicine and oncology.

Exclusion criteria

This review excluded animal studies, *in vitro* investigations, and preclinical research that lack direct relevance to human lung cancer. Additionally, studies exclusively focusing on other cancer types without pertinence to lung

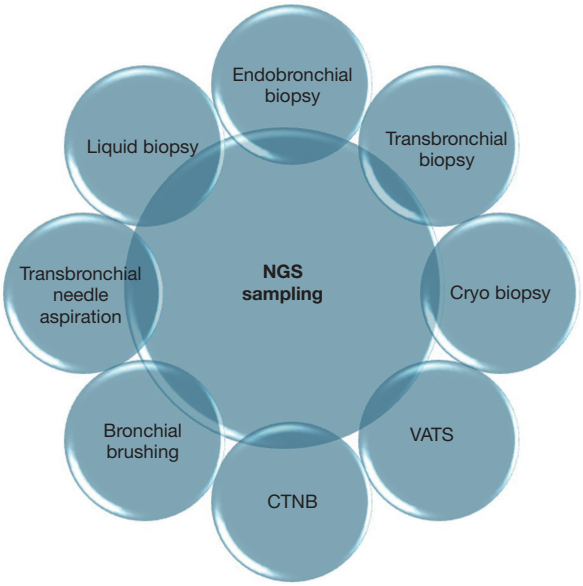

Figure 2 Visual summary of different tissue acquisition methods used for tumor sampling in conjunction with NGS in NSCLC patients. NGS, next-generation sequencing; CTNB, computed tomography-guided needle biopsy; VATS, video-assisted thoracoscopic surgery; NSCLC, non-small cell lung cancer.

cancer are omitted. Conference abstracts, commentaries, and editorials lacking original data are also disregarded. Furthermore, studies lacking comprehensive details on NGS methodologies, patient cohorts, or relevant outcomes are excluded. Publications not in the English language were omitted due to language barriers.

Selection process

Initial screening based on titles and abstracts to identify potentially relevant studies. A full-text assessment of selected studies was performed to determine eligibility based on inclusion and exclusion criteria. Studies that met eligibility criteria were included (*Figure 1*).

Minimally invasive tissue acquisition

Overall, the ability of NGS to successfully detect mutations in surgical specimens is higher compared to other methods (*Figure 2*). This was evidenced in a study by Furuya *et al.*, where tissue biopsy from surgery was compared to cytology brushing in bronchoscopic samples. The success rate of biopsy for DNA sequencing via NGS was 100% in small cell lung cancer histology (32). One key approach for nodal tissue is mediastinoscopy, an operative technique which enables surgical and visual access to the superior mediastinum. Prior to modern endosonographic methods, it represented the primary "gold standard" approach to mediastinal lymph node stations with sampling and lymph node dissection, at the expense of increased morbidity (33). Parasternal mediastinotomy (Chamberlain procedure) also affords surgical entry to the mediastinum via incision in the parasternal second left intercostal space which provides assessment of the above-mentioned lymph nodes, while extended cervical mediastinoscopy is an alternative method to assess the aorto-pulmonary window (APW) (33). Video-assisted thoracoscopic surgery (VATS) is a commonly used minimally invasive procedure for mediastinal lymph node biopsy or tumor sampling. In this method an incision is created in the intercostal space parallel to long access of intercostal space (34). In a unique example, a patient with three synchronous primary adenocarcinomas underwent biopsy with NGS. The tissue samples from wedge resections and VATS each demonstrated driver mutations, thereby ruling out metastasis (35).

Needle techniques

Tissue obtained for NGS testing can be acquired either surgically or with needle-based approaches. The success

rate of NGS analysis in needle-based tissue acquisition depends on various factors such as the techniques employed, needle size, and subsequent procurement of tumor cell containing specimen. A key example of a needle-based technique is TTNA, which is an image guided percutaneous sampling method which can be performed as ultrasound guided, or computed tomography (CT) guided. While TTNA methods are considered minimally invasive insofar as they avoid surgery, they carry a variety of risks such as pneumothorax, hemorrhage, and air embolism (36,37). Recently, bronchoscopic sampling methods have emerged as safe, effective and evidence-based means of acquiring tissue for lung cancer. Current society American College of Chest Physicians (ACCP) guidelines denote the non-inferiority of EBUS with TBNA (EBUS-TBNA) to mediastinoscopy (13).

This tissue acquisition methodology has been well studied in the context of non-small cell lung cancer (NSCLC), with current guidelines supportive of EBUS-TBNA (23). Wang (conventional) and EBUS-TBNA are needle aspiration procedures and can be performed under moderate or deep sedation, often on an outpatient basis, and with minimal morbidity. TBNA methods can sample primary lesions, as well as allow mediastinal staging via evaluation of the paratracheal and hilar lymph node stations. With a high sensitivity and negative predictive value, bronchoscopic mediastinal evaluation via TBNA has become the standard of care for tissue acquisition in lung cancer evaluation. In endoscopic ultrasound with needle aspiration (EUS-NA), cytologic samples are collected from the mediastinum through the esophagus. This method is minimally invasive with rare complications and is practical for nodes that are less than 10 mm in size. EUS-NA can also be used in metastasis extended to celiac lymph and liver (38).

Currently EBUS sampling is preferred over more invasive surgical approaches in the evaluation of mediastinal metastasis (39). In a study conducted by Wallace *et al.*, it was demonstrated that combined utilization of EUS-NA with EBUS-NA can provide a sensitivity of 93% (38). More recently, bronchoscopic cryobiopsy can provide a large high quality sample for both endobronchial and peripheral tumors. Transbronchial lung cryobiopsy (TBLC) for peripheral lesions can be performed utilizing flexible or rigid bronchoscopy, and may be aided by robotic, electromagnetic navigation, and endobronchial ultrasound (40). The use of cryobiopsy in lung disease was initially applied in benign pulmonary pathology such as interstitial lung disease as a means for obtaining larger specimens, with special consideration for the diagnosis of usual interstitial pneumonia (UIP). Here, a transbronchial 1.9 mm cryoprobe is used to enable bronchoscopic biopsy to achieve larger sample sizes with fewer passes. A prospective clinical trial, the COLDICE study, 65 patients undergoing both surgical lung biopsy and cryobiopsy demonstrated that the pathological samples were non inferior to VATS in terms of size and quality (41).

Poletti *et al.* found that EBUS guided cryobiopsy for diagnosis of lung cancer was noninferior in tissue yield as compared to traditional biopsy (42). In this study, 48 patients underwent both EBUS-TBNA using 22 G needle and endobronchial ultrasound-transbronchial mediastinal cryobiopsy (EBUS-TMC) using a 1.1 mm flexible cryoprobe on the same day. The diagnostic success rate for rare mediastinal tumors such as Hodgkin lymphoma, sarcoidosis, and Hamartoma was significantly higher with EBUS-TMC compared to EBUS-TBNA (57% to 100% respectively) (42). There were no reported postoperative major complications, including pneumothorax, pneumomediastinum, or massive bleeding, in either cohort (42). With regard to peripheral pulmonary lesions, Arimura *et al.* (43) showed the feasibility of using EBUS with guide-sheath, finding that the acquired samples consisted of specimens with sufficient size and quality for NGS testing, and were pathologically concordant with gold standard biopsies. Finally, the analysis of circulating tumor DNA (ctDNA) continues to emerge, complemented by other techniques such as the examination of circulating tumor cells (CTCs), microRNAs (miRNAs), and exocellular vesicles using plasma samples. Circulating DNA can be isolated in varying levels from tissues, peripheral blood, pleural fluid, or sputum. Circulating cell-free DNA (cfDNA) isolated from specific tissues, like peripheral blood, sputum, and pleural fluid, contains variable levels of tumor-derived DNA that is known as ctDNA. Revealing the genetic material in target tissues can be difficult, owing to the small percentage of ctDNA in total cfDNA, making techniques that are highly sensitive like NGS of paramount importance. Identifying driver genomic alterations is critical especially in advanced NSCLC and can positively impact survival rates through matched targeted therapies. Although tumor tissue biopsy is the gold standard for biomarker testing, NGS-based cfDNA analysis offers an alternative, less invasive methodology of obtaining tumor tissue that is easily repeatable with short turnaround time and offers early detection of resistance to targeted therapy (44). While analysis of liquid samples has been documented for assessing treatment response, further investigation is

necessary to evaluate its ability to independently diagnose malignancy (*Table 3*).

Sample size and yield according to method

The NCCN recommended use of EBUS-TBNA, a widely available modality, enabling a convenient and accessible means of tissue acquisition for NGS. A study comparing endobronchial, transbronchial, endobronchial ultrasound with guide-sheath (EBUS-GS) and EBUS-TBNA showed that a tissue surface area of at least 1 mm^2 was adequate for NGS testing (15). A study of cytology specimens from needle aspiration of adrenal glands in patients with stage IV metastatic lung cancer was able to show over 95% successful sequencing by NGS when samples contain approximately 5,000 viable cells with over 5 ng/µL DNA concentration (16). In another study, employing a more stringent criterion of 10 ng/µL, approximately 51% of samples acquired through fine needle aspiration (FNA) met the threshold for adequacy. Notably, despite this criterion, a 100% concordance was observed across all samples when compared with conventional testing methods and underscores the potential for accommodating extraction methodologies tailored specifically for NGS purposes (17). Another study was able to show over 99% concordance of DNA utilizing NGS from FNA specimens (18). Investigations into the feasibility of procedures for use in NGS have been conducted with improved results. In 2020, a retrospective study by Kage *et al.* compared CT-guided needle biopsy (CTNB), transbronchial biopsy (TBB) and EBUS-TBNA against surgical resection showing feasibility of small biopsies in NGS (19). DNA NGS analysis was successful in 80% of CTNB samples, 82% of TBB samples, 100% in EBUS-TBNA and 93% in surgical resection specimens. For analysis of RNA, successful analysis was achieved in 100% of CTNB samples, 67% of TBB samples, 64% in EBUS-TBNA and 92% in resection (19). In fact, a meta-analysis published in 2022 of over 1,100 patients was supportive of EBUS-TBNA, with a pooled yield of 86.5% [95% confidence interval (CI): 80.9% to 91.4%] with increasing yield up to 94.9% at a mean of 6 passes (20). Meanwhile, preliminary results of a 2022 prospective study in patients with resectable NSCLC showed biopsies from core needle biopsies when compared to surgical resection were adequate enough to run NGS with an 83.9% concordance (21). Recently, in a study which examined the use of robotic-assisted bronchoscopy (RAB) for sample acquisition, Yu Lee-Mateus *et al.* showed that bronchoscopic samples acquired by robotic bronchoscopy met pre-specified criteria for NGS testing in the diagnosis of early-stage lung cancer (52) confirming the feasibility of use of RAB to acquire satisfactory biopsy material for NGS.

In addition to identifying actionable target mutations and population level analysis, the real potential strength of NGS could be its ability to detect novel mechanisms of resistance to mainline immunologic therapy such as EGFR tyrosine kinase inhibitors (EGFR TKIs), KRAS inhibitors, and ALK inhibitors (53,54). For example, EGFR TKIs were arguably one of the key success stories of targeted therapy in NSCLC. Numerous studies have shown that these agents, especially third generation TKIs like osimertinib, significantly increase progression-free survival (PFS) compared to platinum chemotherapy agents (55-57). However, even patients on osimertinib often acquire resistance and NGS can help clinicians understand this resistance better.

Exon 19 deletions are some of the most common *EGFR* mutations and it is generally thought that such mutations respond well to osimertinib. Clinical guidelines treat them as one group, but multiple analyses have shown that various exon19 deletions are associated with different levels of treatment resistance and different survival rates (58,59). Thus, NGS can help clinicians predict patient responsiveness early in the treatment course. Repeat NGS screening after treatment initiation can also detect the such as heterogeneous ways in which treatment resistance arises. *KRAS* mutations including KRASG12C are common in western populations with NSCLC (*Table 1*) and can be treated with small molecule inhibitors such as adagrasib. However, adragasib also encounters resistance. In a recent study of patients with acquired resistance to adagrasib, it was demonstrated that the majority of patients developed multiple forms of resistance, with at least one that was not related the *KRAS* gene (60).

Conclusions

NGS technology has clearly superseded more primitive methods of sequencing genomic data because of its speed, accuracy, efficiency and cost. Evidenced by the literature described, it is clear that NGS is irreplaceable for the analysis of driver mutations in lung cancer, and has transformed molecular approach to its diagnosis. NGS sequencing of mutations can be readily applied through a variety of approaches, including RAB, bronchoscopic

Table 3 Comparison of methods of tissue acquisition for NGS success in different studies

Study	Year of publication	Country	Type of study	Number of patients	Method of tissue acquisition	% of adequate sample for testing	Median % of tumor cells	NGS platform	Main findings
Ben *et al.* (21)	2022	China	Prospective pilot	17	CNB using 18-gauge Max-Core needle	100%	60%	Nextseq500 sequencer by Illumina	CNB samples obtained intraoperatively from NSCLC were reliable for NGS profiling
Tone *et al.* (45)	2021	Japan	Retrospective cohort	37	TBLC, EBUS-TBNA	66.7%, 10.6%	80%, 12.2%	Oncomine Dx Target Test CDx System	TBLC shows its superiority over EBUS-TBNA for NGS analysis
Sakata *et al.* (46)	2022	Japan	Multi center retrospective	533	Surgical biopsy bronchoscopic CT guided, US guided pleural effusion sample	80%, 76.8%, 76.6% other methods[¶]	30%[◇]	Oncomine Dx Target Test Multi-CDx System	No significant difference was found based on sample collection method
Ariyasu *et al.* (47)	2021	Japan	Retrospective	167	Surgical, TBB, EBUS-TBNA, CTNB	100%, 76.8–90.6%, 78.6%, 84.6–100%	At least 20%	Oncomine Dx	Feasibility of Oncomine Dx improves by obtaining larger tissue samples
Takeyasu *et al.* (48)	2021	Japan	Retrospective	235 in 2 cohorts[†]	CNB, EBUS-TBNA, TBB	Cohort 1: 78.6%, 71.8%, 80.4%. Cohort 2: 0%, 8.8%, 5.1%	At least 20%	Oncomine Dx Target Test CDx System	Sample size of 4 mm^2 or higher with tumor cell content of 20% or higher (regardless of method of acquisition) has a higher rate of success NGS testing
Nemoto *et al.* (49)	2021	Japan	Retrospective observational	119	EBUS-TBNA, TBB, EBUS-GS	13.2%, 12.2%, 69.38%	At least 40%	Oncomine Dx Target Test	This study shows sample size >1.04 mm^2 and tumor cell count >375 (or >40%) has direct association with successful analysis
Uchimura *et al.* (50)	2021	Japan	Retrospective	156	EBUS-TBNA using 22 or 25 G needle	90.3%	At least 30%	Oncomine Dx Target Test	The cut-off value for the number of core tissue required for successful NGS is 4
Aboudara *et al.* (51)	2023	United State	Retrospective	66	EBUS-TBNA 22 G Vizishot needle (FNA) and the 22 G Acquire Franseen needle (fine core biopsy-FNB)	47% in FNA, 76% in FNB	More than 20%	–	FNB needle has higher diagnostic yield in benign lymphadenopathy and greater tumor cell number for NGS testing, compared to FNA
Murakami *et al.* (15)	2021	Japan	Retrospective	184	EBB, EBUS-GS: small; large, EBUS-TBNA	62.5%, EBUS-GS: small: 57.1%; large: 93.4%, 100%	At least 30%	Oncomine Dx Target Test Multi-CDx System	A tumor content of ≥30% and a tissue surface area of ≥1 mm^2 were identified as significant factors for successful NGS analysis
Furuya *et al.* (32)	2021	Japan	Prospective	222	Surgical, thoracoscopy, CNB, EBUS-GS (bronchial brushing), EBUS-TBNA (needle washing), pleural effusion	DNA: 100%, 100%, 100%, 97.9%, 100%, 100%. RNA: 100%, 100%, 81.1%, 84.4%, 80.4%, 100%	Not provided	Oncomine Comprehensive Assay	Success rate of NGS for DNA sequencing was 99.1% regardless of method of biopsy and for RNA sequencing was lower 85.1%. However, regarding RNA sequencing the success rate of cytology sample from bronchoscopy was relatively lower compared with tissue sample

[†], Cohort 1 samples were examined for *BRAF* mutations, Cohort 2 samples were examined for mutations in 46 genes; [¶], no data was available for CT guided biopsy; [◇], macro-dissection was performed in 57.4% of cases where the tumor cell content was less than or equal to 30%. These details suggest that the median percentage of tumor cells may be around 30% or lower, but a specific median value was not provided in the study. NGS, next-generation sequencing; CNB, core needle biopsy; NSCLC, non-small cell lung cancer; TBLC, transbronchial lung cryobiopsy; EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; CT, computed tomography; US, ultrasound; TBB, transbronchial biopsy; CTNB, computed tomography-guided needle biopsy; EBUS-GS, endobronchial ultrasonography with guided sheath; FNA, fine needle aspiration; FNB, fine needle biopsy; EBB, endobronchial biopsy.

cryo-biopsy, cryo-EBUS as well as NGS applied to VATS. Nonetheless, we should not rest on the laurels of these exciting advances. Additional basic, translational and clinical studies are still needed to validate and substantiate the findings and apply them through novel investigations using NGS approaches across sampling methods and the role of cfDNA in serum should be further explored. There is little remaining doubt that NGS is now an indispensable tool in the armamentarium of lung cancer diagnostic pathways, and it plays an increasingly important role in the investigation of immunological and targeted approaches which rely on tumor specific modifications in both molecular structure and sequence.

Acknowledgments

We are grateful for the technical and clinical expertise provided in support of this manuscript by Dr. Joe Zein, Professor of Medicine, Division of Pulmonary and Sleep Medicine, Mayo Clinic, FL, USA.

Footnote

Reporting Checklist: The authors have completed the Narrative Review reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-488/rc

Peer Review File: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-488/prf

Funding: None.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-488/coif). S.J.K. reports the travel payment to Auris Surgical Robotics 2021 (currently J&J Medtech) and patents of method and apparatus for sequential deployment of intra-tumoral agents (US US10869996B2, 12/2020), which is not currently marketed or licensed, but could be perceived as related to the use of NGS. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Cancer Stat Facts: Lung and Bronchus Cancer. National Cancer Institute, Surveillance, Epidemiology, and End Result Program. Available online: https://seer.cancer.gov/statfacts/html/lungb.html
2. Dietel M. Molecular Pathology: A Requirement for Precision Medicine in Cancer. Oncol Res Treat 2016;39:804-10.
3. Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. Nature 2001;409:860-921.
4. Chang JT, Lee YM, Huang RS. The impact of the Cancer Genome Atlas on lung cancer. Transl Res 2015;166:568-85.
5. Gibbs RA. The Human Genome Project changed everything. Nat Rev Genet 2020;21:575-6.
6. Singer DS. A new phase of the Cancer Moonshot to end cancer as we know it. Nat Med 2022;28:1345-7.
7. About the Cancer MoonshotSM. Available online: https://www.cancer.gov/research/key-initiatives/moonshot-cancer-initiative/about
8. Cho JH. Immunotherapy for Non-small-cell Lung Cancer: Current Status and Future Obstacles. Immune Netw 2017;17:378-91.
9. Lievense LA, Sterman DH, Cornelissen R, et al. Checkpoint Blockade in Lung Cancer and Mesothelioma. Am J Respir Crit Care Med 2017;196:274-82.
10. Kchouk M, Gibrat JF, Elloumi M. Generations of Sequencing Technologies: From First to Next Generation. Biol Med (Aligarh) 2017;9:1000395.
11. Sabour L, Sabour M, Ghorbian S. Clinical Applications of Next-Generation Sequencing in Cancer Diagnosis. Pathol Oncol Res 2017;23:225-34.
12. Lim SM, Kim EY, Kim HR, et al. Genomic profiling of lung adenocarcinoma patients reveals therapeutic targets and confers clinical benefit when standard molecular testing is negative. Oncotarget 2016;7:24172-8.
13. Rivera MP, Mehta AC, Wahidi MM. Establishing the

diagnosis of lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2013;143:e142S-65S.
14. Kemper M, Krekeler C, Menck K, et al. Liquid Biopsies in Lung Cancer. Cancers (Basel) 2023;15:1430.
15. Murakami S, Yokose T, Nemoto D, et al. Suitability of Bronchoscopic Biopsy Tissue Samples for Next-Generation Sequencing. Diagnostics (Basel) 2021;11:391.
16. Gleeson FC, Kipp BR, Levy MJ, et al. Lung cancer adrenal gland metastasis: Optimal fine-needle aspirate and touch preparation smear cellularity characteristics for successful theranostic next-generation sequencing. Cancer Cytopathol 2014;122:822-32.
17. Kanagal-Shamanna R, Portier BP, Singh RR, et al. Next-generation sequencing-based multi-gene mutation profiling of solid tumors using fine needle aspiration samples: promises and challenges for routine clinical diagnostics. Mod Pathol 2014;27:314-27.
18. Karnes HE, Duncavage EJ, Bernadt CT. Targeted next-generation sequencing using fine-needle aspirates from adenocarcinomas of the lung. Cancer Cytopathol 2014;122:104-13.
19. Kage H, Kohsaka S, Shinozaki-Ushiku A, et al. Small lung tumor biopsy samples are feasible for high quality targeted next generation sequencing. Cancer Sci 2019;110:2652-7.
20. Zhao JJ, Chan HP, Soon YY, et al. A systematic review and meta-analysis of the adequacy of endobronchial ultrasound transbronchial needle aspiration for next-generation sequencing in patients with non-small cell lung cancer. Lung Cancer 2022;166:17-26.
21. Ben X, Tian D, Zhuang W, et al. Accuracy of next-generation sequencing for molecular profiling of small specimen of lung cancer: a prospective pilot study of side-by-side comparison. Diagn Pathol 2022;17:78.
22. Blumenthal GM, Mansfield E, Pazdur R. Next-Generation Sequencing in Oncology in the Era of Precision Medicine. JAMA Oncol 2016;2:13-4.
23. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines). Non-Small Cell Lung Cancer. Version 3.2017. November 16, 2016. Accessed November 28th, 2024. Available online: https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf
24. Passaro A, Attili I, Rappa A, et al. Genomic Characterization of Concurrent Alterations in Non-Small Cell Lung Cancer (NSCLC) Harboring Actionable Mutations. Cancers (Basel) 2021;13:2172.
25. Tanaka K, Hida T, Oya Y, et al. Unique prevalence of oncogenic genetic alterations in young patients with lung adenocarcinoma. Cancer 2017;123:1731-40.
26. Hou H, Zhu H, Zhao H, et al. Comprehensive Molecular Characterization of Young Chinese Patients with Lung Adenocarcinoma Identified a Distinctive Genetic Profile. Oncologist 2018;23:1008-15.
27. Kuang S, Fung AS, Perdrizet KA, et al. Upfront Next Generation Sequencing in Non-Small Cell Lung Cancer. Curr Oncol 2022;29:4428-37.
28. Zheng R, Yin Z, Alhatem A, et al. Epidemiologic Features of NSCLC Gene Alterations in Hispanic Patients from Puerto Rico. Cancers (Basel) 2020;12:3492.
29. Hondelink LM, Ernst SM, Atmodimedjo P, et al. Prevalence, clinical and molecular characteristics of early stage EGFR-mutated lung cancer in a real-life West-European cohort: Implications for adjuvant therapy. Eur J Cancer 2023;181:53-61.
30. Judd J, Abdel Karim N, Khan H, et al. Characterization of KRAS Mutation Subtypes in Non-small Cell Lung Cancer. Mol Cancer Ther 2021;20:2577-84.
31. Arbour KC, Rizvi H, Plodkowski AJ, et al. Treatment Outcomes and Clinical Characteristics of Patients with KRAS-G12C-Mutant Non-Small Cell Lung Cancer. Clin Cancer Res 2021;27:2209-15.
32. Furuya N, Matsumoto S, Kakinuma K, et al. Suitability of transbronchial brushing cytology specimens for next-generation sequencing in peripheral lung cancer. Cancer Sci 2021;112:380-7.
33. Silvestri GA, Gonzalez AV, Jantz MA, et al. Methods for staging non-small cell lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2013;143:e211S-e250S.
34. Mehrotra M, D'Cruz JR, Bishop MA, et al. Video-Assisted Thoracoscopy. Treasure Island, FL, USA: StatPearls Publishing; 2024.
35. Zhang X, Feng J, Su X, et al. Next Generation Sequencing Reveals a Synchronous Trilateral Lung Adenocarcinoma Case with Distinct Driver Alterations of EGFR 19 Deletion or EGFR 20 Insertion or EZR-ROS1 Fusion. Onco Targets Ther 2020;13:12667-71.
36. DiBardino DM, Yarmus LB, Semaan RW. Transthoracic needle biopsy of the lung. J Thorac Dis 2015;7:S304-16.
37. Chockalingam A, Hong K. Transthoracic needle aspiration: the past, present and future. J Thorac Dis 2015;7:S292-9.
38. Wallace MB, Pascual JM, Raimondo M, et al. Minimally invasive endoscopic staging of suspected lung cancer.

JAMA 2008;299:540-6.
39. Wahidi MM, Herth F, Yasufuku K, et al. Technical Aspects of Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration: CHEST Guideline and Expert Panel Report. Chest 2016;149:816-35.
40. Simon M, Simon I, Tent PA, et al. Cryobiopsy in Lung Cancer Diagnosis-A Literature Review. Medicina (Kaunas) 2021;57:393.
41. Cooper WA, Mahar A, Myers JL, et al. Cryobiopsy for Identification of Usual Interstitial Pneumonia and Other Interstitial Lung Disease Features. Further Lessons from COLDICE, a Prospective Multicenter Clinical Trial. Am J Respir Crit Care Med 2021;203:1306-13.
42. Poletti V, Petrarulo S, Piciucchi S, et al. EBUS-guided cryobiopsy in the diagnosis of thoracic disorders. Pulmonology 2024;30:459-65.
43. Arimura K, Tagaya E, Akagawa H, et al. Cryobiopsy with endobronchial ultrasonography using a guide sheath for peripheral pulmonary lesions and DNA analysis by next generation sequencing and rapid on-site evaluation. Respir Investig 2019;57:150-6.
44. Esposito Abate R, Frezzetti D, Maiello MR, et al. Next Generation Sequencing-Based Profiling of Cell Free DNA in Patients with Advanced Non-Small Cell Lung Cancer: Advantages and Pitfalls. Cancers (Basel) 2020;12:3804.
45. Tone M, Inomata M, Awano N, et al. Comparison of adequacy between transbronchial lung cryobiopsy samples and endobronchial ultrasound-guided transbronchial needle aspiration samples for next-generation sequencing analysis. Thorac Cancer 2021;12:251-8.
46. Sakata S, Otsubo K, Yoshida H, et al. Real-world data on NGS using the Oncomine DxTT for detecting genetic alterations in non-small-cell lung cancer: WJOG13019L. Cancer Sci 2022;113:221-8.
47. Ariyasu R, Uchibori K, Ninomiya H, et al. Feasibility of next-generation sequencing test for patients with advanced NSCLC in clinical practice. Thorac Cancer 2021;12:504-11.
48. Takeyasu Y, Yoshida T, Motoi N, et al. Feasibility of next-generation sequencing (Oncomine™ DX Target Test) for the screening of oncogenic mutations in advanced non-small-cell lung cancer patients. Jpn J Clin Oncol 2021;51:1114-22. Erratum in: Jpn J Clin Oncol 2021;51:1183.
49. Nemoto D, Yokose T, Katayama K, et al. Tissue surface area and tumor cell count affect the success rate of the Oncomine Dx Target Test in the analysis of biopsy tissue samples. Thorac Cancer 2021;12:194-200.
50. Uchimura K, Yanase K, Imabayashi T, et al. The Impact of Core Tissues on Successful Next-Generation Sequencing Analysis of Specimens Obtained through Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration. Cancers (Basel) 2021;13:5879.
51. Aboudara MC, Saettele T, Tawfik O. Endobronchial ultrasound bronchoscopy Franseen fine needle biopsy tool versus standard fine needle aspiration needle: Impact on diagnosis and tissue adequacy. Respir Med 2023;208:107131.
52. Yu Lee-Mateus A, Sawal N, Hartley C, et al. Efficacy of Robotic Bronchoscopy for Molecular Marker Analysis in Primary Lung Cancer. Clin Lung Cancer 2024;25:e11-7.
53. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
54. Wu YL, Zhou C, Hu CP, et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. Lancet Oncol 2014;15:213-22.
55. Zhao Z, Li L, Wang Z, et al. The Status of the EGFR T790M Mutation is associated with the Clinical Benefits of Osimertinib Treatment in Non-small Cell Lung Cancer Patients: A Meta-Analysis. J Cancer 2020;11:3106-13.
56. Mok TS, Wu YL, Ahn MJ, et al. Osimertinib or Platinum-Pemetrexed in EGFR T790M-Positive Lung Cancer. N Engl J Med 2017;376:629-40.
57. Soria JC, Ohe Y, Vansteenkiste J, et al. Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. N Engl J Med 2018;378:113-25.
58. Zheng Q, Huang Y, Zhao H, et al. EGFR mutation genotypes affect efficacy and resistance mechanisms of osimertinib in T790M-positive NSCLC patients. Transl Lung Cancer Res 2020;9:471-83.
59. Huang LT, Zhang SL, Han CB, et al. Impact of EGFR exon 19 deletion subtypes on clinical outcomes in EGFR-TKI-Treated advanced non-small-cell lung cancer. Lung Cancer 2022;166:9-16.
60. Awad MM, Liu S, Rybkin II, et al. Acquired Resistance to KRAS(G12C) Inhibition in Cancer. N Engl J Med 2021;384:2382-93.

Cite this article as: Figueroa A, Gholi SS, Jayant G, Wadgaonkar R, Gubran A, Kuperberg SJ. Improving diagnostic capabilities in lung cancer through next-generation sequencing: a narrative review. J Thorac Dis 2025;17(1):476-486. doi: 10.21037/jtd-24-488