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The Role of Next Generation Sequencing Panels in Personalized medicine of Lung Cancer: A Review Study

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Abstract:
Lung cancer remains a leading cause of cancer-related deaths globally, with high mortality rates due to late-stage diagnosis. Early detection is crucial but challenging due to the asymptomatic nature of early-stage disease. Next-generation sequencing (NGS) has revolutionized oncology by enabling comprehensive genomic profiling, which can identify various genetic alterations from small samples. This review highlights the role of NGS panels in early lung cancer detection within personalized medicine. NGS allows the identification of actionable biomarkers, facilitating precision therapy and improving patient outcomes. Its efficiency in analyzing multiple genes simultaneously makes it a valuable tool for identifying therapeutic targets and resistance mechanisms. NGS is also cost-effective, reducing the need for multiple diagnostic tests, and its rapid data processing capabilities have led to increased adoption in clinical practice. As personalized approaches to cancer treatment gain traction, NGS is expected to play a key role in early diagnosis, prognosis, and monitoring of lung cancer. The ongoing development of advanced NGS panels and bioinformatics tools will enhance its clinical utility, positioning NGS as a cornerstone technology in lung cancer management.

INTRODUCTION

Lung cancer (LC) is a prevalent form of cancer on a global scale, with the distinction of being the primary cause of cancer-related mortality among males and the second most significant cause among females (1). Individuals diagnosed with LC generally have a poor prognosis, as evidenced by a 5-year survival rate of 19%. It is estimated that LC is responsible for 13% of

cancer cases and 24% of cancer deaths (2). LC is the second most prevalent cancer in both men and women in the United States. In Europe, this particular cancer ranks as the third most prevalent in both genders, with the greatest mortality rate (3). The incidence of LC is highly influenced by the geographic region, with greater rates observed in developed nations compared to undeveloped ones. Additionally, the prevalence is increasing in Asian countries (4). Central Africa exhibited the lowest prevalence of LC, whereas North America demonstrated the greatest occurrence. By 2030, it is estimated that LC would rank as the seventh leading cause of mortality, accounting for 3% of all deaths (5). The incidence rate of LC, the fifth most common cancer in Iran, ranges from 4.7 to 9.2 per 100,000 people. According to another study, the age-standardized rate (ASR) of this particular cancer was reported to be 9.7 in 2014 and 27 in 2030 (6).

The prevalence of LC in Iran is increasing, with the North West and West provinces of Iran having a higher incidence compared to other regions, particularly among males (7, 8). The rising prevalence of lung cancer in Iran can be attributed to the escalating rates of urbanization, the prevalence of smoking, and environmental pollution in the country (7). Small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) are the two primary classifications of lung cancer. SCLC makes up around 15% of bronchogenic carcinomas, while NSCLC makes up the remaining 85%. These carcinomas are categorized into squamous cell carcinoma (SCC), adenocarcinoma (ADC), and large cell carcinoma (9, 10). Smoking and tobacco use are significant risk factors for the development of LC. Furthermore, exposure to second-hand smoking can elevate the likelihood of developing LC by up to 26%. Additional risk factors for LC encompass dietary choices, occupational asbestos exposure, a familial predisposition to LC, gender, air pollution, and exposure to hazardous substances such as polycyclic aromatic hydrocarbons, heavy metals, and radon gas (11-13). The elevated mortality rate associated with LC can be related to multiple factors. Initially, the majority of individuals diagnosed with LC are often in an advanced stage of the disease. Furthermore, even in cases of early detection, the efficacy of treatments for LC is comparatively lower than that of other forms of cancer. The mutation burden of LC patients with a smoking history is shown to be significantly higher compared to people with malignancies that are considered “age-related” (14). Due to the elevated death rate associated with LC, researchers are increasingly focusing on its diagnosis and prognosis. Early detection and treatment are crucial in effectively reducing the mortality rate among LC patients. Tumor markers are crucial in the timely identification of lung cancer and hold significant importance in the realms of early detection, personalized treatment, and clinical prognosis.

Next-generation sequencing (NGS) is a valuable method for the identification of tumor markers and plays a significant role in the early detection of lung cancer. The purpose of this study was to examine the significance of NGS panels in early detection of lung cancer through the use of personalized medicine strategies.

Genetic of Lung Cancer

The etiology of LC involves the accumulation of genetic alterations within the cellular composition of lung tissue. The primary focus of the LC genetic analysis consists of the discovery of mutations (15). The presence of genetic modifications in the LC has been widely acknowledged in over 60% of the situations (16). LC is distinguished by a wide range of genetic abnormalities, including mutations, chromosomal area gains and losses, gene rearrangements, and copy number gain or amplifications in oncogenes and tumor suppressor genes (17).

The proto-oncogenes that undergo mutation most frequently in LC belong to the MYC, RAS, and HER families. Additionally, the most commonly observed changes in tumor suppressor genes include mutations in TP53, RB, and p16 (15,18-20). The initial genetic alterations observed in LC were identified in the KRAS and TP53 genes. The identification of KRAS and EGFR mutations is predominantly observed in LC, similar to the rearrangements involving ALK and ROS1, as reported in 2007. Several significant oncogenic alterations have been found in LC, such as B-Raf proto-oncogene (*BRAF*), Erb-B2 Receptor Tyrosine Kinase 2 (*ERBB2*), mesenchyme-epithelial transition factor (MET), and rearranged during transfection (RET). These modifications have been utilized as tumor markers for diagnostic purposes (21-27). Other studies have uncovered a variety of recurrent alterations in lung cancer, including gene amplifications (such as CCND1-3, CDK4, FGFR1-3, MET, PDGFRA, PIK3CA, and SOX2), gene fusions (like FGFR3-TACC3), tumor suppressor mutations (such as PTEN and TP53), and point mutations (including EPHA2, AKT1, and DDR2). Some research indicates that certain driver gene variants—such as mutations in EGFR, KRAS, and BRAF; mutations or amplifications of HER2; rearrangements in ALK, ROS1, and RET; and MET copy number amplifications or splice variants in MET exon 14—form a “core gene list” crucial for lung cancer. Table 1 presents genes involved in the carcinogenesis of lung tissue. The advent of next-generation sequencing has enabled a more detailed understanding of the specific genomic alterations relevant to lung cancer diagnosis.

Next Generation Sequencing

Sanger sequencing, a pioneering technology, was

Table 1. The known genes involved in the development of lung cancer.
LC: Lung Cancer, ADC: Adenocarcinomas, SCC: Squamous Cell Carcinoma.

Gene	Type of LC	Genomic Aberrations	Frequency [%]
EGFR	ADC	*EGFR* exon 21, *EGFR* exon 19, *G719X*, *L861Q* point mutations and copy number variations	**30-40**
KRAS	ADC	G12C mutation in *KRAS* gene	**20–30**
MET	ADC	MET exon 14 mutation (MET ex14), skipping mutations, overexpression, amplifications	**2–5**
ALK	ADC	ALK fusions	**3–7**
BRAF	ADC	V600E mutation in *BRAF* gene	**0.5–5**
ROS1	ADC	ROS fusions	**2-3**
RET	ADC	RET rearrangements, gene fusion of KIF5B-RET; point mutations	**1-2**
NTRK	ADC	NTRK rearrangements, gene fusions of *NTRK1 (NTRKA), NTRK2 (NTRKB), NTRK3 (NTRKC)*	**1-2**
HER2	ADC	mutations in the kinase domain (exon 20), the most frequent is p.A775_G776insYVMA insertion amplifications	**1-5**
PTEN	ADC	mutations copy number variations	**1.7**
PDGFRA	ADC	mutations copy number variations	**6-7**
PIK3CA	ADC	mutations copy number variations	**5**
TP53	ADC	mutations copy number variations	**52**
ERBB2	ADC	mutations copy number variations	**2-5**
TERT	ADC	mutations copy number variations	**75**
CDKN2A	ADC	mutations copy number variations	**7**
FGFR	SCC	gene fusion of FGFR3-TACC3, mutations of *FGFR1*, *FGFR2*	**23**
TP53	SCC	tumor suppressor mutations	**79**
NF1	SCC	mutations of *NF1*	**10**
DDR2	SCC	point mutations of DDR2	**2-3**
PDGFRA	SCC	amplification	**4**
PIK3CA	SCC	amplification	**15**
PTEN	SCC	tumor suppressor mutations	**10**
SOX2	SCC	copy number variations	**8**
CDKN2A	SCC	amplification and copy number variation	**15**

pioneered by Fred Sanger in 1977 and has since been extensively employed in the field of clinical genetics for several decades. After a span of thirty years, NGS technologies have seen significant advancements, resulting in the development of second, third, and fourth-generation sequencing technologies (34). In Figure 1, types of sequencing generation technologies are introduced. There are two distinct methods in first-generation sequencing, specifically the Sanger method and the Maxam-Gilbert approach. The second

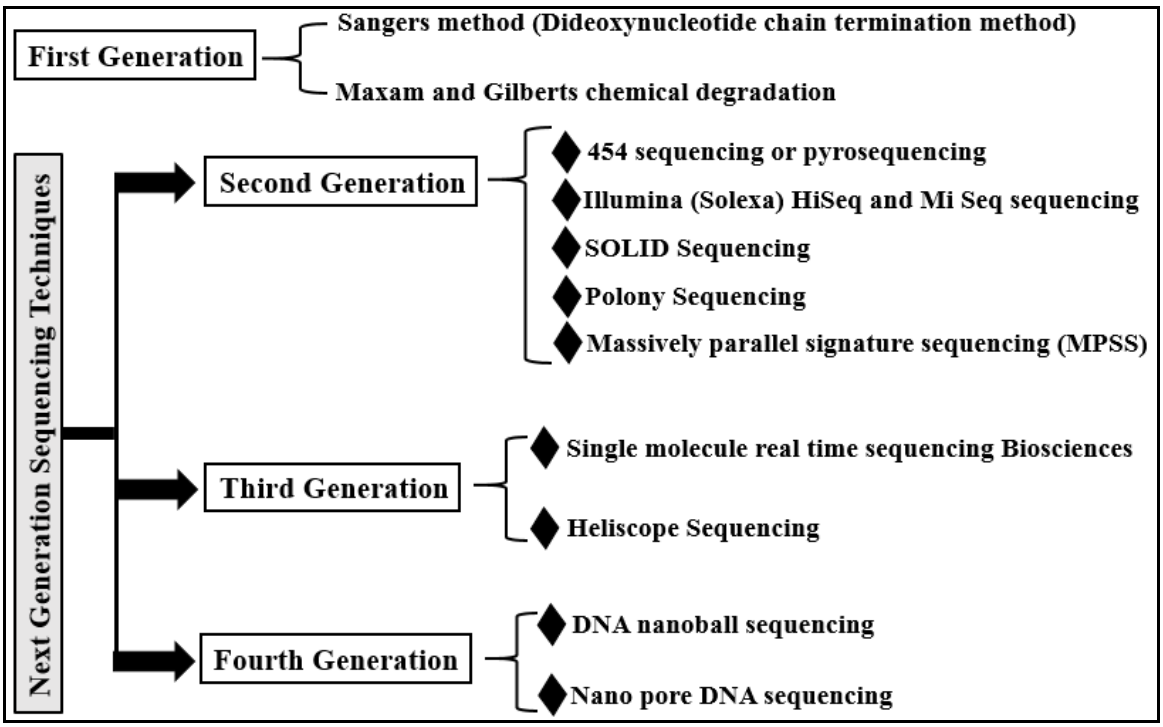

Fig 1. Classification of types of sequencing generation technologies.

generation of sequencing techniques encompasses many methods including pyrosequencing, Illumina, SOLID, polony, and massively parallel signature sequencing (MPSS). third-generation sequencing methods, such as Heliscope and single-molecule real-time sequencing by Biosciences, have advanced DNA analysis capabilities. High-throughput technologies, including DNA nano-ball sequencing and nanopore DNA sequencing, are regarded as fourth-generation sequencing (35). Next-generation sequencing generally encompasses second-, third-, and fourth-generation sequencing technologies, all of which are significantly more efficient than first-generation sequencing (36).

The Sanger sequencing method is the gold standard for detecting single nucleotide variants and modest insertions/deletions. However, its efficacy is limited when it comes to detecting gross insertions/deletions and major rearrangements. NGS is capable of detecting all types of mutations in target genes and abnormalities in chromosomes (37, 38). In essence, NGS is a DNA sequencing methodology that employs parallel sequencing of several small DNA fragments to identify particular sequences (39). The NGS technology can simultaneously identify single nucleotide variants, small insertions, deletions, copy number alteration, structural variations, gene fusions, and chromosomal rearrangements, and has significantly transformed the fields of personal medicine and genomic research (35). The NGS methodologies encompass a range of techniques that focus on the analysis of tumoral DNA and RNA (41). NGS involves several types of sequencing techniques, such as whole genome sequencing (WGS), whole-exome sequencing (WES), whole-transcriptome sequencing (RNA-seq), and targeted sequencing (both DNA and RNA). These techniques are mostly used to detect genetic alterations (42). WGS enables the sequencing of the entire genome, while whole exome sequencing (WES) specifically targets the coding portions of a genome to identify both rare and common mutations that are linked to a disease or phenotype. The utilization of RNA sequencing provides the potential to identify alternative gene-spliced transcripts, posttranscriptional modifications, gene fusion, mutations, single-nucleotide polymorphisms, and alterations in gene expression. The RNA that has been isolated is initially concentrated and then converted into complementary DNA (cDNA) using reverse transcription. Furthermore, the use of the. NGS methodology enables the examination of epigenetic modifications, including promoter methylation, microRNAs, and the expression of additional small RNAs (41).

The flexibility of NGS technology allows for the simultaneous analysis of mutational hotspots in many gene targets across different cancer patients (43). Various methodologies are employed across diverse platforms. HiSeq 2000 Illumina-Solexa is primarily utilized for whole-genome sequencing (WGS), whole-exome sequencing (WES), and RNA sequencing. On the other hand, Miseq Illumina-Solexa is primarily employed for WES and targeted sequencing (44). The two comprehensive molecular profiling NGS tests

are the FoundationOne CDx (F1CDx) and the MSK-IMPACT, which were approved by the Food and Drug Administration (FDA) in 2017. These tests examine a larger number of genes simultaneously. Several NGS tests have received approval from the FDA. These include the Oncomine Dx Target Test for lung cancer, the Illumina Extended RAS Panel for colon cancer, and the Foundation Focus CDx BRCA LOH in 2018. Additionally, there are various NGS diagnostics now in development. One such test is the Caris MI Transcriptome CDx, which is an in vitro diagnostic test based on next-generation sequencing. This test utilizes RNA extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue to identify structural rearrangements. The Breakthrough Device Designation for Illumina's pan-cancer assay, TruSight Oncology 500, was granted by the FDA in 2019. This assay uses DNA and RNA extracted from tumor samples to detect small DNA variants, fusions, and splice variants. NGS technologies have significantly contributed to the comprehension of the modified genomic pathways implicated in human cancer. Utilizing various panels of NGS technologies in both research and clinical settings can play a crucial role in the early detection and treatment of LC (41).

NGS technologies have facilitated the precise and effective identification of somatic mutations (46). NGS exhibits several advantages in comparison to genome-sequencing approaches. Firstly, this technology is classified as high-throughput since it enables the extensive sequencing of several targeted genomic areas in numerous samples simultaneously. This allows for the detection of contemporaneous mutations in the same run. Another significant benefit of routine tumor sequencing is the decreased time required for analysis, resulting in a shorter duration for clinical reporting. Furthermore, NGS analysis necessitates a minimal amount of DNA/RNA input, as opposed to previous sequencing techniques. It is possible to concurrently identify a range of genetic alterations with a high level of accuracy and sensitivity. NGS exhibits greater sensitivity compared to Sanger sequencing, with the ability to identify allele frequencies ranging from 2% to 10% and 15% to 25% respectively. Additionally, NGS enables quantitative assessment of the mutant allele (41). Furthermore, NGS enables the identification of simultaneous genetic changes in a particular subset of patients with molecular classifications, so providing a more comprehensive understanding of the genomic intricacy and prognosis of patients with LC. NGS also decreases the cost and duration of a comprehensive genetic evaluation (47-49). One of the limitations of NGS is the requirement for robust bioinformatics tools and skilled individuals to do both experimental and data analysis (50). NGS will generate hundreds of megabytes of data. Filtering redundant and large volumes of data is a challenging and intricate undertaking for bioinformatics personnel. Most NGS initiatives require the use of specialized applications. Furthermore, the storage, processing, and analysis of NGS data need the utilization of a high-performance computer (51, 52). Additional benefits and constraints of Next-Generation Sequencing (NGS) are illustrated in Figure 2.

The role of NGS in lung cancer diagnosis

Diagnosing LC in its early stages might be challenging due to the absence of symptoms in patients. Furthermore, prior methodologies, such as first-generation sequencing, have yielded numerous false-negative outcomes in the detection of lung cancer due to various factors, including the quality and amount of the samples, as well as the sensitivity of the test (53). NGS technologies have facilitated the precise and effective identification of somatic mutations. NGS has proven to be an effective method for identifying new mutations in LC (46), as it enables the sequencing of several genomic areas within a single test and platform. A technique based on NGS has the potential to offer a more thorough genetic analysis of LC, which could have an impact on the available diagnostic choices and the prognosis of patients (27). Furthermore, NGS has emerged as a prominent methodology employed in clinical practice to acquire thorough genetic profiling in individuals diagnosed with LC (54). NGS offers superior performance in terms of throughput, sensitivity, and specificity compared to traditional PCR testing. This enables the simultaneous amplification of a predetermined set of genes in a single reaction, allowing for multiplex PCR (27, 55, 56). Numerous studies have indicated that NGS might be advantageously utilized to identify particular LC mutations in circulating tumor DNA inside a liquid biopsy sample (57-59).

Several studies have shown that the utilization of NGS in plasma analysis yields a genomic profile of LC patients that is comparable to tissue testing. Furthermore, the incorporation of plasma NGS assays into regular management practices has been found to enhance the identification of clinically relevant mutations (60-62). Additional research has demonstrated that NGS is employed in LC to uncover potential biomarkers for early detection and identify mutations in clinical situations (36).

The advantages of NGS in examining changes linked to lung cancer diagnosis have opened up new possibilities for developing targeted commercial kits aimed at early LC detection. Most genomic profiling data from lung cancer patients who have had cfDNA NGS testing has been gathered using the Guardant360® panel, created by Guardant Health. The panel is equipped to

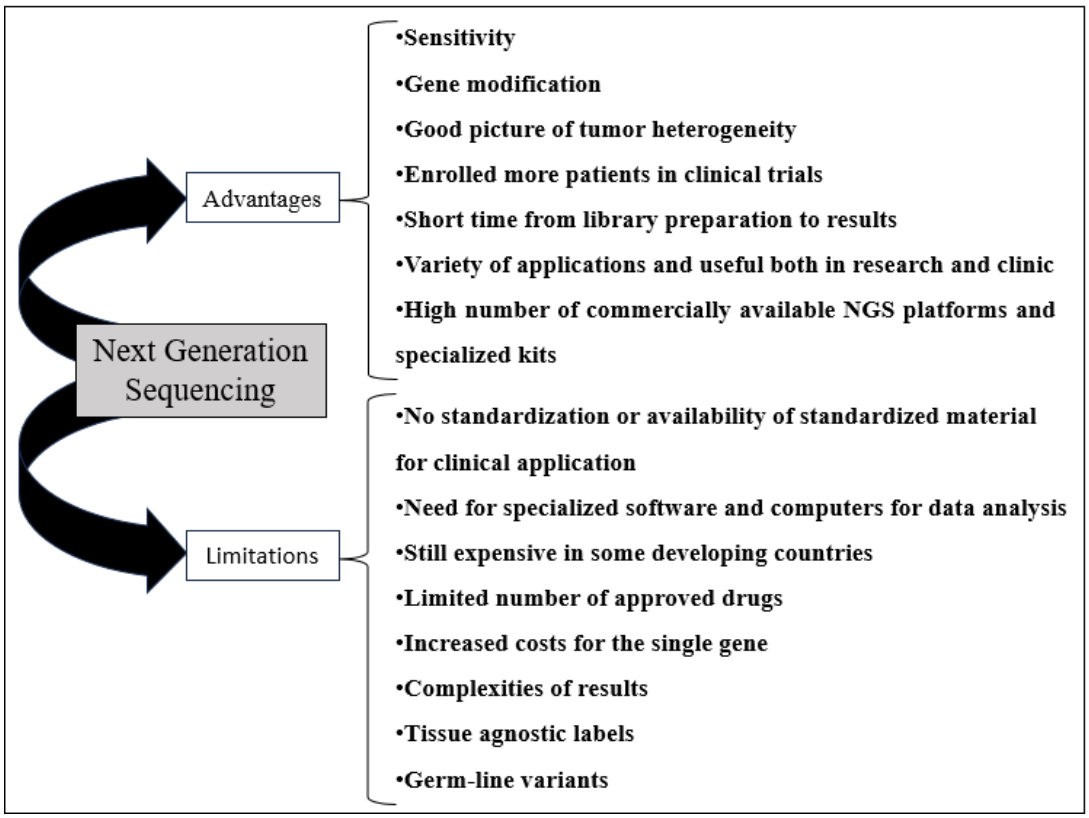

Fig 2. Advantages and limitation of Next Generation Sequencing
Also, NGS offers many benefits in comparison with traditional sequencing methods.

detect genetic alterations, including single nucleotide variants (SNVs), insertions/deletions (indels), copy number variations (CNVs), and gene fusions, across 73 genes. Recently published findings highlight the analysis of cfDNA using the Guardant360® panel, which encompasses over 8,000 plasma samples from lung cancer patients. Additionally, the Department of Public Health at the University of Naples Federico II in Italy has developed the SiRe® NGS panel, designed to assess 568 mutations in six specific genes—EGFR, KRAS, NRAS, BRAF, cKIT, and PDGFR³—in lung cancer tissue samples. Numerous studies have validated the high clinical performance of NGS-based cfDNA analysis, especially regarding success rates and mutation detection accuracy. Consequently, the SiRe® NGS panel is recognized as a valuable diagnostic tool for cfDNA analysis. (43, 65).

In 2013, Scarpa et al. highlighted the diagnostic value of the Ion AmpliSeq Colon and Lung Cancer Panel for lung adenocarcinoma samples (66). The initial version of this panel targeted 504 regions with high mutation rates across 22 cancer-related genes and was capable of detecting variants with an allele frequency as low as 1%, representing approximately 2% of cancer cells in a sample (67). Using the Ion AmpliSeq Colon and Lung Cancer Panel is essential for identifying the EGFR deletion type, a detail not provided by in vitro diagnostic molecular testing on Rotor-Gene real-time PCR. (41).

The TruSeq Amplicon-Cancer Panel, developed by Illumina, Inc. in the United States, is a targeted resequencing assay that exhibits a high level of multiplexing. It is designed to detect somatic mutations in cancer genomes by identifying numerous mutational hotspots. The presented panel offers an efficient workflow, encompassing a quality control assay designed for DNA extracted from FFPE samples. Additionally, this panel facilitates the detection of very sensitive mutations in significant genes, including *BRAF*, *KRAS*, and *EGFR*. LC is associated with mutations in these genes. The assay possesses a distinctive capability to evaluate significant FFPE samples for these crucial variations, hence enabling the extraction of a substantial amount of genomic data from LC tumors (68).

The mutations of *EGFR* exon 18, 19, 20, 21, *KRAS* exon 2, 3, *PIK3CA* exon 9, 20, and *BRAF* exon 11, 15 were detected using the NextDaySeqLung panel, developed by Beijing ACCB Biotech in Beijing, China. According to previous studies (69), the NextDaySeq-Lung panel has exhibited superior outcomes compared to Sanger sequencing or qRT-PCR.

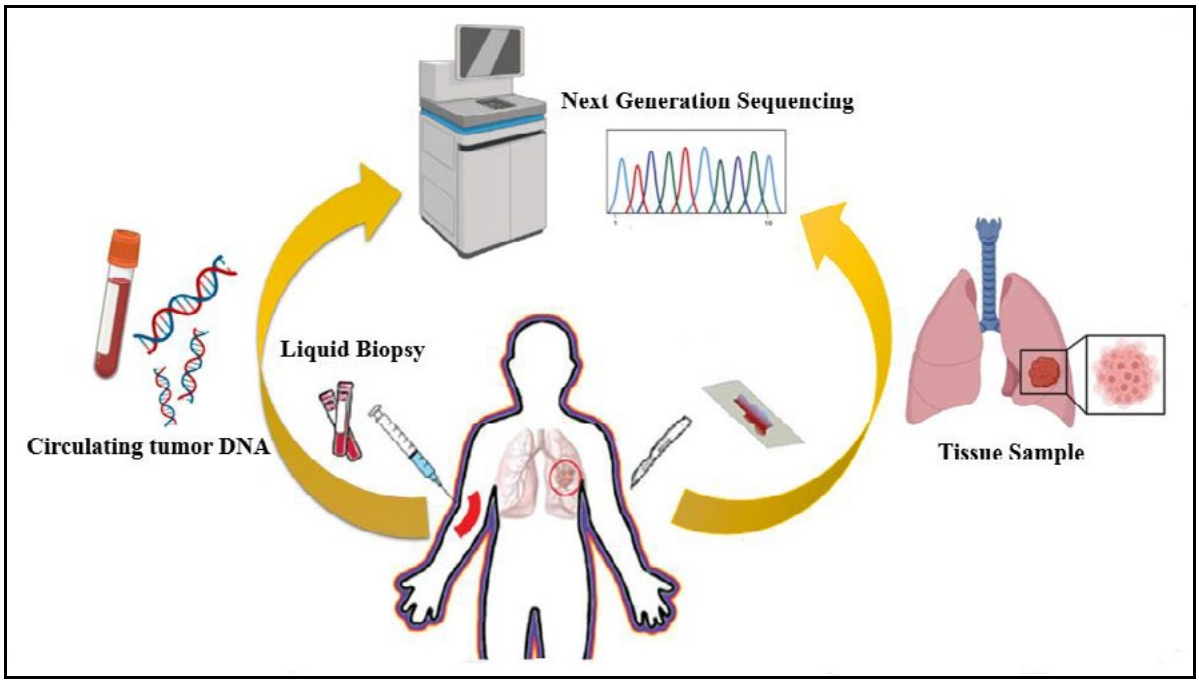

Fig 3. NGS analysis of liquid biopsy and tissue sample for detect of specific mutations.

Furthermore, several studies have demonstrated that the NextDaySeq-Lung panel exhibits notable technical benefits compared to Sanger and qPCR tests, indicating its promising potential as a molecular diagnostic panel for lung cancer (70, 71). LC gene panels utilizing RNA sequencing have been developed to examine gene fusion, translocations, chromosomal inversions, and interstitial deletions. The Ion Ampliseq RNA fusion lung cancer panel, developed by ThermoFisher Scientific in Waltham, USA, is an example of such a panel. This panel specifically targets 70 well-known fusion transcripts of *ALK, RET, ROS1,* and *NTRK*. This panel has exhibited a high level of sensitivity and a strong agreement with the conventional techniques employed for fusion testing (72). Displays the NGS panels that are currently accessible to diagnose LC. As previously said, cancer is known as a genetic disorder (16). LC is a multifaceted illness characterized by a wide range of somatic mutations (73). It arises from a process of polyphase carcinogenesis, wherein genetic alterations gradually accumulate over time. The utilization of screening for the distinctive genetic mutation as a biomarker has the potential to facilitate the early detection of LC (15). NGS technologies have significantly contributed to the identification of modified genomic pathways implicated in cancer. Consequently, the utilization of diverse panels of NGS technology has proven valuable in both research and clinical settings (41).

NGS is primarily used for the early detection of lung cancer by identifying mutations in genes such as *EGFR, BRAF, KRAS, TP53, HER2, ROS, ALK, PIK3CA, NTRK, RET*, and *MET*. These genes are considered potential candidates in the development of lung cancer (74, 75). NGS techniques are extensively employed in the field of oncology, particularly for the early detection of biomarkers and the identification of driving mutations in LC. Numerous studies have been conducted to examine the significance of NGS in the diagnosis of lung cancer and to compare it with conventional testing methods. The study conducted by Gabriela et al. (2019) aimed to compare the efficacy of a NGS method with a Sanger sequencing and Fluorescence In Situ Hybridization (FISH) sequential strategy in detecting actionable genomic alterations in a cohort of 117 patients diagnosed with advanced lung cancer. Patients were categorized as EGFR-mutated (n = 22, 18.8%), ALK-mutated (n = 9, 7.7%), and unclassifiable (n = 86, 73.5%) using Sanger and FISH techniques. On the other hand, the study using NGS resulted in the detection of at least one genetic variation in 56 patients (47.9%), resulting in a total of 68 variants across all samples. The study's findings indicate that the NGS-assay is a viable approach for conducting genomic profiling in individuals diagnosed with advanced lung adenocarcinoma (27). Gao et al. (2015) conducted a study to assess the practicality of using NGS-based assays to analyze mutations in important driver genes of lung cancer in a clinical environment. A total of 138 FFPE samples of lung cancer were analyzed simultaneously with NGS assays, quantitative PCR (qPCR), and Sanger sequencing platforms to detect the mentioned somatic mutations. The findings revealed that the NGS assays exhibited significantly greater

sensitivity compared to Sanger. Additionally, the NGS test demonstrated additional benefits compared to qPCR in terms of delivering precise data on allele sequence and mutation frequency, as well as identifying non-hotspot alterations. The findings indicate that the NGS assay possesses notable technical benefits compared to Sanger and qPCR assays, making it a promising molecular diagnostic panel for lung cancer (70, 71). In Lim et al.'s (2016) investigation, a NGS-based assay was employed to detect 51 FFPE samples of lung cancer. The efficiency of this assay was compared to that of a traditional technique. The study found that 58% of wild-type patients exhibited mutations in one of these genes when the NGS approach was used (53). In previous studies, NGS has been utilized to diagnose lung cancer, as conventional approaches are not adequate for the limited availability of tissue samples (55). Another study has demonstrated that existing tools are limited to identifying a single hot spot at a time. However, NGS offers various advantages over traditional sequencing methods, including increased throughput and reduced testing time. NGS allows for the simultaneous sequencing of multiple hot spots within a short timeframe. Therefore, NGS decreases expenses and produces valuable genetic data on lung cancer, helping clinicians in the diagnostic process (36). Moreover, another study compared an NGS panel, Sanger sequencing, and qRT-PCR in the detection of mutation in 138 lung cancer FFPE samples showed that the NGS and qRT-PCR have a higher sensitivity than Sanger sequencing. Also, NGS is better than qRT-PCR and identifies mutations that are not in the hotspot area (70). In the study by Park et al. (2019) the single-gene assays, such as real-time PCR, IHC, and FISH were compared with the NGS method.

The NGS data indicate that EGFR PCR had a sensitivity of 80.3% and specificity of 99.4%, ALK FISH had a sensitivity of 71.4% and specificity of 100%, and ROS1 FISH had a sensitivity of 100% and specificity of 99.5%. The observed outcomes are associated with the diminished sensitivity of single-gene assays when compared to deep-targeted NGS (76). Numerous studies have demonstrated various transformations resulting from the integration of NGS into clinical practice for the diagnosis of lung cancer. These studies have reported a notable level of sensitivity in detecting alterations through the utilization of a gene panel-based NGS approach on lung cancer specimens (77-79). For instance, Lim et al. (2016) found that 58% of patients who tested for EGFR/KRAS/ALK using standard methods exhibited alterations that were identified by NGS (53). The research conducted by de Leng et al. (2016) and Jing et al. (2018) demonstrates a strong concurrence between NGS and single gene assays in identifying driver-gene mutations in individuals with lung cancer. These studies exhibit notable levels of sensitivity and specificity, indicating the potential for the widespread adoption of NGS assays in informing clinical decision-making (80, 81). The examination of fusion alterations to lung cancer revealed that NGS has greater sensitivity and specificity compared to FISH or IHC. Lin et al. (2019) reported a positive rate of 92.7% for ALK rearrangement when employing NGS, 82.4% for FISH, and 94.5% for Immunohistochemistry (IHC). Additionally, they observed a concordance of 87.3% between NGS and IHC results (82). The NGS technique has facilitated the development of liquid biopsy testing for the diagnosis of lung cancer. Leighl et al. (2019) reported a significant level of agreement between the NGS results obtained from cfDNA and tissue DNA in lung cancer cases. The benefits of NGS have facilitated the creation of various assays utilizing liquid biopsy samples for the timely detection of lung cancer (83, 84). A study has demonstrated that NGS-based methodologies are very suitable options for achieving optimal characterization of circulating tumor DNA in individuals diagnosed with lung cancer. Various NGS techniques have been created and verified for identifying anomalies in lung cancer ctDNA. These methods have the benefit of being able to discover uncommon mutations that were previously undetectable using conventional methods (85). The meta-analysis of 1639 lung cancer patients from 21 studies found that the combined sensitivity and specificity of NGS-based ctDNA T790M testing were 0.87 (95% CI 0.76–0.95) and 0.89 (95% CI 0.82–0.94), respectively. These values were higher than those of other detection methods such as Real-Time PCR and ddPCR, indicating that NGS is more efficient (86). In addition, a tag-based NGS panel was utilized to detect T790M in plasma samples obtained from patients with lung cancer. This approach yielded a notably higher detection rate of NGS in comparison to Real-Time PCR, with rates of 42.85% and 21.4% respectively. In addition, NGS demonstrated the capability to detect mutations at extremely low AFs (up to 0.07%), resulting in a drop in false negative instances necessitating repeated tumor biopsies and a reduction in turnaround times compared to alternative approaches (87). In a recent study, the effectiveness of an 11-gene NGS panel in detecting target mutations in two separate groups of lung cancer patients was compared to conventional approaches and focused NGS. NGS has demonstrated the ability to accurately detect the EGFR T790M mutation in liquid samples of lung cancer patients with a high level of sensitivity and specificity. Reckamp et al. (2016) conducted a study. NGS assays were employed to investigate the presence of EGFR activating mutations and the T790M resistance mutation in urine or plasma samples obtained from patients diagnosed with advanced NSCLC who tested positive for EGFR mutants. The NGS assay conducted on urine and

plasma samples revealed a higher prevalence of EGFR mutated positive results compared to tissue samples subjected to RT-PCR testing. The urine specificity is 94% and the plasma specificity is 96-100%. The urine and plasma sensitivity rates are 80-93% and 87-100%, respectively (89). In their study, Liu et al. (2021) offer a collection of three cases wherein the utilization of NGS enabled the identification of genomic alteration patterns, hence aiding in the differentiation between various original lung malignancies and intrapulmonary metastases. NGS exhibits unique molecular attributes that distinguish it from conventional disease detection methods. This finding supports the potential of NGS in aiding the diagnosis of lung cancer (90). Jiang et al. (2021) conducted a retrospective investigation. Both IHC and NGS were utilized to identify changes in the *ALK, EGFR, KRAS, BRAF, RET, ROS1, V-Erb-B2, CerbB-2*, and *MET* genes in 19 cases of FFPE lung adenocarcinoma. The findings of this investigation indicate a strong agreement between the NGS assay and the IHC technique (91). In their study, Pekar-zlotin et al. (2014) conducted a thorough analysis of FISH and IHC to detect *EML4-ALK* rearrangement in 51 patients with lung cancer. If there was any disagreement, they resorted to NGS. The findings of this study indicate that the FISH-based approach for identifying *EML4-ALK* rearrangement in lung cancer may overlook a considerable number of patients who could potentially benefit from targeted ALK therapy. Therefore, it is highly recommended to strongly consider screening for EML4-ALK rearrangement through IHC and to consider NGS in cases that are on the borderline (92). The study conducted by Clavé et al. (2019) evaluated the identification of ALK and ROS1 rearrangements in a retrospective cohort of forty patients with non-small cell lung cancer who had known FISH data. This assessment was performed using NGS and immunohistochemical techniques. The findings indicate that the presence of a 3 isolated signal FISH pattern in cases of ALK and ROS1 may indicate a false positive outcome.NGS appears to be a reliable technique for evaluating ALK and ROS1 rearrangements, with the benefit of discovering additional molecular changes that may have therapeutic significance, in comparison to immunohistochemistry (93). In their study, Dacic et al. (2016) conducted a comparative analysis between different ALK-FISH patterns and NGS for gene fusion detection and ALK immunohistochemistry. The authors propose that relying solely on ALK FISH may not be the most dependable method for detecting ALK gene rearrangements. Instead, they suggest that a combination of ALK IHC and NGS should be employed to detect gene fusions and mutations in lung cancer (94). The studies conducted by Moskalev et al. (2014) and Beadling et al. (2016) showed a strong agreement between the NGS assay and conventional methods (95, 96). Drilon et al. (2015) showed that employing NGS assay is a superior and more effective approach in identifying actionable mutations in lung adenocarcinomas, as compared to non-NGS testing (97). A multitude of clinical trials have been conducted to investigate the utilization of NGS technology in the diagnosis and treatment of LC. A search conducted on clinicaltrial.gov on 24 March 2022 revealed a total of 117 trials focused on lung cancer that employ NGS.

Certain of these trials are formerly complete and some others are recruiting or enrolling.

CONCLUSIONS

In the new era of precision oncology, detecting genomic alterations is the priority and guiding principle for personalized care. The next-generation sequencing method is an ideal technique than other standard techniques for investigating different types of lung cancer in a short period and at a low price. NGS has allowed the obtainment of a wide spectrum of genomic alterations occurring within lung cancer. NGS is widely used in the clinic for the detection of a greater number of driver genes and leading to early diagnosis of lung cancer. NGS maximises the identification of clinically related genomic alterations using a limited amount of tissue, thus reducing time and costs. Molecular biomarkers can be useful in diagnosis at an early and non-invasive stage of lung cancer. It is essential to develop innovative approaches using NGS panels to generation biomarkers in lung cancer screening programs for early diagnosis. The application of a standard NGS panel for lung cancer diagnosis is more efficient at a lower cost point than other methods such as Sanger-Sequencing and provides a much better resolution compared to microarrays. NGS has been used to identify new biomarker candidates for the early diagnosis of lung cancer. The different clinical studies were developed to obtain specific NGS panels for lung cancer diagnosis. Different NGS panels should be used to develop clinical tests in personalized medicine for early diagnosis of lung cancer. The apply of NGS-specific panels in the early diagnosis of lung cancer can play an important role in reducing mortality, increasing response to treatment and reducing treatment costs from this cancer. All the mention advantages, make NGS the preferred method to extend clinical tests for personalized medicine and early diagnosis of lung cancer.

Abbreviations

LC: Lung cancer, ASR: Age-standard rate, NSCLC: Non-small cell lung cancer, SCLC: Small cell lung cancer, SCC: Squamous cell carcinoma, ADC: Adenocarcinoma, NGS: Next generation sequencing, *BRAF*: *B-Raf* proto-oncogene, *ERBB2*: Erb-B2 receptor tyrosine kinase2, MET: Mesenchyme-epithelial transition factor, RET: Rearranged during transfection,

HER2: Human epidermal growth factor receptor2, MPSS: Massively parallel signature sequencing, WES: Whole-exome sequencing, WGS: Whole-genome sequencing, RNA-seq: RNA sequencing, DNA-seq: DNA sequencing, FFPE: Formalin-fixed paraffin-embedded, MSI: Microsatellite instability, TMB: Total number of mutations, PDL-1: Programmed death-ligand 1, SNVs: Single-nucleotide variants, Indels: Insertion–deletion mutations, cfDNA: Cell-free DNA, FISH: Fluorescence in situ hybridization, IHC: Immunohistochemistry, ctDNA: Circulating free DNA.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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