Review

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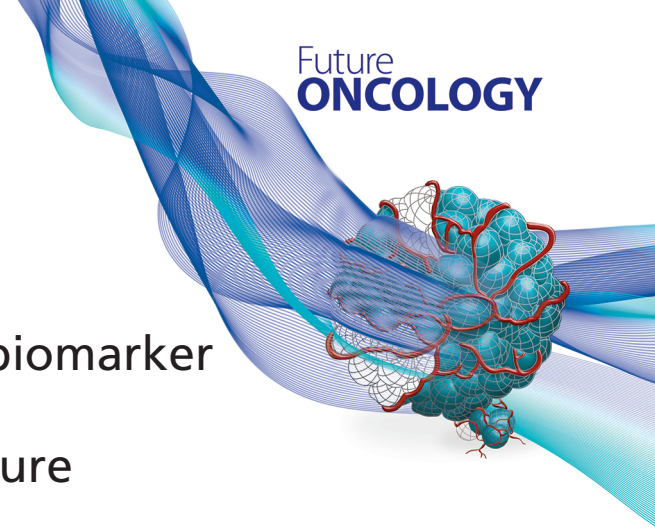

Diagnostic and economic value of biomarker testing for targetable mutations in non-small-cell lung cancer: a literature review

Ying Zheng[1], Helene Vioix[2], Frank X Liu*[,1], Barinder Singh[3], Sakshi Sharma[3] & Deepti Sharda[3]

[1]US Health Economics and Outcomes Research, EMD Serono, Inc., an affiliate of Merck KGaA, Rockland, MA 02370, USA

[2]Global Evidence & Value Development, Merck Healthcare KGaA, Darmstadt, Germany

[3]HEOR, Parexel, Access Consulting, Mohali, Punjab, India

*Author for correspondence: frank.liu@emdserono.com

We aimed to assess the diagnostic and economic value of next-generation sequencing (NGS) versus single-gene testing, and of liquid biopsy (LBx) versus tissue biopsy (TBx) in non-small-cell lung cancer biomarker testing through literature review. Embase and MEDLINE were searched to identify relevant studies (n = 43) from 2015 to 2020 in adults with advanced non-small-cell lung cancer. For NGS versus single-gene testing, concordance was 70–99% and sensitivity was 86–100%. For LBx versus TBx, specificity was 43–100% and sensitivity was ≥60%. Turnaround times were longer for NGS versus single-gene testing (but not vs sequential testing) and faster for LBx versus TBx. NGS was cost-effective, and LBx reduced US per-patient costs. NGS versus single-gene testing and LBx versus TBx were concordant. NGS and LBx may be cost-effective for initial screening.

Plain language summary: Patients with lung cancer with specific genetic mutations can benefit from medications that are specific to those mutations, known as targetable mutations. There are many methods to test for specific genetic mutations in patients with lung cancer. To detect genetic mutations, doctors can test the blood or urine, or they can test biopsy tissue; a small piece of the tumor removed from the lung. These tests can either look for mutations in one specific gene at a time, or they can use technology that reads the entire DNA sequence to observe multiple genes at once. In this review, we examined scientific reports to answer important questions about using genetic testing to find targetable mutations in patients with lung cancer. How accurate are different genetic tests? How fast can doctors get results from different genetic tests? How much do different genetic tests cost? We found that reading the entire DNA sequence was as accurate as testing one specific gene. Reading the entire DNA sequence takes more time than testing one specific gene, but it might reduce overall costs. Testing blood or urine was not as accurate as testing tissue, but it took less time for doctors to receive genetic test results and reduced costs.

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Keywords: biomarker testing • cancer mutation • diagnostic value • economic value • literature review • non-small-cell lung cancer

Lung cancer is the most common cancer worldwide and is the leading cause of cancer death for men and the second leading cause for women [1]. Non-small-cell lung cancer (NSCLC) affects approximately 85% of all patients with lung cancer [2]. These patients typically have a poor prognosis, with 5-year survival rates of 24% for all NSCLC patients and of only 6% among those with metastatic disease [3].

Targeted therapy is an important treatment option for patients with NSCLC who have driver mutation-positive tumors. Current treatment guidelines recommend tyrosine kinase inhibitor therapy for patients with actionable driver mutations, including *EGFR*, *BRAF* and *RAS* mutations, *ALK*, *ROS1*, *RET* or *NTRK* fusions and

MET tyrosine kinase abnormalities (i.e., high-level *MET* amplification and *MET* exon 14 skipping mutation) [4,5]. Genetic testing is thus required, either to guide appropriate selection of available therapies or to assess patient suitability for a clinical trial of a new therapy [6]. However, with each biomarker testing procedure performed using a biopsy from an individual patient, the amount of tissue available for further biomarker testing is reduced. As the number of potential genetic targets increases, prioritization of limited tissue is essential [7].

Strategies to ensure maximum testing yield from available tissue and to limit invasive procedures for the patient include multigene sequencing with next-generation sequencing (NGS) and liquid biopsy (LBx) techniques, respectively. NGS is tissue-sparing compared with conventional sequencing because it allows identification of a panel of genes using a single sample, but it has not replaced conventional sequencing despite progressive cost reduction [8]. LBx techniques can be used to test for circulating tumor DNA (ctDNA), circulating tumor cells, exosomes, platelets and microRNAs [9]. The role of these biomarker techniques in NSCLC, including their diagnostic and economic value, has not been clearly defined.

The overall objectives of this literature review were to assess the diagnostic evidence and economic impact of first, NGS versus single-gene testing and second, LBx compared with standard tissue biopsy (TBx) in adults with unresectable, advanced or metastatic NSCLC.

Methods

A literature review was conducted to identify publications related to NGS and LBx in adult patients (aged ≥18 years) with advanced, recurrent and/or metastatic NSCLC. The review was guided by the population, intervention, comparison, outcome, study type (PICOS) framework [10].

Data sources

Embase® and MEDLINE® were searched for records from 2015 to 2020 using targeted keyword searches (Supplementary Table 1). To supplement the database searches, conference abstracts (2017–2020) from American Society of Clinical Oncology (ASCO), European Society for Medical Oncology (ESMO), International Society for Pharmacoeconomics and Outcomes Research (ISPOR), American Association for Cancer Research (AACR), World Conferences on Lung Cancer (WCLC), International Association for the Study of Lung Cancer (IASLC) North American Congress on Lung Cancer (NACLC), IASLC Targeted Therapies and International Lung Cancer Congress were searched manually. Bibliographies of review articles were also searched manually, and the Tufts Cost–Effectiveness Analysis (CEA) Registry was searched for economic evidence [11,12].

Study selection

Eligible studies were controlled clinical trials (including both randomized and non-randomized), single-arm studies, observational studies (excluding case reports/series), systematic reviews, surveys and economic evaluations published in English. Studies had to include ≥100 adult patients of any race or sex with unresectable, advanced and/or metastatic NSCLC, regardless of histology, for any biomarker or mutation. Any study meeting the above criteria and reporting biomarker types, molecular testing methods and application, and their associated challenges and advantages in NSCLC, were considered.

Review procedure

All articles were downloaded into a systematic review database. First screening (titles and abstracts) and second screening (full-text papers) were conducted by a single reviewer, followed by a quality check from a second independent reviewer (Supplementary Figure 1). Data were extracted by a single reviewer and verified by an independent reviewer. Where more than one publication was identified describing a single trial, the data were compiled into a single entry in the data extraction table to avoid double counting of patients and studies.

Results

Literature review

A total of 2602 records were identified, from which 375 full-text studies were screened (Figure 1). Of these 375 publications, the review included 43 relevant publications describing 42 studies. Fourteen studies examined NGS versus standard molecular testing techniques in NSCLC. Twenty-eight studies reported evidence on LBx versus TBx (29 publications describing 28 studies).

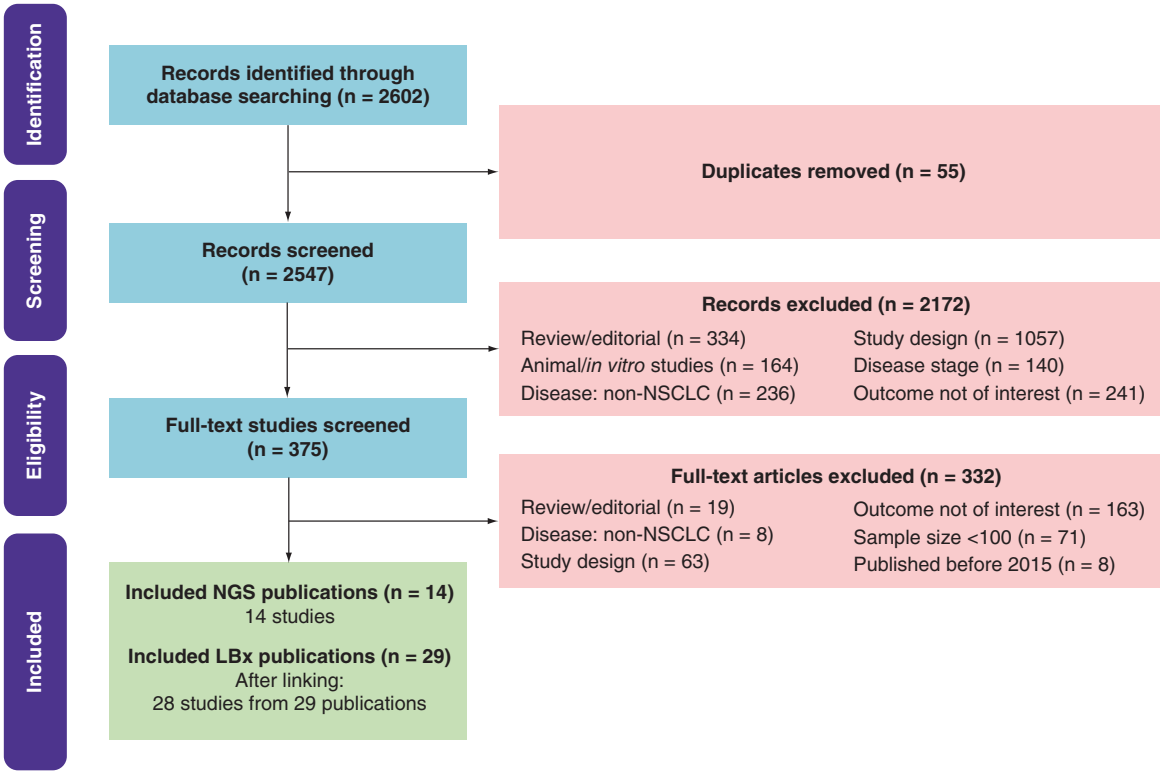

Figure 1. Flow of studies included in the literature review.
LBx: Liquid biopsy; NGS: Next-generation sequencing; NSCLC: Non-small-cell lung cancer.

NGS versus standard molecular testing

Six studies, two of them USA-based, reported diagnostic outcomes [13–18], while nine (five USA-based) reported economic evidence [17,19–27]. Of these, one study conducted in Singapore by Tan *et al.* reported both diagnostic and economic outcomes [17].

Diagnostic evidence

All studies reporting diagnostic outcomes were observational; two were retrospective and four were prospective. Among studies reporting demographic data (n = 4 studies with a range of 174–533 patients), the median age of patients ranged from 67 to 70 years, and 38–62% of patients were male [13,15–17]. Two studies from Europe [15,16] and one from Asia [17] reported concordance rates ranging from 70 to 99% for NGS versus standard molecular testing across clinically actionable mutations, including *EGFR* and *ALK* fusion (Table 1). Sensitivity for NGS versus standard molecular testing ranged from 86 to 100% for clinically actionable mutations and was reported as 55.6% in one study based on acquired resistant mutations [13–15,17]. Of the two USA-based studies included, one (Dagogo-Jack *et al.*) reported sensitivity and specificity of 98 and 100%, respectively, for a rapid *EGFR* PCR assay using NGS as the reference assay [13]. Other studies included did not report specificity data. The second USA-based study (Yu *et al.*) reported higher rates of test initiation and completion using less tissue compared with single-gene testing for four or more biomarkers [18]. Based on the two USA-based studies, median turnaround times were longer using NGS than with single-gene testing (14–17 vs 7–11 days), but this was not the case if multiple sequential single-gene tests were required (e.g., three single-gene tests ordered in sequence would require ~21–~33 days total turnaround time) [13,18].

Economic impact

Among the ten economic studies, six assessed cost–effectiveness [17,19,23–26], one reported costs [27], two assessed budget impacts [20,21] and one reported a cost–consequence analysis (Table 2) [22].

Among the five USA-based studies that reported economic evidence, two found tumor tissue NGS versus sequential exclusionary testing or hotspot panel testing (excluding treatment costs) to be cost saving [20,22], with

Table 1. Diagnostic accuracy of next-generation sequencing versus single-gene testing for clinically actionable mutations in patients with non-small-cell lung cancer.

Study (year)†	Country	Study design	Test and comparator	Biomarker tests	Sample size	Sensitivity %§,¶	Concordance %	Ref.
Dagogo Jack (2018)	USA	Prospective observational	*EGFR*-specific PCR assay vs NGS	$EGFR_{L858R}$ and exon 19 deletion	NR	98	NR	[13]
Garcia (2018)	France	Prospective observational	NGS vs OncoBEAM‡	$EGFR_{T790M}$	196	55.6#	NR	[14]
Fernandes (2019)	Portugal	Prospective observational	NGS vs Sanger sequencing	*EGFR*	117	100¶	99.1	[15]
Lindquist (2017)	Sweden	Prospective observational	NGS vs single-gene testing	*EGFR, KRAS, NRAS* and *BRAF*	81	NR	96	[16]
Tan (2020)	Singapore	Retrospective observational	NGS vs standard molecular testing	*EGFR* exon 19 deletion	NR	93.9	70	[17]
				ALK fusion	NR	85.7	94	

†One USA-based study (Yu, 2017) identified in the literature review did not report sensitivity or concordance for NGS vs conventional molecular testing [18].
‡ctDNA was used as a reference standard.
§Specificity for NGS vs conventional molecular testing was reported in only one study (Dagogo-Jack, 2018), which reported a specificity of 100%.
¶*EGFR*+ cases were assumed to be true positives. One patient was classified as *EGFR*+ by NGS but was unclassified by Sanger sequencing.
#The detection rates for $EGFR_{T790M}$ were 10.2% and 18.3% by NGS and OncoBEAM, respectively. Sensitivity of NGS assumed all T790M+ cases were true positives, and thus describes the detection of an acquired resistance mutation.
NGS: Next-generation sequencing; NR: Not reported.

one (Dalal *et al.*) reporting the shortest wait time, earlier initiation of effective targeted therapy and lower costs in patients receiving upfront NGS [22]. Two studies found tumor tissue NGS versus single-gene testing (including treatment costs) to be associated with increased budget, although that was balanced by evidence of cost–effectiveness for NGS testing [19,21]. In the study conducted by Yu *et al.* the budget increase over single-gene testing was minimal, at US$0.0072 per member per month, but NGS was expected to identify more patients with activating mutations, enabling better selection for targeted therapy [21]. Another study, by Steuten *et al.*, found ctDNA NGS versus standard of care molecular testing (no additional genomic testing) to be cost-effective, with 8% more patients identified with targetable mutations and expected survival increasing by 0.06 years versus single-gene testing [19].

Studies conducted in other regions (e.g., Europe, Asia) were aligned with USA-based studies regarding economic and diagnostic outcomes. In Spain, Simarro *et al.* reported that NGS implementation was feasible and could be done at reasonable cost [27]. In Singapore, Tan *et al.* found that routine upfront NGS was cost-effective compared with sequential sequencing [17].

LBx versus TBx

Of the 25 studies included that compared LBx with TBx (both NGS and single-gene assays) [28–52], four were from the USA [32–35], five were from Europe [36–40], 12 were from Asia [41–52] and four were global [28–31]. The majority (88%) of diagnostic studies were observational. Among studies reporting demographic data (n = 16 studies with a range of 102–1026 patients), the median age of patients ranged from 57 to 70 years (n = 11), and 32–70% of patients were male (n = 16) [32–38,40,45–51].

Three studies comparing economic outcomes between LBx and TBx were a cost study by Arnaud *et al.* from the USA [53], a cost–consequence analysis from Italy by Gancitano *et al.* [54] and a Canadian cost–effectiveness and budget impact analysis by Ontario Health [55].

Diagnostic evidence

Measures of diagnostic accuracy, including sensitivity, concordance, positive predictive values (PPVs) and negative predictive values (NPVs), for LBx versus TBx in detecting clinically actionable genes were examined across studies (Table 3).

The range of specificity values reported across mutations and across studies was 42.5–100%, with 11 of 17 studies reporting a specificity ≥90% for all tests [28,30–33,35–37,40–43,47–49,51,52]. Specificity values <90% for $EGFR_{T790M}$ were observed in two of two studies reporting specificity specifically for this variant [28,31]. Sensitivity values varied widely across mutations and across studies (range: 0–100%), and 14 of 18 studies reported sensitivity of ≥60% for all tests [28,30–33,35–37,40–43,47–52].

Concordance rates using LBx to detect targetable mutations were reported in 18 studies [28–30,34–43,45–49]. Concordance rates for all mutations tested were ≥70% in 16 studies and >90% in seven [28–30,35–37,39–43,45–49].

Table 2. Details of studies reporting the economic impact of next-generation sequencing (base-case analysis) in advanced non-small-cell lung cancer.

Study (year)	Country	Study design	Test vs comparator	Biomarker tests	Cost year	Study results	Ref.
Steuten (2019)	USA	Cost–effectiveness analysis	MGPS vs SMGT	*EGFR, ALK, BRAF, RET, ROS1, HER2, MET*	2017	MGPS vs SMGT ICER: US$148,478 per LYG LYs: 1.2 vs 1.14 Incremental cost: US$67,110 vs US$58,297	[19]
Pennell (2019)	USA	Budget impact analysis†	NGS vs sequential testing, exclusionary testing and hotspot panel testing	PD-1/PD-L1, *EGFR, ALK, ROS1, KRAS, MET, RET, NTRK1, BRAF*	2017	CMS (2066 patients) Cost savings for NGS vs: • Exclusionary testing: US$1,393,678 • Sequential testing: US$1,530,869 • Hotspot panel testing: US $2,140,795 Commercially insured (156 patients) Cost savings for NGS vs: • Exclusionary testing: US$3809 • Sequential testing: US$127,402 • Hotspot panel testing: US$250,842	[20]
Yu (2018)	USA	Budget impact analysis‡	NGS vs single-gene testing	*EGFR, ALK, ROS1, BRAF, MET, HER2, RET*	2016	Budget increase for NGS vs single-gene testing in 1-million-member plan model: • Over 5 years: US$432,554 • PMPM: US$0.0072	[21]
Dalal (2017)	USA	Cost–consequence analysis	Sequential testing Exclusionary mutation (i.e., *KRAS*) testing followed by sequential *BRAF* testing Upfront NGS, including *BRAF* testing	$BRAF_{V600E}$	NR	CMS reimbursement: • NGS cost: US$623 • Cost saving: US$980 vs sequential and mutations panel • Cost saving: US$1238 vs exclusionary strategy Sensitivity analysis (based on amounts reimbursed by third-party payers [commercial claims data]): • NGS (US$2860) remained the least expensive option by US$894–1044	[22]
Lopes (2020)	USA	Cost–effectiveness analysis	ctDNA NGS vs SoC (no additional genomic testing)	NSCLC with incomplete tissue genotyping	NR	Conservative drug costs: • ctDNA NGS increased the number of patients who received guideline-adherent treatment decisions • Clinical outcomes improved and were accompanied by meaningful cost savings • Per-patient cost savings were US$1943 Patients receiving NGS had, on average, an increased: • RR of 7.57% • PFS of 0.75 month • OS of 1.24 months	[23]
Ho (2019)	Brazil	Cost–effectiveness analyses	NGS panel of ctDNA vs ctDNA *EGFR* testing	*EGFR, ALK, ROS1, BRAF*	NR	Annual cost savings for NGS: *1. EGFR* and *ALK*: -4138.67 BRL$ *2. EGFR, ALK* and *ROS*: -6245.10 BRL$ 3. *EGFR, ALK, ROS-1* and *BRAF*: -5720.48 BRL$ ICERs§: 1: -15,595.77 (BRL$/PFS) 2: -21,399.29 (BRL$/PFS) 3: -18,006.42 (BRL$/PFS)	[24]

†Budget impact was calculated for a hypothetical 1,000,000-member health plan, with an expected 2066 Medicare-insured and 156 commercially insured testing-eligible patients with metastatic NSCLC.
‡Budget impact was calculated for a hypothetical 1,000,000-member health plan, with an expected 316 testing-eligible patients with advanced NSCLC.
§ICERs reported in congress abstract. Units of PFS were not explicitly reported in the abstract. The horizon time for the model was 1 year.
CMS: Centers for Medicare & Medicaid Services; ctDNA: Circulating tumor DNA; ddPCR: Droplet digital polymerase chain reaction; FISH: Fluorescence *in situ* hybridization; ICER: Incremental cost–effectiveness ratio; LY: Life-year; LYG: Life-year gained; MGPS: Multigene panel sequencing; NGS: Next-generation sequencing; NR: Not reported; NSCLC: Non-small-cell lung cancer; OS: Overall survival; PFS: Progression-free survival; PMPM: Per member per month; RR: Response rate; SMGT: Single-marker genetic testing; SoC: Standard of care.

Table 2. Details of studies reporting the economic impact of next-generation sequencing (base-case analysis) in advanced non-small-cell lung cancer (cont.).

Study (year)	Country	Study design	Test vs comparator	Biomarker tests	Cost year	Study results	Ref.
Ferreira (2018)	Brazil	Cost–effectiveness analyses	NGS vs sequential testing (RT-PCR and ddPCR)	*EGFR*$_{T790M}$	NR	ICER (per positive T790M detected): Plasma PCR then tissue NGS if plasma negative vs plasma NGS + tissue NGS: US$21,193.66	[25]
Schluckebier (2017)	Brazil	Cost–effectiveness analyses	NGS vs diagnostic tests (RT-PCR and FISH)	*EGFR, ALK, ROS1*	2016	ICER (per correct case detected): NGS vs sequential: US$3381.82	[26]
Simarro (2019)	Spain	Cost analyses	NGS with Oncomine solid tumor (ThermoFisher) vs conventional methods	*EGFR, ALK, ROS1*	NR	Cost for NGS: Total: €3369.84 Per sample: €421.23 Cost for conventional: Total: €2941.27 Per sample: €367.66	[27]
Tan (2020)	Singapore	Cost–effectiveness analyses	Targeted NGS panels (sequential singleplex, sequential multiplex and NGS-only testing) vs traditional assay (SoC)	*EGFR, ALK, ROS1, MET, RET*	2018	Cost per patient: SoC only: SGD$ 2224.6 Sequential (singleplex): SGD$ 1469.2 NGS only: SGD$ 1579.4 Sequential (multiplex): SGD$ 2622.7 ICER (per percent increase in patients on targeted therapy): Sequential (singleplex): Dominant NGS only: SGD$ 110 Sequential (multiplex): SGD$ 261	[17]

†Budget impact was calculated for a hypothetical 1,000,000-member health plan, with an expected 2066 Medicare-insured and 156 commercially insured testing-eligible patients with metastatic NSCLC.
‡Budget impact was calculated for a hypothetical 1,000,000-member health plan, with an expected 316 testing-eligible patients with advanced NSCLC.
§ICERs reported in congress abstract. Units of PFS were not explicitly reported in the abstract. The horizon time for the model was 1 year.
CMS: Centers for Medicare & Medicaid Services; ctDNA: Circulating tumor DNA; ddPCR: Droplet digital polymerase chain reaction; FISH: Fluorescence *in situ* hybridization; ICER: Incremental cost–effectiveness ratio; LY: Life-year; LYG: Life-year gained; MGPS: Multigene panel sequencing; NGS: Next-generation sequencing; NR: Not reported; NSCLC: Non-small-cell lung cancer; OS: Overall survival; PFS: Progression-free survival; PMPM: Per member per month; RR: Response rate; SMGT: Single-marker genetic testing; SoC: Standard of care.

In ten studies reporting PPV for LBx versus TBx, the range of PPVs for clinically actionable mutations was 77.8–100% [32,34–37,41,45,47,48,51].

In two USA-based studies, 100% PPV was reported for all clinically actionable mutations except *EGFR*$_{T790M}$ (79%) [32,35]. In seven studies that reported NPV of LBx versus TBx, the range of NPVs for clinically actionable mutations was 52–98% [34,35,41,45,47,48,51]. Faster turnaround times were reported for LBx versus TBx (range across three studies: 2–10 vs 5–25 days) [29,32,35].

Economic impact

Three economic studies found that incorporating LBx into the treatment pathway was associated with lower testing cost per patient (Table 4) [53–55].

A USA-based study conducted from a Medicare reimbursement perspective compared the clinical costs and complications of LBx with TBx (computed tomography [CT]-guided biopsy and navigational bronchoscopy) in a hypothetical biomarker case that tested for *EGFR*, *KRAS*, *ALK* and *BRAF* mutations [53]. The authors found that LBx was significantly less expensive, having a lower total cost of biopsy and biomarker testing (US$836) compared with CT-guided biopsy (US$4130) and navigational bronchoscopy (US$8284). Overall, LBx was associated with cost reductions of ≥US$3200 per patient. LBx also reduced time from screening to treatment and was associated with fewer complications for patients compared with CT-guided biopsy or navigational bronchoscopy, which was associated with pneumothorax, lung collapse, hemorrhage and respiratory distress in some patients.

A cost–consequence analysis was performed in a hypothetical cohort with *EGFR* mutations in Italy [54]. This model compared three different diagnostic pathways: TBx (for first- and second-line treatment), combined (TBx for first line and LBx for second line if the outcome was unknown) and potential (TBx or LBx for tissue-ineligible patients as first line and LBx as second line). The potential pathway provided the greatest number of correctly identified cases. The average cost per correctly identified case in the potential pathway (€685) was lower than for the combined pathway (€732) or the TBx pathway (€1004). Overall, the addition of LBx was associated with a cost reduction of approximately €300 per patient in this setting. These authors concluded that a correct diagnostic

Table 3. Diagnostic accuracy of liquid biopsy versus tissue biopsy for clinically actionable mutations in patients with non-small-cell lung cancer.

Study name[†]	Country	Study design	Intervention/assay[‡]	Sample size	Specificity %	Sensitivity %	Concordance %	PPV %	NPV %	Ref.
Goldman (2018)	Global (USA, Poland, France, Australia)	Non-randomized trial	Tumor (*EGFR* exon 19 deletions) vs plasma	475	97.2[§]	84.6[¶]	88.8	NR	NR	[28]
			Tumor ($EGFR_{L858R}$) vs plasma		97.7[§]	81[¶]	92.7	NR	NR	
			Tumor ($EGFR_{T790M}$) vs plasma		42.5[§]	80.9[¶]	77.0	NR	NR	
Tu (2019)	Global (USA, Australia)	Prospective observational	Concordance between plasma and tissue NGS (*ROS1, RET, BRAF, MET* and *HER2*)	399	NR	NR	94.7	NR	NR	[29]
Schwartzberg (2018)	Global[#]	Prospective observational	*EGFR* alterations	140 (LBx), 117 (concurrent TBx)	100[§]	76.7[¶]	94	NR	NR	[30]
			ALK alterations		NR	NR	95.7	NR	NR	
Zhou (2017)	Global (Australia, China, Korea)	Single-arm trial	$EGFR_{T790M}$ (ddPCR)	249	73	56	NR	NR	NR	[31]
			$EGFR_{L858R}$ (ddPCR)		99	62	NR	NR	NR	
			EGFR exon 19 deletions (ddPCR)		98	66	NR	NR	NR	
			$EGFR_{T790M}$ (Cobas plasma)	240	83	42	NR	NR	NR	
			$EGFR_{L858R}$ (Cobas plasma)		100	65	NR	NR	NR	
			EGFR exon 19 deletions (Cobas plasma)		97	86	NR	NR	NR	
			$EGFR_{T790M}$ (SuperARMS)	249	78	49	NR	NR	NR	
Sacher (2016)	USA	Prospective observational	*EGFR* exon 19 deletion	50	100	82	NR	100	NR	[32]
			$EGFR_{L858R}$	32	100	74	NR	100	NR	
Li (2019)	USA	Prospective observational	Confirmed mutation status of actionable alternations (*EGFR, KRAS, ALK, MET, ERBB2, BRAF, ROS1* and *RET*)	91	100	75	NR	NR	NR	[33]
Thompson (2016)	USA	Prospective observational	*EGFR* variants; ≤2 weeks between LBx and TBx	50	NR	NR	100	NR	NR	[34]
			EGFR variants; ≤2 months between LBx and TBx		NR	NR	92	NR	NR	
			EGFR variants; ≤6 weeks between LBx and TBx		NR	NR	94	NR	NR	
			EGFR variants; >6 weeks between LBx and TBx		NR	NR	60	NR	NR	
Leighl (2019)	USA	Prospective observational	cfDNA vs tissue; *EGFR* exon 19 deletion	282	100	81.8	98.2	100	98	[35]
Remon (2019)	France	Prospective observational	Core gene variants (36-gene panel, including *EGFR, KRAS, ALK, MET, ERBB2, BRAF* and *ROS1*)	35 (tissue and liquid), 8 (tissue), 10 (liquid)	96.7	81.4	95.2	77.8	97.6	[36]

[†]Studies with available specificity, sensitivity, concordance, PPV and/or NPV data are shown.
[‡]Summary outcomes and/or outcomes associated with key clinically actionable mutations are shown. One study from China (Cao, 2020) identified in the literature review did not report quantitative sensitivity or specificity data [44].
[§]Reported as negative percent agreement.
[¶]Reported as positive percent agreement.
[#]The geographic location of the patient population was not clear; the study was categorized as global based on the authors having affiliations with institutions in multiple countries.
ARMS: Amplification-refractory mutation system; BALF: Bronchoalveolar lavage fluid; cfDNA: Cell-free DNA; ctDNA: Circulating tumor DNA; EV: Extravesicular; FFPE: Formalin-fixed paraffin-embedded; LBx: Liquid biopsy; NGS: Next-generation sequencing; NPV: Negative predictive value; NR: Not reported; NSCLC: Non-small-cell lung cancer; PPV: Positive predictive value; TBx: Tissue biopsy; TKI: Tyrosine kinase inhibitor.

Table 3. Diagnostic accuracy of liquid biopsy versus tissue biopsy for clinically actionable mutations in patients with non-small-cell lung cancer (cont.).

Study name†	Country	Study design	Intervention/assay‡	Sample size	Specificity %	Sensitivity %	Concordance %	PPV %	NPV %	Ref.
Papadopoulou (2019)	Greece	Prospective observational	Clinically significant genes (23-gene panel, including *EGFR, KRAS, ALK, MET, ERBB2, BRAF* and *MET*)	121	88.24	84.21	86.11	88.89	NR	[37]
Minari (2020)	Italy	Retrospective observational	$EGFR_{T790M}$	52	NR	NR	46.1	NR	NR	[38]
Spasic (2019)	Serbia	Prospective observational	*EGFR* mutation testing	104	NR	NR	93	NR	NR	[39]
Mayo de Las Casas (2017)	Spain	Prospective observational	Serum cfDNA vs tissue (*EGFR*)	268	100	57	90	NR	NR	[40]
			Plasma cfDNA vs tissue (*EGFR*)		100	70	90	NR	NR	
Mok (2015)	China	RCT	Overall *EGFR* mutation positive	96	96	75	88	94	85	[41]
Yang (2018)	China	Prospective observational	ctDNA samples (*EGFR*)	114	77	59	70	NR	NR	[42]
Ma (2016)	China	Prospective observational	*EGFR* mutation	129	97	60	NR	93	78	[34]
Zhang (2017)	China	Prospective observational	Overall (*EGFR*)	106	100	94.87	99.06	NR	NR	[43]
Zhang (2018)	China	Prospective observational	$EGFR_{L588R}$ (biopsy vs plasma DNA)	62	NR	NR	82	NR	NR	[45]
			EGFR exon 19 deletions (biopsy vs plasma DNA)	68	NR	NR	75	NR	NR	
			$EGFR_{T790M}$ (biopsy vs plasma DNA)	3	NR	NR	100	NR	NR	
			All *EGFR* mutations (biopsy vs plasma DNA)	NR	NR	NR	NR	100	51.7	
He (2017)	China	Prospective observational	$EGFR_{L858R}$	58	NR	NR	83	NR	NR	[46]
			EGFR exon 19 deletion	48	NR	NR	72	NR	NR	
			EGFR+	106	NR	NR	78	NR	NR	
			$EGFR_{T790M}$	26	NR	NR	100	NR	NR	
Veldore (2018)	India	Prospective observational	Overall *EGFR* mutated	45	100	91.11	96.97	100	95.6	[47]
Wulandari (2020)	Indonesia	Prospective observational	*EGFR* ctDNA	124	90.9	48.3	70.97	82.35	66.67	[48]
Takahama (2016)	Japan	Prospective observational	TKI-sensitizing mutations (*EGFR*)	41	87.5	75.8	78	NR	NR	[49]
Itotani (2019)	Japan	Prospective observational	cfDNA gene mutation (*EGFR, KRAS, BRAF, ERBB2, MET, ALK, RET* and *ROS1*)	121	NR	75§	NR	NR	NR	[50]
Hur (2019)	South Korea	Prospective observational	BALF EV-based *EGFR* genotyping, overall	137	86.7	75.9	NR	78.8	84.7	[51]

†Studies with available specificity, sensitivity, concordance, PPV and/or NPV data are shown.
‡Summary outcomes and/or outcomes associated with key clinically actionable mutations are shown. One study from China (Cao, 2020) identified in the literature review did not report quantitative sensitivity or specificity data [44].
§Reported as negative percent agreement.
¶Reported as positive percent agreement.
#The geographic location of the patient population was not clear; the study was categorized as global based on the authors having affiliations with institutions in multiple countries.
ARMS: Amplification-refractory mutation system; BALF: Bronchoalveolar lavage fluid; cfDNA: Cell-free DNA; ctDNA: Circulating tumor DNA; EV: Extravesicular; FFPE: Formalin-fixed paraffin-embedded; LBx: Liquid biopsy; NGS: Next-generation sequencing; NPV: Negative predictive value; NR: Not reported; NSCLC: Non-small-cell lung cancer; PPV: Positive predictive value; TBx: Tissue biopsy; TKI: Tyrosine kinase inhibitor.

Table 4. Details of studies reporting the economic evidence for liquid biopsy in advanced non-small-cell lung cancer.

Study (year)	Country	Study design	Test and comparator	Mutations	Cost year	Study results	Ref.
Arnaud (2016)	USA	Cost analyses	• Tissue-based biopsy: CT-guided fine-needle aspiration • Tissue-based biopsy: electromagnetic navigational bronchoscopy) • Blood-based GeneStrat test	*EGFR* sensitizing and resistance, *ALK*, *KRAS* and *BRAF*	NR	CT-guided fine-needle aspiration: • Procedure Medicare costs: US$3253.52 • Pathology cost: US$876.57 • Total cost of biopsy and biomarker testing: US$4130.09 Electromagnetic navigational bronchoscopy: • Procedure Medicare costs: US$7407.05 • Pathology cost: US$876.57 • Total cost of biopsy and biomarker testing: US$8283.62 GeneStrat: • Procedure Medicare costs: US$3.00 • Pathology cost: US$833.45 • Total cost of biopsy and biomarker testing: US$836.45	[53]
Ontario Health (2020)	Canada	Cost/cost–effectiveness/ budget impact analyses	• LBx as a triage test • LBx alone • TBx	$EGFR_{T790M}$	2018	Estimated costs: • LBx: CAN$700 • TBx: CAN$2500 Long-term cost of treatment and care: • LBx as a triage test was the most effective and most costly strategy, followed by LBx alone • ICERs of LBx as a triage test compared with LBx alone and of LBx alone compared with TBx alone were >CAN$100,000 per QALY Budget impact of LBx as a triage test: • Approximately CAN$60,000 in Year 1 to CAN$3 million in Year 5 in treatment costs	[55]
Gancitano (2018)	Italy	Cost–consequence analyses	• Tissue pathway • Combined pathway† • Potential pathway‡	*EGFR*	NR	Costs per correctly identified case: • The potential pathway had the greatest number of correctly identified cases and the lowest average cost per correctly identified case • Tissue pathway: €1004 • Combined pathway: €732 • Potential pathway: €685	[54]

† Combined pathway, first line: TBx, if outcome is unknown proceed with LBx; second line: LBx, if outcome negative proceed with TBx.
‡ Potential pathway, first line: TBx and LBx for tissue-ineligible patients, if outcome of TBx is unknown proceed with LBx; second line: LBx, if outcome is negative proceed with TBx.
CT: Computed tomography; ICER: Incremental cost–effectiveness ratio; LBx: Liquid biopsy; NR: Not reported; QALY: Quality adjusted life year; TBx: Tissue biopsy.

pathway is essential to optimize cancer therapies. This analysis also highlights the value of upfront LBx in the diagnostic pathway for both first- and second-line treatment.

A recent Canadian Health Technology Assessment reported the cost–effectiveness of TBx alone, LBx alone or LBx as a triage test in a cohort with *EGFR*$_{T790M}$ mutations [55]. LBx alone or as a triage test was less costly and more effective (i.e., resulted in fewer tissue biopsies and more correct decisions) than TBx in terms of test-related costs and effects only. With regard to lifetime costs, LBx as a triage test was the most effective and produced the most life-years and quality-adjusted life-years, but it was the costliest of the three options. However, this was largely driven by higher long-term treatment cost as more patients were correctly identified to receive targeted therapy [55].

Discussion

This targeted literature search conducted from January 2015 to March 2020 identified studies reporting diagnostic and economic aspects of NGS versus single-gene testing and LBx versus TBx. The studies were global, with most conducted in North America (including the USA and Canada), followed by Europe and Asia. Overall, the studies suggest that NGS is highly concordant with conventional molecular testing in patients with NSCLC. Two studies reporting <90% concordance (55.6% [14] and 82% [37]) between NGS and single-gene tests used specialized single-gene tests for *EGFR* testing, and the discordances (missed by NGS) were mainly in the low mutant allelic fractions. While less sensitive than conventional methods, NGS resulted in broader genomic coverage, which may reveal diverse mechanisms of resistance among patients with advanced NSCLC.

NGS can also measure tumor mutational burden (TMB), an emerging biomarker to select patients for immunotherapy, and will likely need to be used in conjunction with PD-L1 immunohistochemistry [5]. The sequencing of targeted therapies and immunotherapies as recommended in treatment guidelines will continue to evolve as the treatment landscape changes, but clinicians may make use of the wider range of genetic information available with NGS to facilitate the selection of the right therapy for an individual patient. The potential role of additional genetic screening – via NGS or single-gene testing – in a patient whose disease develops resistance to initial therapy needs to be clarified in future studies.

In terms of cost and cost–effectiveness, NGS leads to a greater proportion of patients assigned to targeted therapy and increased life-years gained while being cost neutral or cost saving. NGS was generally found to be cost-effective at typical thresholds. With current treatment guidelines recommending targeted therapies for eight specific genetic biomarkers plus additional recommendations based on PD-L1 and TMB status [5], the additive costs of multiple single-gene tests should be considered. This review provides indirect evidence on the question of how the costs of NGS compare with those of multiple single-gene tests, and future studies on the costs of biomarker testing in NSCLC may provide clearer evidence.

In all studies, concordance between LBx and TBx for all mutations tested was generally high (≥70%), with six studies reporting >90% concordance. LBx exhibits high specificity to detect targetable mutations in patients with NSCLC, but it may have lower sensitivity than TBx. Overall, the LBx studies reported shorter turnaround times from blood sample collection to report delivery compared with TBx. The faster turnaround time and high PPVs of LBx enable faster treatment decisions in patients with NSCLC who have targetable mutations. LBx may also provide additional genetic material for subsequent testing at the time when a patient develops resistance to initial therapy, limiting invasive procedures and potentially improving patient experience and outcomes. Upfront cost savings may be achieved using LBx as an initial screening method in complement to TBx, although identification of a greater number of cases may lead to increased treatment costs.

Several limitations to this review should be noted. This review includes only English-language papers published as journal articles in the last 5 years. While unpublished or non-English-language studies may contain valid results that may conflict with the conclusions of this review, the broad search strategy used here and the large number of citations screened make this unlikely. The review excludes diagnostic studies with small sample size (≤100 patients); however, these are likely to be exploratory studies that could introduce bias. Additional parameters that inform the quality of biomarker testing (e.g., test failure rate) were not included in the data extraction. Only three economic evaluations were found comparing LBx and TBx, suggesting that data may be limited in this area.

Future perspective

The findings of our review may have implications regarding recommendations for the timing of LBx and NGS in future NSCLC treatment guidelines. Among studies included in this review, the results of NGS and conventional single-gene testing were highly concordant. Comparisons of TBx and LBx indicated that these techniques also

generally have high concordance. NGS and LBx separately showed benefits in terms of correctly identifying more patients for targeted therapy, enabling faster turnaround and quicker treatment decisions. In terms of cost and cost–effectiveness, these methods were associated with reductions in short-term treatment-related costs. In the long term, increased use of NGS could result in a minimal increase of budget largely driven by more patients receiving targeted treatment.

Executive summary

Concordance & turnaround time for next-generation sequencing versus standard molecular testing

- High concordance was found between next-generation sequencing (NGS) and single-gene testing methods.
- Turnaround times were longer for NGS versus single-gene testing, but not longer versus sequential testing.

Economic impact of NGS versus standard molecular testing

- NGS was found to be cost-effective in the USA in identifying patients with non-small-cell lung cancer with targetable mutations.

Concordance & turnaround time for liquid biopsy versus tissue biopsy

- Liquid biopsy has high specificity but lower sensitivity for targetable mutations than tissue biopsy.
- Turnaround times were faster with liquid biopsy versus tissue biopsy.

Economic impact of liquid biopsy versus tissue biopsy

- Liquid biopsy was found to reduce per-patient costs in the USA and may be cost-effective as an initial screening method.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/fon-2021-1040

Author contributions

Y Zheng, H Vioix and F Liu contributed to conceptualization, methodology, formal analysis and writing of this manuscript. B Singh, S Sharma and D Sharda contributed to the methodology, investigation, data curation, formal analysis and writing of this manuscript.

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