ACE BRIEF FOR NEW AND EMERGING HEALTH TECHNOLOGIES

Guardant360 CDx for Patients with Locally Advanced or Metastatic Non-Small Cell Lung Cancer

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Summary of Key Points

- Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer diagnoses, with 50% of patients in stage III (locally advanced) or IV (metastatic) NSCLC at diagnosis. Notably, targetable driver mutations are found in a substantial proportion of NSCLC.
- At present, tissue-based testing is the gold standard to genotype tumours for actionable mutations. It is limited by its invasive nature, sampling failure, patient selection and the presence of spatiotemporal tumour heterogeneity.
- Guardant360 CDx (G360; Guardant Health, Inc.) is a liquid biopsy test that detects genetic mutations in circulating tumour DNA through a less invasive manner while overcoming limitations of tissue-based tests. It is intended for use as a companion diagnostic (CDx) to identify patients with NSCLC with specific mutations who may benefit from treatment with targeted therapy. It may also be used as a comprehensive genomic profiling tool for all solid malignant neoplasms.
- Given the disease burden and high rate of point mutations in NSCLC among other cancers, along with international NSCLC guidelines supporting the use of liquid biopsy, this brief focused on the use of G360 for patients with NSCLC.
- Overall, G360 was found to be safe and effective.
    - No major safety concerns were expected.
    - Compared to tissue-based tests, G360 had a high specificity (≥86.9%) with a moderate sensitivity (≥54%). This should be interpreted in view of the limitations of tissue-based tests as reference standard.
    - Multiple studies showed that complementary testing with G360 improved turnaround time, increased detection of driver mutations by 15% to 65%, guided a shift to targeted therapy in 9.8% to 44% of patients with similar clinical outcomes in terms of response rates, overall and progression-free survival compared to tissue-guided treatments.
    - Besides patient benefit, the test may potentially benefit the healthcare system by improving resource utilisation and decentralising biopsy services.
- However, the results are limited by the applicability of findings to low-prevalence mutations and varying clinical judgement in interpreting results and initiating treatment plans across studies.
- There is uncertainty on the cost-effectiveness of G360 as a CDx to guide therapy selection in the treatment of NSCLC. While it may potentially reduce the use of inappropriate treatment regimens in some patients, the use of other high-cost targeted therapies with locally advanced or metastatic NSCLC may drive up costs.
- G360 was found to be priced between S$4,174 to S$6,803.
- Key implementation considerations include infrastructure requirements, shift in testing model and staff training.
- There are various similar liquid biopsy technologies in ongoing development. A number of ongoing trials investigating G360 are expected to be completed in the next two to three years.

I. Background

Lung cancer is a heterogeneous disease with wide-ranging clinicopathological characteristics.[1] It is broadly classified as non-small cell lung cancer (NSCLC) or small cell lung cancer, of which NSCLC accounts for around 85% of all lung cancer diagnoses.[1] The severity of NSCLC is classified based on stages, including early (stages I and II), locally advanced (stage III) and metastatic (stage IV) NSCLC, with around 50% of patients in stage III or IV NSCLC at time of diagnosis.[2,3]

In Singapore, lung cancer is the third most common cancer with 8,292 new cases reported from 2015 to 2019.[4] During this period, lung cancer accounted for the highest (25.6%) and third highest (15.5%) cancer mortality in males and females, respectively.[4] Patients with lung cancer experience a poor prognosis with a five-year age-standardised relative survival rate of 12.2% for males and 29.3% for females.[4] Symptoms of lung cancer usually manifest in later stages, including respiratory symptoms or symptoms related to common metastatic sites including the brain, adrenal glands and liver.[5]

In particular, targetable driver mutations are found in a substantial proportion of NSCLC and represent attractive targets for therapeutic interventions.[6] Oncogenic driver mutations associated with NSCLC include epidermal growth factor receptor (*EGFR)*, anaplastic lymphoma kinase (*ALK*), ROS proto-oncogene 1 (*ROS1*) and Kristen rat sarcoma (*KRAS*).[7] Globally, the frequency of *EGFR* mutation in patients with NSCLC remains the highest in the Asia-Pacific region, which accounts for 40% of patients with NSCLC in Singapore.[8] Other notable mutations in NSCLC include *KRAS* and *ALK*, with a frequency of 10% and 5% in the Asian population.[9,10]

The emergence of novel targeted therapies in NSCLC, although promising, depends on the detection of clinically relevant targetable driver mutations. At present, tissue-based biomarker assessment remains the gold standard for genetic tumour profiling despite various limitations. These include its invasive nature, substantial failure rates, patient ineligibility and inaccurate sampling arising from spatial and temporal tumour heterogeneity.[11] Together, this may lead to missed diagnostic and subsequent treatment opportunities. A simple, less invasive and more comprehensive approach to detect genetic alterations (GAs) would be beneficial to address this clinical gap.

II. Technology

Guardant360 CDx (G360; Guardant Health, Inc.) is a liquid biopsy test that detects genetic mutations in circulating tumour DNA (ctDNA) through next-generation sequencing (NGS) using a high throughput hybridisation-based capture technology. The test process involves whole blood collection and shipping to a laboratory, plasma isolation and cell free DNA extraction, library preparation and enrichment, followed by DNA sequencing, data analysis and reporting. G360 is able to sequence 74 genes and report on 55 genes across several classes of GAs, including single nucleotide variations (SNVs), indels, copy number amplifications (CNAs) and genomic fusions or rearrangements (Table A1 in Appendix A).[12]

There are two intended uses of G360. It is used as a companion diagnostic (CDx) to identify patients with NSCLC harbouring specific driver mutations who may benefit from treatment

with targeted therapy as summarised in Table 1. In addition, G360 can provide comprehensive genomic profiling (CGP) to be used by qualified healthcare professionals for patients with solid malignant neoplasms.

Table 1: Intended uses of Guardant360 CDx

Indication	Biomarker(s)	Therapy
Non-small cell lung cancer (NSCLC)	*EGFR* exon 19 deletion, L858R, T790M	Osimertinib
	EGFR exon 20 insertion	Amivantamab-vmjw
	KRAS G12C	Sotorasib
All solid malignant neoplasms, including NSCLC	SNVs and indels in 55 genes, CNAs in 2 genes, fusion in 4 genes	—
Abbreviations: CNA, copy number amplification; EGFR, Epidermal Growth Factor Receptor; KRAS, Kristen Rat Sarcoma Viral Oncogene Homolog; NSCLC, non-small cell lung cancer; SNV, single nucleotide variation.		

Although G360 can be used for multiple cancer types, this brief focused on its use for patients with NSCLC. This is based on the high disease burden of NSCLC and its highest rate of point mutations amongst other cancer types.[13] In addition, various international guidelines, including those published by the National Comprehensive Cancer Network (NCCN), International Association for the Study of Lung Cancer (IASLC) and the American Society of Clinical Oncology (ASCO), have recommended the use of liquid biopsy for patients with NSCLC.[14-16]

Tumour genotyping with liquid biopsy obviates limitations presented with tissue biopsy and expands precision oncology to patients who are previously ineligible as a result of the barriers associated with tissue sampling.[11] Compared to tissue biopsy, liquid biopsies are less invasive and only require a small sampling of blood. This reduces procedural risk to patients and cost of sample collection in comparison with surgical biopsies. In addition, liquid biopsies overcome the spatial limitations of tissue biopsy, allowing serial testing to evaluate cancer progression to better inform treatment decisions.[17] However, it should be noted that liquid biopsy may be limited by low ctDNA shedding arising from low total body tumour burden, low extrathoracic metastatic spread, or the involvement of sanctuary sites such as the brain.[16] Moreover, liquid biopsy does not provide information on histological subtypes and histological changes. As such, liquid biopsy may substitute tissue testing in certain situations, while in other circumstances, tissue testing may still be preferred. Regardless, results from both liquid biopsy and tissue testing may be complementary.

III. Regulatory and Subsidy Status

G360 received a Breakthrough Device Designation from the US Food and Drug Administration (FDA) in 2018 and was approved by the FDA in 2021 for the intended uses listed in Table 1. It was also granted the CE mark in March 2021 for use as a CGP tool for all solid neoplasms and as a CDx to identify patients with NSCLC harbouring the *EGFR* Ex19del, L858R or T790M mutations who may benefit from treatment with osimertinib. In the United States, reimbursement for G360 is provided by Medicare, as well as several private payers including Cigna and the Blue Shield of California.

Of the three targeted therapies listed in Table 1, osimertinib has been approved by the Health Sciences Authority (HSA). In patients with locally advanced or metastatic NSCLC, it is indicated

for use as a first-line treatment for *EGFR* Ex19del or L858R mutations, or for the treatment of patients with *EGFR* T790M mutation who disease has progressed on or after *EGFR* tyrosine kinase inhibitor (TKI) therapy. Osimertinib is also indicated for adjuvant treatment after tumour resection in patients with NSCLC harbouring the *EGFR* Ex19del or L858R mutations. In addition, osimertinib is eligible for subsidy under the Medication Assistance Fund for the treatment of patients with locally advanced or metastatic *EGFR* T790M mutation-positive NSCLC whose disease has progressed on or after *EGFR*-TKI therapy.

IV. Stage of Development in Singapore

The G360 assay is currently under review by HSA and is locally available through Guardant Health AMEA as a laboratory developed test. Clinicians may request for a kit from the company and the plasma samples will be couriered to the Guardant Health laboratory in the United States for testing to be performed. In addition, the assay is locally investigated in a trial conducted at the National Cancer Centre Singapore (ClinicalTrials.gov identifier: NCT04087473).

☐	Yet to emerge	☐	Established
☒	Investigational / Experimental (subject of clinical trials or deviate from standard practice and not routinely used)	☐	Established *but* modification in indication or technique
☐	Nearly established	☐	Established *but* should consider for reassessment (due to perceived no/low value)

V. Treatment Pathway

Based on local practice, chemoradiotherapy is the standard treatment for patients with locally advanced unresectable stage III NSCLC. Systemic therapy, including conventional chemotherapy as well as targeted therapies and immunotherapies, is used in patients with stage IV metastatic NSCLC, and is recommended based on results of upfront broad-based genomic testing, particularly in non-squamous NSCLC. Of note, targeted therapies are used as a first-line treatment in patients with oncogene-driven NSCLC while chemotherapy and/or immunotherapy serves as a first-line option in patients with non-oncogene driven (wild-type) NSCLC. In these patients, eligibility for targeted therapies is conventionally determined by tissue-based genotyping (Personal communication: Oncologist from National Cancer Centre Singapore, 5 May 2022).

When integrated into local clinical pathways, plasma ctDNA testing can be used to complement tissue-based tests as recommended by the NCCN, IASLC and ASCO.[14,15] In brief, it may be used when patients are ineligible for invasive tissue sampling, or in the initial diagnostic setting when there is insufficient tissue material or incomplete tissue-based assessment of recommended biomarkers.[14] Besides, recent updates by the IASLC acknowledged plasma ctDNA as a valid tool for biomarker evaluation at the point of diagnosis while a plasma-first approach could be used to evaluate mechanisms of resistance and to

monitor treatment efficacy in real-time, with reflex to tissue testing due to uninformative plasma test result.[16,18] The National Institute of Health and Care Excellence (NICE) recommended that plasma ctDNA testing for *EGFR* may be performed before or in place of tissue testing.[19]

VI. Summary of Evidence

This assessment was conducted based on the Population, Intervention, Comparison and Outcome (PICO) criteria presented in Table 2. Literature search was conducted in PubMed and Embase, including pearling of retrieved publications. One HTA report[20] conducted by the Blue Shield of California was identified, which evaluated multiple liquid biopsy assays including G360. Of note, the HTA report consists of six comparative studies[21-26] and a FDA Summary of Safety and Effectiveness Data (SSED)[12] for G360. Furthermore, two additional FDA SSEDs[27,28] and seven comparative studies[29-35] reporting on clinical effectiveness were also included. Of the seven studies, one[31] pooled results across 11 studies. Additionally, four other non-comparative studies[11,36-38] were referenced as supplementary evidence for analytical accuracy and clinical utility. The evidence base, the inclusion and exclusion criteria were listed in Table B1 (Appendix B), while the study design and characteristics of the included studies were presented in Table B2 (Appendix B).

Table 2: Summary of PICO criteria

Population	Patients with locally advanced or metastatic non-small cell lung cancer
Intervention	Guardant360 CDx, alone or as an add-on to tissue-based test
Comparison	Tissue-based tests
Outcome	Safety, effectiveness (test accuracy and clinical utility) and cost effectiveness

Safety

The studies included did not report on adverse events related to the use of a liquid biopsy test. As liquid biopsy involves a phlebotomy procedure that is routinely performed, no major safety concern is expected. Compared to tissue biopsy, liquid biopsy presents a potentially better safety profile with the avoidance of invasive tissue sampling. However, there is a risk of false test results that may impact treatment decision, although this may not be easily quantified.

Effectiveness

Accuracy

The HTA report[20], five additional studies[11,30,33,36,39] and two FDA SSEDs[27,28] reported on the accuracy of the G360 test. Briefly, the test was technically accurate, with variant detection as low as 0.02% to 0.1% of variant allele frequency, good precision and analytical concordance with external assays (Table C1 in Appendix C).[11,12,27,28,36] In addition, the diagnostic accuracy of the G360 test was reported for clinically relevant biomarkers such as *EGFR*, *ALK*, *MET*, *KRAS*, *BRAF*, *ROS1* and *RET* across various studies[20,21,27,28,30,33,39], either individually or as a composite of biomarkers (Tables C2 and C3 in Appendix C). Compared to tissue-based tests, G360 generally showed a moderate to high sensitivity (range, 54% to 100%) and a consistently high specificity (range, 86.9% to 100%), except for the *EGFR* T790M variant (specificity, 67.1%; Table 3). This points to a high false positive rate in detecting T790M which was postulated to

be related to the limitations of tissue biopsy, where the T790M status of the tissue biopsied site may not represent the heterogenous mutational status of all tumours in the patient.[20] Nevertheless, guidelines by the College of American Pathologists, IASLC and the Association for Molecular Pathology, as well as NCCN, recommended the use of liquid biopsy to identify the T790M variant in patients with progression or resistance to *EGFR* TKIs, with tissue testing for negative liquid biopsy results.[20]

The positive and negative predictive values (PPV, NPV) varied for the composite biomarkers (PPV, 62.3% to 91%; NPV, 62.1% to 94.1%) and were consistently high for the individual biomarkers (PPV, 80% to 100%; NPV, 98.1% to 100%) where reported, although they should be interpreted with caution as they are affected by the prevalence of mutations which vary across demographic groups.

Table 3: Diagnostic accuracy of G360 compared to tissue-based genotyping for patients with NSCLC

Biomarkers	Comparison of G360 with tissue-based genotyping				
	Sensitivity	Specificity	PPV	NPV	Concordance
Composite biomarkers					
Composite of clinically relevant biomarkers	67% to 86.3%	86.9% to 92.9%	62.3% to 91%	62.1% to 94.1%	77.6% to 81.3%
Individual biomarkers					
EGFR variants	54% to 100%	67.1% to 100%*	100%	98% to 99.5%	98.2% to 99.6%
KRAS variants	71.6%	100%	—	—	88%
ALK fusion	62.5%	100%	100%	99%	99.1%
ROS1 fusion	—	100%	—	98.7%	98.7%
BRAF V600E	100%	100%	100%	100%	100%
*MET*ex14	80%	98.1%	80%	98.1%	96.5%
RET fusion	—	100%	—	100%	100%

Note: The full list of clinically relevant biomarkers and its corresponding diagnostic accuracy data were listed in Tables C2 and C3 in Appendix C.

* Specificity generally ranged between 90% to 100%, except for *EGFR* T790M where a specificity of 67.1% was reported.

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

In addition, G360 was found to be non-inferior to standard tissue-based tests in the detection of clinically relevant biomarkers (G360 *vs.* tissue, 27.3% *vs.* 21.3%; p<0.0001).[20,21] The test further increased the rate of driver mutations detected by 15% to 65% in patients with negative or failed tissue findings.[31,39] Similarly, in a tissue-first model, the use of G360 as an add-on test led to an increment of 12% to 33% of therapeutically targetable mutations detected (Table 4).[21,33] However, it remains ambiguous if a tissue- or plasma-first strategy would result in a greater initial biomarker discovery rate with conflicting results reported in the North American[21] and Korean[33] cohorts (Table 4). Nevertheless, these findings support the value-added benefit of complementary testing with both tissue and plasma tests. The HTA report concluded that using liquid biopsy (including G360) as a triage test to detect *EGFR* TKI-sensitising variants followed by reflex tissue-based test for patients with a negative plasma result would lead to a testing strategy with a sensitivity equivalent to tissue-based tests and a high overall specificity (95% to 100%).[20] This may lead to the avoidance of invasive tissue

biopsy in approximately two-thirds of patients with *EGFR* TKI-sensitising variants, especially in the Asian population with a relatively higher prevalence of *EGFR* mutations.[20]

Table 4: Percentage of guideline-recommended biomarkers detected based on a tissue-first *vs.* plasma-first approach

Study	N	Tissue *vs.* plasma first	Percentage of GAs detected	Incremental
Leighl et al. (2019)[21]	89	Tissue first	67.0%	33.0%
		Plasma first	87.0%	13.0%
Park et al. (2021)[33]	250*	Tissue (NGS) first	88.0%	12.0%
		Plasma first	60.4%	39.6%
	232†	Tissue (non NGS) first	73.3%	26.7%
		Plasma first	65.1%	34.9%

* Patients who had NCCN-recommended genomic biomarkers detected by either tissue-based NGS test (Oncomine Focus Assay) or plasma-based NGS test (G360).

† Patients who had NCCN-recommended genomic biomarkers detected by either standard-of-care testing (conventional tissue genotyping methods, excluding NGS) or plasma-based NGS test (G360).

Abbreviations: GA, genomic alteration; NGS, next-generation sequencing.

Clinical utility

As evident from the HTA report[20], seven additional studies[29-35] and two FDA SSEDs[27,28], G360 was found to improve genotyping and treatment time, influence treatment plans and impact health outcomes. Compared to tissue-based tests, G360 improved turnaround time to obtain genotyping results (median, 9 to 10 days *vs.* 11 to 20 days; Table 5).[21,29,34] Moreover, time to treatment was reported to be significantly faster with G360 than tissue-based tests (median, 18 *vs.* 31 days; p=0.0008).[32]

Table 5: Time to result and treatment with G360 compared to tissue-based tests in patients with NSCLC

Study	G360	Tissue-based tests	p-value
Median time to result			
Leigh et al. (2019)[21]	9 days	15 days	<0.0001
Bonanno et al. (2020)[29]	10 days (range, 4 to 29 days)	11 days (range, 1 to 31 days)	NR
Peled et al. (2020)[34]	9 days (range, 7 to 12 days)	20 days (range, 9 to 34 days)	NR
Median time to treatment			
Page et al. (2021)[32]	18 days	31 days	0.0008
Abbreviation: NR, not reported.			

Multiple studies also reported that G360 could guide treatment decisions in patients with advanced or metastatic NSCLC at different lines of therapy, including those at diagnosis,[30,32,34,39,40] upon progression[37,39,40] or those with unavailable or failed tissue-based testing,[33,35,38] with some studies[29,34,37] conducted in real-world settings. The use of G360 either alone or complementary to tissue-based test resulted in an overall switch to targeted therapy in 9.8% to 44% of patients (see Table C4 in Appendix C),[24,30,33,35,38-40] which was translated into improved patient health outcomes. Various studies[26,29,31,32,35], including a pooled analysis of 11 studies, reported favourable G360-guided overall survival (OS), progression-free survival (PFS), objective response rate (ORR) and disease control rate (see Table 6 and Table C5 in Appendix C). In particular, there were no meaningful difference in clinical outcomes such as OS, PFS and ORR from treatments guided by G360 or tissue-based tests in the FDA pivotal studies for patients with *ALK* or *ROS* fusions, *KRAS* G12C, *EGFR*

activating mutations or *EGFR* T790M-altered NSCLC, in line with findings by other studies[32,40] (see Table 7 and Figure C1 in Appendix C).[12,20,27,28] To further add, the HTA report concluded that a reflex testing strategy for *EGFR* TKI-sensitising variants with G360 should lead to similar outcomes to tissue testing and result in meaningful improvement in net health outcomes.[20] Notably, patients detected with driver mutations by G360 who did not receive targeted therapy had poorer outcomes than those who had a treatment switch (Table C6 in Appendix C).[24,29,35,38]

Table 6: Clinical outcomes of G360-guided treatment

Study	N*	Clinical endpoint	Clinical outcome
Mack et al. (2020)[31]	48†	ORR (95% CI)	68.8% (53.6% to 80.9%)
		DCR (95% CI)	93.8% (81.8% to 98.4%)
	82‡	ORR (95% CI)	58.5% (47.1% to 69.2%)
		DCR (95% CI)	86.6% (76.9% to 92.8%)
Zatarain-Barrón et al. (2021)[35]	24	DCR (95% CI)	85.7% (NR)
		Median OS (95% CI), months	40.3 (95% CI, 27.1 to 53.6)
		Median PFS (95% CI), months§	11.1 (95% CI, 7.6 to 14.6)
Page et al. (2021)[32]	33	DCR (95% CI)	94% (NR)
		ORR (95% CI)	58% (NR)
		EFS (95% CI) at 12 months	52% (NR)
Villaflor et al. (2016)[26]	8	Median PFS (95% CI), months#	11.5 (95% CI, 5.7 to 28.7)
Bonanno et al. (2020)[29]	16	Median OS (95% CI), months	Not reached (NE)^

Notes:

1. Results from Mack et al. (2020)[31] were pooled from 11 studies.
2. Outcomes from Page et al. (2021)[32], Villaflor et al. (2016)[26] and Bonanno et al. (2020)[29] were based on treatment guided by G360 and/or tissue testing.

* Patients who were evaluable; † First-line treatment; ‡ Second-line and beyond treatment; § Patients treated with TKIs; # Data available for 6 of 8 patients; ^ Based on a median follow-up of 11.7 months.

Abbreviations: DCR, disease control rate; EFS, event-free survival; NE, not evaluable; NR, not reported; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; TKI, tyrosine kinase inhibitor.

Table 7: Clinical response of G360-guided *vs.* tissue-guided treatment in the FDA pivotal studies

Study	Biomarker(s)	Treatment arm(s)	Diagnostic method	N	Clinical endpoint	Treatment response
FDA SSED[12] (FLAURA RCT)	*EGFR* Ex19del or L858R	Osimertinib *vs.* SOC *EGFR* TKI*	G360	304	PFS, HR† (95% CI)	0.41 (0.31 to 0.54)
			Tissue	556		0.46 (0.37 to 0.57)
FDA SSED[12] (AURA3 RCT)	*EGFR* T790M	Osimertinib *vs.* chemotherapy	G360	191		0.34 (0.22 to 0.53)
			Tissue	419		0.30 (0.23 to 0.41)
Helman et al. (2018)[22] (TIGER-X and TIGER-2 studies)		Rociletinib	G360	63	ORR#, % (95% CI)	28.6 (17.9 to 41.3)
			Tissue	77		29.9 (20.0 to 41.4)
			G360	63	PFS, months (95% CI)	4.1 (3.9 to 5.6)
			Tissue	77		4.2 (3.9 to 5.7)
FDA SSED[27] (CHRYSALIS study)	*EGFR* Ex20ins	Amivantamab-vmjw	G360	62	ORR‡, % (95% CI)	38.7 (26.6 to 51.9)
			Tissue	81		39.5 (28.8 to 51.0)
			G360	62	DoR, months§ (range)	8.31 (1.3+ to 21.7)
			Tissue	81		11.14 (1.3+ to 21.7)

FDA SSED[28] (CodeBreaK 100 study)	*KRAS* G12C	Sotorasib	G360	77	ORR#, % (95% CI)	38.0 (27.0 to 49.0)
			Tissue	124		36.0 (28.0 to 45.0)
			G360	77	DoR, months§ (range)	7.1 (1.3 to 8.4)
			Tissue	124		10.0 (1.3 to 11.1)

* Include gefitinib or erlotinib; † HR <1 favours osimertinib; ‡ Refer to overall response rate; # Refer to objective response rate; § Median values reported; ^ p-value corresponds to the hazard ratio comparing the two treatment arms.

Abbreviations: DoR, duration of response; EGFR, epidermal growth factor receptor; FDA; US Food and Drug Administration; G360, Guardant360 CDx; HR, hazard ratio; KRAS, Kirsten rat sarcoma viral oncogene homolog; ORR†, overall response rate; ORR‡, objective response rate; PFS, progression free survival; SOC, standard of care; SSED, summary of safety and effectiveness data; TKI, tyrosine kinase inhibitor.

Potential healthcare system benefits

Besides benefit to patient health outcomes, the use of G360 may bring potential benefits to the healthcare system. It may reduce the need for resource intensive tissue sampling, while potentially allowing blood sampling to be performed at primary or community care settings. This decentralises biopsy services and free up resources at specialty centres. Notably, Guardant Health has reported that its revenue growth for G360 in the community settings was double that of academic centres.[41]

Cost effectiveness

A cost effectiveness analysis (CEA) performed by Health Quality Ontario (HQO) found that liquid biopsies (not G360 specific) used either alone or as a triage test compared to tissue-based test alone in patients with *EGFR* T790M NSCLC resulted in high incremental cost effectiveness ratios (ICERs) exceeding C$100,000 per quality-adjusted life-year (QALY).[3] Notably, the high cost of osimertinib was a key driver of the ICERs.[3] It is also worthwhile to note that a local CEA performed by ACE found an ICER of S$418,839 per QALY for osimertinib as a first-line treatment of locally advanced or metastatic NSCLC compared to first or second generation TKIs.[42]

Limited details were reported in two abstracts suggesting potential cost savings with the use of G360 as CDx in patients with stage IV[43] or stage IIIB/IV[44] NSCLC. In both abstracts, cost savings were driven by minimising the inappropriate use of immuno- and/or chemotherapies in patients with oncogene-driven NSCLC. However, the driver mutations identified and matched therapies used were unclear.

Overall, G360 leads to improved identification of patients with oncogene-driven NSCLC to maximise treatment benefits. However, the ICER of diagnostic strategies incorporating the use of liquid biopsies may be unfavourably high, largely driven by the downstream costs from certain high-cost targeted therapies.

Ongoing trials

Six ongoing clinical trials were identified from the ScanMedicine database involving patients with NSCLC (NIHR Innovation Observatory; Table 8). Two studies seek to investigate the effectiveness of the G360 test while the other four studies involve the G360 test as a screening tool for trial inclusion, including one trial conducted in Singapore (NCT04087473). Findings from the VALUE study, which includes cost analysis as a secondary endpoint, may provide additional information to inform on the cost effectiveness of G360.

Table 8: Ongoing clinical trials

Study (Trial ID)	Estimated enrollment	Brief description	Estimated study completion date
Studies investigating the effectiveness of G360			
VALUE (NCT03576937)	207 (actual enrollment)	A multi-centre prospective cohort study to compare G360 to standard of care tissue-based profiling within the Canadian system for patients with NSCLC.	September 2021
A prospective observational study by G360 in NSCLC patients whose gene alterations are not detected by tissue-based singleplex assays (WJOG13620L)	72	A prospective observational study conducted in Japan to examine the detection rate of major genomic alterations by Guardant360 in patients whose gene alterations are not detected by tissue-based singleplex assays with Stage 3B-4 and recurrent non-squamous NSCLC that is inoperable and chemoradiation therapy ineligible.	No completion date reported
Studies involving G360 as a screening tool for trial inclusion			
Poziotinib in EGFR Exon 20 Mutant Advanced NSCLC (NCT03066206)	80	A phase II trial investigating how well poziotinib works in treating patients with non-small lung cancer with an EGFR or HER2 exon 20 mutation that is stage IV or has come back (recurrent).	March 2023
POZITIVE20-1 (NCT03318939)	603	A phase II study to evaluate the Objective Response Rate (ORR) to poziotinib in patients with NSCLC with EGFR or HER2 (ErBB2) exon 20 insertion mutations.	December 2023
A Phase II Study of Poziotinib and Ramucirumab in EGFR Exon 20 Mutant Advanced NSCLC (NCT05045404)	36	A phase II trial to investigate the effectiveness of poziotinib and ramucirumab in patients with *EGFR* Exon 20 gene mutant stage IV non-small cell lung cancer.	December 2024
Plasma Molecular Profiling in ALK Inhibitor Resistant NSCLC (NCT04087473)	50	This study seeks to provide a better understanding of ALK resistance in the treatment of Asian lung cancer to allow for improved clinical outcomes with genotype-matched ALK inhibitor.	August 2022
Abbreviations: EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; NSCLC, non-small cell lung cancer.			

Summary

G360 was found to be safe with no major safety issues anticipated with blood sampling. Compared to tissue-based tests in detecting clinically relevant biomarkers, G360 generally has a high specificity (≥87%) with a moderate to high sensitivity (≥54%). It remains unclear if a tissue- or plasma-first strategy would result in a greater initial biomarker discovery rate although there is some evidence supporting the complementary use of G360 to tissue-based testing in reducing turnaround time and increasing the yield of actionable mutations detected by 15% to 65%. When used alone or complementary to tissue-based tests, G360 led to a therapeutic shift towards genotype-matched therapies in 9.8% to 44% of patients at initial diagnosis or at progression, with favourable clinical responses similar to outcomes observed in the tissue-guided FDA pivotal trials. Besides patient benefits, the test may potentially bring healthcare system benefits by improving resource utilisation and decentralising biopsy services to primary or community care settings. The cost-effectiveness of G360 as a CDx to guide therapy selection in the treatment of NSCLC is uncertain.

There were several key limitations across the studies. Due to spatiotemporal tumour heterogeneity, tissue-based tests serve as an imperfect reference standard which raises

uncertainty in its comparative diagnostic accuracy with G360. This was further confounded by the lack of a consistent methodology for tissue-genotyping used across studies as well as a time lag bias between tissue and plasma testing in some studies. Nevertheless, treatment outcome studies validated the G360 test, where similar patient outcomes were found between G360- and tissue-guided treatments. In addition, applicability of the clinical accuracy and utility findings of the G360 test may be relatively weaker for the lower prevalence biomarkers, such as *MET*, *RET* and *BRAF*, in contrast to *EGFR* in line with its higher prevalence in patients with NSCLC. Also, varying clinical practices and clinician judgement in interpreting the results and initiating treatment plans may vary across studies, potentially affecting the rate of patients who had a treatment switch and subsequently their clinical outcomes.

VII. Estimated Costs

Based on information listed on Guardant Health's website, the out-of-pocket cost of G360 for patients without insurance would be US$5,000 (S$6,803)[a]. Information listed on the website of a European distributor (TherapySelect) listed a cost of €3,500 (S$5,217)[a] for the first application and €2,800 (S$4,174)[a] for treatment monitoring.[45] As a reference, the local cost of tissue-based tests ranges from around [redacted] for fluorescence in situ hybridization (FISH) to [redacted] for a limited NGS panel (Personal communication: Oncologist from National Cancer Centre Singapore, 5 May 2022).

A budget impact analysis performed by HQO estimated that the public funding of liquid biopsy (not limited to G360) as a triage test in Ontario would cost the healthcare system C$60,000 to C$3 million over the next five years for patients with *EGFR* T790M-altered NSCLC.[3] The majority of these costs were attributed to costs of treatment, adverse events and patient care while testing-related costs were minimal in the first year with cost savings over time.

VIII. Implementation Considerations

The clinical implementation of a multigene liquid biopsy test based on NGS requires a coordinated effort amongst various stakeholders, including policy makers, regulatory bodies and healthcare providers. At the broader level, reimbursement may serve as a barrier in the utilisation of liquid biopsy in the face of a resource-limited healthcare setting where clinicians have to prioritise the type of tests to use for patients with NSCLC.[7] As observed in Europe, limited reimbursement was found to reduce the use of molecular testing which may be further complicated by differences in reimbursement provided for liquid biopsy compared to tissue-based tests.[7] In addition, rapid advancement in precision oncology allows the development of novel targeted therapies for driver mutations. To reap the benefit gained from identifying therapeutically targetable mutations with liquid biopsies, the corresponding regulatory approval and subsidy consideration for new therapies may be imperative to ensure both access and affordability of such drugs to patients identified with actionable mutations.

Besides, various implementation considerations exist in introducing the G360 test into existing clinical workflows. At present, the G360 test may be ordered locally, and the plasma

[a] Based on the Monetary Authority of Singapore exchange rate as of 28 March 2022: €1=S$1.4906, US$1=S$1.3606. Figures were rounded to the nearest dollar.

samples are sent to Guardant Health's laboratory in the United States for processing and testing. The feasibility of shipping the plasma samples to overseas centres, including potential shipment delays, increased turnaround time that may affect treatment plans, and cost, should be taken into consideration. To mitigate these implementation roadblocks, a central laboratory may be established similar to how the test is offered in the United States. As a result, there would be a change in care delivery arising from a paradigm shift in cancer diagnostics from a decentralised model of conducting tumour analysis in individual hospital's pathology laboratory to a centralised model of an off-site, central laboratory.[46] This paradigm shift reflects the high level of expertise and resources required to perform a multigene assay such as G360, including the investment in NGS systems, sufficient cloud storage for the sequencing data, technical and bioinformatics expertise.[46] The shift to a centralised model may also lead to infrastructure considerations, although a consolidation of sample testing at the national or cluster level may lead to potential cost savings derived from economies of scale.[46]

Training of healthcare providers also serve as a key enabler for the clinical implementation of the G360 test. With the use of NGS, oncologists and pulmonologists require appropriate training to read and interpret the assay report to make treatment decisions.[46] The need for further training was reflected in a survey which found that oncologists were least confident in using whole genome sequencing to guide patient care.[7] Further, it may be difficult for clinicians to keep abreast of the rapid advancements of treatment strategies arising from new therapies.[46] To this end, the establishment of a multidisciplinary tumour board consisting of clinicians, molecular pathologists, clinical molecular biologists, geneticist and bioinformaticians would be useful to interpret results and improve care guided by G360.

IX. Concurrent Developments

The rapid development of liquid biopsy technologies represents a step forward to precision oncology. Similar to G360, there are various multi-panel liquid biopsy assays in ongoing development that uses NGS to detect for driver mutations in patients with NSCLC (Table 9).

Table 9: Multi-gene NGS liquid biopsy assays for patients with NSCLC

Technology (Manufacturer)	Brief description	Status
FoundationOne Liquid CDx (Foundation Medicine, Inc.)	For the detection of specific mutations in patients with NSCLC, breast, ovarian and prostate cancer.	FDA approved
LiquidPlex Dx (Invitae)	A CGP that examines 29 genes for solid malignant neoplasms as well as serving as a CDx to identify patients diagnosed with NSCLC with *MET*ex14 skipping alterations	CE-IVD marked
Resolution ctDx Lung assay (Resolution Bioscience, Inc.)	The Resolution ctDx Lung assay targets actionable, somatic SNVs, indels, fusions, and copy number variants in 22 genes in NSCLC	Commercially available in the United States
GeneStrat NGS (Biodesix)	A blood-based, 52-gene panel composed of guideline recommended variants that helps identify patients with advanced stage NSCLC who may be eligible for targeted therapy or clinical trial enrollment.	
OncoGxOne (Admera Health)	A comprehensive 364 gene NGS assay for profiling all solid tumor types. Input includes both DNA and RNA for optimal fusion detection.	
OncoBEAM Lung2 (Sysmex Inostics, Inc.)	OncoBEAM uses highly-sensitive BEAMing technology to accurately detect ctDNA.	

Abbreviations: CDx, companion diagnostic; CE, Conformité Européenne; CGP, comprehensive genomic profiling; ctDNA, circulating tumour DNA; FDA, US Food and Drug Administration; IVD, in-vitro diagnostics; LDT, laboratory developed test; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer.

X. Additional Information

As briefly discussed in Section V, various oncology professional bodies have supported the use of liquid biopsy as a standard diagnostic tool concurrent to tissue-based testing, or as a plasma-first approach, for patients with advanced or metastatic NSCLC.[14-16] In addition, ctDNA testing is currently implemented as a CDx for patients with advanced NSCLC to identify those who may benefit from *EGFR* TKIs across the National Health Service (NHS) in England.[18]

Although liquid biopsies have been recommended to be used as a standard-of-care in patients with NSCLC, there are also ethical considerations owing to the inadvertent harm that liquid biopsy may bring. The moderate sensitivity of the G360 test implies a higher false negative rate, which may lead to delayed treatment and result in a poorer prognosis that could have been preventable. However, as previously discussed, the risk of a false negative result with G360 may be averted with a reflex tissue-based test for patients with a negative plasma test result.

Besides ethical concerns, legal and social implications may arise with the increasing use of liquid biopsy and precision oncology in healthcare practices. The rising amount of genomic information obtained through CGP results in additional responsibilities for clinicians, which increases the possibility of legal liability as a result of medical malpractice. Similarly, the profusion and availability of genomic data may lead to privacy and discrimination issues. Furthermore, the use of personalised therapeutics, such as targeted therapies, may exacerbate existing health disparities arising from ease of access and affordability.[47]

In addition, most of the studies included in this brief reported conflict of interests, where the study was either funded by Guardant Health or the authors were affiliated to or received a fee from Guardant Health.

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Appendix

Appendix A: Additional information regarding Guardant360 CDx

Table A1: Genetic alterations detected by Guardant360 CDx

Alteration Type	Genes
Single nucleotide variation	*AKT1, ALK, APC, AR, ARAF, ATM*, BRAF, BRCA1**, BRCA2**, CCND1, CDH1, CDK4, CDK6, CDK12*, CDKN2A, CTNNB1, EGFR, ERBB2, ESR1, FGFR1, FGFR2, FGFR3, GATA3, GNA11, GNAQ, HRAS, IDH1, IDH2, KIT, KRAS, MAP2K1, MAP2K2, MET, MLH1, MTOR, MYC, NF1, NFE2L2, NRAS, NTRK1, NTRK3, PDGFRA, PIK3CA, PTEN, RAF1, RET, RHEB, ROS1, SMAD4, SMO, STK11, TERT, TSC1, VHL*
Indels	*AKT1, ALK, APC, ATM*, BRAF, BRCA1**, BRCA2**, CDH1, CDK12*, CDKN2A, EGFR, ERBB2, ESR1, FGFR2, GATA3, HNF1A, HRAS, KIT, KRAS, MET, MLH1, NF1, PDGFRA, PIK3CA, PTEN, RET, ROS1, STK11, TSC1, VHL*
Copy number amplifications	*ERBB2, MET*
Fusions	*ALK, NTRK1, RET, ROS1*
* Reporting is enabled for pathogenic germline alterations only. Somatic alterations will not be reported ** Reporting is enabled for both germline and somatic alterations.	

Appendix B: Studies identified and study design

Table B1: List of included studies

Type of study	Number of studies included
Health technology assessment (HTA) report	1
FDA Summary of Safety and Effectiveness Data (SSED)	2
Published studies (key evidence base)	7
Published studies (supplementary evidence)	4
Note: 1. Inclusion criteria a. Studies that fulfil the PICO criteria listed in Table 2. 2. Exclusion criteria a. Studies only available in the abstract form.	

Table B2: Characteristics of included studies

Author (year)	N	Study design	Population	Timing between G360 and tissue-based test
HTA report				
FDA SSED[12] (P200010; FLAURA study)	304*	Retrospective	Patients with advanced and metastatic NSCLC with *EGFR* exon 19 deletions or exon 21 L858R mutations confirmed by the cobas *EGFR* Mutation Test enrolled in the FLAURA phase 3 study assessing the efficacy of osimertinib vs standard *EGFR* TKI therapy; patients enrolled in the NILE study were used to estimate the prevalence of CDx-positive, tissue-negative patients as no plasma from FLAURA tissue negative patients was available	Unclear
FDA SSED[12] (P200010; AURA3 study)	191*	Retrospective	Adult patients with centrally confirmed *EGFR* T790M-positive locally advanced/metastatic NSCLC and radiological evidence of progression following treatment with a first-line EGFR-TKI; Guardant360	Unclear

			plasma-positive patients was also performed using 150 randomly selected samples derived from the screened population of AURA3 that failed screening due to a negative *EGFR* T790M tissue test result	
Leighl et al. (2019)[21]	282	Prospective	Patients with biopsy proven, previously untreated, nonsquamous NSCLC (stage IIIB/IV) enrolled in the NILE study	Unclear
Schwaederle et al. (2017)[24]	88	Retrospective	Patients with lung adenocarcinoma (86% with metastatic disease)	Median time of 0.8 months (range not provided)
Thompson et al. (2016)[25]	102	Prospective	Patients with NSCLC or suspected NSCLC (96% stage IV)	Range from 0 days to >2 years
Villaflor et al. (2016)[26]	68	Retrospective	Patients with NSCLC (68% stage IV)	Median time of 1.4 years (range, 0 days to 7 years)
Papadimitrakopoulou et al (2020)[23]	891	Retrospective	Patients harboring T790M mutation with locally advanced or metastatic NSCLC who had progressed on EGFR TKI therapy enrolled in AURA3 trial	Tissue and plasma sample collected concurrently
Helman et al. (2018)[22]	77	Prospective	Patients who received rociletinib in second-line or later *EGFR* T790M-postiive or T790M-negative NSCLC patients.	Unclear
Additional studies (key evidence base)				
FDA SSED[27] (P200010/S001)	62*	Retrospective	Patients with metastatic NSCLC having an in-frame base pair insertion mutation in *EGFR* exon 20 whose disease has progressed on or after platinum-based chemotherapy in the CHRYSALIS study; patients enrolled in the NILE study were used to estimate the prevalence of CDx-positive, tissue-negative patients as no plasma from CHRYSALIS tissue negative patients was available	Unclear
FDA SSED[28] (P200010/S002)	78*	Retrospective	Patients with pathologically documented locally advanced or metastatic NSCLC harbouring *KRAS* G12C mutation in the CodeBreak 100 study; supplemental matched tissue and plasma samples were obtained from subjects in other Amgen clinical studies and commercial vendors using subject selection criteria similar to those of the CodeBreak 100 clinical study and used to estimate the prevalence of patients positive for KRAS G12C mutations by Guardant360 CDx but negative by tissue testing	Unclear
Page et al. (2021)[32]	282	Prospective	Patients with previously untreated, stage IIIB/IV non-squamous advanced NSCLC	Unclear
Park et al. (2021)[33]	421	Retrospective	Patients with advanced NSCLC, including treatment-naïve and previously treated patients	Tissue and plasma sample collected concurrently
Bonanno et al. (2020)[29]	235	Prospective	Patients with stage IIIB/IV NSCLC who were recruited for the VISION trial (NCT02864992)	Unclear
Bustamante Alvarez et al. (2021)[30]	143	Retrospective	Patients with histologically confirmed stage IV NSCLC	12 weeks

Zatarain-Barrón et al. (2021)[35]	54	Prospective	Hispanic patients with stage IIIB/IV lung adenocarcinoma, age >18 years, WHO performance status of 0 to 2, unsuitable for curative treatment irrespective of systemic treatment previously received	Unclear
Peled et al. (2020)[34]	10	Prospective	Patients with treatment-naïve stage IV adenocarcinoma NSCLC	Within 2 days
Mack et al. (2020)[31]	1288†	Retrospective	Patients with advanced (defined on the test request form as stage IIIB-IV) lung adenocarcinoma (LUAD) or NSCLC not otherwise specified who underwent ctDNA analysis using clinical Guardant360 testing between June 2014 and October 2016	Unclear
Supplementary evidence				
Odegaard et al. (2018)[11]	—	—	—	—
Lanman et al. (2015)[36]	—	—	—	—
Zugazagoitia et al. (2019)[37]	53	Prospective	Patients with *EGFR, ALK* or *ROS1*-altered advanced-stage NSCLC who experience clinical or radiological progression on prior TKI therapy	—
Zugazagoitia et al. (2019)[38]	93	Prospective	Patients with advanced-stage lung adenocarcinomas with insufficient or inadequate tumour samples for standard care EGFR, ALK or ROS1 genotyping	—

***** Number of patients in the G360 primary clinical efficacy analysis set (gCEAS).

† Subset of patients for whom tissue biomarker analysis results were available or reported as incomplete due to insufficient tissue.

Abbreviations: CDx, companion diagnostic; ctDNA, circulating tumour DNA; NSCLC, non-small cell lung cancer; TKI, tyrosine kinase inhibitor; WHO, World Health Organisation.

Appendix C: List of supplementary tables and figures.

Table C1: Technical accuracy of G360

Accuracy measure	Outcomes
Analytical sensitivity	Variant detection as low as 0.02% to 0.1% mutant allele frequency
Analytical specificity	>99.9999%
Test precision	CDx variants: PPA of 96.7% to 100% All genetic alterations: PPA of 86.7% to 100%
Analytical concordance with external comparator assays	Panel wide indels: PPA of 82.5%, NPA >99% SNVs: PPA: 91.4%, NPA: >99% CDx variants: PPA of 95% to 100%, NPA of 86.9% to 99.9%

Abbreviations: CDx, companion diagnostic; NPA, negative predictive agreement; PPA, positive predictive agreement; SNV, single nucleotide variant.

Table C2: Overall comparison of G360 and tissue-based molecular profiling in patients with NSCLC with guideline-recommended genomic alterations

Study	N	Composite biomarkers	Sensitivity	Specificity	PPV	NPV	Concordance
Leighl et al. (2018)[21]	282	*EGFR* mutations, *ALK* fusion, *ROS1* fusion, *BRAF* V600E, *RET* fusion, *MET* amplification, *MET*ex14, *ERBB2* mutation	80.0%	86.9%	62.3%	94.1%	NR
	64*		86.3%	92.9%	86.4%	92.9%	NR

Park et al. (2021)[33]	262	*EGFR* mutations, *ALK* fusions, *MET* amplification, *MET*ex14, *RET* fusions, *ROS1* fusions, *KRAS* mutations	67.7%	88.8%	91.0%	62.1%	77.6%
Bustamante Alvarez et al. (2021)[30]	94	*EGFR, ALK, ROS, BRAF* mutations, *RET* fusions, *MET* amplification, *MET*ex14, *NTRK* fusions	67.0%	89.0%	76.0%	83.0%	NR
Aggarwal et al. (2019)[39]	128†	*EGFR, ALK, MET, BRCA1, ROS1, RET, ERBB2, BRAF, KRAS*	NR	NR	NR	NR	81.3%
* Subcohort of patients who attempted or completed assessment of all eight guideline-recommended biomarkers. † Subcohort of patient with both plasma and tissue results.							

Table C3: Variant-specific comparison of G360 and tissue-based molecular profiling in patients with NSCLC

Genomic alteration	Study	N	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV	Concordance
EGFR Ex19del	Leigh et al. (2018)[21]	223	81% (60% to 95%)†	100% (98% to 100%)†	100%	98%	98.2%
	FDA SSED[12]	380	77.7% (71.9% to 82.9%)	99.3% (96.1% to 100%)	—	—	—
	Papadimitrakopoulou et al. (2020)[23]	208	79% (72% to 86%)	99% (92% to 100%)	—	—	—
EGFR L858R	Leigh et al. (2018)[21]	223	90% (56% to 100%)†	100% (98% to 100%)†	100%	99.5%	99.6%
	FDA SSED[12]	380	70.6% (62.2% to 78.1%)	99.2% (97.1% to 99.9%)	—	—	—
	Papadimitrakopoulou et al. (2020)[23]	208	63% (50% to 74%)	100% (98% to 100%)	—	—	—
EGFR T790M	FDA SSED[12] (AURA3 study)	447	67.4% (61.6% to 72.8%)	67.1% (58.9% to 74.7%)	—	—	—
	Papadimitrakopoulou et al. (2020)[23]	207	66% (59% to 72%)	—	—	—	—
EGFR Ex20ins	FDA SSED[27] (CHRYSALIS study)	261	80.4% (71.4% to 87.1%)	100% (97.7% to 100%)	—	—	—
EGFR sensitising variants	FDA SSED[12] (FLAURA study)	380	75.1% (70.4% to 79.4%)	—	—	—	—
	FDA SSED[12] (NILE study)	88	100% (76.8% to 100%)	98.7% (92.7% to 100%)	—	—	—
	Thompson et al. (2016)[25]	50	79%	100%	—	—	—
	Villaflor et al. (2016)[26]	31	63%	96%	—	—	—
EGFR variants (various)	Schwaederle et al. (2017)[24]	34	54% (25% to 81%)	90% (70% to 99%)	—	—	—
KRAS G12C	FDA SSED[28] (CodeBreaK 100 study)	181	71.6% (62.1% to 79.8%)	100% (95% to 100%)	—	—	—
KRAS mutations*	Bustamante Alvarez et al. (2021)[30]	83	—	—	—	—	88%

ALK fusion	Leigh et al. (2018)[21]	215	63% (24% to 91%)†	100% (98% to 100%)†	100%	99%	99.1%
ROS1 fusion	Leigh et al. (2018)[21]	153	—	100% (98% to 100%)†	—	98.7%	98.7%
BRAF V600E	Leigh et al. (2018)[21]	92	100% (16% to 100%)†	100% (96% to 100%)†	100%	100%	100%
	Thompson et al. (2016)[25]	50	100%	100%	—	—	—
*MET*ex14	Leigh et al. (2018)[21]	57	80% (30% to 99%)†	98% (88% to 100%)†	80%	98.1%	96.5%
RET fusion	Leigh et al. (2018)[21]	57	—	100%	—	100%	100%

* *KRAS* G12C was the most common variant detected.

† 95% CI derived from the HTA report[20].

Note: For the FDA SSED[12] (FLAURA and AURA3 studies), data derived from Guardant360 CDx and Guardant360 LDT were combined.

Abbreviations: NPV, negative predictive value; PPV, positive predictive value; SSED, Summary of Safety and Effectiveness Data.

Table C4: Impact of G360 on switch in treatment strategy in the overall patient cohort

Study	N	Patients who switched to targeted therapy based on G360 alone or concurrent with tissue-based tests, n (%)
Bustamante Alvarez et al. (2021)[30]	82*	8 (9.8%)
Aggarwal et al. (2019)[39]	323	67 (20.7%)
Park et al. (2021)[33]	50†	10 (20.0%)
Laufer-Geva et al. (2018)[40]	116	30 (25.9%)
Zatarain-Barrón et al. (2021)[35]	54	24 (44.4%)
Zugazagoitia et al. (2019)[38]	93	12 (12.9%)
Schwarderle et al. (2017)[24]	88	25 (28.4%)

* Based on patients tested with G360 at the time of diagnosis.

† Based on a subcohort of patients with failed tissue-based NGS results.

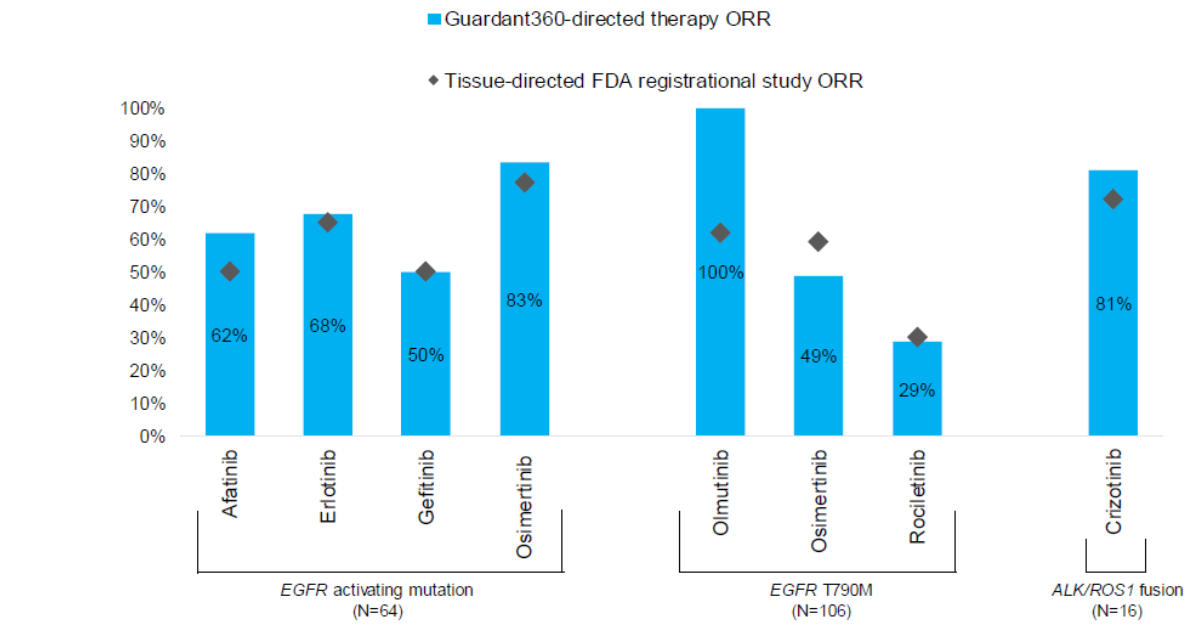
Table C5: Additional information and supplementary studies supporting G360-guided clinical outcomes

Study	Clinical outcomes*
Bustamante Alvarez et al. (2021)[30]	• Of 8 patients who started on targeted therapy based on G360 results, 6 patients experienced partial response, 1 patient with complete response and 1 patient with stable disease • Response rate of 88%
Park et al. (2021)[33]	• In patients with failed tissue-based NGS results (n=50), 8 out of 10 patients who received targeted therapy based on G360 results showed partial response
Zatarain-Barrón et al. (2021)[35]	• In patients who switched therapy (n=24) based on G360 results, the disease control rate was 85.7%. There was a partial response of 60.7% and stable disease of 25%. • The median PFS for patients treated with TKIs was 11.1 months (95% CI, 7.6 to 14.6 months) • The median OS was 40.3 months (95% CI, 27.1 to 53.6 months) in patients who switched therapy following positive ctDNA result, compared to a median OS of 22.3 months (95% CI, 8.3 to 36.5; p=0.14) in patients who did not switch therapy despite bring ctDNA-positive
Zugazagoitia et al. (2019)[37]	• In 2 patients with G360-identified *ALK/ROS1*-rearranged NSCLC with resistance to crizotinib and/or next generation *ALK/ROS1* TKIs: o 1 patient received lorlatinib and showed early resistance (3.8 months) o 1 patient received cabozantinib and experience a partial response (8 months)

Zugazagoitia et al. (2019)[38]	• At median follow-up of 8 months, the median OS was not reached in patients (n=12) who received matched targeted therapies based on G360 results
Page et al. (2021)[32]	• Out of 61 patients who received targeted therapy based on G360 and/or tissue-based tests, 33 patients were found to be evaluable. • Among the 33 patients, 25 (76%) and 17 (52%) achieved a durable response of >6 and >12 months, respectively • There was an objective response rate of 58% and disease control rate of 94% • 52% of patients exhibited event-free survival at 12 months
Bonanno et al. (2020)[29]	• Patients detected with a targetable driver mutation detected by tissue NGS or G360 who started matched targeted therapy had a longer median OS (not reached) compared to those who did not (median OS, 9.1 months; 95% CI, 4.6 to 13.6 months; p=0.046) • Although statistically significant difference was found, patients detected with targetable driver mutation who started matched targeted therapy showed a numerically longer median OS (not reached) compared with the entire study cohort (median OS, 21.7 months; 95% CI, 17.4 to 25.9 months, p=0.173)
Villaflor et al. (2016)[26]	• 9 patients with paired tissue and blood samples had *EGFR* mutation detected in plasma and tissue (n=5), plasma only (n=1), or tissue only (n=3) • 8 of these patients were treated with erlotinib or afatinib at first or second line • Of which, 2 patients were still responding to therapy at the time of data analysis • The remaining 6 patients had a median PFS of 11.5 months (range, 7.5 to 29 months; 95% CI, 5.7 to 28.7 months)
* Clinical outcomes were based on patients who were evaluable (for example, RECIST criteria). Abbreviations: DoR, duration of response; NGS, next-generation sequencing; OS, overall survival; PFS, progression-free survival; RECIST, response evaluation criteria in solid tumours; TKI, tyrosine kinase inhibitor.	

Table C6: Comparison of patients tested ctDNA-positive who received targeted therapy and those who did not

Study	Treatment outcome	ctDNA positive patients who switched to targeted therapy		p-value
		Yes	No	
Zatarain-Barrón et al. (2021)[35]	Median PFS	40.3 months (95% CI, 27.1 to 53.6)	22.3 months (95% CI, 8.3 to 36.5)	0.14
Bonanno et al. (2020)[29]*	Median OS	Not reached (Not evaluable)	9.1 months (95% CI, 4.6 to 13.6)	0.046
Zugazagoitia et al. (2019)[38]	Median OS	Not reached†	11.5 months	0.32
Schwarderle et al. (2017)[24]	Median PFS	14.7 months (95% CI, 3.7 to 25.7 months)	7.8 months	0.28
	Median OS	Not reached‡	36.7 months	0.928
* Include patients with therapeutically targetable mutations detected by tissue NGS or G360. † Median follow-up of 8 months. ‡ Median follow-up time of 18.6 months. Abbreviations: CI, confidence interval; ctDNA, circulating tumour DNA; OS, overall survival; PFS, progression free survival.				



Guardant360 Data								
Tested positive (N)	21	31	6	6	4	39	63	16
PR/CR (N)	13	21	3	5	4	19	18	13
ORR (%)	62%	68%	50%	83%	100%	49%	29%	81%
95% CI	37-81%	48-83%	14-86%	36-99%	40-100%	33-65%	18-41%	54-95%

Figure C1: Pooled response rate of patients with G360-guided therapy compared with tissue-guided FDA pivotal study. No statistically significant differences in the overall response rate were identified between the G360-guided therapy and the FDA pivotal studies (p>0.12 for each therapy). Adapted from Mack et al. (2020)[31].