

***Small gene panel
testing for non-
squamous non-
small cell lung
cancer***

August 2022

MSAC application no. 1721

Assessment report

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The technical information in this document is used by the Medical Services Advisory Committee (MSAC) to inform its deliberations. MSAC is an independent committee established to provide advice to the Minister for Health on the strength of evidence available on new and existing medical technologies and procedures in terms of their safety, effectiveness and cost-effectiveness. This advice will help to inform government decisions about which medical services should attract funding through the Medicare Benefits Schedule (MBS) or alternative funding programs/arrangements.

MSAC's advice does not necessarily reflect the views of all individuals who participated in the MSAC evaluation.

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Australian Government

Medical Services Advisory Committee

DCAR Executive Summary

Application No. 1721 Small gene panel testing for NSCLC

Applicant: Royal College of Pathologists of Australasia

Date of MSAC consideration: MSAC 86th Meeting, 24-25 November 2022

Main issues for MSAC consideration

Clinical issues:

- The applicants requested up to 3 new items be added to the MBS (1 for a combined DNA/RNA panel, and/or 2 for sequential DNA and RNA panels). There was insufficient evidence to guide MSAC on the differential effectiveness of one versus two panels.
- This assessment focused on patients with non-squamous or not otherwise specified non-small cell lung cancer (NSCLC). Although the majority of studies on the concordance of testing included both squamous and non-squamous tumours, the key evidence was focused on non-squamous NSCLC, as was the economics and financial analysis.
- The evidence available on the proportion of samples successfully tested, (i.e. the inverse of the test failure rate) using NGS versus sequential single-gene testing, was not explicit about the method of NGS testing (i.e. whether a simultaneous DNA/RNA panel was used, or sequential DNA ± RNA panels were used). This reduces the certainty that the reported benefits would be replicated in the Australian setting, where sequential panels are expected to be more common than simultaneous DNA/RNA testing in the near future.
- NGS panels were found to be superior to sequential single-gene testing in detecting *EGFR* variants, due to a higher level of analytical sensitivity (i.e. finding in-scope variants at a low allelic frequency), and/or due to being more comprehensive (i.e. detecting variants outside of the scope of single-gene testing), out of which only some would be considered to confer sensitivity to *EGFR* tyrosine kinase inhibitors (TKIs). The additional cases detected may not respond to targeted treatments to the same extent as those with common *EGFR* variants (L858R and exon 19 deletions, or resistant variant T790M), or in those with higher variant allele frequency.
- There was no comparative evidence to support or negate a conclusion that NGS panel testing would reduce the rate of tissue rebiopsy, as international evidence (where liquid biopsies are recommended if insufficient tissue is available) is not applicable to the Australian setting (where PBS restrictions require alterations to be found in tumour tissue). Note, liquid biopsies are out of scope of this application.

Main issues for MSAC consideration

Economic issues:

- Small gene panel testing was associated with a small incremental cost per patient receiving testing. This was driven by the cost of proposed panel testing, and was offset by a reduction in use and cost of current tests and rebiopsies. Incremental costs were driven by the assumption that RNA panels cannot be used where *KRAS* and *BRAF* activating variants are identified (which do not have associated targeted therapies) and assumptions regarding the use and cost of rebiopsy.
- While small gene panel testing was associated with identifying more patients with actionable variants eligible for targeted therapy, this was driven by an increase in patients with incremental actionable variants identified, including “in scope” variants due to detection at lower variant allelic frequency and “beyond restriction” variants, which were unable to be distinguished from one another in the analysis. Interpretation of this outcome is therefore difficult due to the uncertain effectiveness, and therefore cost-effectiveness, of targeted therapy in these additional cases.
- Given the uncertainties in the effectiveness of targeted therapies in patients with incremental actionable variants identified, the model presented was truncated at the point of treatment.

Financial issues:

- A market-share approach based on current utilisation of *EGFR* services was used to estimate the financial impact of listing small gene panel testing on the MBS. No market growth was assumed. The analysis was sensitive to variations in the distribution of use of combined or sequential small gene panels and whether separate RNA panels are allowed in those found to have *KRAS* or *BRAF* activating variants.
- If there is substantial growth in the market due to the listing of small gene panels (e.g. if some testing currently is being funded through the state system, and this shifts to the MBS), then the net impact to the MBS may be higher. However, the extent of this shift is unknown.

1. Purpose of application

An application requesting Medicare Benefits Schedule (MBS) listing for small next generation sequencing (NGS) panels for biomarker testing of patients with non-squamous (or histology not otherwise specified) non-small cell lung cancer (NSCLC), was received from the Royal College of Pathologists of Australasia (RCPA) by the Department of Health and Aged Care. In this case, biomarker testing is for the purposes of determining suitability for targeted treatments for non-squamous NSCLC, available through the Pharmaceutical Benefits Scheme (PBS).

The clinical claim is that the use of either a small combined deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) NGS panel, or sequential use of a DNA panel and (if required) an RNA panel, would be superior in effectiveness and safety compared to sequential single-gene testing for biomarkers in patients with NSCLC. This is due to a small panel (or two) making more efficient use of tumour tissue, resulting in fewer re-biopsies being required, a more rapid turnaround time, and faster initiation of targeted treatment.

This Department Contracted Assessment Report (DCAR) assessed the safety, effectiveness, and cost-effectiveness of small NGS panels compared to sequential single gene testing, to provide the evidence-base for the Medical Services Advisory Committee (MSAC) to decide its advice regarding funding on the MBS.

2. Background

MSAC has not previously considered any panel testing for NSCLC.

A similar assessment for a somatic tumour panel test (that was not histology-specific) was initiated in 2018 (MSAC assessment 1495), but was withdrawn prior to being considered by MSAC, as no single somatic tumour panel test could appropriately assess epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase (*ALK*) and ROS proto-oncogene 1 (*ROS1*) variants at the time. In the same year, an application (MSAC assessment 1634) was also made by Roche Diagnostics for MBS listing of a comprehensive gene panel of over 300 genes for use in squamous and non-squamous NSCLC, which was revised in 2020 to focus on non-squamous NSCLC. During the PICO development, the applicant for 1634 nominated the application would proceed as an ADAR for consideration at an MSAC meeting in late 2022. However, in May 2022, the applicant notified the Department that it would be delaying the submission of its ADAR.

MSAC has considered individual single gene tests for biomarker assessment in patients with NSCLC, and in November 2017, *“MSAC noted that the sequential testing of EGFR, ALK and ROS1 yield mutually exclusive treatment pathways and that sequential testing wastes tissue sample, time and is more expensive than a single panel of tests. MSAC recommended that the Department conduct a cost-utility review of gene panel and/or next generation sequencing (NGS) test options to inform these first-line therapy options”*. *“MSAC advised that any MBS funding should be based on a gene panel or NGS test of equivalent or better analytical performance to sequential IHC and FISH testing and assurance that the average gene panel or NGS test is no more costly than the average cost of the sequential testing that it would replace. MSAC noted that overall testing may still require more than one gene panel test due to differences in lung cancer gene aberrations as somatic mutations are tested in genomic DNA, whereas gene fusions (such as ROS1) are usually tested in cDNA [complementary DNA] prepared from RNA.”* ([Public Summary Document, ADAR 1454](#), November 2017, p3).

A summary of how this DCAR has addressed the suggestions by MSAC is shown in Table 1.

Table 1 Summary of key matters of concern from MSAC 1454 PSD, November 2017, p3

Component	Matter of concern	How the current assessment report addresses it
Overarching DCAR	MSAC recommended that the Department conduct a cost-utility review of gene panel and/or next generation sequencing (NGS) test options to inform these first-line therapy option.	Addressed. Current DCAR assessing small NGS panel.
Intervention	MSAC noted that overall testing may still require more than one gene panel test due to differences in lung cancer gene aberrations as somatic mutations are tested in genomic DNA, whereas gene fusions (such as <i>ROS1</i>) are usually tested in complementary DNA prepared from RNA.	Addressed. Intervention proposed as both DNA and RNA testing, or sequential DNA then RNA testing.
Test performance	Any MBS funding should be based on a gene panel or NGS test of equivalent or better analytical performance to sequential IHC and FISH testing.	Addressed. NGS has superior or equivalent analytical performance compared to single-gene assays or IHC and FISH testing.
Cost-minimisation	The average gene panel or NGS test is no more costly than the average cost of the sequential testing that it would replace.	At the proposed items fees, small gene panel testing is associated with additional costs. This may be reasonable if the claim of superior effectiveness is accepted.

DCAR = Department Contracted Assessment Report; DNA = deoxyribonucleic acid; FISH = fluorescence *in situ* hybridisation; IHC = immunohistochemistry; MBS = Medicare Benefits Schedule; MSAC = Medical Services Advisory Committee; NGS = next generation sequencing; PSD = Public Summary Document; *ROS1* = ROS proto-oncogene 1; RNA = ribonucleic acid.

3. Prerequisites to implementation of any funding advice

Small DNA/RNA or DNA and RNA NGS panel testing would occur in a National Association of Testing Authorities (NATA) accredited laboratory in accordance with National Pathology Accreditation Advisory Council (NPAAC) guidelines: '[Requirements for human medical genome testing utilising massively parallel sequencing technologies \(First Edition 2017\)](#)'.

Currently, there are no NGS assays approved by the Therapeutic Goods Administration (TGA) for the purposes of detecting biomarkers for targeted treatment of patients with NSCLC. There are several NGS assays available in Australia for 'Research Use Only' (RUO), and local laboratories will be able to purchase RUO products and develop an *in vitro* test medical device approved by the National Association of Testing Authorities (NATA) as per the framework in '[Requirements for the development and use of in-house *in vitro* diagnostic medical devices \(IVDs\) \(Fourth Edition 2018\)](#)'.

Currently, the PBS restrictions for most of the drugs targeting *ALK* or *ROS1* gene rearrangements (all except second-line lorlatinib) specify the method of determining the variants and the threshold separating a positive result from a negative result (i.e., patients must have evidence of an *ALK* gene rearrangement or *ROS1* gene rearrangement in tumour material, defined as 15% (or greater) positive cells by fluorescence *in situ* hybridisation (FISH) testing). If the proposed items for small DNA ± RNA NGS panels are listed on the MBS, coordinated amendments to the restrictions listed on the PBS would be required to allow for biomarkers to be detected using either FISH (with the current restriction to ≥15% of positive cells) or NGS (without the same threshold) in the criteria for crizotinib, ceritinib, alectinib, and entrectinib. If this application is supported by MSAC, the necessary coordination may most efficiently be achieved by MSAC referring the related amendments to the PBAC for consideration at its December 2022 Intracycle meeting.

4. Proposal for public funding

The proposal is for up to three new MBS items to be listed: one for a nucleic acid-based test of both DNA and RNA for simultaneous testing, and two additional items for separate DNA and RNA testing (as not many laboratories currently have the capacity to perform simultaneous testing). Consistent with current items for *EGFR* testing, IHC testing for *ALK* and *ROS1*, and FISH testing for *ALK* and *ROS1*, the items are proposed to be pathologist-determinable.

The proposed fees are based on the cost of delivering the tests, including extraction, pathologist assessment, quality control, curation and reporting ([MSAC application 1721](#)).

Table 2 Applicant proposed MBS items with suggested modifications

Category 6 – Genetics P7	
AAAA	<p>A nucleic acid-based multi-gene panel test of tumour tissue from a patient diagnosed with non-small cell lung cancer, shown to have non-squamous histology or histology not otherwise specified, requested by, or on behalf of, a specialist or consultant physician, to detect:</p> <ol style="list-style-type: none"> variants in at least <i>EGFR</i>, <i>BRAF</i>, <i>KRAS</i> and <i>MET</i> exon 14 to determine access to specific therapies listed on the Pharmaceutical Benefits Scheme (PBS); and the fusion status of at least <i>ALK</i>, <i>ROS1</i>, <i>RET</i>, and <i>NTRK</i> to determine access to specific therapies listed on the PBS; or if the requirements relating to <i>EGFR</i>, <i>ALK</i> and <i>ROS1</i> status for access to a PD-(L)1 immunotherapy pembrolizumab under the PBS are fulfilled. <p>Maximum one test per episode of disease This item cannot be claimed in addition to MBS items BBBB, CCCC, 73337, 73341, 73344, or MBS item for <i>MET</i>ex14sk testing</p> <p>Fee: \$1,247 Benefit: 75% = \$935.25 85% = \$1,159.10^a</p>
BBBB	<p>A DNA-based multi-gene panel test of tumour tissue from a patient diagnosed with non-small cell lung cancer, shown to have non-squamous histology or histology not otherwise specified, requested by, or on behalf of, a specialist or consultant physician, to detect:</p> <ol style="list-style-type: none"> variants in at least <i>EGFR</i>, <i>BRAF</i>, <i>KRAS</i> and <i>MET</i> exon 14 to determine access to specific therapies listed on the Pharmaceutical Benefits Scheme (PBS); or if the requirements relating to <i>EGFR</i> status for access to a PD-(L)1 immunotherapy pembrolizumab under the PBS are fulfilled. <p>Maximum one test per episode of disease This item cannot be claimed in addition to MBS item AAAA, 73337, or MBS item for <i>MET</i>ex14sk testing</p> <p>Fee: \$682.35 Benefit: 75% = \$511.75 85% = \$594.45^a</p>
CCCC	<p>A nucleic acid-based multi-gene panel test of tumour tissue from a patient diagnosed with non-small cell lung cancer, shown to have non-squamous histology or histology not otherwise specified, and with documented absence of activating mutations variants of the <i>EGFR</i> gene, <i>KRAS</i>, <i>BRAF</i> and <i>MET</i> exon14, requested by, or on behalf of, a specialist or consultant physician, to detect:</p> <ol style="list-style-type: none"> the fusion status of at least <i>ALK</i>, <i>ROS1</i>, <i>RET</i>, and <i>NTRK</i> to determine access to specific therapies listed on the Pharmaceutical Benefits Scheme (PBS) are fulfilled; or if the requirements relating to <i>ALK</i> and <i>ROS1</i> status for access to a PD-(L)1 immunotherapy pembrolizumab under the PBS are fulfilled. <p>Maximum one test per episode of disease This item can only be claimed if the result from MBS item number BBBB is negative, and cannot be claimed in addition to MBS items AAAA, 73341, 73344</p>

Category 6 – Genetics P7		
Fee: \$682.35	Benefit: 75% = \$511.75	85% = \$ 594.45 ^a

^a Reflects the 1 November 2021 Greatest Permissible Gap (GPG) of \$87.90. All out-of-hospital Medicare services which have an MBS fee of \$586.20 or more will attract a benefit that is greater than 85% of the MBS fee – being the schedule fee less the GPG amount. The GPG amount is indexed annually on 1 November in line with the Consumer Price Index (CPI) (June quarter). Suggested changes to the MBS items are shown in red and strikethrough text.

The proposal is that item CCCC (for RNA testing) would only be used if targetable biomarkers are not already detected by item BBBB (DNA testing). Although additional genes may be tested as part of the panels (as those listed are the minimum), a variant identified in other genes on the DNA panel (for which there is not a PBS-listed treatment available) is not intended to prohibit further testing of the RNA.

Note, the proposed items include testing of genes which currently do not have PBS-listed specific therapies for NSCLC (i.e. *KRAS*, *BRAF*, *MET* exon 14, *RET* and *NTRK*), although *MET* exon 14 has a PBAC-recommended specific therapy, which is not yet PBS-listed. The applicants justified the additional genes by referencing international guidelines, which recommend the inclusion of the specified genes as a minimum (given targeted therapies are available for NSCLC tumours with variants in the specified genes, even if they are not PBS-listed). This should future-proof the items in case the targeted therapies become PBS-listed in the near future. Concurrent variants in the listed genes are rare, so identifying pathogenic variants in the *KRAS*, *BRAF*, *RET* or *NTRK* genes is highly likely to rule out the presence of rearrangements in *ALK* or *ROS1* genes. The additional genes are therefore reasonable to include, although it may result in a very small number of patients with *ALK* or *ROS1* variants in their tumour not being identified, and consequently missing out on receiving an appropriate targeted therapy.

The proposal to refer to a PD-(L)1 immunotherapy rather than pembrolizumab reflects the fact that the PBS restriction for NSCLC of several of these medicines require that the “condition must not have evidence of an activating epidermal growth factor receptor (EGFR) gene or an anaplastic lymphoma kinase (ALK) gene rearrangement in tumour”. If MSAC supports this suggestion, then it is requested that MSAC also support the related changes to existing MBS items 73337, 73341 and 73344.

Sensitivity analyses were performed to assess the impact of allowing patients with *KRAS* or *BRAF* variants to undergo RNA testing.

5. Population

The target population are those diagnosed with non-squamous or not otherwise specified (NOS) NSCLC. It is estimated that in 2021, there were 11,738 newly diagnosed cases of NSCLC in Australia.

There are a number of different somatic variants which are important to identify in NSCLC tumours, as they may be the primary cause of the cancer growing and dividing. For many variants in NSCLC tumours, there are targeted treatments which have been found effective, and identification of the biomarker can therefore allow optimal treatment of the tumour. The targeted treatments currently listed on the PBS are:

- erlotinib, gefitinib, afatinib, osimertinib (for *EGFR* activating variants),
- osimertinib (for *EGFR* T790M variant after prior *EGFR* targeted treatment),
- crizotinib, ceritinib, alectinib, brigatinib, lorlatinib (for *ALK* rearrangements),

- crizotinib and entrectinib (for *ROS1* rearrangements), and
- PD-(L)1 immunotherapies (for those with an absence of activating *EGFR* variants, *ALK* rearrangements or *ROS1* rearrangements).

Tepotinib has also been recommended for those with *MET*ex14sk alterations.

Currently, the testing for the relevant biomarkers is done in a sequential manner, with *EGFR* variants the first to be tested (testing pathologist-determinable, and *EGFR* testing may occur as soon as NSCLC which is non-squamous or NOS is diagnosed). As small NGS gene panels are expected to replace the use of single gene testing, the projected number of patients who would use the proposed intervention can be estimated based on historical use of *EGFR* testing under MBS item 73337. A survey performed for the purposes of [PICO confirmation 1669](#) reported that most laboratories are already using small DNA panels. If the proposed separate DNA and RNA panels are added to the MBS, then the small DNA panel item is likely to be able to be used by most laboratories from the time of listing (Table 4). However, capacity to perform small RNA panels is more restricted, and in the near future, laboratories may either transfer the tissue to another laboratory for RNA testing or continue to use IHC and FISH for the assessment of *ALK* and *ROS1*. The applicants have also stated that some patients will have insufficient tumour tissue available for RNA to be extracted, so 5-10% of cases may continue to be tested using FISH rather than an NGS panel.

Prior testing, and projections of use only consider testing in patients with non-squamous (or not otherwise specified) histology, and do not consider the utilisation if patients with squamous NSCLC are also tested. This is estimated to increase the projections by 15%.

Table 3 Use of MBS item 73337, 2015–2021

	2015	2016	2017	2018	2019	2020	2021
No. services	3,368	3,419	3,863	4,147	4,603	4,697	4,854

Source: Services Australia

Table 4 Projected use of small gene panel testing (assuming 100% market share)

	2023	2024	2025	2026	2027	2028
Projected use of item 73337	5,521	5,797	6,074	6,351	6,628	6,905

If a patient has a biomarker identified by the small NGS gene panel (or the comparator), they may then be eligible for targeted treatment if they have locally advanced (stage IIIB) or metastatic (stage IV) NSCLC at the point of diagnosis, or once they progress to having locally advanced or metastatic disease.

If patients progress while on treatment, they may be suspected of having developed intolerance to treatment due to resistance-variants and may be tested for the *EGFR* T790M resistance variant. This use of test is not expected to alter with the introduction of small NGS panels.

The studies in the systematic review were included if at least 80% of the patients had non-squamous NSCLC (i.e. studies with a small proportion of squamous NSCLC were allowed as it was considered they would not influence the results significantly).

6. Comparator

The comparator to (one or two) small NGS panels is the use of sequential testing of biomarkers for targeted therapies for NSCLC using items currently available on the MBS (or in the near future). Specifically, this is:

- Testing of *EGFR* activating variant status (MBS item 73337)
- Immunohistochemistry (IHC) testing as triage ALK testing and triage ROS1 testing (most likely included under MBS item 72846 at the time of initial diagnosis)
- Testing of *ALK* gene rearrangement status by FISH (MBS item 73341)
- Testing of *ROS1* gene rearrangement status by FISH (MBS item 73344)
- Testing of *METex14* skipping alterations (recommended by MSAC)

At the point of diagnosis, patients are tested for *EGFR* activating variants using a single gene test and with IHC for ALK and ROS1 protein expression. MSAC has recommended that testing for *METex14* skipping (*METex14sk*) alterations be performed without the absence of other NSCLC biomarkers being a pre-requisite (Public Summary Document, ADAR 1660, p1). Although *METex14sk* testing is limited to patients with locally advanced or metastatic disease, the majority of patients meet this criteria at the point of diagnosis, so are assumed to be tested for *METex14sk* at the point of diagnosis.

If the patient's tumour is *EGFR* activating variant negative, but positive or equivocal on ALK IHC triage testing (staining intensity score >0), they may undergo confirmatory *ALK* gene rearrangement testing using FISH if/when they have locally advanced or metastatic disease.

Likewise, if the patient's tumour is *EGFR* variant negative, but positive or equivocal on ROS1 IHC triage testing (staining intensity score of 2+ or 3+), they may undergo confirmatory *ROS1* gene rearrangement testing using FISH if/when they have locally advanced or metastatic disease.

If patients do not have locally advanced or metastatic disease at the time of diagnosis, then a block retrieval item (MBS item 72860) may be required if referral to an outside laboratory is required for the FISH testing.

Small NGS panels are expected to replace all of these separate genetic tests. Other tests which co-occur at the point of diagnosis (but will not be affected by the introduction of NGS), are IHC to determine programmed death-ligand 1 (PD-L1) levels (noting that the PBS restriction for pembrolizumab in NSCLC is now agnostic for PD-L1 status).

7. Summary of public consultation input

Placeholder in case public consultation received prior to MSAC.

8. Characteristics of the evidence base

A total of 49 studies were identified from the systematic review, assessing the direct from test to health outcomes evidence, test performance, and change in management. The majority of the evidence was on test performance (k=40), with 30 studies reporting on the concordance of NGS with single gene tests. Conclusions on the concordance of the tests could therefore be made with high certainty. The proportion of samples successfully tested (based on having sufficient tissue/DNA/RNA for testing) was assessed in only one between-patient study, although this was

large (n=4040) and had low to moderate risk of bias. However, the evidence may not be directly applicable, as the healthcare setting in the Netherlands used a combination of DNA NGS with fusion testing performed by IHC or FISH or RNA NGS rather than just DNA and RNA NGS.

Change in management data (predictive yield and uptake of rebiopsy) and test-to-health outcomes data (clinical utility) were very limited. However, the last step of linked evidence (assessing the impact of the change of management) was supplemented by targeted (non-systematic) searches, which provided reasonable certainty in regards to the harms associated with rebiopsy, and low certainty evidence that the additional targetable variants identified by NGS are likely to respond to targeted therapies. The economic analysis incorporates the test performance data (concordance and proportion of tests performed successfully) and proportion of samples rebiopsied.

Although the target population was non-squamous NSCLC (or NOS), studies were included if no more than 15% of the included samples were squamous. Where data could be extracted separately for patients with non-squamous tumours, this was done (such as for some of the key evidence provided by Steeghs et al. (2022)), but the majority of studies did not provide subgroup analyses.

Table 5 Key features of the included evidence

Criterion	Type of evidence supplied	Extent of evidence supplied	Overall risk of bias in evidence base
Accuracy and performance of the test (cross-sectional accuracy)	Evidence that NGS is highly concordant with single-gene testing, and detects more extra cases than it misses Evidence that NGS has a higher proportion of samples successfully tested (better use of tumour tissue)	☒ k=30 n=4081 ☒ k=1 n=4040	Low to moderate risk of bias (QUADAS 2)
Change in patient management	Evidence that shows that use of NGS influences the treatments given in those with discordant results. Evidence that shows that some patients with insufficient tissue are rebiopsied	☒ k=6 n=99 ☒ k=2 n=225	Low to moderate risk of bias (QUADAS 2) (However, very small heterogeneous studies)
Health outcomes	Evidence that extra cases detected by NGS are likely to respond to TKIs Evidence that avoiding rebiopsies is safer than undergoing rebiopsy	☒ k=8 n=2921 ☒ k=16 n=2326	Moderate risk of bias (NHLBI for case series, AMSTAR 2 for SRs)
Predictive effect (treatment effect variation)	Evidence that NGS-selection of patients for ALK TKIs is superior to IHC- or FISH-selection for ALK TKIs	☒ k=1 n=50	Moderate to high risk (QUIPS checklist)

ALK = anaplastic lymphoma kinase; AMSTAR 2 = Assessing the Methodological Quality of Systematic Reviews; FISH = fluorescent *in situ* hybridisation; IHC = immunohistochemistry; k=number of studies, n=number of patients; NGS = next generation sequencing; QUADAS 2 = Quality assessment tool for diagnostic accuracy studies; QUIPS = Quality of Prognostic Studies tool; SRs = systematic reviews; TKI = tyrosine kinase inhibitor (therapy)

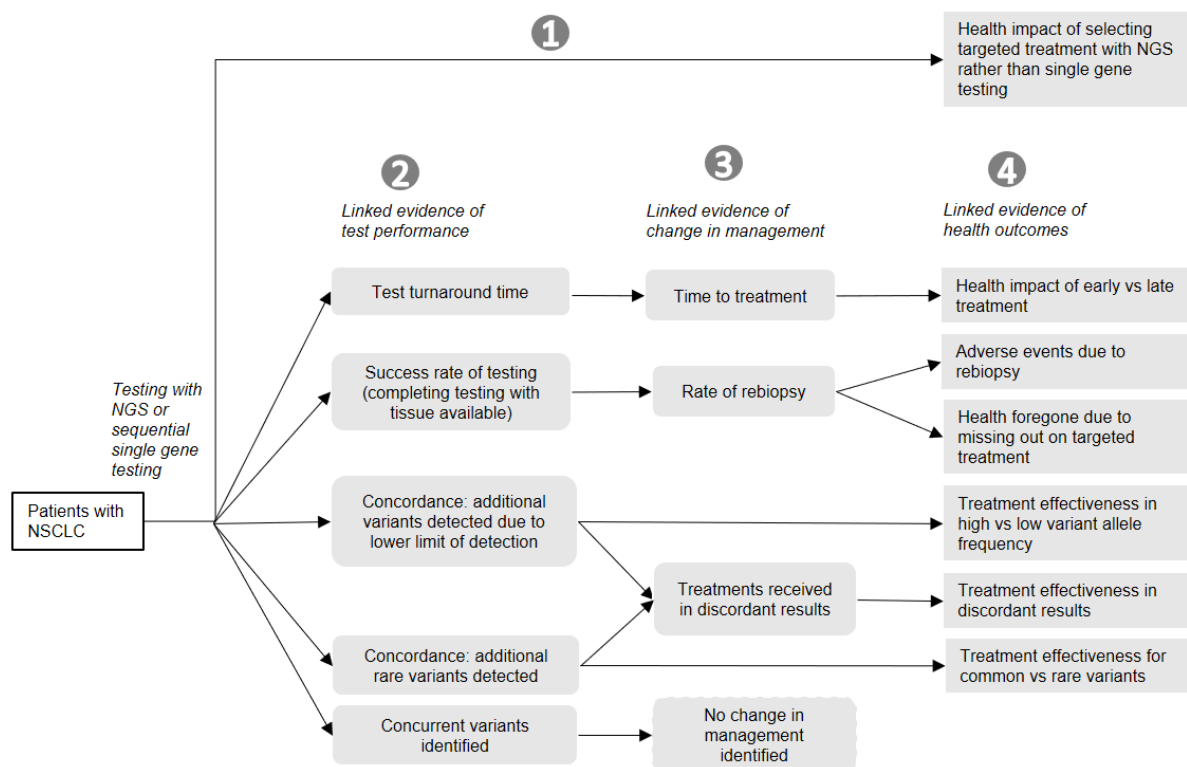


Figure 1 Assessment framework for small DNA/RNA NGS panel vs sequential single gene testing in patients with non-squamous (or NOS) NSCLC

Figure notes: 1: direct from test to health outcomes evidence; 2: test performance; 3: change in treatment/management; 4: influence of the change in management on health outcomes

9. Comparative safety

No studies directly compared the safety of NGS testing with sequential single-gene testing. However, the evidence supported the claim that NGS had a higher proportion of samples being successfully tested (i.e. making more efficient use of the available tissue to get a test result) than sequential single-gene testing, which should correspond to a lower rate of rebiopsy. A single between-patient comparison was identified in a retrospective cohort study with a low to moderate risk of bias¹ (a further three studies provided within-patient comparisons, but these were considered to not be as informative, as the volume of tissue used for one method of testing would influence the volume of tissue remaining for the alternative method of testing, and the ordering of testing would highly bias the proportion of samples successfully tested). Steeghs et al. (2022) reported that NGS methods were successful in 97.2% of cases, whereas non-NGS methods were successful in 94.6% of cases (Figure 2).

¹ Steeghs, EMP, Groen, HJM, Schuurin, E, Aarts, MJ, Damhuis, RAM, Voorham, QJM, Ligtenberg, MJL & Grunberg, K 2022, 'Mutation-tailored treatment selection in non-small cell lung cancer patients in daily clinical practice', *Lung Cancer*, vol. 167, May, pp. 87-97.

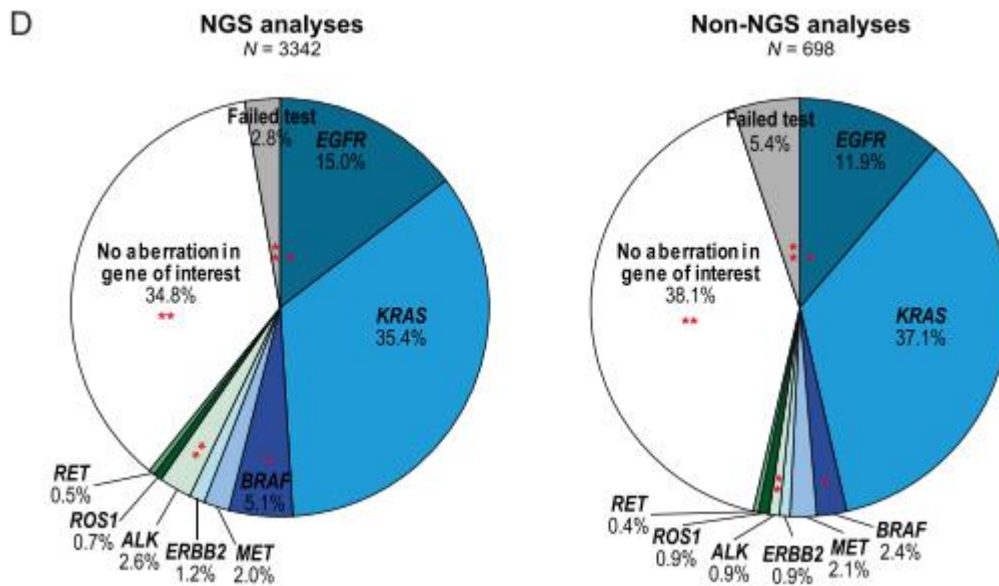


Figure 2 Comparison of pathogenic variants identified by NGS vs non-NGS methods (Sanger sequencing, HRM, MassARRAY, Pyrosequencing, Idylla, Cobas, ddPCR, FISH, IHC and/or RNA-based sequencing) in patients with adenocarcinoma.

Source: Steeghs et al, 2022, p91. Reproduced with permission under Creative Common CC-BY license.

ALK = anaplastic lymphoma kinase; BRAF = proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B; ddPCR = digital droplet polymerase chain reaction; EGFR = epidermal growth factor receptor; ERBB2 = erb-b2 receptor tyrosine kinase 2; FISH = fluorescent *in situ* hybridisation; HRM = high resolution melting; IHC = immunohistochemistry; KRAS = Kirsten rat sarcoma; MET = mesenchymal-epithelial transition; NGS = next generation sequencing; RET = rearranged during transfection; RNA = ribonucleic acid; ROS1 = ROS proto-oncogene 1; If multiple variants were identified in one patient, only the first variant was included in the pie chart (so that the sum = 100%), **p<0.01, *p<0.05

The relationship between insufficient tissue for testing, or test failure due to insufficient DNA or RNA and subsequent rebiopsy is uncertain, as some international guidelines now recommend the use of liquid biopsy (i.e., using a blood sample) when tumour tissue is insufficient, rather than performing a second biopsy of tumour tissue/cytology. Two case series reported that 13% and 43% of patients with insufficient tissue for NGS or testing EGFR and ALK (by an unspecified method) had a rebiopsy performed, with the remainder either having plasma NGS, or not having their tumour further biomarker-tested². No Australian guidelines were able to be identified on the role of rebiopsy versus liquid biopsies in the absence of sufficient tissue from the initial biopsy. However, these case series data are unlikely to be relevant to the current Australian setting, as the PBS restrictions for targeted therapies in NSCLC require the biomarkers to be identified in *tumour tissue*. A higher proportion of failed tests are therefore likely to proceed to rebiopsy than reported in these case series.

With each rebiopsy, there is a risk of additional adverse events. A systematic review of 16 studies in patients with NSCLC undergoing percutaneous transthoracic needle biopsies (PTNBs) or rebiopsies for biomarker testing, reported that the risk of any adverse event was 17% (95%CI 12%, 23%). The most common complication was pneumothorax (collapsed lung), with a pooled

² Gutierrez, ME, Choi, K, Lanman, RB, Licitra, EJ, Skrzypczak, SM, Pe Benito, R, Wu, T, Arunajadai, S, Kaur, S, Harper, H, Pecora, AL, Schultz, EV & Goldberg, SL 2017, 'Genomic Profiling of Advanced Non-Small Cell Lung Cancer in Community Settings: Gaps and Opportunities', *Clin Lung Cancer*, vol. 18, no. 6, Nov, pp. 651-659.

Li, W, Li, Y, Guo, L, Liu, Y, Yang, L & Ying, J 2021, 'Metastatic NSCLCs With Limited Tissues: How to Effectively Identify Driver Alterations to Guide Targeted Therapy in Chinese Patients', *JTO Clin Res Rep*, vol. 2, no. 5, May, p. 100167.

incidence of 9.2% (95%CI 4.0%, 15.7%)³. Severe adverse events (pneumothorax requiring chest tube, massive haemoptysis, air embolism and death) occurred in less than 1%. Although the authors of meta-analysis reported that PTNBs were safe, it is clear that a reduction in the need for rebiopsy would reduce the risk of adverse events associated with biopsies.

Linked evidence (of proportion of samples successfully tested, the frequency of rebiopsy, and risk of adverse events due to rebiopsy) therefore supported the claim that NGS has superior safety to sequential single-gene testing. The key uncertainties are the extent to which patients in Australia currently undergo rebiopsy when the volume of tissue available is insufficient, and whether practice in Australia will change in the near future to incorporate liquid biopsy as an alternative to tissue rebiopsy.

10. Comparative effectiveness

The claims made by the applicant was that NGS is superior to sequential single-gene testing, as it makes more efficient use of tumour tissue. This results in a higher proportion of patients being successfully tested, having biomarkers identified, and able to receive targeted treatment (which should result in superior health outcomes). NGS may also detect concurrent variants, which is unlikely with sequential single-gene testing as testing is halted once a targetable biomarker is identified (concurrent variants may influence treatment or provide prognostic information). NGS may also provide faster results than sequential single-gene testing (resulting in faster access to targeted treatment and superior health outcomes). The evidence addressing these claims was examined.

As outlined in the safety section, a single between-patient study was identified which provided the proportion of samples successfully tested, favouring NGS over sequential single-gene testing (97.2% vs 94.6%).

In order to test how concordant NGS and sequential single-gene testing are, within-patient studies were required, which provide data to compile a 2x2 table. A total of 30 relevant studies were identified in patients with NSCLC, with results separated per gene (rather than per person or per variant). The positive percent agreement (akin to the concept of sensitivity) and negative percent agreement (akin to the concept of specificity) were meta-analysed (where possible). These data were then transformed back into 2x2 data (per 1000 patients), using prevalence figures appropriate to Australia. The summary of these results is shown in Table 6.

In cases successfully tested by both testing strategies, NGS and sequential single-gene testing were highly concordant, with 95.7% of cases receiving the same test result from both strategies (22.4% with a biomarker, and 73.3% without biomarkers). Overall, NGS was estimated to result in an additional 35 cases per 1000 tested with variants identified which would have been missed by single-gene testing, with 8 cases per 1000 having a biomarker missed by NGS, which would have been detected by single-gene testing.

The largest impact which NGS would have (in raw numbers), is an additional 2.5% of patients being found with *EGFR* variants. This was due in part to NGS having a higher level of analytical sensitivity (a lower threshold of detection) than Sanger sequencing, the cobas assay and some other PCR tests, although NGS had a higher threshold of detection than ARMS-PCR. The population criteria for *EGFR* TKIs on the PBS do not specify a threshold for positivity, so the use of tests with a higher level of sensitivity would identify more patients eligible for TKIs, despite

³ Nam, BD, Yoon, SH, Hong, H, Hwang, JH, Goo, JM & Park, S 2021, 'Tissue Adequacy and Safety of Percutaneous Transthoracic Needle Biopsy for Molecular Analysis in Non-Small Cell Lung Cancer: A Systematic Review and Meta-analysis', *Korean J Radiol*, vol. 22, no. 12, Dec, pp. 2082-2093.

these patients potentially having a different spectrum of disease than those in the key trials used to establish the clinical utility of the test-drug codependency. In addition, NGS detected some rare variants not able to be detected by all the methods of single-gene testing. For example, Tan et al. (2020) reported that NGS identified an additional 12 variants, of which 7 (58%) were common variants (ex19del, L858R or T790M), and 5 (42%) were rare variants. Similarly, Park et al. (2020) reported that of the 16 incremental *EGFR* variants identified by NGS, 8 were in hotspot locations (in regions tested by PCR, but below the sensitivity threshold), and the remaining 8 were in locations not tested by PCR, although half of the rare variants identified were considered actionable, and *EGFR* TKIs were administered. The majority (but not all) of the additional 2.5% with *EGFR* variants would therefore be considered to have “activating variants” conferring sensitivity to *EGFR* TKIs.

The largest relative difference was the number of patients identified with *ALK* rearrangements. Four studies used the same threshold for positivity as the PBS restrictions for *ALK* TKIs ($\geq 15\%$ of cells with staining on FISH), and had similar results (PPA 91%, NPA 99%) to studies which used a lower threshold ($\geq 10\%$, $k=2$) or did not specify the threshold for positivity ($k=5$) (PPA 92%, NPA 99%). Results for *ROS1* and *MET*ex14sk were highly concordant between testing methods.

Table 6 Summary of concordance data between NGS and single-gene testing

Gene	Evidence base	PPA (95%CI)	NPA (95%CI)	Prevalence	Per 1000 successfully tested (95%CI)				PPV (95%CI)	NPV (95%CI)
					NGS+ /SG+	NGS+ /SG-	NGS- /SG+	NGS- /SG-		
<i>EGFR</i>	n=2611 k=22	0.98 (0.95, 0.99)	0.97 (0.95, 0.99)	15% ^a	147 (143, 149)	25 (8, 42)	3 (1, 7)	825 (808, 842)	0.85	1.00
<i>ALK</i>	n=1464 k=11	0.92 (0.77, 0.97)	0.99 (0.93, 1.00)	3% ^b	28 (23, 29)	10 (0, 68)	2 (1, 7)	960 (902, 97)	0.74	1.00
<i>ROS1</i>	n=830 k=6	0.86 (0.63, 0.96)	1.00 (0.99, 1.00)	1.61% ^c	14 (10, 15)	0 (0, 10)	2 (1, 6)	984 (974, 984)	1.00	1.00
<i>MET</i> ex14sk	n=99 k=1	0.98 (0.89, 1.00)	1.00 (0.93, 1.00)	3.6% ^d	35 (32, 36)	0 (0, 69)	1 (0, 4)	964 (895, 964)	1.00	1.00
Total					224	35	8	733		

^aBased on p18 [MSAC 1161 PSD](#), November 2012

^bBased on p5 [MSAC 1250.1 PSD](#), November 2014

^cBased on p12 [MSAC 1454 PSD](#), July 2018

^dBased on Table 11, p27 [Tepotinib PBAC PSD](#), November 2021

ALK = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; *MET* = mesenchymal-epithelial transition; NGS = next generation sequencing; NPA = negative percent agreement; NPV= negative predictive value; PPA = positive percent agreement; PPV = positive predictive value; *ROS1* = ROS proto-oncogene 1; SG = single-gene testing

The superiority of targeted therapies over non-targeted therapies for those with biomarkers has been demonstrated in submissions to the PBS for erlotinib, gefitinib, afatinib, osimertinib, crizotinib, ceritinib, alectinib, brigatinib, lorlatinib, entrectinib, and tepotinib. Steeghs et al. (2022) reported that NGS had a higher success rate, higher sensitivity, and was more comprehensive than sequential single-gene testing, and consequently, a higher yield of actionable variants. This should therefore result in a higher proportion of patients receiving targeted therapies, and result in superior health outcomes.

Change in management data were scant (six small before-and-after case series) but suggested that in cases where NGS identified actionable variants missed by sequential single gene testing, targeted treatment was initiated in a median of 50% of cases (range 17.6% to 100%). (Note,

insufficient information was provided to determine whether those variants considered actionable in the studies would also be considered eligible for PBS-listed targeted treatments).

Targeted searches were performed to identify evidence on whether patients with low allele frequency or rare variants responded to *EGFR* TKIs in the same manner as patients selected by the clinical utility standards. A systematic review was identified comparing *EGFR* TKI treatment effectiveness in those with common sensitising variants (ex19del or L858R⁴), the common resistance conferring variant (T790M) and rare variants (any other variants)⁵. The results were heterogeneous, and not meta-analysed due to differences in the method of grouping variants. Those with exon 20 variants were less likely to respond to the listed *EGFR* TKIs than those with common sensitising variants, and a number of exon 20 insertions were considered to have some evidence of conferring resistance to *EGFR* TKIs. Those with variants in exon 18 (such as variant G719X) frequently responded well to *EGFR* TKIs, so this variant may now be considered likely to confer sensitivity to *EGFR* TKIs. Therefore, currently, the benefit of having additional rare variants identified due to using NGS is mixed. Eligibility for *EGFR* TKIs will depend on whether the report provided by pathologists to the treating clinician, defines the actionability of the identified variants. In the future, it is expected that targeted treatment for those with exon 20 insertions will become available in Australia, which should increase the proportion of patients who benefit from having rare variants identified.

⁴ These common sensitising variants align with the inclusion criteria for studies such as the EURTAC RCT of erlotinib vs chemotherapy (2012) which was part of the evidentiary basis of the test-drug co-dependency approved by MSAC/PBAC.

⁵ John, T, Taylor, A, Wang, H, Eichinger, C, Freeman, C & Ahn, MJ 2022, 'Uncommon EGFR mutations in non-small-cell lung cancer: A systematic literature review of prevalence and clinical outcomes', *Cancer Epidemiol*, vol. 76, Feb, p. 102080.

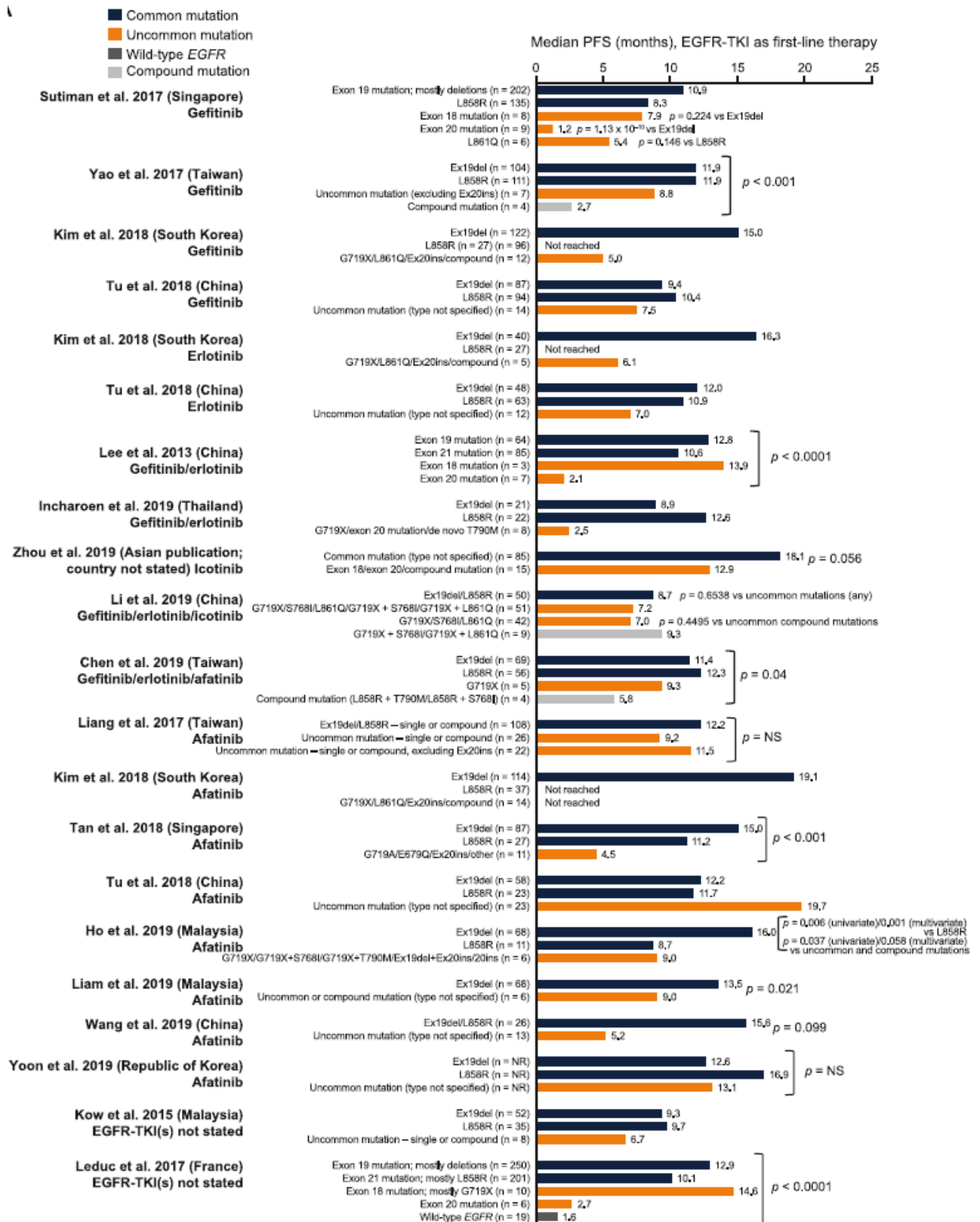


Figure 3 Progression free survival in patients with common or uncommon *EGFR* variants receiving *EGFR*-TKI as first-line therapy

NB: p-values denote comparison between common and uncommon variants.

Liang et al. "compound mutations" refers to two uncommon variants. Wu et al. (2018): L858R cohort includes four patients with both L858R and Ex19del. Ho et al. (2019): exact uncommon mutations are G719X/G719X + S768I/G719X + T790M/Ex19del + Ex20ins/Ex20ins. Lee et al. (2013) and Leduc et al. (2017): "Exon 20 mutations" do not include T790M.

Targeted (non-systematic) searches were performed to assess whether patients with low allele frequency in tumour tissue (likely only detected by high sensitivity testing methods), responded to EGFR TKIs in the same manner as those with high allele frequency. Note that for EGFR TKIs, no threshold is defined for positivity in PBS restrictions. Three observational studies were identified, which suggested that those with a high allele frequency (i.e., a high proportion of tumour cells which have the variant identified) responded better to EGFR TKIs than those with low allele frequency. Low levels of T790M variants identified concurrently with activating EGFR variants did not significantly impact on treatment effectiveness of EGFR TKIs. Some of the additional variants detected by small NGS panels may therefore not respond to targeted therapy in the same manner as those detected by sequential single-gene testing.

Table 7 Association between variant allele frequency and response to treatment

Study	Population	Intervention	Outcome	Results
Friedlaender et al. (2021) ⁷ Switzerland	42 patients with NSCLC and EGFR variants Threshold for high vs low allelic frequency: 0.30	NGS using IonAmpliseq Hotspot Panel V2 Treatment with EGFR TKI	PFS	High vs low: HR = 0.27 (95%CI 0.09, 0.79, p=0.017)
			OS	High vs low: HR = 0.47 (95%CI 0.17, 1.30, p=0.14)
Gieszer et al. (2021) ⁸ Hungary	89 Caucasian patients with NSCLC (adenocarcinomas), and EGFR variants Adjusted VAF (aVAF) = VAF/TC% x 100	Therascreen EGFR Pyro assay Erlotinib or gefitinib as first- or second-line treatment	PFS	Positive linear correlation between aVAF and PFS: r = 0.319, p=0.003, Spearman's correlation
			PFS	Adjusting for clinicopathological variables (age, gender, variant, treatment, treatment line): HR = 0.991 (95%CI 0.982, 0.999, p=0.042)
			OS	High vs low aVAF median 94 vs 57 weeks, p=0.011
Ye et al. (2021) ⁹ Australia	64 patients with NSCLC and EGFR variants, with stage IV disease 14 VAF <0.1% 28 VAF ≥0.1% 1 detectable by SS, VAF = 28.5%	Digital PCR Erlotinib or gefitinib	PFS	No significant difference by T790M status (log rank test p = 0.897), or T790M allele frequency (<0.1 vs ≥0.1%, p=0.515)

HR = hazard ratio; NSCLC = non-small cell lung cancer; OS = overall survival; PCR = polymerase chain reaction; PFS = progression free survival; SS = Sanger sequencing; TC = estimated percentage of neoplastic cells; VAF = variant allele frequency, percentage of alleles determined by the assay to have EGFR variants

One of the claims made by the applicant was that NGS returns results faster than sequential single-gene testing. Three cohort studies were identified which compared turnaround times and reported that NGS was 0 to 3 days faster than sequential single-gene testing strategies. The 3-

⁶ John, T, Taylor, A, Wang, H, Eichinger, C, Freeman, C & Ahn, MJ 2022, 'Uncommon EGFR mutations in non-small-cell lung cancer: A systematic literature review of prevalence and clinical outcomes', *Cancer Epidemiol*, vol. 76, Feb, p. 102080.

⁷ Friedlaender, A, Tsantoulis, P, Chevallier, M, De Vito, C & Addeo, A 2021, 'The Impact of Variant Allele Frequency in EGFR Mutated NSCLC Patients on Targeted Therapy', *Front Oncol*, vol. 11, p. 644472.

⁸ Gieszer, B, Megyesfalvi, Z, Dulai, V, Papay, J, Kovalszky, I, Timar, J, Fillinger, J, Harko, T, Pipek, O, Teglas, V, Regos, E, Papp, G, Szallasi, Z, Laszlo, V, Renyi-Vamos, F, Galffy, G, Bodor, C, Dome, B & Moldvay, J 2021, 'EGFR variant allele frequency predicts EGFR-TKI efficacy in lung adenocarcinoma: a multicenter study', *Transl Lung Cancer Res*, vol. 10, no. 2, Feb, pp. 662-674.

⁹ Ye, L, Mesbah Ardakani, N, Thomas, C, Spiisbury, K, Leslie, C, Amanuel, B & Millward, M 2020, 'Detection of Low-level EGFR c.2369 C > T (p.Thr790Met) Resistance Mutation in Pre-treatment Non-small Cell Lung Carcinomas Harboring Activating EGFR Mutations and Correlation with Clinical Outcomes', *Pathol Oncol Res*, vol. 26, no. 4, Oct, pp. 2371-2379.

day saving in turnaround time was reported when a combined DNA and RNA panel was used¹⁰. In a large study from the Netherlands, no difference to sequential single gene testing was reported, when a DNA panel was used in combination with either IHC, FISH or an RNA panel¹¹. These data are likely to be more applicable to the Australian setting in the near future, as not many laboratories are currently able to use NGS on both DNA and RNA simultaneously. However, as more laboratories develop the ability to perform simultaneous NGS testing, and as more biomarkers are deemed relevant by MSAC/PBAC, the difference in turnaround time between NGS and sequential single gene testing is expected to increase.

Table 8 Turnaround time for NGS vs sequential single-gene testing strategy

Study	Population	Intervention (NGS)	Comparator (SG)	Turnaround time for NGS	Turnaround time for comparator	Difference
Dall'Olio et al. (2020)	N=537 Consecutive NSCLC (adenocarcinoma) patients	Oncomine Focus Assay on DNA and RNA	Single gene (<i>EGFR</i> , <i>KRAS</i> , <i>BRAF</i> , <i>MET</i> or <i>HER2</i>), IHC and FISH	Mean 10 working days	Mean 13.15 days	-3.15 days
Li et al. (2021) ¹²	884 newly diagnosed, treatment-naïve metastatic NSCLC patients with limited tissue sample	NGS on DNA only	ARMS-PCR and IHC/FISH	Median 12 business days (range 5 - 79 days)	Median 13 business days (range 9 – 86)	-1 day
Steeghs et al. (2022) ¹³	Stage IV NSCLC patients. 3343 NGS patients, 698 non-NGS patients	NGS on DNA, plus fusions tested by IHC, FISH or RNA NGS	Various non-NGS single gene testing such as ICH and FISH used throughout clinical practice in the Netherlands	Median 10 days (range 0 - 495; IQR 7 – 14)	Median 10 days (range 2 – 63; IQR 7 – 13)	0 days

ARMS-PCR = amplification-refractory mutation system polymerase chain reaction; DNA = deoxyribonucleic acid; *EGFR* = epidermal growth factor receptor; FISH = fluorescent *in situ* hybridisation; IHC = immunohistochemistry; *KRAS* = Kirsten rat sarcoma; NGS = next generation sequencing; NSCLC = non-small cell lung cancer; RNA = ribonucleic acid; SG = single-gene testing

The clinical implications of faster initiation of targeted treatment are unclear. No studies could be found which focused on the health implications of the timeliness of targeted treatments (i.e. prompt vs delayed targeted treatment). However, a systematic review was identified which compared health outcomes in those with advanced NSCLC who received timely vs untimely first-line untargeted treatment (surgery, radiotherapy, chemotherapy, or any treatment), and reported that those treated faster had worse outcomes¹⁴. This “waiting time paradox” is likely due to

¹⁰Dall'Olio, FG, Conci, N, Rossi, G, Fiorentino, M, De Giglio, A, Grilli, G, Altamari, A, Gruppioni, E, Filippini, DM, Di Federico, A, Nuvola, G & Ardizzoni, A 2020, 'Comparison of Sequential Testing and Next Generation Sequencing in advanced Lung Adenocarcinoma patients - A single centre experience', *Lung Cancer*, vol. 149, November, pp. 5-9.

¹¹ Steeghs, EMP, Groen, HJM, Schuurin, E, Aarts, MJ, Damhuis, RAM, Voorham, QJM, Ligtenberg, MJL & Grunberg, K 2022, 'Mutation-tailored treatment selection in non-small cell lung cancer patients in daily clinical practice', *Lung Cancer*, vol. 167, May, pp. 87-97.

¹² Li, W, Li, Y, Guo, L, Liu, Y, Yang, L & Ying, J 2021, 'Metastatic NSCLCs With Limited Tissues: How to Effectively Identify Driver Alterations to Guide Targeted Therapy in Chinese Patients', *JTO Clin Res Rep*, vol. 2, no. 5, May, p. 100167.

¹³ Steeghs, EMP, Groen, HJM, Schuurin, E, Aarts, MJ, Damhuis, RAM, Voorham, QJM, Ligtenberg, MJL & Grunberg, K 2022, 'Mutation-tailored treatment selection in non-small cell lung cancer patients in daily clinical practice', *Lung Cancer*, vol. 167, May, pp. 87-97.

¹⁴ Hall, H, Tocock, A, Burdett, S, Fisher, D, Ricketts, WM, Robson, J, Round, T, Gorolay, S, MacArthur, E, Chung, D, Janes, SM, Peake, MD & Navani, N 2021, 'Association between time-to-treatment and outcomes in non-small cell lung cancer: a systematic review', *Thorax*, Aug 17.

patients with more symptoms (and worse prognosis) being treated faster, and those treated palliatively receiving more timely care than those treated with curative intent.

Clinical claim

The evidence supported the clinical claim of superior effectiveness due to more patients being identified with variants by small NGS panels than sequential single gene testing (moderate level of confidence). The majority of these additional patients would be considered eligible for PBS-listed targeted treatments with the proposed changes to restrictions. The additional patients with actionable variants identified are then able to be managed with targeted treatment, which may result in superior health outcomes (low confidence).

The evidence also supported the clinical claim of superior safety, due to the likelihood that the more efficient use of tumour tissue by NGS than sequential single gene testing (moderate confidence) would result in fewer rebiopsies being performed (low confidence) and avoiding biopsies would reduce the risk of adverse events associated with biopsies (moderate confidence).

11. Economic evaluation

The clinical claim of superiority was made based on:

- an improvement in the test success rate (i.e., more samples with sufficient quantity and/or quality to be able to be successfully tested for variants); and
- an improvement in the yield of variants identified due to being more comprehensive (identifying “in scope” and “beyond restriction” variants) and more sensitive (detecting in-scope variants at a lower variant allelic frequency).

Therefore, a cost-effectiveness analysis was presented based on the results of the linked evidence approach. No evidence was identified to enable modelling changes in treatment, and given the following uncertainties, the model presented was truncated at the point of treatment:

- Variants that can be identified by either current or proposed testing (referred to in the analysis as “common” variants) result in the use of targeted therapies. In patients with common variants that are missed by proposed small gene panel testing (due to discordant results or unsuccessful testing and unsuccessful rebiopsy) or those that are missed by current testing (due to IHC triage or unsuccessful testing and unsuccessful rebiopsy), patients may receive standard of care (SoC) in place of targeted therapies. Quantifying the foregone benefit associated with the treatment of common variants with SoC is difficult because, in many cases, SoC has evolved since the initial trials of targeted therapies. No evidence was identified in the clinical evaluation to quantify the benefit of targeted therapies compared with SoC in patients with common variants.
- Variants that can only be identified through small gene panel testing are referred to in the analysis as “incremental” variants, and include variants both within the current scope of eligibility to PBS-listed targeted therapies (due to detection of lower allelic frequencies and some additional *EGFR* variants known to confer sensitivity to TKIs), and those beyond current PBS restrictions. Best estimates from the clinical evidence base suggest that the majority of these patients would be eligible for PBS-listed targeted treatments; however, these patients may have a different spectrum of disease than those in the key trials of the targeted therapies. Therefore, treatment response and duration of treatment in patients with these incremental variants to both targeted therapy and SoC is uncertain,

and so the incremental benefits and costs that may be associated with changing treatment from SoC to targeted therapy are also uncertain.

- In addition to the issues regarding quantifying the differences in outcomes with proposed small gene panel testing, the modelled costs of any analyses that attempt to capture outcomes due to changes in treatment would likely be affected by existing special price arrangements for targeted therapies and immunotherapies. Analyses based on the published prices would not reflect the accepted cost-effectiveness of the included therapies, and the cost-effectiveness of proposed small gene panel testing would be influenced by confidential discounts applied to both targeted therapies and immunotherapies.

The analysis presented was therefore a cost-effectiveness analysis where the primary outcome reported was the net change in patients determined to be eligible for targeted therapy. This outcome was disaggregated by type of actionable variant identified (i.e. common or incremental).

A stepped approach was used to generate the base case analysis that incorporated different aspects of the linked evidence separately to distinguish the effect of each of these on the results. Further, incremental yield data with proposed panel testing have been adjusted in the economic analysis to reflect some IHC ± FISH expected in practice and to reflect comparisons to the clinical utility standard. Test success data have also been transformed to reflect implications of rebiopsies due to insufficient quantity or quality of tissue. These translations of the clinical evidence for use in the model have been added in separate steps. Other key model assumptions – RNA panel use restricted to an absence of *KRAS* and *BRAF* variants, and use of testing in patients who do not progress to advanced disease – have also been incorporated in separate steps.

A summary of the key components of the economic evaluation is presented in Table 9.

Table 9 Summary of the economic evaluation

Component	Description
Perspective	Health care system perspective
Population	Patients with non-squamous or NOS NSCLC
Prior testing	Histopathology testing to confirm tumour histology
Comparator	Single gene testing (reflex <i>EGFR</i> , ALK IHC and ROS1 IHC, followed by, if relevant, reflex ALK FISH and/or <i>ROS1</i> FISH, and <i>MET</i> ex14sk testing)
Type(s) of analysis	Cost-effectiveness analysis
Outcomes	Primary: Patients eligible for targeted therapy, disaggregated by patients with common and incremental variants identified Additional: Patients with actionable (i.e. common and incremental variants) variants identified, patients with known biomarker status; changes in rebiopsies required
Time horizon	Time to first-line treatment decisions in the advanced NSCLC setting
Computational method	Decision analytic
Generation of the base case	Modelled stepped analysis, incorporating different aspects of the linked evidence, translations of the clinical evidence and other key model assumptions separately to distinguish the effect of each of these on the results.
Transition probabilities	Yield of actionable variants: Accepted estimates of variant yield as identified by the clinical utility standard ('common' variants), adjusted for additional variants identified by small gene panel testing in the same biomarker ('incremental' variants) using concordance estimates derived in the clinical evaluation. Yield estimates were adjusted to reflect some IHC ± FISH use following small DNA panel testing (in instances where tissue quantity or quality is insufficient for RNA panel testing). Success of testing was also based on estimates presented in the clinical evaluation.
Discount rate	Not applicable
Software	TreeAge Pro and Microsoft Excel

ALK = anaplastic lymphoma kinase; DNA = deoxyribose nucleic acid; *EGFR* = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; IHC = immunohistochemistry; *MET*ex14sk = MET proto-oncogene, receptor tyrosine kinase exon 14 skipping alterations; NOS = not otherwise specified; NSCLC = non-small cell lung cancer; RNA = ribonucleic acid; *ROS1* = ROS proto-oncogene 1.

The results of the stepped analysis to generate the base case economic evaluation is presented in Table 10. The steps that had the most effect on the results of the analysis included restricting RNA-only panel testing to those without *KRAS* and *BRAF* activating variants; applying an increase in variant yield with panel testing, the inclusion of patients tested with early-stage disease who do not progress; and including costs and outcomes related to rebiopsy.

Table 10 Results of the stepped economic analysis

	Small gene panel testing	Single gene testing	Increment
Step 1: Test cost difference only			
No difference in success or yield between current and proposed testing. In two-stage panel testing, patients with <i>KRAS</i> or <i>BRAF</i> variants receive RNA testing.			
Total cost	\$1,240.55	\$894.72	\$345.83
Step 2: RNA panel testing restricted to <i>KRAS</i> and <i>BRAF</i> negatives			
As per the proposed small RNA gene panel test item, where two-stage panel testing is used, patients found with <i>KRAS</i> or <i>BRAF</i> variants <u>cannot</u> receive RNA testing.			
Total cost	\$1,093.43	\$894.72	\$198.72

	Small gene panel testing	Single gene testing	Increment
Step 3: Incorporate differences in test success across model arms			
Sufficient sample is available for testing in 97.2% of patients tested with small gene panels, compared to 94.6% with single gene testing, based on Steeghs et al. (2022) ^a (Section 10). As proposed testing can only be claimed once per episode of disease and cannot be claimed in addition to single gene items, where testing is not successful due to insufficient sample, no cost of testing is assumed to apply in either model arm.			
Total cost	\$1,062.82	\$846.40	\$216.42
Proportion with an actionable variant identified ^b	0.2256	0.2196	0.0060
ICER per additional patient with an actionable variant identified			\$35,862
Step 4: Incorporate differences in yield across model arms			
Concordance data of small gene panel testing, relative to the respective single gene test, is incorporated (Table 6, Section 10). Where PPA < 1, some variants that may have otherwise been identified through single gene testing may be missed, and where NPA < 1 additional "in scope" and "beyond restriction" variants are identified. As the majority of small gene panel testing uses the two-step method, with more variants identified on the small DNA panel, fewer small RNA panels may be required (and so a reduction in small gene panel test cost is observed).			
Total cost	\$1,052.71	\$846.40	\$206.30
Proportion with an actionable variant identified ^b	0.2517	0.2196	0.0321
ICER per additional patient with an actionable variant identified			\$6,425
Step 5: Adjust ALK concordance for comparison to clinical utility standard			
The concordance of small gene panel testing to single gene test methods for ALK in Table 6 was based on a comparison of NGS to FISH ± IHC, whereas the clinical utility standard used in the trials for ALK targeted therapy was FISH (≥15% positive cells). Only one study that compared small gene panel testing to FISH reported using this same definition of positivity (Park and Shim 2020) ^c . PPA of ALK and ROS1 IHC relative to FISH was also incorporated.			
Total cost	\$1,052.71	\$846.40	\$206.30
Proportion with an actionable variant identified ^b	0.2526	0.2184	0.0342
ICER per additional patient with an actionable variant identified			\$6,026
Step 6: Adjust for some IHC ± FISH use with proposed testing			
The applicant expected that 5–10% of tests would require current testing methods. MSAC have previously considered that small DNA panels are currently being used for EGFR testing (MSAC 1669 PSD, March 2022 MSAC Meeting) and so this has been assumed to apply to small RNA gene panels only, as RNA panels may have larger sampling requirements. This reduces both the cost of proposed testing and also yield (as additional "in scope" and "beyond restriction" variants would not be identified in this proportion of patients)			
Total cost	\$1,035.55	\$846.40	\$189.15
Proportion with an actionable variant identified ^b	0.2523	0.2184	0.0339
ICER per additional patient with an actionable variant identified			\$5,582
Step 7: Incorporate patients with early disease who do not progress			
Small gene panel testing is proposed to occur on diagnosis of non-squamous or NOS NSCLC. While current EGFR and ALK and ROS1 IHC testing also occur at diagnosis, ALK and ROS1 FISH and proposed METex14sk testing do not occur until the development of advanced disease. The analysis therefore has been adjusted to reflect that not all patients who receive small gene panel testing would develop advanced disease (and so would not be eligible for targeted therapy, currently available only in the advanced setting).			
Total cost	\$1,035.54	\$743.77	\$291.76
Proportion eligible for targeted therapy	0.1913	0.1656	0.0257
ICER per additional patient eligible for targeted therapy			\$11,352

	Small gene panel testing	Single gene testing	Increment
Step 8: Incorporate rebiopsies			
In those with insufficient sample for testing, rebiopsy is attempted where 20% are assumed to fail (Kelly et al. 2019) ^d .			
Total cost	\$1,173.23	\$1,004.20	\$169.02
Proportion eligible for targeted therapy	0.1957	0.1732	0.0225
ICER per additional patient eligible for targeted therapy			\$7,496

^a Steeghs, EMP, Groen, HJM, Schuurin, E, Aarts, MJ, Damhuis, RAM, Voorham, QJM, Ligtenberg, MJL & Grunberg, K 2022, 'Mutation-tailored treatment selection in non-small cell lung cancer patients in daily clinical practice', Lung Cancer, vol. 167, May, pp. 87-97.

^b Incorporates variants that could be identified by either current or proposed testing, or incremental variants within the current scope of eligibility to PBS-listed targeted therapies, and those beyond current PBS restrictions.

^c Park, E & Shim, HS 2020, 'Detection of targetable genetic alterations in Korean lung cancer patients: A comparison study of single-gene assays and targeted next-generation sequencing', Cancer Research and Treatment, vol. 52(2), pp. 1-9.

^d Kelly, RJ, Turner, R, Chen, YW, Rigas, JR, Fernandes, AW & Karve, S 2019, 'Complications and Economic Burden Associated With Obtaining Tissue for Diagnosis and Molecular Analysis in Patients With Non-Small-Cell Lung Cancer in the United States', J Oncol Pract, vol. 15, no. 8, Aug, pp. e717-e727.

ALK = anaplastic lymphoma kinase; BRAF = proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B; DNA = deoxyribonucleic acid; EGFR = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; IHC = immunohistochemistry; KRAS = Kirsten rat sarcoma viral oncogene homologue; METex14sk = MET proto-oncogene, receptor tyrosine kinase exon 14 skipping alterations; NGS = next-generation sequencing; NOS = not otherwise specified; NPA = negative percent agreement; NSCLC = non-small cell lung cancer; PPA = positive percent agreement; RNA = ribonucleic acid; ROS1 = ROS proto-oncogene 1.

Disaggregated costs and outcomes are presented in Table 11.

Table 11 Disaggregated modelled costs and outcomes

	Small gene panel testing	Single gene testing	Increment
Disaggregated costs			
Cost of testing	\$1,053.65	\$773.59	\$280.06
Cost of rebiopsy	\$119.58	\$230.61	-\$111.03
Total cost	\$1,173.23	\$1,004.20	\$169.02
Disaggregated outcomes			
Eligible for targeted therapy	0.1957	0.1732	0.0225
• Common variants	0.1706	0.1732	-0.0026
• Incremental variants	0.0251	0.0000	0.0251
Actionable variant identified	0.2556	0.2075	0.0481
• Common variants identified	0.2226	0.2075	0.0152
• Incremental variants identified	0.0330	0.0000	0.0330
Patients successfully tested	0.9890	0.9788	0.0102
Proportion with known biomarker status	0.9817	0.7583	0.2234
Proportion undergoing rebiopsy	0.0212	0.0410	-0.0197

The additional patients eligible for targeted therapy was driven by an increase in patients with incremental variants identified. A slight reduction in patients with common variants was also observed (due to PPA < 1 applied for small gene panel testing, offset to some extent by improvement in patients successfully tested). As the incremental variants were not identified using the same testing method as was used in the clinical trials of targeted therapy, it is unclear whether all of these patients would respond to targeted therapies to the same extent as those with common variants.

More patients were identified with actionable variants (i.e. combined common and incremental variants) than those considered eligible for targeted therapy (absolutely and incrementally). This was due to the inclusion of patients tested with early stage disease who do not develop advanced disease (and so are not eligible for targeted therapy). The incremental difference was also higher (and in some cases the direction of the effect changed) due to incomplete current testing performed (i.e. not FISH or *MET*ex14sk testing).

The key drivers of the model are presented in Table 12.

Table 12 Key drivers of the model

Description	Method/Value	Impact Base case: \$7,496 per additional patient eligible for TT
Proportion patients with advanced disease (inc. those who progress)	Distribution of stage at diagnosis was based on a retrospective analysis of Victorian Cancer Registry data (Mitchell et al. 2013) ^a ; 65.5% were advanced at diagnosis. Of those diagnosed with Stages I-IIIa disease, 30% are assumed to experience progression. Therefore, 75.9% of patients diagnosed with non-squamous NSCLC are modelled to have (or reach) an advanced disease stage.	The proportion is uncertain. The ICER is highly sensitive to changes in this estimate. Increasing the proportion to 100% reduces the ICER to \$941 per additional patient eligible for TT, whereas decreasing this to 50%, increases the ICER to \$21,530.
Small gene panel concordance	Based on the systematic literature review of concordance conducted during the clinical evaluation. Given differences between the comparator used for <i>ALK</i> concordance (FISH ± IHC, with varied definitions of FISH positivity) in the meta-analysis, the data most aligned with the clinical utility standard was used in the base economic analysis.	The analyses were highly sensitive to the NPA values used (as these determine the incremental variants identified through small gene panel testing). The ICER was most sensitive to <i>EGFR</i> NPA values, where the range in ICERs observed was \$4,562–\$18,168 per additional patient eligible for TT.
RNA panel use	Where separate DNA then RNA panels are used, only those without <i>EGFR</i> , <i>MET</i> , <i>KRAS</i> and <i>BRAF</i> variants are assumed to receive further RNA panel testing (as per the proposed item descriptor).	The analysis is highly sensitive to this assumption. Where testing is allowed in those with <i>KRAS</i> and <i>BRAF</i> variants, the ICER per additional patient eligible for TT increases to \$13,627.
Test success	Based on Steeghs et al. (2022) ^b : <ul style="list-style-type: none"> • Small gene panel testing: 97.2% • Single gene testing: 94.6% 	The ICER is moderately sensitive to the difference between strategies. Where there is no difference, the ICER increases to \$12,829 per additional patient eligible for TT, however when the difference doubles (from 2.6% to 5.2%), the ICER decreases to \$2,731.
Rebiopsy	100% where testing was not successful. Rebiopsy was associated with a 20% failure rate (Kelly et al. 2019) ^c and a 14% complication rate (1161 PSD, November 2012 MSAC Meeting). The base case assumed all rebiopsies occurred in the outpatient setting, with cost based on AR-DRG E42A, B and C.	The analysis was moderately sensitive to the uptake of rebiopsy and to a lesser extent, cost. Reducing the rebiopsy rate to 60% increased the ICER per additional patient eligible for TT to \$9,161. Assuming all rebiopsies occur in an outpatient setting increased the ICER to \$9,475

^a Mitchell, PL, Thursfield, VJ, Ball, DL, Richardson, GE, Irving, LB, Torn-Broers, Y, Giles, GG & Wright, GM 2013, 'Lung cancer in Victoria: are we making progress?', *Med J Aust*, vol. 199, no. 10, Nov 18, pp. 674-679.

^b Steeghs, EMP, Groen, HJM, Schuurings, E, Aarts, MJ, Damhuis, RAM, Voorham, QJM, Ligtenberg, MJL & Grunberg, K 2022, 'Mutation-tailored treatment selection in non-small cell lung cancer patients in daily clinical practice', *Lung Cancer*, vol. 167, May, pp. 87-97.

^c Kelly, RJ, Turner, R, Chen, YW, Rigas, JR, Fernandes, AW & Karve, S 2019, 'Complications and Economic Burden Associated With Obtaining Tissue for Diagnosis and Molecular Analysis in Patients With Non-Small-Cell Lung Cancer in the United States', *J Oncol Pract*, vol. 15, no. 8, Aug, pp. e717-e727.

ALK = anaplastic lymphoma kinase; *BRAF* = proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B; DNA = deoxyribose nucleic acid; *EGFR* = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; ICER = incremental cost-effectiveness ratio; IHC = immunohistochemistry; *KRAS* = Kirsten rat sarcoma viral oncogene homologue; *MET* = mesenchymal-epithelial

transition; NPA = negative percent agreement; NSCLC = non-small cell lung cancer; RNA = ribonucleic acid; TT = targeted therapy.

The results of key sensitivity analyses are presented in Table 13.

Table 13 Results of the key sensitivity analyses

	Inc. cost	Inc. eligible for targeted therapy	ICER	% change
Base case	\$169.02	0.0225	\$7,496	-
Proportion of patients with advanced disease (base case: 75.9%)				
100%	\$27.97	0.0297	\$941	-87%
50%	\$320.01	0.0149	\$21,530	187%
Timing of <i>MET</i> ex14sk testing (base case: after <i>EGFR</i>)				
At the same time as <i>EGFR</i>	\$109.54	0.0225	\$4,858	-35%
After <i>EGFR</i> (excluding block retrieval and consult costs)	\$252.67	0.0225	\$11,206	49%
Small gene panel testing strategy (base case: mixed)				
All combined DNA/RNA panel testing	\$348.65	0.0228	\$15,277	104%
All two-stage DNA then RNA panel testing	\$116.98	0.0228	\$5,126	-32%
All DNA then IHC/FISH testing	-\$162.34	0.0173	Dominant	-225%
Test success (base case: 97.2% for panels, 94.6% for single-gene testing)				
Both strategies 97.2%	\$273.97	0.0216	\$12,662	69%
97.2% for panels, 95.9% ^a for single-gene testing	\$221.50	0.0221	\$10,026	34%
97.2% for panels, 92.0% ^b for single-gene testing	\$64.07	0.0235	\$2,731	-64%
<i>ALK</i> small gene panel concordance (base case: vs clinical utility standard, FISH ≥15% positivity)				
<i>ALK</i> small gene panel concordance vs FISH ± IHC #1	\$168.99	0.0219	\$7,730	3%
<i>ALK</i> small gene panel concordance vs FISH	\$169.00	0.0360	\$4,697	-37%
Small panel concordance				
<i>ALK</i> NPA, 0.97 (base case: 0.99)	\$169.02	0.0375	\$4,509	-40%
<i>ALK</i> NPA, 1.00 (base case: 0.99)	\$169.02	0.0166	\$10,162	36%
<i>ALK</i> PPA, 0.48 (base case: 1.00)	\$168.81	0.0114	\$14,848	98%
<i>EGFR</i> NPA, 0.95 (base case: 0.97)	\$161.36	0.0354	\$4,562	-39%
<i>EGFR</i> NPA, 0.99 (base case: 0.97)	\$176.69	0.0097	\$18,168	142%
<i>MET</i> ex14sk NPA, 0.93 (base case: 1.00)	\$138.59	0.0734	\$1,887	-75%
Rebiopsy uptake rate (base case: 100%)				
30%	\$254.94	0.0248	\$10,298	37%
60%	\$218.12	0.0238	\$9,161	22%
Average fee charged for <i>EGFR</i> and <i>ALK</i> and <i>ROS1</i> FISH (base case: MBS Schedule Fees)	\$185.26	0.0225	\$8,217	10%
FISH utilisation, use IHC NPA data (base case: calibrated) ^c #2	\$183.78	0.0225	\$8,151	9%
Separate RNA small panel use, allowed with <i>KRAS</i> or <i>BRAF</i> #4 (base case: not allowed)	\$307.24	0.0225	\$13,627	82%
Proportion with <i>KRAS</i> or <i>BRAF</i> activating variants, 52% (base case: 30.8%)	\$73.89	0.0225	\$3,277	-56%
Rebiopsy cost, \$3,369 [all outpatient] #3 (base case: \$5,630 [all inpatient])	\$213.63	0.0225	\$9,475	26%

	Inc. cost	Inc. eligible for targeted therapy	ICER	% change
Base case	\$169.02	0.0225	\$7,496	-
Multivariate analyses				
#1 AND #2	\$183.74	0.0219	\$8,404	12%
#1, #2 AND #3	\$228.35	0.0219	\$10,444	39%
#1, #2, #3 AND #4	\$366.57	0.0219	\$16,767	124%

^a Half the difference between test strategies

^b Double the difference between test strategies

^c Estimates of FISH use in the base case was calibrated to MBS utilisation data on the ratio of *EGFR:ALK* or *ROS1* FISH services. The sensitivity analysis uses estimates based on biomarker prevalence and IHC specificity.

ALK = anaplastic lymphoma kinase; *BRAF* = proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B; DNA = deoxyribose nucleic acid; *EGFR* = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; ICER = incremental cost-effectiveness ratio; IHC = immunohistochemistry; *KRAS* = Kirsten rat sarcoma viral oncogene homologue; *METex14sk* = MET proto-oncogene, receptor tyrosine kinase exon 14 skipping alterations; NPA = negative percent agreement; *NTRK* = neurotrophic tropomyosin receptor kinase; PPA = positive percent agreement; RNA = ribonucleic acid; *ROS1* = ROS proto-oncogene 1.

The analyses were most sensitive to the proportion of patients with advanced disease (as this affects the current costs offset), the small gene panel testing strategy used (including distribution of strategies used), differences in the test success rate, concordance of small gene panel testing (particularly NPA, which is assumed to increase incremental variants with small gene panel testing), and rebiopsy rate. The analysis was also sensitive to the assumption that patients found to have *KRAS* and *BRAF* variants on the DNA panel only would not receive RNA small gene panel testing (and expected yield of these non-actionable variants). Instances of concurrent variants may be more common than previously thought. A prospective case series¹⁵ from Germany reported that of all patients with *ROS1* and *ALK* variants identified, respectively, 23.7% (14/59) and 16.1% (19/118) also had variants in *BRAF* or *KRAS*.

A few assumptions included in the base case analysis may not be the most conservative approach. Justification has been provided to support the use of the estimates in the base case, however multivariate analyses are performed using alternate approaches identified. The results do suggest that the analyses are sensitive to the combined effects of these changes.

12. Financial/budgetary impacts

A market-share approach was used to estimate the extent of use of small gene panel testing in patients with non-squamous NSCLC with MBS listing. This was based on projections of current *EGFR* service use and current use of *ALK* and *ROS1* FISH services relative to *EGFR* services. *METex14sk* testing has also recently been recommended by MSAC in this patient population. Epidemiological estimates are applied to the projections of *EGFR* use to estimate the change in use and cost related to *METex14sk* testing.

The financial implications to the MBS resulting from the proposed listing of small gene panel testing are summarised in Table 14.

¹⁵ Griesinger, F, Eberhardt, W, Nusch, A, Reiser, M, Zahn, MO, Maintz, C, Bernhardt, C, Losem, C, Stenzinger, A, Heukamp, LC, Buttner, R, Marschner, N, Janicke, M, Fleitz, A, Spring, L, Sahlmann, J, Karatas, A, Hipper, A, Weichert, W, Heilmann, M, Sadjadian, P, Gleiber, W, Grah, C, Waller, CF, Reck, M, Rittmeyer, A, Christopoulos, P, Sebastian, M, Thomas, M & Group, CR 2021, 'Biomarker testing in non-small cell lung cancer in routine care: Analysis of the first 3,717 patients in the German prospective, observational, nation-wide CRISP Registry (AIO-TRK-0315)', *Lung Cancer*, vol. 152, Feb, pp. 174-184.

Table 14 Net financial implications of small gene panel testing to the MBS

Parameter	2023	2024	2025	2026	2027	2028
Estimated use and cost of the proposed health technology						
Size of the <i>EGFR</i> testing market	5,521	5,797	6,074	6,351	6,628	6,905
Share of the <i>EGFR</i> testing market (100%)	5,521	5,797	6,074	6,351	6,628	6,905
Number of services of small gene panel testing	7,425	7,798	8,030	8,250	8,458	8,493
• Combined DNA/RNA (MBS benefit: \$1,087.92) ^a	1,380	1,449	1,822	2,223	2,651	3,453
• DNA only (MBS benefit: \$568.17) ^b	4,140	4,348	4,252	4,128	3,977	3,453
• RNA only (MBS benefit: \$568.17) ^b	1,905	2,000	1,956	1,899	1,829	1,588
Cost to the MBS	\$4,936,022	\$5,183,631	\$5,509,717	\$5,842,958	\$6,183,354	\$6,620,116
Change in use and cost of other health technologies						
Reduction in use of comparator testing services						
• <i>EGFR</i> (MBS benefit: \$325.13) ^c	5,521	5,797	6,074	6,351	6,628	6,905
• <i>ALK</i> FISH (MBS benefit: \$325.80) ^d	245	257	270	282	295	308
• <i>ROS1</i> FISH (MBS benefit: \$325.80) ^d	408	428	449	470	491	513
• <i>METex14sk</i> (MBS benefit: \$337.75) ^e	3,559	3,738	3,916	4,095	4,273	4,452
Reduction in use of block retrieval services (MBS benefit: \$72.25) ^f	3,559	3,738	3,916	4,095	4,273	4,452
Net change in costs to the MBS	\$3,466,662	\$3,640,562	\$3,814,751	\$3,988,966	\$4,163,208	\$4,337,803
Net financial impact to the MBS	\$1,469,360	\$1,543,069	\$1,694,966	\$1,853,992	\$2,020,147	\$2,282,313

Source: 'Section 4.4' worksheet of the '1721 financial impact.xlsx' workbook accompanying the DCAR.

ALK = anaplastic lymphoma kinase; DNA = deoxyribose nucleic acid; *EGFR* = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; *METex14sk* = MET proto-oncogene, receptor tyrosine kinase exon 14 skipping alterations; RNA = ribonucleic acid; *ROS1* = ROS proto-oncogene 1.

^a 31.8% × \$935.25 [75% MBS benefit] + 68.2% × \$1,159.10 [85% MBS benefit]. Split of use based on MBS data for use of *EGFR* services.

^b 31.8% × \$511.80 [75% MBS benefit] + 68.2% × \$594.45 [85% MBS benefit]. Split of use based on MBS data for use of *EGFR* services.

^c 31.8% × \$298.05 [75% MBS benefit] + 68.2% × \$337.75 [85% MBS benefit]. Split of use based on MBS data for use of *EGFR* services.

^d 35.5% × \$300.00 [75% MBS benefit] + 64.5% × \$340.00 [85% MBS benefit]. Split of use based on MBS data for use of *ALK* or *ROS1* FISH services.

^e 100% × \$337.75 [85% MBS benefit]. As proposed *METex14sk* testing has not been proposed to be a pathologist determinable test, all services have been assumed to be requested in the outpatient setting. ^f 100% × \$72.25 [85% MBS benefit]. Assumed for each *METex14sk* test which has been assumed to be requested in the outpatient setting.

The net financial impact estimates were most sensitive to the distribution of use of combined or sequential small gene panels and whether separate RNA panels are allowed in those found to have *KRAS* or *BRAF* activating variants. If there is substantial growth in the market due to the listing of small gene panels (e.g. if some testing currently is funded by the states, and this shifts to the MBS), then the net impact to the MBS may be higher. However, the extent of this shift is unknown.

While there may also be a change in the relative use of IHC testing items, PASC considered that the expected reduction in the cost of IHC testing for *ALK* and *ROS1* would not be straightforward to estimate (p10, 1634 Ratified PICO). The total number of IHC services is not likely to change with proposed small gene panel testing (as this is performed on diagnosis of NSCLC), and for

many patients, the item claimed will not change (where the number of antibodies tested does not change the item being charged e.g. from use of ten to eight antibodies tested). A conservative approach has been adopted in the DCAR that assumes no reduction in cost of *ALK* and *ROS1* IHC testing. The budget impact was not sensitive to an assumption that all *EGFR* services would be associated with a change in IHC item use (from 72849 [85% benefit: \$88.70] to 72847 [85% benefit: \$76.00], reduction in cost to the MBS of \$12.70).

13. Other relevant information

Nil.

14. Committee-in-confidence information

Redacted.

Acronyms and abbreviations

AIHW	Australian Institute of Health and Welfare
ALK	anaplastic lymphoma kinase
ARMS-PCR	amplification-refractory mutation system polymerase chain reaction
ARTG	Australian Register of Therapeutic Goods
<i>BRAF</i>	proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B
CI	confidence interval
CGP	comprehensive genomic profiling
CNB	core needle biopsy
DCAR	Department Contracted Assessment Report
DCR	disease control rate
ddPCR	digital droplet polymerase chain reaction
DNA	deoxyribonucleic acid
EBUS-TBNA	endobronchial ultrasound-guided transbronchial needle aspiration
<i>EGFR</i>	epidermal growth factor receptor
<i>ERBB2</i>	erb-b2 receptor tyrosine kinase 2
ESC	Evaluation Sub-Committee
FFPE	formalin-fixed paraffin embedded
FISH	fluorescence <i>in situ</i> hybridisation
FNA	fine needle aspirate
HRQoL	health-related quality of life
HTA	health technology assessment
ICER	incremental cost-effectiveness ratio
IHC	immunohistochemistry
IQR	inter quartile range
ISH	<i>in situ</i> hybridisation
<i>KRAS</i>	Kirsten rat sarcoma
LDTs	laboratory developed tests
MBS	Medicare Benefits Schedule
<i>MET</i>	mesenchymal-epithelial transition

MLPA	Multiplex ligation-dependent probe amplification
MSAC	Medical Services Advisory Committee
NGS	next generation sequencing
NHMRC	National Health and Medical Research Council
NOS	not otherwise specified
NSCLC	Non-small cell lung cancer
NTRK	neurotrophic tyrosine receptor kinase
ORR	overall response rate
PASC	PICO Confirmation Advisory Sub-Committee of the MSAC
PBS	Pharmaceutical Benefits Scheme
PCR	polymerase chain reaction
PFS	progression free survival
PNA-LNA	peptide nucleic acid, locked nucleic acid polymerase chain reaction
PSD	Public Summary Document
PTNB	percutaneous transthoracic needle biopsies
QALY	quality-adjusted life year
<i>RET</i>	rearranged during transfection
RNA	ribonucleic acid
<i>ROS1</i>	ROS proto-oncogene 1
RT-PCR	real time polymerase chain reaction
SG	single-gene testing
SISH	silver <i>in situ</i> hybridisation
SS	Sanger sequencing
SoC	Standard of care
TGA	Therapeutic Goods Administration
TKI	tyrosine kinase inhibitor
VAF	variant allele frequency

Section 1 Context

1.1 Purpose of application

This DCAR assessing small gene panels for the biomarker testing of patients with non-squamous (or histology not otherwise specified), non-small cell lung cancer (NSCLC) is intended for the Medical Services Advisory Committee (MSAC).

MSAC appraises medical services, health technologies and health programs for public funding through an assessment of their comparative safety, clinical effectiveness, cost-effectiveness and total cost, using the best available evidence. This includes, but is not limited to, amendments and reviews of existing services funded on the Medicare Benefits Schedule (MBS) or other non-MBS-funded programs (e.g. blood products, screening programs or prostheses referred to the Prostheses List Advisory Committee).

Adelaide Health Technology Assessment (AHTA), the University of Adelaide, has been commissioned by the Australian Government Department of Health and Aged Care to conduct a systematic literature review and economic evaluation of a small gene panel for testing in NSCLC. This assessment has been undertaken to inform MSAC's decision-making regarding whether the proposed health technology should be publicly funded. The purpose of this assessment report is to synthesise the information most likely to be useful for committee members. Technical appendices provide assurance of the rigour behind the systematic review and construction of the economic and financial analyses.

The proposed use of a small gene panel for testing in NSCLC in Australian clinical practice was considered similar enough to MSAC application 1634 for comprehensive genomic profiling of non-squamous non-small cell lung cancer using next generation sequencing assays, for the ratified PICO confirmation for MSAC application 1634 to be used as the basis of assessment 1721.

The clinical claim is that small gene panel testing results in superior health outcomes compared to sequential single variant testing (the comparator) as it should result in reduced re-biopsy rates, and may have a more rapid turnaround time, which will translate into quicker access to appropriate treatment for patients. Small panel testing may also identify concurrent variants at the same time, which would not be detected by sequential testing, due to the strategy of sequential testing being halted once one variant is identified.

1.2 Background

MSAC has not previously considered any panel testing for NSCLC but has assessed many individual tests for sequential testing within NSCLC (i.e., the comparative test strategy).

In November 2017, *“MSAC noted that the sequential testing of EGFR, ALK and ROS1 yield mutually exclusive treatment pathways and that sequential testing wastes tissue sample, time and is more expensive than a single panel of tests. MSAC recommended that the Department conduct a cost-utility review of gene panel and/or next generation sequencing (NGS) test options to inform these first-line therapy options”*. *“MSAC advised that any MBS funding should be based on a gene panel or NGS test of equivalent or better analytical performance to sequential IHC and*

FISH testing and assurance that the average gene panel or NGS test is no more costly than the average cost of the sequential testing that it would replace. MSAC noted that overall testing may still require more than one gene panel test due to differences in lung cancer gene aberrations as somatic mutations are tested in genomic DNA, whereas gene fusions (such as ROS1) are usually tested in cDNA [complementary DNA] prepared from RNA.” (Public Summary Document, ADAR 1454, November 2017, p3).

In 2018, a DCAR was commenced to assess a somatic tumour panel test (DCAR 1495) for patients with NSCLC, but this was withdrawn by the applicants prior to the DCAR being presented to MSAC. At the time, no single somatic tumour gene panel test performed in Australia was able to appropriately assess the *EGFR*, *ALK* and *ROS1* variants in NSCLC. The panels for *ALK* and *ROS1* identified therapy resistance variants rather than gene rearrangement mutations.

In 2018, Roche Diagnostics lodged an application (MSAC assessment 1634) for MBS listing of a comprehensive gene panel of over 300 genes for use in squamous and non-squamous NSCLC, which was revised in 2020 to focusing just on non-squamous NSCLC. This was considered by PASC in 2020 and 2021. During the PICO development the applicant for 1634 nominated the application would proceed as an ADAR for consideration at an MSAC meeting in late 2022. However, in May 2022 the applicant notified the Department that it would be delaying the submission of its ADAR.

The two most relevant topics that have gone to the PICO Advisory Sub Committee (PASC) are outlined in Table 15.

Table 15 MSAC application history

Committee	MSAC application no.	Meeting date(s)
PASC	1495 Somatic tumour gene panel	April 2018
	1634 Comprehensive genomic profiling of NSCLC	December 2020 April 2021

MSAC = Medical Services Advisory Committee; NSCLC = non-small cell lung cancer; PASC = PICO Advisory Sub-Committee of the MSAC

A summary of how this DCAR addresses the suggestions by MSAC, in response to assessment 1454, is shown below in Table 16.

Table 16 Summary of key matters of concern from 1454 PSD, November 2017, p3

Component	Matter of concern	How the current assessment report addresses it
Overarching DCAR	MSAC recommended that the Department conduct a cost-utility review of gene panel and/or next generation sequencing (NGS) test options to inform these first-line therapy option.	Addressed. Current DCAR assessing small NGS panel.
Intervention	MSAC noted that overall testing may still require more than one gene panel test due to differences in lung cancer gene aberrations as somatic mutations are tested in genomic DNA, whereas gene fusions (such as <i>ROS1</i>) are usually tested in complementary DNA prepared from RNA.	Addressed. Intervention proposed as both DNA and RNA testing, or sequential DNA then RNA testing.
Test performance	Any MBS funding should be based on a gene panel or NGS test of equivalent or better analytical performance to sequential IHC and FISH testing.	Addressed. NGS has superior or equivalent analytical performance compared to single-gene assays or IHC and FISH testing.
Cost-minimisation	The average gene panel or NGS test is no more costly than the average cost of the sequential testing that it would replace.	At the proposed items fees, small gene panel testing is associated with additional costs. This may be reasonable if the claim of superior effectiveness and the ratio of the incremental cost to incremental benefit is accepted.

DCAR = Department Contracted Assessment Report; DNA = deoxyribonucleic acid; FISH = fluorescence in situ hybridisation; IHC = immunohistochemistry; MBS = Medicare Benefits Schedule; MSAC = Medical Services Advisory Committee; NGS = next generation sequencing; PSD = Public Summary Document; ROS1 = ROS proto-oncogene 1; RNA = ribonucleic acid.

1.3 Prerequisites to implementation of any funding advice

Testing would occur in a NATA accredited diagnostic laboratory in accordance with NPAAC guidelines – Requirements for human medical genome testing utilising massively parallel sequencing technologies (NPAAC 2017).

There are currently no NGS assays approved by the TGA for the purpose of detecting biomarkers for targeted treatment of patients with NSCLC. There are several NGS assays available in Australia for use in patients with NSCLC, marked as ‘Research Use Only’ (RUO): (AVENIO tumor tissue targeted panel (17 genes), AVENIO tumor tissue expanded panel (77 genes), TruSight Oncology 170 (170 genes) and TruSight Oncology 500 (523 genes from DNA and RNA).

Local laboratories will be able to purchase RUO products from commercial suppliers and develop an IVD test under the framework of the ‘Requirement for the development of an in-house *in vitro* diagnostic medical devices (IVDs) (NPAAC 2018)’.

Currently, the PBS restrictions for most of the therapies targeting *ALK* or *ROS1* gene rearrangements (all except second-line lorlatinib) specify the method of determining the variants and the threshold separating a positive result from a negative result in order for the patient to be eligible for the therapeutics (i.e., patients must have evidence of an *ALK* gene rearrangement or *ROS1* gene rearrangement in tumour material, defined as 15% (or greater) positive cells by fluorescence *in situ* hybridisation (FISH) testing). If the proposed items for small DNA ± RNA NGS panels are listed on the MBS, coordinated amendments to the restrictions listed on the PBS would be required to allow for either FISH (with the threshold of ≥15%) or NGS (without a specified threshold) in the criteria for crizotinib, ceritinib, alectinib, and entrectinib. If this application is supported by MSAC, the necessary coordination may most efficiently achieved by

MSAC referring the related amendments to the PBAC for consideration at its December 2022 Intracycle meeting.

1.4 Population

The population of interest are those with non-small cell lung cancer (NSCLC), which is non-squamous, or histology not-otherwise-specified (NOS).

In 2021, lung cancer was the fifth most common cancer in Australia, with 13,810 total cases, and an age-standardised rate of 42.6 cases per 100,000 persons (AIHW 2021). Lung cancer is classified into two types which grow and spread differently. They are small cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC), the latter accounting for 85% of all lung cancers. It is therefore estimated that there were 11,738 cases of NSCLC diagnosed in Australia in 2021. In 2021, there were 8,693 deaths from lung cancer, which makes it the most common cause of cancer-related mortality.

NSCLC is classified further as either adenocarcinoma (beginning in mucus-producing cells and is more often found in the outer part of the lungs), squamous cell carcinoma (most commonly develops in the larger airways) or large cell undifferentiated carcinoma. The proposed population are those with non-squamous (i.e., adenocarcinoma or large cell undifferentiated carcinoma) or NOS NSCLC.

The diagnosis may occur at any stage of cancer progression, although most patients are diagnosed in later stages of the disease. There are several treatments available for patients with advanced NSCLC on the Pharmaceutical Benefits Scheme (PBS), but the eligibility criteria depend on the detection or ruling out of particular biomarkers in the tumour tissue. After NSCLC has been diagnosed, it is therefore important that patients undergo biomarker testing, to determine which treatment will best target their tumour.

The estimated number of patients who would use the proposed technology is based on the current use of the *EGFR* testing MBS item. The increase from 2018–19 to 2019–20 was 6.2%, and from 2019–20 to 2020–21 was 6.05%. It is therefore expected that the increase in the number of patients with NSCLC being tested for the purposes of determining eligibility for PBS-subsidised drugs will increase on average by 6.125% per year.

A survey performed for the purposes of [PICO confirmation 1669](#) reported that most laboratories are already using small DNA panels. If the proposed separate DNA and RNA panels are added to the MBS, then the small DNA panel item is likely to be able to be used by most laboratories from the time of listing (Table 4). However, capacity to perform small RNA panels is more restricted, and laboratories may either transfer the tissue to another laboratory for RNA testing or continue to use IHC and FISH for the assessment of *ALK* and *ROS1*. The applicants have also stated that some patients will have insufficient tumour tissue available for RNA to be extracted, so 5- 10% of cases may continue to be tested using FISH rather than an NGS panel.

Prior testing, and projections of use only consider testing in patients with non-squamous (or not otherwise specified) histology, and do not consider the utilisation if patients with squamous NSCLC are also tested. This is estimated to increase the projections by 15%.

Table 17 Number of patients who have claimed MBS item 73337 and projected testing numbers

	2018-19	2019-20	2020-21	Projected 2021-22	Projected 2022-23	Projected 2023-24	Projected 2024-25
EGFR testing	4,371	4,643	4,924	5,226	5,546	5,886	6,247
% increase on previous year	-	6.2%	6.05%	6.125%	6.125%	6.125%	6.125%

EGFR = epidermal growth factor receptor; MBS = Medicare Benefits Schedule

1.5 Intervention

The proposed intervention is one or two next generation sequencing (NGS) panel(s) testing of resected/ biopsied tumour samples. It is proposed to be used at the point of diagnosis of NSCLC which is non-squamous or NOS. The purpose of the intervention is for determining eligibility for NSCLC patients to access specific Pharmaceutical Benefits Scheme (PBS)-listed targeted treatments (the treatments are listed in Table 2).

The NGS panel involves nucleic acid extraction, which then undergoes target enrichment using either hybridisation-based target enrichment or amplicon-based target enrichment. Targeted panels would focus on a certain number of genes or gene regions. The sequence data are processed by a bioinformatics pipeline which includes sequence read alignment and variant calling and annotation. Genomic variants are curated by scientists/pathologists and a clinical report is generated.

The applicants estimate that 5–10% of tumours may not be suitable for NGS testing and will still need a single gene/FISH based approach.

The applicants suggest two potential approaches to small panel testing, the use of which may depend on each individual laboratory’s capacities and infrastructure:

- One MBS item describing a “nucleic acid” panel, combining both DNA/RNA.
- Two separate MBS items for NGS panels:
 - DNA panel for point variants/small indels to test the most common alterations in *EGFR* and *MET* ex14 skipping variants (and possibly *KRAS* and *BRAF V600E*).
 - RNA panel for translocations resulting in fusions in *ALK* or *ROS1* genes (and possibly *RET*, *NTRK* fusions)

A combined panel would be less widely available than separate panels, although it would be faster. The RNA panel is proposed to only be used if no point variants/small indels are identified from the DNA panel (in the genes listed).

Guidelines recommend that a small NGS panel for NSCLC should include at a minimum: *EGFR* (15% of NSCLC harbour *EGFR* exon 19 deletions or exon 21 L858R substitutions), *ALK* (5% of NSCLC have *ALK* rearrangements), *ROS1*, *BRAF*, *MET* ex 14 skipping, and *RET* in a small NGS panel (Ettinger et al. 2021; Lindeman et al. 2018b, 2018a; Mosele et al. 2020).

PASC noted that there may initially be capacity issues for laboratories [to perform RNA panels or combined DNA and RNA panels] potentially requiring samples to be referred [to a central

laboratory for RNA testing], but laboratory capacity will expand naturally with market forces over the next couple of years. (Ratified PICO confirmation, application 1634, p8)..

PASC noted that the applicant foreshadowed that additional biomarkers to be reported on in the near future under the requested MBS item could include MET exon 14 skipping alterations, and NTRK1, NTRK2, NTRK3 and KRAS G12C variants. PASC advised that, for test reporting purposes, the evidentiary standard tests in the trials of the related medicines should be used to identify the specific biomarkers in each case (Ratified PICO confirmation, application 1634, p7).

Table 18 List of biomarker-specific therapies currently available through the PBS

Biomarker	Population criteria in PBS restrictions	Clinical utility standard	PBS therapy	PBS code(s)	Sponsor
EGFR activating variant	Patient must have evidence of an activating EGFR gene mutation known to confer sensitivity to treatment with EGFR TKIs in tumour material	EGFR cobas® real time PCR test	Erlotinib	10014C; 10019H; 10020J; 10025P; 10028T; 11259N; 11260P; 11263T	Roche
			Gefitinib	11264W; 8769M	Astra Zeneca
			Afatinib	11329G; 11335N; 11336P; 11341X; 113147F; 11348G; 11359W	Boehringer Ingelheim
			Osimertinib (first-line)	12232T	Astra Zeneca
EGFR T790M variant positive after prior EGFR targeted treatment	NA		Osimertinib (second-line)	11622Q	Astra Zeneca
ALK gene rearrangement	Patient must have evidence of an ALK gene rearrangement in tumour material, defined as ≥15% positive cells by FISH testing	ALK FISH	Crizotinib	10322G; 10323H	Pfizer
			Ceritinib	11056X	Novartis
	Alectinib		11226W	Roche	
	Brigatinib		11980M; 11974F; 11976H; 11984R	Takeda	
	Patient must have evidence of an ALK gene rearrangement		Lorlatinib (second-line)	12096P; 12091J	Pfizer
ROS1 gene rearrangement	Patient must have evidence of ROS1 gene rearrangement in tumour material, defined as ≥15% positive cells by FISH testing	ROS1 FISH	Crizotinib	11589Y; 11594F	Pfizer
			Entrectinib ^a	12092K	Roche
METex14sk	Proposed criteria: Patient must have evidence of MET exon 14 skipping alterations in tumour material	RNA- or DNA-based testing of liquid biopsy or tissue biopsy	Tepotinib	Recommended by PBAC	Merck
Absence of activating EGFR	The condition must not have evidence of an activating EGFR gene or	-	Pembrolizumab	11492W	Merck Sharp & Dohme

Biomarker	Population criteria in PBS restrictions	Clinical utility standard	PBS therapy	PBS code(s)	Sponsor
variants, <i>ALK</i> gene rearrangement or <i>ROS1</i> gene rearrangement	an <i>ALK</i> gene rearrangement or <i>ROS1</i> gene arrangement in tumour material,		Atezolizumab	11792P	Roche

ALK = anaplastic lymphoma kinase gene; DNA = deoxyribonucleic acid; *EGFR* = epidermal growth factor receptor gene; FISH = fluorescent in situ hybridisation; *MET* = mesenchymal-epithelial transition; NA = not applicable (no population criteria in PBS restriction); PBS = Pharmaceutical Benefits Scheme; PCR = polymerase chain reaction; RNA = ribonucleic acid; *ROS1* = ROS1 receptor tyrosine kinase gene
Source: MSAC application 1634 ratified PICO confirmation

Commercially supplied NGS tests available which may be used as the basis of *in vitro* diagnostic tests under the framework of 'Requirement for the development of an in-house *in vitro* diagnostic medical devices (IVDs) (NPAAC 2018)' are shown in Table 19.

Table 19 List of commercially available assays available for patients with NSCLC

Company	Test	Coverage	DNA and/or RNA
Illumina	Various solid tumour NGS panels including TruSight Oncology 170 (RUO assay)	170 genes	DNA and DNA/RNA options
ThermoFisher Scientific	Various solid tumour NGS panels including AmpliSeq Cancer Panel (RUO assay) Oncomine Dx Target Test	>500 genes <i>EGFR, BRAF and ROS1</i>	DNA and RNA
Roche Products	FoundationOne® CDx assay	324 genes	DNA only
Roche Diagnostics	AVENIO Tumor Tissue targeted panel (RUO assay)	17 genes	DNA only

DNA = deoxyribonucleic acid; NGS = next generation sequencing; NSCLC = non-small cell lung cancer; RNA = ribonucleic acid; RUO = research use only

1.6 Comparator(s)

The comparator to (one or two) small NGS panels is the use of sequential testing of biomarkers for targeted therapies for NSCLC using items currently available on the MBS (or in the near future). Specifically, this is:

- Testing of *EGFR* activating variant status (MBS item 73337)
- Immunohistochemistry (IHC) testing as triage *ALK* testing and triage *ROS1* testing (most likely included under MBS item 72846 at the time of initial diagnosis)
- Testing of *ALK* gene rearrangement status by FISH (MBS item 73341)
- Testing of *ROS1* gene rearrangement status by FISH (MBS item 73344)
- Testing of *MET*ex14 skipping alterations (recommended by MSAC)

At the point of diagnosis, patients are testing for *EGFR* activating variants using a single gene panel and tested with IHC for *ALK* and *ROS1*. MSAC has recommended that testing for *MET*ex14 skipping (*MET*ex14sk) alterations be performed without the absence of other NSCLC biomarkers being a pre-requisite (Public Summary Document, ADAR 1660, p1). Although *MET*ex14sk testing is limited to patients with locally advanced or metastatic disease, the majority of patients meet

this criteria at the point of diagnosis, so are assumed to be tested for *MET*ex14sk at the point of diagnosis.

If the patient's tumour is *EGFR* activating variant negative, but positive or equivocal on ALK IHC triage testing (staining intensity score >0), they may undergo confirmatory *ALK* gene rearrangement testing using FISH if/when they have locally advanced or metastatic disease.

Likewise, if the patient's tumour is *EGFR* variant negative, but positive or equivocal on ROS1 IHC triage testing (staining intensity score of 2+ or 3+), they may undergo confirmatory *ROS1* gene rearrangement testing using FISH if/when they have locally advanced or metastatic disease.

If patients are not locally advanced or metastatic at the time of diagnosis, then a block retrieval item (MBS item 72860) may be required if referral to an outside laboratory is required for the FISH testing.

The MBS items relevant to the comparator are summarised below.

Table 20 Relevant MBS items for the comparator

Category 6 – PATHOLOGY SERVICES	Group P7 - Genetics
72846	Immunohistochemical examination of biopsy material by immunofluorescence, immunoperoxidase or other labelled antibody techniques with multiple antigenic specificities per specimen - 1 to 3 antibodies Fee: \$59.60 Benefit: 75% = \$44.70 85% = \$50.70
73337	A test of tumour tissue from a patient diagnosed with non-small cell lung cancer, shown to have non-squamous histology or histology not otherwise specified, requested by, or on behalf of, a specialist or consultant physician, to determine: <ul style="list-style-type: none"> a. if the requirements relating to epidermal growth factor receptor (<i>EGFR</i>) gene status for access to EGFR tyrosine kinase inhibitor under the Pharmaceutical Benefits Scheme are fulfilled; or b. if the requirements relating to EGFR status for access to pembrolizumab under the Pharmaceutical Benefits Scheme are fulfilled. Fee: \$397.35 Benefit: 75% = \$298.05 85% = \$337.75
73341	Fluorescence in situ hybridisation (FISH) test of tumour tissue from a patient with locally advanced or metastatic non-small cell lung cancer, which is of non-squamous histology or histology not otherwise specified, with documented evidence of anaplastic lymphoma kinase (<i>ALK</i>) immunoreactivity by immunohistochemical (IHC) examination giving a staining intensity score > 0, and with documented absence of activating mutations of the epidermal growth factor receptor (<i>EGFR</i>) gene, requested by a specialist or consultant physician, to determine: <ul style="list-style-type: none"> a. if requirements relating to <i>ALK</i> gene rearrangement status for access to an anaplastic lymphoma kinase inhibitor under the Pharmaceutical Benefits Scheme (PBS) are fulfilled; or b. if requirements relating to <i>ALK</i> status for access to pembrolizumab under the Pharmaceutical Benefits Scheme are fulfilled. Fee: \$400.00 Benefit: 75% = \$300.00 85% = \$340.00
73344	Fluorescence in situ hybridization (FISH) test of tumour tissue from a patient with locally advanced or metastatic non-small-cell lung cancer (NSCLC), which is of non-squamous histology or histology not otherwise specified, with documented evidence of ROS proto-oncogene 1 (<i>ROS1</i>) immunoreactivity by immunohistochemical (IHC) examination giving a staining intensity score of 2+ or 3+; and with documented absence of both activating mutations of the epidermal growth factor receptor (<i>EGFR</i>) gene and anaplastic lymphoma kinase (<i>ALK</i>) immunoreactivity by IHC, requested by a specialist or consultant physician, to determine: <ul style="list-style-type: none"> a. if requirements relating to <i>ROS1</i> gene rearrangement status for access to crizotinib or entrectinib under the Pharmaceutical Benefits Scheme are fulfilled; or

<p>b. if requirements relating to <i>ROS1</i> status for access to pembrolizumab under the Pharmaceutical Benefits Scheme are fulfilled.</p> <p>Fee: \$400.00 Benefit: 75% = \$300.00 85% = \$340.00</p>
<p>72860</p> <p>Retrieval and review of one or more archived formalin fixed paraffin embedded blocks to determine the appropriate samples for the purpose of conducting genetic testing, other than:</p> <p>(a) a service associated with a service to which item 72858 or 72859 applies; or</p> <p>(b) a service associated with, and rendered in the same patient episode as, a service to which an item in Group P5, P6, P10 or P11 applies</p> <p>Applicable not more than once in a patient episode</p> <p>Fee: \$85.00 Benefit: 75% = \$63.75 85% = \$72.25</p>
<p>MSACs supported MBS item for <i>MET</i>ex14sk testing (<i>with amendments proposed by HTA group to be consistent with PBAC recommendations</i>)</p> <p>A test of tumour tissue from a patient diagnosed with locally advanced or metastatic non-small cell lung cancer, shown to have non-squamous histology or histology not otherwise specified, requested by, or on behalf of, a specialist or consultant physician, to determine:</p> <p>a. if the requirements relating to <i>MET</i> exon 14 skipping alteration status for access to tepotinib are fulfilled under the Pharmaceutical Benefits Scheme (PBS)</p> <p>b. if requirements relating to <i>ROS1</i> status for access to pembrolizumab under the Pharmaceutical Benefits Scheme are fulfilled.</p> <p>Fee: \$397.35 Benefit: 85% = \$337.75</p>

Likely changes to the proposed MBS for *MET*ex14sk testing are shown in red (the PBAC considered it would be appropriate for tepotinib to be available for all patients with a *MET*ex14sk alteration, regardless of histology).

For application 1634 (Comprehensive genomic profiling of patients with non-squamous NSCLC) PASC noted that the applicants requested that near market comparators of MET exon 14 skipping alterations, and NTRK1, NTRK2, NTRK3 and KRAS G12C variants be incorporated into the list of eligible comparators. PASC considered that it would be necessary to identify the related evidentiary standards from the studies of the related codependent medicines to be considered by PBAC, and to extend the related comparative analytical performance assessments to include these additional biomarkers.

PASC advised that the near market comparators should not be included as part of the base case for the economics and financial analyses but may be considered as part of their sensitivity analyses. This will require the applicants to make a judgement call regarding what the near market comparator costs would likely to be.

1.7 Summary of the PICO criteria

The Prior tests, Population, Investigation/Index test, Comparator and Outcomes (PPICO) that were prespecified at the start of the DCAR to guide the systematic literature review are presented in Table 21. More details (separate PPICO criteria for elements of the linked evidence approach) are provided in Appendix A.

Table 21 PPICO criteria for assessing small NGS DNA/RNA panel(s) for non-squamous NSCLC

Component	Description
Patients	Patients with non-squamous (or histology not otherwise specified) non-small cell lung cancer (NSCLC)

Prior tests	Disease staging and histology workup. This is part of routine management and there would be no change between the intervention and comparator
Intervention	1. Small NGS panel to simultaneously test DNA/RNA for relevant point variants/small indels or fusions in the following genes: <i>EGFR</i> , <i>METex14sk</i> , <i>KRAS</i> , <i>BRAF</i> , <i>ALK</i> , <i>ROS1</i> , <i>RET</i> , <i>NTRK</i> ; or 2. NGS DNA panel for relevant point variants/small indels in the following genes: <i>EGFR</i> , <i>METex14sk</i> , <i>KRAS</i> , and <i>BRAF</i> . If negative, then an RNA panel for fusions in the following genes: <i>ALK</i> , <i>ROS1</i> , <i>RET</i> , and <i>NTRK</i> .
Comparator	Sequential single gene testing for activating mutations in the <i>EGFR</i> gene, <i>METex14sk</i> , <i>KRAS</i> ALK IHC and <i>ROS1</i> IHC, with subsequent <i>ALK</i> FISH and/or <i>ROS1</i> FISH as appropriate
Evidentiary standards	<i>EGFR</i> cobas® real time PCR test <i>ALK</i> FISH <i>ROS1</i> FISH <i>METex14sk</i> by RNA or DNA testing on plasma or tumour tissue
Outcomes	<p>Test outcomes</p> <p>Positive percent agreement and negative percent agreement of small DNA/RNA panels against the evidentiary standards</p> <p>Positive predictive value and negative predictive value of small DNA/RNA panels against the evidentiary standards</p> <p>Concordance between small DNA/RNA panels and comparator biomarker assays</p> <p>Test turnaround time</p> <p>Rebiopsy rate / test failure rate / inadequate sample rate (e.g. from an inadequate cytological specimen)</p> <p>Safety outcomes</p> <p>Any adverse events related to treatment, repeated biopsies, adverse effects of delayed treatment due to time taken to test</p> <p>Effectiveness outcomes</p> <p>Direct health outcomes (Disease-free and/or overall survival, disease-related or and/or all-cause mortality, disease progression, tumour control (regression/remission), incidence of metastases, tumour recurrence, quality of life, and other patient-relevant outcomes)</p> <p>Healthcare resources</p> <p>Cost</p> <p>Cost-effectiveness</p> <p>Net Australian Government healthcare costs</p>
<p>Systematic review question:</p> <p>What is the safety, effectiveness and cost-effectiveness of small NGS DNA/RNA panel(s) compared to sequential single gene testing in patients with non-squamous (or histology not otherwise specified) non-small cell lung cancer?</p> <p><i>NB: questions related to the linked evidence approach are shown in Appendix A.</i></p>	

ALK = anaplastic lymphoma kinase; *DNA* = deoxyribonucleic acid; *EGFR* = epidermal growth factor receptor; *FISH* = fluorescence *in situ* hybridisation; *IHC* = immunohistochemistry; *KRAS* = Kirsten rat sarcoma; *MET* = mesenchymal-epithelial transition; *NGS* = next generation sequencing; *NTRK* = neurotrophic tyrosine receptor kinase; *RET* = rearranged during transfection; *RNA* = ribonucleic acid; *ROS1* = ROS proto-oncogene 1

1.8 Alignment with the PICO confirmation

No PICO confirmation was created for MSAC assessment 1721, as the topic was deemed similar enough to MSAC assessment 1634, to use that as the basis. The key difference to PICO confirmation 1634 were the inclusion of smaller NGS panels (rather than comprehensive NGS panels).

1.9 Clinical management algorithms

Clinical management algorithms for the current scenario and the two proposed options are shown in Figure 4 to Figure 6. Patients have the same treatment options in all three scenarios, the only differences between algorithms are the number of tests which patients may have. If insufficient tumour tissue is available due to sequential testing, more rebiopsies may be required in the current clinical management algorithm.

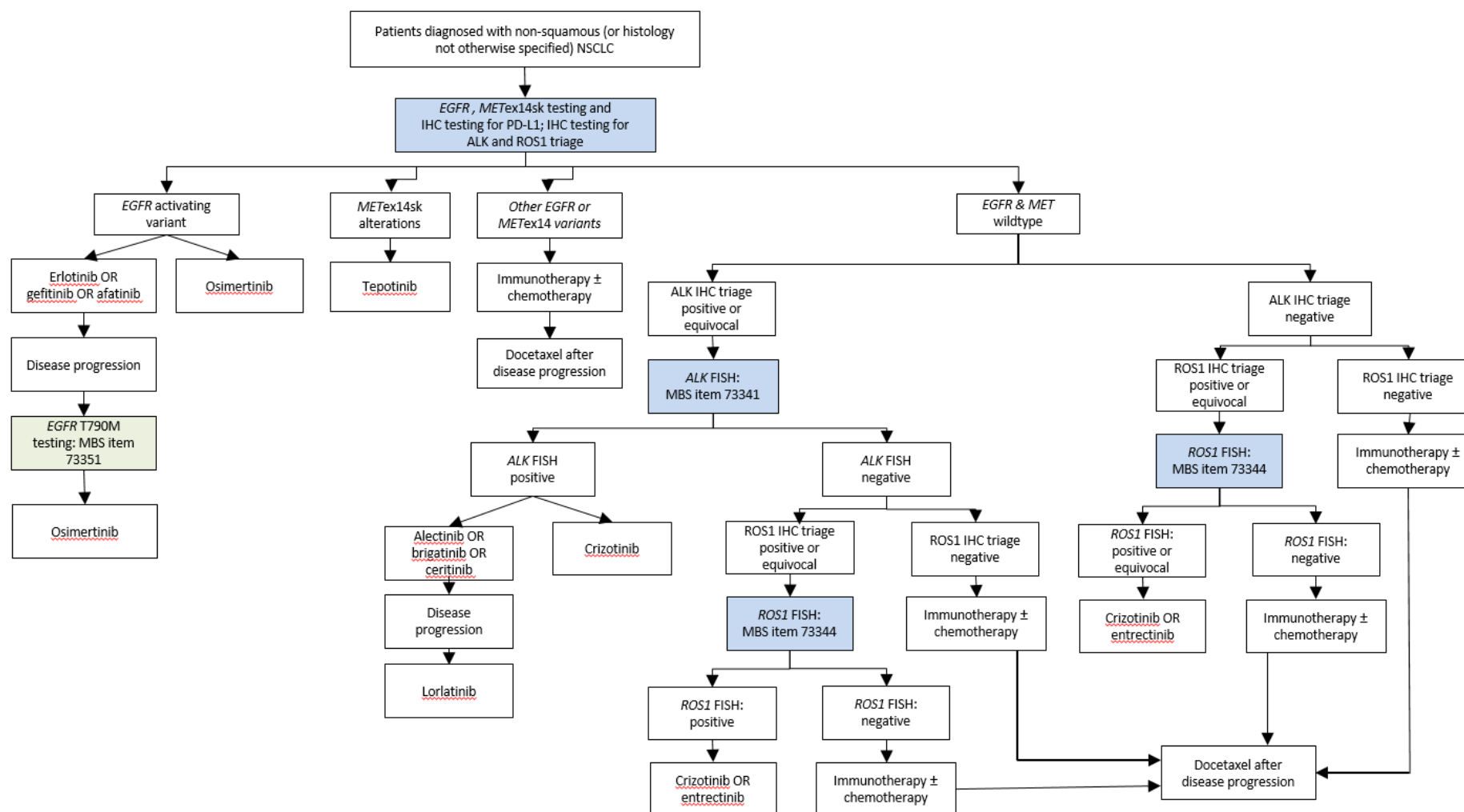


Figure 4 Current clinical management algorithm, showing sequential testing

Source: based on clinical algorithm from ratified PICO confirmation for MSAC application 1669. Blue boxes show the position of testing to be replaced (NB IHC testing for PD-L1 not proposed to be replaced). *EGFR* T790M testing not proposed to be replaced. *ALK* = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; IHC = immunohistochemistry; *MET* = mesenchymal-epithelial transition; NGS = next generation sequencing; *ROS1* = ROS proto-oncogene 1

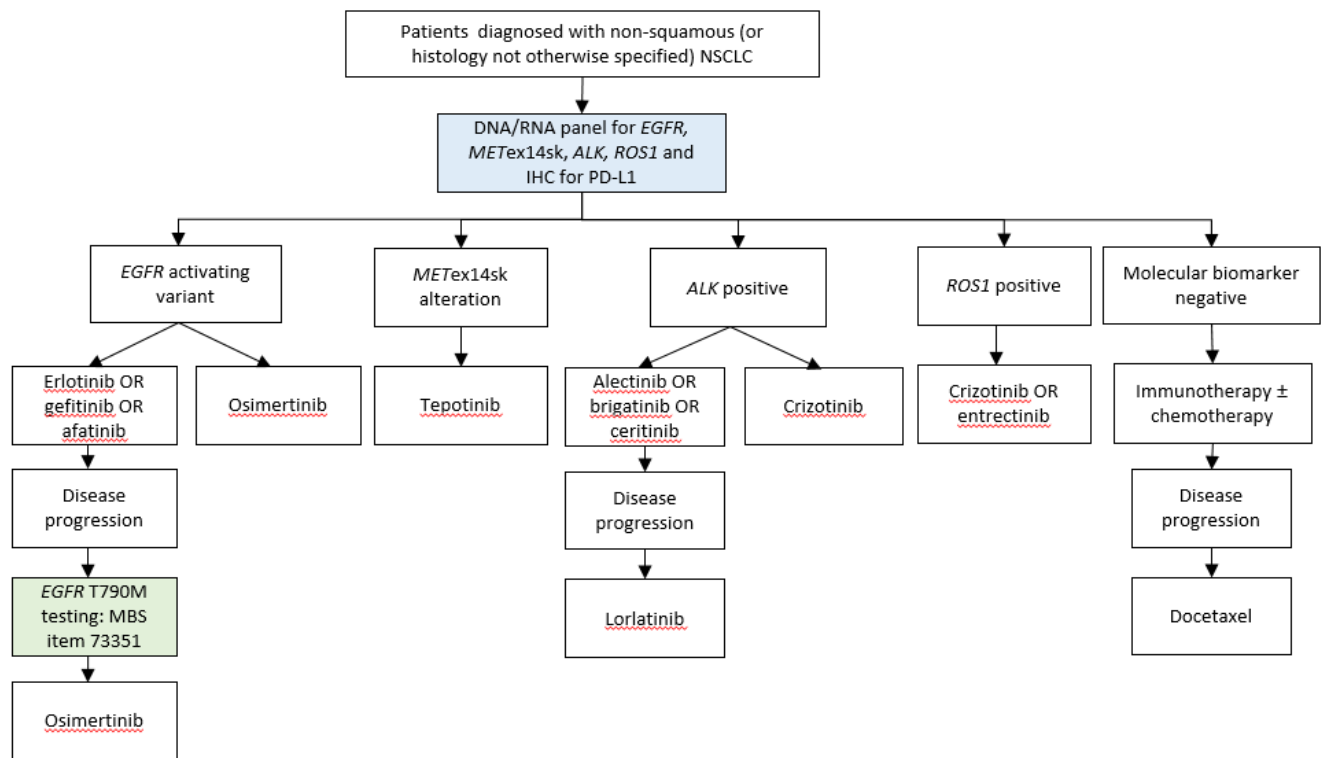


Figure 5 Proposed clinical management algorithm, showing simultaneous testing of DNA and RNA (option 1)

Source: based on clinical algorithm from ratified PICO confirmation for MSAC application 1634. *EGFR* T790M testing not proposed to be replaced. *ALK* = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; IHC = immunohistochemistry; *MET* = mesenchymal-epithelial transition; NGS = next generation sequencing; *ROS1* = ROS proto-oncogene 1

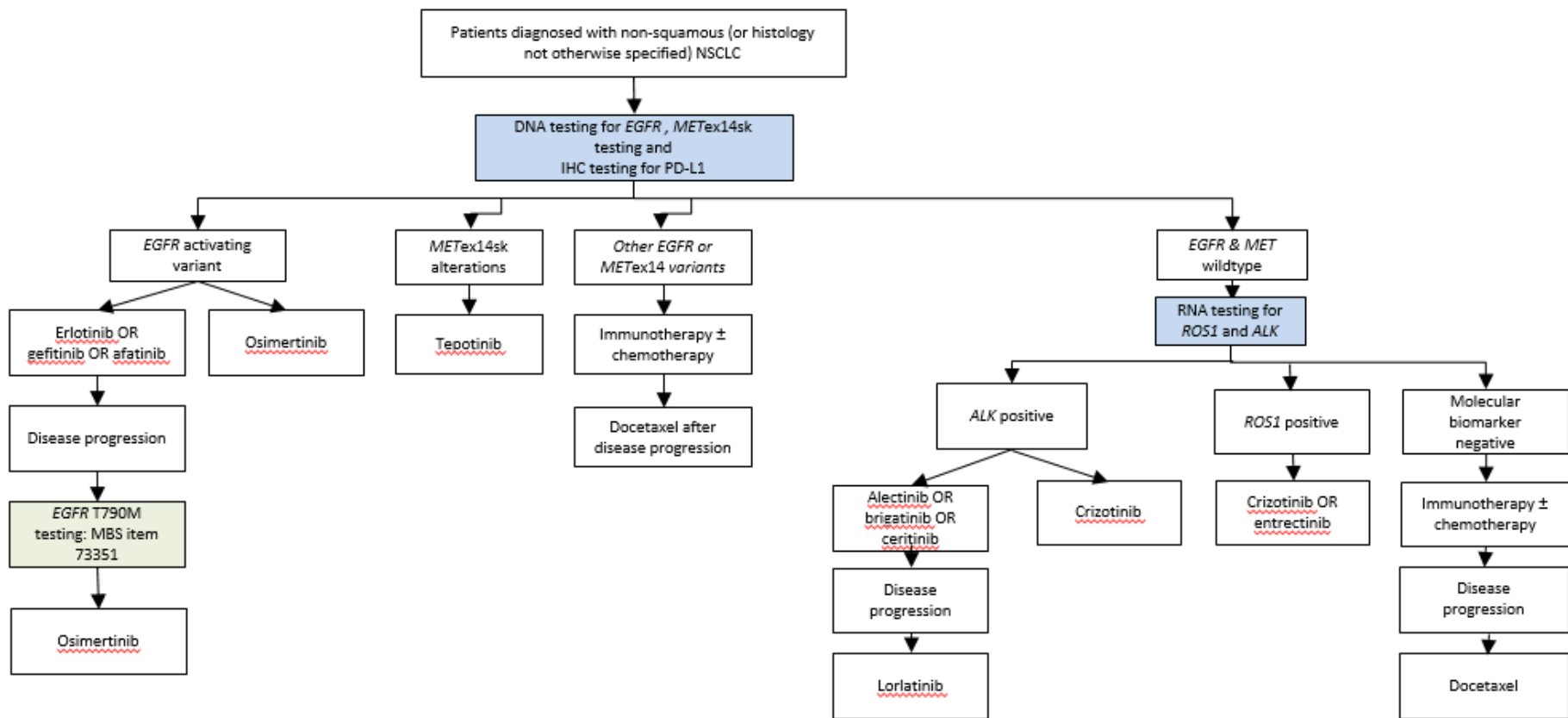


Figure 6 Proposed clinical management algorithm, showing separate DNA and RNA panels (option 2)

Source: based on clinical algorithm from ratified PICO confirmation for MSAC application 1669. *EGFR* T790M testing not proposed to be replaced. *ALK* = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; *IHC* = immunohistochemistry; *MET* = mesenchymal-epithelial transition; NGS = next generation sequencing; *ROS1* = ROS proto-oncogene 1

1.10 Proposal for public funding

Three new MBS items are proposed: one for a nucleic acid-based test of both DNA and RNA for simultaneous testing. However, not many laboratories have the capacity to perform simultaneous testing. Two additional MBS items are therefore proposed, for separate DNA and RNA panels. All three items are proposed to be pathologist determinable (as per *EGFR* testing, IHC testing for *ALK* and *ROS1*, *ALK* FISH and *ROS1* FISH).

The proposed fees were based on those currently in use for private patients, and reflects the costs of delivering the tests, including extraction, pathologist assessment, quality control, curation and reporting (MSAC application 1721).

Table 22 Applicant proposed MBS item for DNA/RNA panel with suggested modifications

Category 6 – Genetics P7	
AAAA	
A nucleic acid-based multi-gene panel test of tumour tissue from a patient diagnosed with non-small cell lung cancer, shown to have non-squamous histology or histology not otherwise specified, requested by, or on behalf of, a specialist or consultant physician, to detect:	
<ul style="list-style-type: none"> iv. variants in at least <i>EGFR</i>, <i>BRAF</i>, <i>KRAS</i> and <i>MET</i> exon 14 to determine access to specific therapies listed on the Pharmaceutical Benefits Scheme (PBS); and v. the fusion status of at least <i>ALK</i>, <i>ROS1</i>, <i>RET</i>, and <i>NTRK</i> to determine access to specific therapies listed on the PBS; or vi. if the requirements relating to <i>EGFR</i>, <i>ALK</i> and <i>ROS1</i> status for access to a PD-(L)1 immunotherapy pembrolizumab under the PBS are fulfilled. 	
Maximum one test per episode of disease	
This item cannot be claimed in addition to MBS items 73337, 73341, 73344, or MBS item for <i>MET</i> ex14sk testing	
Fee: \$1,247	Benefit: 75% = \$935.25 85% = \$1,159.10 ^a

^aTaking into account the Greatest Permissible Gap

Suggested changes are shown in red, blue and strikethrough text. Changes in red are to prevent co-claiming with single-gene testing

Table 23 Applicant proposed MBS items for separate DNA and RNA panels with suggested modifications

Category 6 – Genetics P7		
BBBB		
A DNA-based multi-gene panel test of tumour tissue from a patient diagnosed with non-small cell lung cancer, shown to have non-squamous histology or histology not otherwise specified, requested by, or on behalf of, a specialist or consultant physician, to detect:		
<ul style="list-style-type: none"> iii. variants in at least <i>EGFR</i>, <i>BRAF</i>, <i>KRAS</i> and <i>MET</i> exon 14 to determine access to specific therapies listed on the Pharmaceutical Benefits Scheme (PBS); or iv. if the requirements relating to <i>EGFR</i> status for access to a PD-(L)1 immunotherapy pembrolizumab under the PBS are fulfilled. 		
Maximum one test per episode of disease		
This item cannot be claimed in addition to MBS item AAAA, 73337, or MBS item for METex14sk testing		
Fee: \$682.35	Benefit: 75% = \$511.75	85% = \$594.45 ^a
CCCC		
A nucleic acid-based multi-gene panel test of tumour tissue from a patient diagnosed with non-small cell lung cancer, shown to have non-squamous histology or histology not otherwise specified, and with documented absence of activating mutations variants of the <i>EGFR</i> gene, <i>KRAS</i> , <i>BRAF</i> and <i>MET</i> exon14, requested by, or on behalf of, a specialist or consultant physician, to detect:		
<ul style="list-style-type: none"> iii. the fusion status of at least <i>ALK</i>, <i>ROS1</i>, <i>RET</i>, and <i>NTRK</i> to determine access to specific therapies listed on the Pharmaceutical Benefits Scheme (PBS) are fulfilled; or iv. if the requirements relating to <i>ALK</i> and <i>ROS1</i> status for access to a PD-(L)1 immunotherapy pembrolizumab under the PBS are fulfilled. 		
Maximum one test per episode of disease		
This item can only be claimed if the result from MBS item number BBBB is negative, and cannot be claimed in addition to MBS items AAAA, 73341, 73344		
Fee: \$682.35	Benefit: 75% = \$511.75	85% = \$594.45 ^a

^a Reflects the 1 November 2021 Greatest Permissible Gap (GPG) of \$87.90. All out-of-hospital Medicare services which have an MBS fee of \$586.20 or more will attract a benefit that is greater than 85% of the MBS fee – being the schedule fee less the GPG amount. The GPG amount is indexed annually on 1 November in line with the Consumer Price Index (CPI) (June quarter)

Suggested changes are shown in red and strikethrough text.

Suggested changes to the MBS items are shown in red text. MBS items 73337, 73341 and 73344 for testing tumour tissue from patients with NSCLC use the phrase “under the Pharmaceutical Benefits Scheme”. However, other items on the MBS use the phrase “on the Pharmaceutical Benefits Scheme” (MBS items 11219, 13761, 13762, 73343), so the use of “on” has therefore not been changed.

The suggested wording in item CCCC only allows it to be claimed if item BBBB is “negative”. The intention is that patients can only be tested if the *variants specified* are wildtype (as per the description of the population). ESC and MSAC may consider whether there is a risk that if other genes are included in the panel for item BBBB and have variants identified (e.g., *TP53*, which is frequently concurrent with other variants), whether this could be misinterpreted as a positive result.

Note, the proposed items include testing of genes which currently do not have PBS-listed specific therapies for NSCLC (i.e. *KRAS*, *BRAF*, *MET*exon 14, *RET* and *NTRK*), although *MET*ex14sk has a PBAC-recommended specific therapy, which is not yet PBS-listed. The applicants justified the additional genes by referencing international guidelines, which recommend the inclusion of the specified genes as a minimum (given targeted therapies are available for patients with tumours with variants in the specified genes, even if they are not PBS-listed). This should future-proof the items in case the targeted therapies become PBS-listed in the near future. Concurrent variants in

the listed genes are rare, so identifying pathogenic variants in the *KRAS*, *BRAF*, *RET* or *NTRK* genes is highly likely to rule out the presence of rearrangements in *ALK* or *ROS1* genes. The additional genes are therefore reasonable to include, although it may result in a very small number of patients with *ALK* or *ROS1* variants in their tumour not being identified, and consequently missing out on receiving an appropriate targeted therapy.

The proposal to refer to a PD-(L)1 immunotherapy rather than pembrolizumab reflects the fact that the PBS restriction for NSCLC of several of these medicines require that the “condition must not have evidence of an activating epidermal growth factor receptor (EGFR) gene or an anaplastic lymphoma kinase (ALK) gene rearrangement in tumour”. If MSAC supports this suggestion, then it is requested that MSAC also support the related changes to existing MBS items 73337, 73341 and 73344.

Sensitivity analyses have been performed to assess the impact of allowing patients with *KRAS* and/or *BRAF* variants to access RNA testing, given there are currently no targeted treatments available for patients with *KRAS* or *BRAF* variants.

Section 2 Clinical evaluation of investigative technologies

Methods for undertaking the assessment

The application from the RCPA claimed that a small DNA/RNA NGS panel or DNA ± RNA NGS panels are superior to sequential single-gene testing in patients with NSCLC. The aim of the health technology assessment was to assess whether the evidence supported this claim, which required a full health technology assessment through to health outcomes.

Scoping searches indicated that there was insufficient direct from test to health outcomes evidence (comparing health outcomes in those tested by NGS against to those tested sequentially by single-gene tests) on which to base the assessment. Therefore, a linked evidence assessment was also performed. PICO criteria were developed a priori for both the direct from test to health outcomes evidence, and the 'test performance' and 'change in management' steps of a linked evidence assessment. The protocol was registered on PROSPERO (CRD42022334620). PubMed, Embase, Cochrane, Australian Clinical Trials Registry, ClinicalTrials.gov, International clinical trials registry platform, INAHTA database and PROSPERO were searched to identify potentially relevant primary and secondary research. Search terms were developed, and tested using the SearchRefinery tool (Scells & Zuccon 2018), using relevant articles from DCAR 1495 and application 1721 as the seed citations. Relevant citations were downloaded into Endnote, where one assessor reviewed every citation based on title/abstract. A second reviewer assessed the most relevant 50% citations as determined by an algorithm in Rayyan. Full text articles determined potentially relevant by either reviewer were retrieved for assessment by one reviewer. Those which met the PICO criteria had their references and citations assessed, using Citation Chaser (Haddaway, Grainger & Gray 2021). A PRISMA flowchart was developed to summarise the number of citations identified at each step.

Each included study had its risk of bias assessed, based on the outcomes it provided and the study design. Concordance studies were evaluated using the QUADAS 2 checklist (substituting assessment of the reference standard with assessment of the comparator). Studies on the success rate of testing or turnaround time, or studies on the effectiveness of targeted treatment in non-randomised controlled studies were assessed using a checklist for cohort studies developed by SIGN. The certainty of evidence was judged using GRADE for the key outcomes which fed into the assessment framework. A full description of the methods is provided in Appendix A.

The methods used for assessing the last step of the linked evidence approach is discussed in section 2.4.1 Methods for undertaking the assessment.

Assessment framework

A single study provided direct from test to health outcomes evidence, assessing progression free survival, overall response rate and disease control rate for those testing positive or negative for *ALK* fusions by IHC, FISH or NGS (Lin, C et al. 2019) (component 1 in [Figure 7](#)).

One additional study was identified which compared health outcomes between NGS (which tested *EGFR*, *ALK*, *ROS1* and other genes) and *EGFR* and *ALK* testing by sequential testing (Presley et al. 2018). However, this study was excluded as any health differences observed could have been due to the additional genes being tested, rather than the method of testing.

A linked evidence approach was therefore used to supplement the limited direct evidence. Evidence of the comparative success rate of testing and test concordance (component 2 in Figure 7) is reported in Section 2.2. Evidence of the impact of testing on the management of patients (component 3 in Figure 7) is reported in Section 2.3. Evidence regarding the impact of how the change in management results in health benefits (component 4 in Figure 7) is reported in Section 2.4.

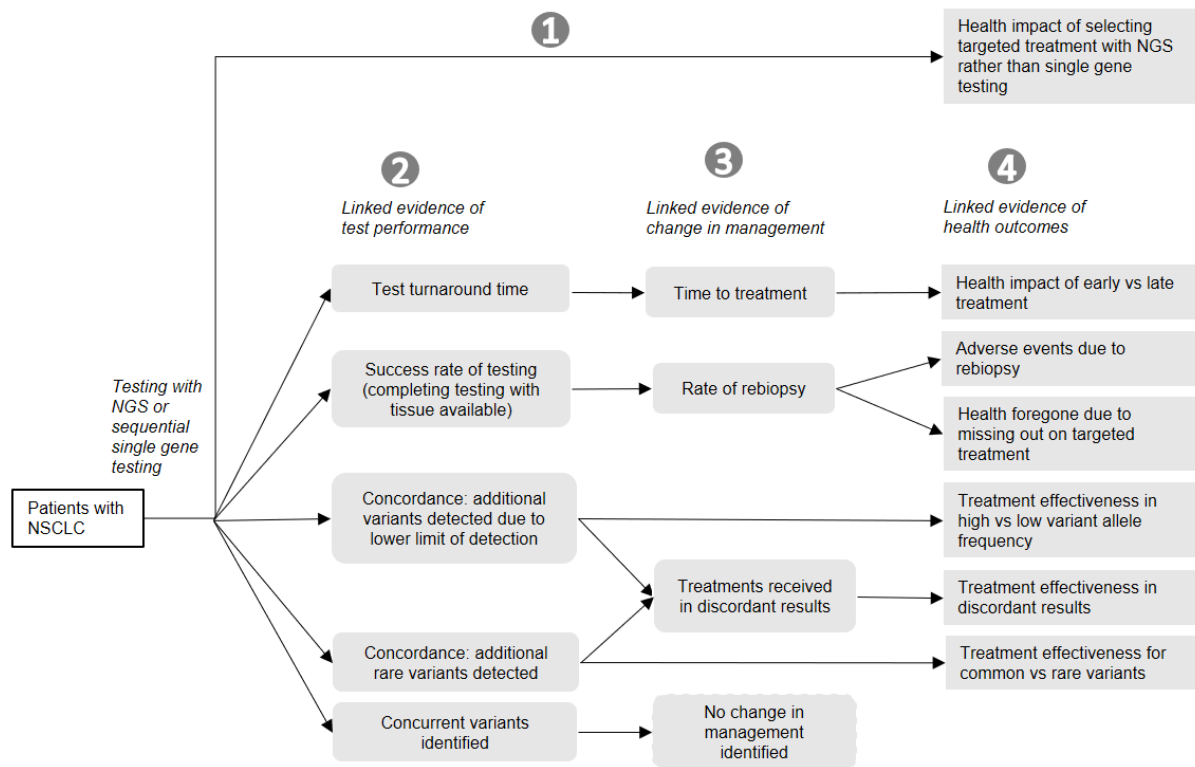


Figure 7 Assessment framework for small DNA/RNA NGS panel for NSCLC

Figure notes: 1: direct from test to health outcomes evidence; 2: test performance; 3: change in treatment/management; 4: influence of the change in management on health outcomes

2.1 Direct from test to health outcomes evidence

2.1.1 Methods for undertaking the assessment

The methods for identifying direct evidence are described at the start of section 2.

2.1.2 Characteristics of the evidence base

One small study provided direct evidence from testing to health outcomes (Table 24). It was a predictive study, retrospectively comparing health outcomes by those with and without the *ALK* biomarker, as determined by different testing methods.

Table 24 Key features of the included evidence comparing NGS against IHC and FISH for selecting patients to receive crizotinib

Study	N	Study design Risk of bias	Population	Intervention	Comparator	Key outcome(s)	Result used in economic model
(Lin, C et al. 2019) China	40	Retrospective cohort, within-patient comparison Moderate to high risk of bias (QUIPS)	Patients with advanced NSCLC (adenocarcinoma, or NSCLC NOS) who were <i>ALK</i> positive by at least one testing method, and received crizotinib for first- or second-line treatment	NGS for <i>ALK</i>	IHC for <i>ALK</i> FISH for <i>ALK</i>	PFS, ORR, DCR	No

ALK = anaplastic lymphoma kinase; DCR = disease control rate; FISH = fluorescent *in situ* hybridisation; IHC = immunohistochemistry; NGS = next generation sequencing; NOS = not otherwise specified; NSCLC = non-small cell lung cancer; ORR = overall response rate; PFS = progression free survival; QUIPS = Quality in Prognostic Studies tool

2.1.3 Results

Lin et al. (2019) reported on a retrospective cohort of patients with advanced NSCLC, treated at Zhejiang Cancer Hospital in China between 2014 and 2017. A total of 55 patients were positive for *ALK* by at least one testing method (IHC, FISH and/or NGS), and of these, 40 received crizotinib.

The authors explored which testing method separated those likely to respond to crizotinib most accurately. The difference between NGS *ALK*+ and *ALK*- was larger than either IHC or FISH (Table 25; Figure 8). Given all the patients received crizotinib, it is unknown whether the differences in health outcomes were due to differences in response to the targeted drug (i.e., NGS was better at predicting which patients were likely to respond well to crizotinib than either IHC or FISH), or whether there were prognostic differences (i.e., NGS was better at predicting who would have good health outcomes regardless of the type of treatment given). The study was also very small, and there was only a single study, so the results would be considered hypothesis-generating rather than conclusive. Furthermore, as the sample only includes those positive by at least one testing method, there is a risk that those classified as negative (by one or more methods) would not reflect the health outcomes of patients classified as negative by all testing methods.

Table 25 Response to crizotinib by NGS, IHC and FISH results

Study	Outcome measure	NGS <i>ALK</i> +	IHC <i>ALK</i> +	FISH <i>ALK</i> +	Triple <i>ALK</i> +	NGS <i>ALK</i> -	IHC <i>ALK</i> -	FISH <i>ALK</i> -
(Lin, C et al. 2019)	mPFS	11.1m	10.3m	8.8m	8.3m	4.6m	11.7m	14.8m
	ORR	75%	68.4%	70.6%	68.8%	25%	100%	75%
	DCR	97.2%	94.7%	94.1%	81.3%	50%	100%	100%

ALK = anaplastic lymphoma kinase; DCR = disease control rate; FISH = fluorescent *in situ* hybridisation; IHC = immunohistochemistry; NGS = next generation sequencing; ORR = overall response rate; mPFS = median progression free survival (months)
NB: those *ALK*- on one testing method had to have discordant results and be *ALK*+ on at least one other method.

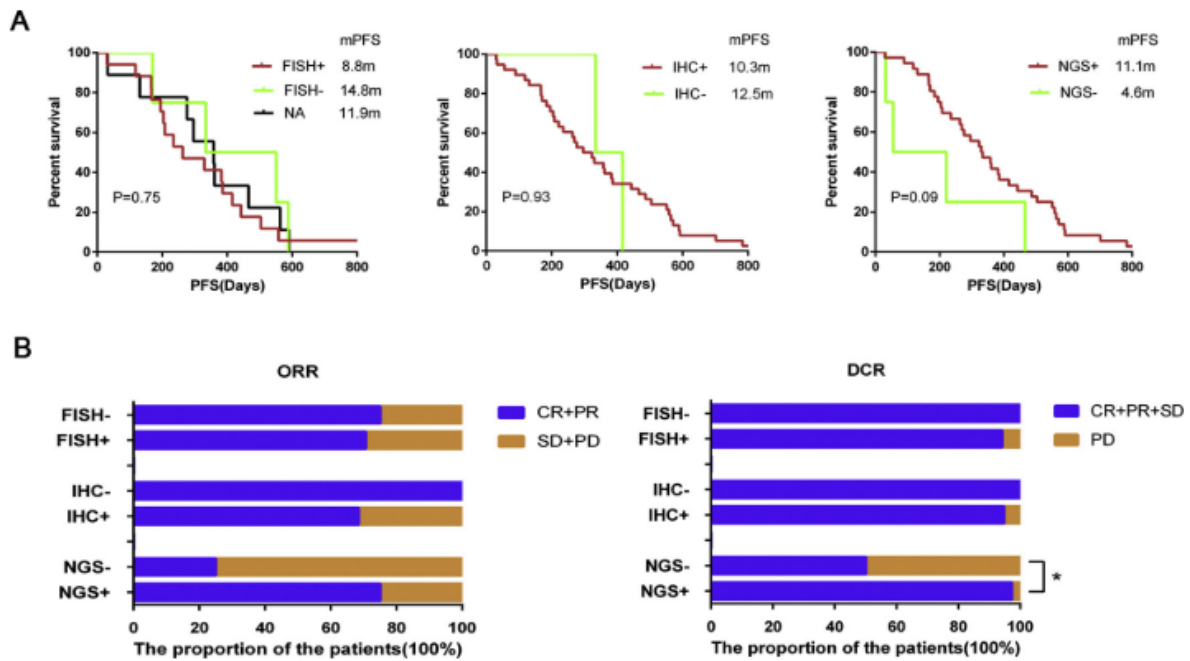
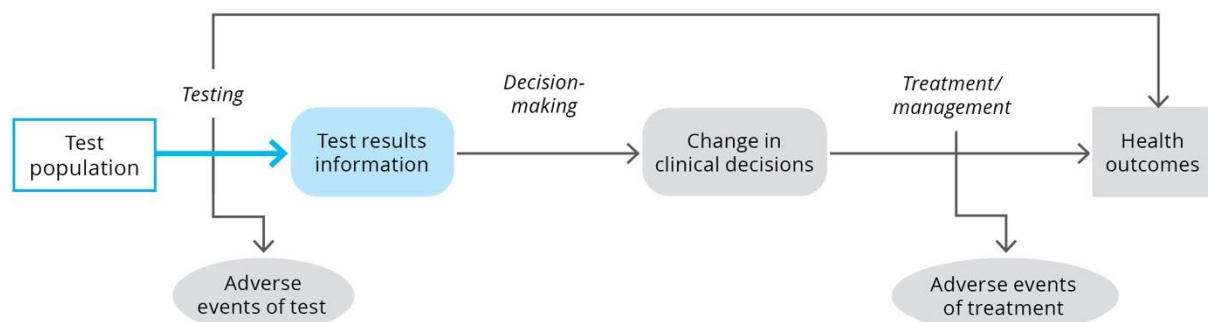


Figure 8 Comparison of clinical efficacy between ALK+ and ALK- patients by FISH, IHC and NGS

Source: (Lin, C et al. 2019) Reprinted from Lung Cancer, volume 131, Lin, C et al. "Comparison of ALK detection by FISH, IHC and NGS to predict benefit from crizotinib in advanced non-small-cell lung cancer", p65, Copyright (2019), with permission from Elsevier
 DCR = disease control rate; FISH = fluorescent *in situ* hybridisation; IHC = immunohistochemistry; mPFS = mean progression free survival; ORR = overall response rate; *p<0.05

The certainty of the evidence is low, as it was an observational study with a moderate to high risk of bias, with a population which represents only a small percentage of the target population (i.e., only those positive for ALK rearrangements by at least one method, rather than all those with NSCLC who are tested for biomarkers) (GRADE ⊕⊖⊖⊖).

2.2 Linked evidence of test performance



2.2.1 Methods for undertaking the assessment

The overarching methods for the systematic review have been summarised in Section 2. As per the PICO criteria, studies were included in section 2.2 if they assessed the following outcomes:

- Test failure rate / inadequate sample rate (e.g., from an inadequate cytological specimen)

- Concordance between DNA/RNA assays and comparator biomarker assays
- Positive percent agreement and negative percent agreement of DNA/RNA assays against the clinical utility standards
- Positive predictive value and negative predictive value of DNA/RNA assays against the clinical utility standards
- Test turnaround time

Additional outcomes of comparative yield, and the frequency of concurrent variants being identified were also added to the review.

For the comparison of test performance of NGS and sequential single-gene testing, only comparative studies were included, as single-arm studies would not have increased the certainty of the evidence. This restriction also applied to the outcome of “identification of concurrent variants” for pragmatic reasons, despite comparative data not being available for this outcome. For positive percent agreement and negative percent agreement, only studies which provided 2x2 data were included.

2.2.2 Clinical utility standard

There are multiple targeted drugs available for patients with NSCLC, available through the PBS. Each time a codependent application was put in for funding of a new MSAC test to determine eligibility for a PBS-listed drug, the type of test used in the key trial, is called the ‘clinical utility standard’. The clinical utility standard is defined, so that any other tests which could potentially be used using the same MBS item are compared against the clinical utility standard. The clinical utility standards relevant to this application are listed below.

Table 26 Clinical utility standards for tests to determine eligibility for targeted drugs for NSCLC

Variant	Clinical utility standard
<i>EGFR</i>	Cobas PCR on tumour tissue
<i>ALK</i>	FISH on tumour tissue
<i>ROS1</i>	FISH on tumour tissue
<i>MET</i> ex14sk	DNA or RNA analysis on blood or tumour tissue

ALK = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; *MET* = mesenchymal-epithelial transition; PCR = polymerase chain reaction; *ROS1* = ROS proto-oncogene 1

2.2.3 Characteristics of the evidence base

A total of 41 studies met the inclusion criteria, comparing the test performance of NGS (using a DNA panel, combined DNA or RNA panel, or RNA panel, or combination) against sequential single-gene testing. The most common outcome was concordance, reported by 30 studies.

The characteristics of these studies have been provided in Table 78 in Appendix D. The majority of these studies were classified as having a low to moderate risk of bias on the QUADAS 2 checklist. Most concerns related to the selection of the patient population, or with the flow and timing of the intervention and comparator. A summary of the key features of studies reporting on test performance outcomes other than concordance is shown in Table 78. Full study profiles and a PRISMA flowchart are presented in Appendix B.

The two key outcomes for test performance, are how successful the testing strategies are at providing test results (from the tissue/cytology samples available), and from the test results, how concordant NGS was compared to sequential single-gene testing. These outcomes were used in the economic model, and the evidence base for these is summarised in Table 27.

Table 27 Key features of the included studies comparing NGS with sequential single-gene testing

Study	N	Study design Risk of bias	Population	Intervention	Comparator	Key outcome(s)	Result used in economic model
(Steeeghs et al. 2022) The Netherlands	4040	Between-patient retrospective cohort study Level III-2 interventional levels of evidence Low to moderate risk of bias (QUADAS 2)	Stage IV NSCLC patients. 3343 NGS patients, 698 non-NGS patients	NGS (no further information given)	Various non-NGS single gene testing such as ICH and FISH used throughout clinical practice in the Netherlands	Success rate of testing Comparative yield Turnaround time	Yes
k=30 For more details, see Table 78	4081	Within-patient cohort studies (retrospective or prospective), or diagnostic case-control studies Level III-2 and level III-3 diagnostic levels of evidence Predominantly low to moderate risk of bias (QUADAS 2)	Patients with NSCLC, with sufficient tumour tissue/ cytology sample available for both tests (i.e. only including samples with results on both tests)	NGS (hybrid-capture NGS, Compact NGS panel, Ion Ampliseq cancer hotspot panel, TruSight 170, Lung core 56 panel, OncoAim, Oncomine Dx Target Test, SNUH FIRST cancer panel, K-MASTER Cancer panel, 454 GS-Junior NGS, IonTorrent Lung panel or not specified)	<i>EGFR</i> : RT-PCR, ARMS-PCR, MLPA, PNA-LNA clamp, PCR, SS, cobas PCR, Idylla <i>EGFR</i> mutation test, or pyrosequencing <i>ALK</i> : IHC + FISH or FISH <i>ROS1</i> : FISH or real-time PCR or reverse-transcription PCR <i>METex14sk</i> : Archer <i>MET</i> test	Concordance between testing methods on <i>EGFR</i> , <i>ALK</i> , <i>ROS</i> and <i>METex14sk</i>	Yes

ALK = anaplastic lymphoma kinase; *ARMS-PCR* = amplification-refractory mutation system polymerase chain reaction; *EGFR* = epidermal growth factor receptor; *FISH* = fluorescent *in situ* hybridisation; *IHC* = immunohistochemistry; *MLPA* = Multiplex ligation-dependent probe amplification; *MET* = mesenchymal-epithelial transition; *NGS* = next generation sequencing; *NSCLC* = non-small cell lung cancer; *ROS1* = ROS proto-oncogene 1; *RT-PCR* = real time polymerase chain reaction; *SS* = Sanger sequencing

2.2.4 Results

Success rate of testing/rate of sufficient tissue

One of the claims made regarding the use of a small NGS panel rather than sequential testing, is that it makes more efficient use of the tumour sample, requiring fewer re-biopsies (and is therefore safer). In the majority of the literature identified in the systematic review, rebiopsies were only performed at the point of progression. If patients had insufficient tissue for testing, then international practice is to use circulating tumour DNA extraction and evaluation, rather than rebiopsy (Griesinger et al. 2021; Li, W, Li, Y, et al. 2021). However, assessing use to circulating tumour DNA extraction and evaluation is out of scope of this assessment. For more discussion on testing of circulating tumour DNA, see 'Section 5 Other relevant

information'. However, in Australian practice, access to PBS-listed drugs for targetable variants in NSCLC is restricted to those which have been identified in tumour tissue or cytology. There is the possibility that patients with insufficient tumour tissue for testing would therefore have a rebiopsy.

The best evidence identified on success rates of testing was published by Steeghs et al. (2022) in a large retrospective cohort study from the Netherlands (with a comparison between cohorts of patients rather than within-patient comparisons) (Steeghs et al. 2022). The other studies which provided comparisons of the success rate of sequential testing and NGS were within-patient (where patients were tested samples using both an NGS panel and with sequential testing). Comparing the rate of insufficient tissue from this style of study may not reflect results achieved in clinical practice where NGS is expected to replace sequential testing, as the prior use of one type of test would influence the volume of tissue left for subsequent tests (for more information on the results from within-study comparisons, see Appendix D).

Steeghs et al. (2022) reported that NGS had a higher rate of success of testing all the genes of interest in patients with NSCLC, than the comparative sequential single-gene strategy (97.2% vs 94.6%; Table 28, Figure 9) (Steeghs et al. 2022). Therefore, if NGS replaced sequential gene-testing, for every 1000 patients tested, an additional 26 patients would receive results for the genes of interest.

It is unknown how applicable the data from this study are to Australia, as biomarkers other than *EGFR*, *ALK*, *ROS1* and *MET* were also assessed in some patients, and gene fusions were tested with a mix of NGS on RNA, FISH and IHC. The certainty of the evidence was rated as moderate, due to the difference between the proposed intervention (Small DNA/RNA panel or DNA ± RNA panels) and the study (which included the use of IHC and FISH for gene fusions), the observational study design, and the low to moderate risk of bias in the study (GRADE ⊕⊕⊖⊖).

Table 28 Success rates of sequential single-gene testing and NGS (between-patient comparison)

Study	Population	Intervention	Comparator	Genes	Success of single gene testing	Success of NGS
(Steeghs et al. 2022)	4233 patients with adenocarcinoma	NGS of DNA Gene fusions examined by FISH, IHC and/or RNA-based NGS	Predominantly traditional single gene tests (e.g. Sanger sequencing). High sensitive single-gene tests (i.e. ddPCR) only, plus IHC and/or FISH	<i>EGFR</i>	827/833 (99.3%) ^a	Overall 3248/3342 (97.2%) success ^b
				<i>KRAS</i>	682/689 (99.0%) ^a	
				<i>BRAF</i>	516/523 (98.7%) ^a	
				<i>ALK</i>	459/488 (94.1%) ^a	
				<i>ROS1</i>	302/336 (89.9%) ^a	
				<i>MET</i> _{ex14sk}	82/88 (93.2%) ^a	
				Overall	660/698 (94.6%) success ^b	

ALK = anaplastic lymphoma kinase; *BRAF* = proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B; ddPCR = digital droplet polymerase chain reaction; *EGFR* = epidermal growth factor receptor; FISH = fluorescent *in situ* hybridisation; IHC = immunohistochemistry; *KRAS* = Kirsten rat sarcoma; *MET* = mesenchymal-epithelial transition; NGS = next generation sequencing; RNA = ribonucleic acid; *ROS1* = ROS proto-oncogene 1

^a Calculated from supplementary data (counting “insufficient quantity” and “insufficient quality” as test failures, but excluding those “not analysed” or “not performed” although the reasons for not performing testing were not stated).

^bReported in the journal article

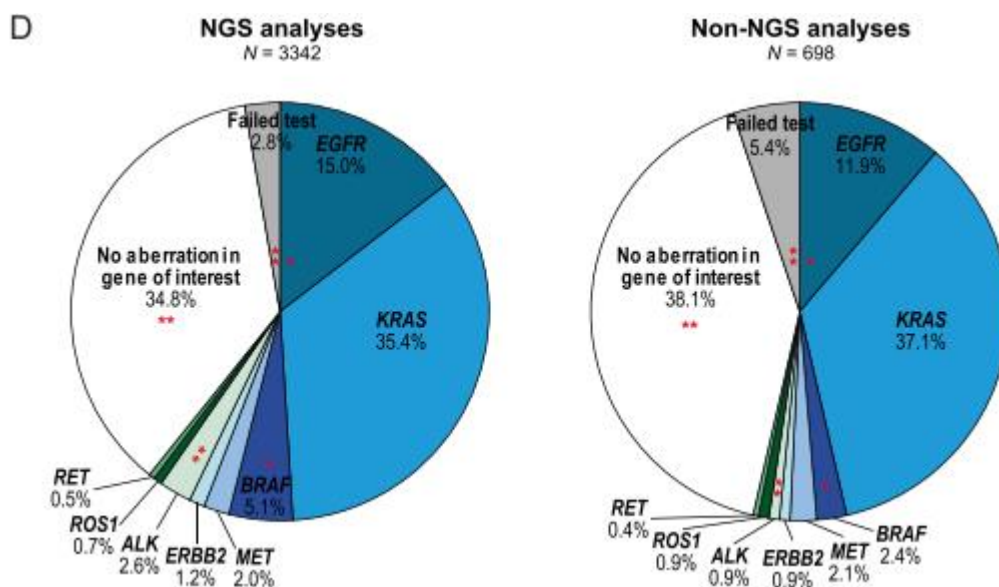


Figure 9 Comparison of pathogenic variants identified by NGS vs non-NGS methods (Sanger sequencing, HRM, MassARRAY, Pyrosequencing, Idylla, Cobas, ddPCR, FISH, IHC and/or RNA-based sequencing) in patients with adenocarcinoma.

Source: (Steeghs et al. 2022), p91. Reproduced with permission under Creative Common CC-BY license.

ALK = anaplastic lymphoma kinase; BRAF = proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B; ddPCR = digital droplet polymerase chain reaction; EGFR = epidermal growth factor receptor; ERBB2 = erb-b2 receptor tyrosine kinase 2; FISH = fluorescent *in situ* hybridisation; HRM = high resolution melting; IHC = immunohistochemistry; KRAS = Kirsten rat sarcoma; MET = mesenchymal-epithelial transition; NGS = next generation sequencing; RET = rearranged during transfection; RNA = ribonucleic acid; ROS1 = ROS proto-oncogene 1; If multiple variants were identified in one patient, only the first variant was included in the pie chart (so that the sum = 100%), **p<0.01, *p<0.05

If multiple variants were identified in one patient, only the first variant was included in the pie chart (so that the sum = 100%), **p<0.01, *p<0.05

Concordance, positive percent agreement and negative percent agreement

To assess the concordance of the proposed test, compared to the comparator (single-gene testing), studies were only included if they provided data that could be extracted into a classic 2-by-2 table by patient (rather than by variant), in which the results of the NGS were cross-classified against the results of single-gene testing and Bayes' Theorem was applied.

Table 29 explains how the key concepts of the positive percent agreement (PPA) and negative percent agreement (NPA) were calculated. The data on concordance were meta-analysed to provide the PPA and NPA (akin to the concepts of sensitivity and specificity but compared against the comparator or clinical utility standard rather than a reference standard), and the forest plots are shown in Appendix D. The results were then transformed to the prevalence of the target variants in the Australian population with non-squamous NSCLC.

Table 29 Diagnostic accuracy data extraction

		Single gene testing / clinical utility standard		
		Variant +	Variant –	
NGS	Variant +	a. Positive concordance	b. Incremental variants identified by NGS	Positive predictive value = $a/(a+b)$
	Variant –	c. Variants identified by single-gene testing but not NGS	d. Negative concordance	Negative predictive value = $d/(c+d)$
		Positive percent agreement = $a/(a+c)$	Negative percent agreement = $d/(b+d)$	

A total of 30 studies were identified which provided 2x2 data per gene assessed, and were able to be included on the outcome of concordance. The summary of the concordance results are shown in Table 30.

For those tested with DNA NGS ± IHC, FISH or RNA NGS instead of single-gene testing, the vast majority of patients (95.7%) would be classified the same (due to concordant variants identified in 22.4% of patients and being found to have no pathogenic variants in *EGFR*, *ALK*, *ROS1* or *METex14sk* by both forms of testing in 73.3% of patients). In the 4.3% of cases where the tests were discordant, the majority (3.5%) were due to NGS identifying an additional variant in one of the four specified genes, whereas in a very small proportion of patients (0.8%), the NGS did not detect a variant identified by single-gene testing. Li et al. (2016) suggest a non-inferiority margin of 5% difference in PPA and NPA for concordance (Li et al. 2016), suggesting that NGS is non-inferior to single-gene testing.

Discordant classifications on *EGFR* variants were due to the NGS panels frequently being more comprehensive than single-gene testing (including variants not included on single-gene tests), or due to a difference in the level of analytical sensitivity between the tests (NGS had a higher level of sensitivity, and lower threshold of detection, than Sanger sequencing, the cobas assay, and some PCR tests, whereas ARMS-PCR was set at a higher-level sensitivity than NGS in several studies). Comments on the cases of discordance are compiled in Table 83 in Appendix D. Tan et al. (2020) reported that NGS identified an additional 12 variants, of which 7 (58%) were common variants (ex19del, L858R or T790M), and 5 (42%) were rare variants. Similarly, Park et al. (2020) reported that of the 16 incremental *EGFR* variants identified by NGS, 8 were in hotspot locations (in regions tested by PCR, but below the sensitivity threshold), and the remaining 8 were in locations not tested by PCR, although half of the rare variants identified were considered actionable, and *EGFR* TKIs were administered.

For *EGFR* and *ROS1*, there were insufficient studies comparing NGS against the clinical utility standard (cobas assay and FISH) to provide results for NGS against the clinical utility standard, separate from other tests potentially used in Australia. For *ALK*, NGS was able to be compared to the clinical utility standard (FISH) in six studies, with a PPA of 93% (95%CI 72%, 98%) and NPA of 97% (79%, 100%).

The negative predictive value (NPV; the likelihood that someone classified as being wildtype by NGS would also be classified as wildtype on single-gene testing) was very high (ranging from 99.6% for *EGFR* to 99.9% for *METex14sk*). The positive predictive values (PPV; the likelihood that someone with an actionable variant identified by NGS would also be classified as having an actionable variant by single-gene testing) was less consistent, with the PPV for *ALK* rearrangements being 73.7%, and for *ROS1* and *METex14sk* being 100%. These results suggest

that although the largest discordance (in raw numbers) would be the additional *EGFR* variants detected, the largest relative difference would be in the patients being identified with *ALK* rearrangements.

Only four studies reported using a threshold of 15% to determine a positive result on *ALK* FISH testing. These four studies reported similar results to the studies overall (PPA 91%; 95%CI 42, 99; NPA 99%; 95%CI 95, 100). There were insufficient details provided in the studies with discordance between *ALK* (FISH ± IHC) and NGS to determine whether the additional cases identified by NGS would potentially allow *ALK* TKIs to be used in patients who currently are not eligible based on the current PBS criteria.

Table 30 Summary of concordance data between NGS and single-gene testing

Gene	Evidence base	PPA (95%CI)	NPA (95%CI)	Prevalence	Per 1000 successfully tested (95%CI)				PPV	NPV
					NGS+ /SG+	NGS+ /SG-	NGS- /SG+	NGS- /SG-		
<i>EGFR</i>	n=2611 k=22	0.98 (0.95, 0.99)	0.97 (0.95, 0.99)	15% ^a	147 (143, 149)	25 (8, 42)	3 (1, 7)	825 (808, 842)	0.85	1.00
<i>ALK</i>	n=1464 k=11	0.92 (0.77, 0.97)	0.99 (0.93, 1.00)	3% ^b	28 (23, 29)	10 (0, 68)	2 (1, 7)	960 (902, 97)	0.74	1.00
<i>ROS1</i>	n=830 k=6	0.86 (0.63, 0.96)	1.00 (0.99, 1.00)	1.61% ^c	14 (10, 15)	0 (0, 10)	2 (1, 6)	984 (974, 984)	1.00	1.00
<i>MET</i> ex14s k	n=99 k=1	0.98 (0.89, 1.00)	1.00 (0.93, 1.00)	3.6% ^d	35 (32, 36)	0 (0, 69)	1 (0, 4)	964 (895, 964)	1.00	1.00
Total				23.21%	224	35	8	733		

^aBased on p18 [MSAC 1161 PSD](#), November 2012

^bBased on p5 [MSAC 1250.1 PSD](#), November 2014

^cBased on p12 [MSAC 1454 PSD](#), July 2018

^dBased on Table 11, p27 [Tepotinib PBAC PSD](#), November 2021

ALK = anaplastic lymphoma kinase; CI = confidence interval; *EGFR* = epidermal growth factor receptor; k = number of studies; *MET* = mesenchymal-epithelial transition; n = number of patients; NGS = next generation sequencing; NPA = negative percent agreement; NPV = negative predictive value; PPA = positive percent agreement; PPV = positive predictive value; *ROS1* = ROS proto-oncogene 1; SG = single-gene testing

The main sources of bias in the included studies were the lack of clarity regarding whether the interpretation of the tests was independent of each other, and how directly relevant the patient samples were. Most studies included a small proportion of patients with squamous NSCLC, who are not proposed to be eligible for testing with the proposed MBS items for NGS in Australia but were a small enough proportion (<20%) that they were considered to have a small impact on the results. Although the prevalence of biomarkers varies a large amount between populations, the transformation of the data to accepted Australian prevalence data should mean that the results per 1000 patients test are likely to be applicable to the target population.

The overall certainty of the evidence on concordance is rated as moderate (for concordance of *EGFR*, *ALK* and *ROS1* variants), as most of the studies used designs applicable to the study question, the populations were appropriate, the studies relatively consistent, and there were sufficient studies that the results were relatively precise (GRADE ⊕⊕⊕⊖).

Yield of targetable variants

The best published evidence for comparative yield data were provided by Steeghs et al. (2022), who compared the success rate of testing and the yield of therapeutically relevant alterations detected by NGS and non-NGS-approaches in patients with NSCLC (Table 28). The authors suggested that the higher alteration rate reported by NGS was due to lower failure rates (2.8% vs 5.4%), a higher frequency rate of *ALK*-rearrangements being reported by NGS than non-NGS approaches (IHC and/or FISH), and differences in tissue availability (there was insufficient tissue for *ALK* testing in 3.7% of samples which were being tested with single-gene testing, and 1.1% of samples tested with NGS) (Steeghs et al. 2022).

Table 31 Comparative diagnostic yield of NGS and sequential single-gene testing (between patient comparison), taking into account comparative success rate

Study	Intervention	Comparator	Yield of therapeutically relevant alterations			
			Genes	NGS	Single-gene testing	Difference
(Steeghs et al. 2022)	NGS	Predominantly traditional single gene tests (e.g. Sanger sequencing), plus IHC and/or FISH	Overall	62.4%	56.5%	p=0.004
			<i>EGFR</i> , <i>MET</i> , <i>ALK</i> , <i>ROS1</i> only	20.3%	15.8%	not stated

ALK = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; FISH = fluorescent *in situ* hybridisation; IHC = immunohistochemistry; *MET* = mesenchymal-epithelial transition; NGS = next generation sequencing; *ROS1* = ROS proto-oncogene 1

Comparative yields were also calculated using the 2x2 data collated for concordance (applying Australian-relevant prevalence figures). The difference between the yield of NGS and single-gene tested in these studies was much lower than reported by Steeghs et al. (2022). However, the concordance data only included results for patients in whom testing was successful for both NGS and single-gene testing.

Table 32 Comparative diagnostic yield of NGS and single-gene testing (in those who were successfully tested by both)

Gene	Evidence base	PPA (95%CI)	NPA (95%CI)	Prevalence	Per 1000 successfully tested	
					Yield by NGS	Yield by SG
<i>EGFR</i>	n=2611 k=22	0.98 (0.95, 0.99)	0.97 (0.95, 0.99)	15% ^a	172	150
<i>ALK</i>	n=1464 k=11	0.92 (0.77, 0.97)	0.99 (0.93, 1.00)	3% ^b	38	30
<i>ROS1</i>	n=830 k=6	0.86 (0.63, 0.96)	1.00 (0.99, 1.00)	1.61% ^c	14	16
<i>MET</i> ex14sk	n=99 k=1	0.98 (0.89, 1.00)	1.00 (0.93, 1.00)	3.6% ^d	35	36
Total					259 (25.9%)	232 (23.2%)

^aBased on p18 MSAC 1161 PSD, November 2012

^bBased on p5 MSAC 1250.1 PSD, November 2014

^cBased on p12 MSAC 1254 PSD, July 2018

^dBased on Table 11, p27 Tepotinib PBAC PSD, November 2021

ALK = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; *MET* = mesenchymal-epithelial transition; NGS = next generation sequencing; NPA = negative percent agreement; PPA = positive percent agreement; *ROS1* = ROS proto-oncogene 1; SG = single-gene testing

Proportion of samples with concurrent variants detected

One of the claims made in the application was that an advantage of NGS is the ability to detect concurrent variants. Nine cohort studies were identified that reported on the proportion of samples with concurrent variants detected by NGS panels. However, the clinical implications of the identification of concurrent variants are unclear. These data are shown in Appendix D.

Turnaround time

Three studies compared the turnaround time for NGS versus a sequential single-gene testing strategy in patients with NSCLC (Table 33), with eight studies reporting mean or median turnaround time for individual types of testing (Table 87). Although NGS took more time than individual single-gene tests (see Appendix D), when the entire strategy was taken into account (i.e. on a per person analysis rather than per gene), NGS was as fast or marginally faster at providing test results. Dall'Olio et al. (2020) used the OncoPrint Focus Assay which analyses both DNA and RNA at the same time (Dall'Olio et al. 2020). Conversely, the panel used by Li et al. (2021) (Li, W, Li, Y, et al. 2021) appears to have only used DNA, so is less relevant to the proposed panel(s) for use in Australia, while Steeghs et al. (2022) did not provide details of whether the RNA testing was incorporated into the same panel as the DNA testing or tested separately (Steeghs et al. 2022).

None of the studies explicitly reflected the scenario of a small DNA NGS panel followed by reflex testing of a small RNA NGS panel if the DNA panel was negative. It is expected that a combined DNA/RNA panel would have a longer turnaround time than a DNA-only panel, but a shorter turnaround time than a DNA panel, followed by an RNA panel if no pathogenic variants are identified on the DNA panel. It is therefore uncertain what the difference in turnaround time is likely to be in Australia, with the expected mix of DNA/RNA or DNA ± RNA panels. The certainty of the evidence is rated as very low (GRADE ⊕⊖⊖⊖).

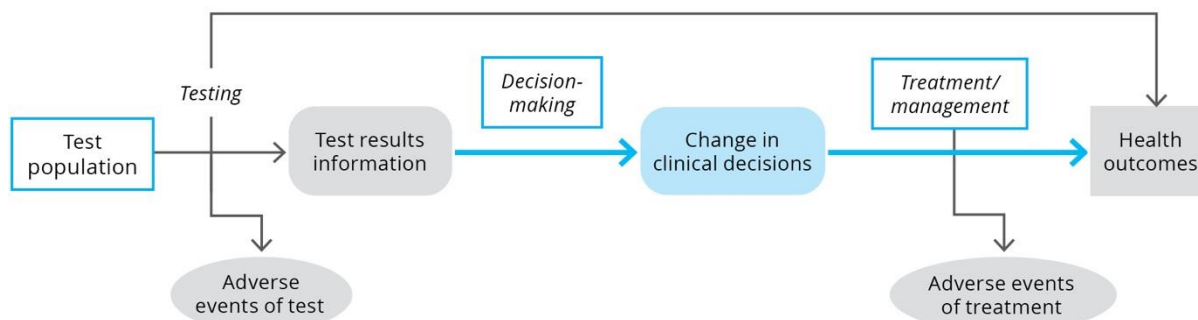
Table 33 Turnaround time for NGS vs sequential single-gene testing strategy

Study	Population	Intervention (NGS)	Comparator (SG)	Turnaround time for NGS	Turnaround time for comparator	Difference
(Dall'Olio et al. 2020)	N=537 Consecutive NSCLC (adenocarcinoma) patients Histology and cytology samples	OncoPrint Focus Assay on DNA and RNA	Single gene (EGFR, KRAS, BRAF, MET or HER2), IHC and FISH	Mean 10 working days	Mean 13.15 days	-3.15 days
(Li, W, Li, Y, et al. 2021)	884 newly diagnosed, treatment-naïve metastatic NSCLC patients with limited tissue sample FFPE samples from core biopsy, fine-needle aspiration, bronchoscopic	NGS on DNA only	ARMS-PCR and IHC/FISH	Median 12 business days (range 5 - 79 days)	Median 13 business days (range 9 – 86)	-1 day

Study	Population	Intervention (NGS)	Comparator (SG)	Turnaround time for NGS	Turnaround time for comparator	Difference
	biopsy, pleural effusion (cytology specimen), and excisional biopsy					
(Steeeghs et al. 2022)	Stage IV NSCLC patients. 3343 NGS patients, 698 non-NGS patients	NGS on DNA, plus fusions tested by IHC, FISH or RNA NGS	Various non-NGS single gene testing such as ICH and FISH used throughout clinical practice in the Netherlands	Median 10 days (range 0 - 495; IQR 7 - 14)	Median 10 days (range 2 - 63; IQR 7 - 13)	0 days

ARMS-PCR = amplification-refractory mutation system polymerase chain reaction; DNA = deoxyribonucleic acid; EGFR = epidermal growth factor receptor; FISH = fluorescent *in situ* hybridisation; IHC = immunohistochemistry; KRAS = Kirsten rat sarcoma; NGS = next generation sequencing; NSCLC = non-small cell lung cancer; RNA = ribonucleic acid; SG = single-gene testing

2.3 Linked evidence of change in management



2.3.1 Methods for undertaking the assessment

The overarching methods for the systematic review have been summarised in Section 2. The outcomes sought to assess whether the use of NGS would result in differences in the way patients are managed, as compared to sequential single-gene testing were:

- Any changes in management between small DNA/RNA panel(s) and sequential testing (e.g. rebiopsy rate, timing of treatment initiation, different treatments received due to false positives (FP) and false negatives (FN))

2.3.2 Characteristics of the evidence base

A total of 9 studies met the inclusion criteria for assessing change in management following small NGS panel testing. Full study profiles and a PRISMA flowchart are shown in Appendix B.

A summary of the key features of the change in management evidence is provided in Table 34.

Table 34 Key features of the included change in management evidence comparing NGS with sequential single-gene testing

Study	N	Study design Risk of bias	Population	Intervention	Comparator	Key outcome(s)	Result used in economic model
(Ali et al. 2016) United States	11	Retrospective cohort study Level III-2 Moderate risk of bias (QUADAS 2)	11/41 Patients with <i>ALK</i> rearrangements whose results were discordant between NGS and FISH	Hybrid-capture based CGP using NGS, 236 cancer related genes, plus 47 introns from 19 genes frequently rearranged in cancer	<i>ALK</i> FISH testing	Treatment received in <i>ALK</i> discordant cases	No
(de Biase et al. 2013) Italy	14	Cohort study Level III-2 Low risk of bias (QUADAS 2)	14/80 NSCLC samples randomly selected from patients underwent diagnostic workup	NGS, targeting <i>EGFR</i> exon 18-21, using a 454 GS-Junior Next Generation sequencer (Roche Diagnostics)	Sanger sequencing carried out using the GenomeLab DTCS Kit (Beckman Coulter, U.S.A.) and a CEQ2000 XL automatic DNA sequencer (Beckman Coulter) and the BigDye Terminator kit (version 3.1; Life Technologies)	Treatment received in <i>EGFR</i> discordant cases	No
(DiBardino et al. 2016) United States	49	Retrospective cohort study Level III-2 Low to moderate risk of bias (QUADAS 2)	N=49 NSCLC patient case series, 30 (61%) metastatic	Hybridisation capture and sequencing of exons of 236 genes and 19 rearrangement	Single gene assay for <i>EGFR</i> (n=25), FISH for <i>ALK</i> (n=20)	Management changes due to NGS (<i>EGFR</i> , <i>ALK</i> and <i>ROS1</i>)	No
(Gutierrez et al. 2017) United States	53	Retrospective cohort study Level III-2 Low risk of bias (SIGN for cohorts)	N=53/814 Patients with non-squamous NSCLC in 2013 to 2015, who had insufficient tissue for testing <i>EGFR/ALK</i>	Full panel NGS testing for 7 genes	-	Rate of rebiopsy	No
(Hamblin et al. 2017) United Kingdom	4	Prospective cohort Level III-2 Low risk of bias (SIGN for cohorts)	N=4/108 NSCLC patients who treating clinicians thought might benefit from more extensive genetic analysis, who had discordant results between NGS and cobas assays	NGS using 46-gene Ion AmpliSeq Cancer Hotspot Panel (Thermo Fisher Scientific)	Roche cobas <i>EGFR/KRAS/BRAF</i> (for NSCLC samples)	Treatment received in discordant cases	No

Study	N	Study design Risk of bias	Population	Intervention	Comparator	Key outcome(s)	Result used in economic model
(Li, W, Li, Y, et al. 2021) China	172	Cohort study (retrospective or prospective unknown) Level III-2 Low to moderate risk of bias (QUADAS 2)	N=172/1,392 newly diagnose, treatment-naïve metastatic NSCLC patients with limited tissue sample, who had insufficient tissue for NGS	Tissue NGS panel designed against 56 cancer-related genes (Burning Rock Biotech, China), sequenced on the NextSeq N500 platform (Illumina)	ARMS-PCR for <i>EGFR/KRAS/BRAF</i> , IHC for <i>ALK</i> FISH: <i>ROS1</i> and <i>RET</i>	Rate of rebiopsy	No
(Robert et al. 2022) United States	3474	Retrospective cohort study Level III-2 Low risk of bias	N=3474 patients with mNSCLC that initiated 1 st line treatment and had not received diagnosis or treatment for another cancer	Time from testing order to testing result for NGS	Time from testing order to testing result for single biomarker testing of <i>EGFR</i> , <i>ALK</i> , <i>ROS1</i> , <i>BRAF</i> , and PD-L1	Timing of treatment initiation	No
(Sakaguchi et al. 2021) Japan	4	Retrospective cohort study Level III-3 Moderate risk of bias (QUADAS 2)	N=4/116 NSCLC samples that underwent NGS and conventional screening for <i>EGFR</i> mutations simultaneously and had discordant results	Oncomine Dx target test	PNA-LNA PCR (Rt-qPCR) clamp test for <i>EGFR</i> mutations.	Treatment received in <i>EGFR</i> discordant cases	No
(Schrock et al. 2016) United States and Israel	17	Case series Level IV Moderate to high risk of bias (NHLBI for case series)	N=17/400 consecutive NSCLC cases with <i>EGFR</i> exon 19 deletions identified by CGP but negative on single gene testing	Hybrid capture-based CGP using NGS	Non-hybrid based capture testing	Treatment received in <i>EGFR</i> discordant cases	No

ALK = anaplastic lymphoma kinase; ARMS-PCR = amplification-refractory mutation system polymerase chain reaction; *BRAF* = proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B; CGP = comprehensive genomic profiling; DNA = deoxyribonucleic acid; *EGFR* = epidermal growth factor receptor; FFPE = formalin-fixed paraffin embedded; FISH = fluorescent *in situ* hybridisation; IHC = immunohistochemistry; *KRAS* = Kirsten rat sarcoma; *MET* = mesenchymal-epithelial transition; NGS = next generation sequencing; NSCLC = non-small cell lung cancer; PCR = polymerase chain reaction; PFS = progression free survival; PNA-LNA = peptide nucleic acid, locked nucleic acid polymerase chain reaction; *RET* = rearranged during transfection; RNA = ribonucleic acid; *ROS1* = ROS proto-oncogene 1; RT-PCR = real time polymerase chain reaction

2.3.3 Results

Rate of rebiopsy

The rate of rebiopsy is an important safety outcome for patients undergoing testing. Although the rate of test failure may be a proxy for the rate of rebiopsy, the use of liquid biopsy is becoming more common, so studies were specifically sought to determine the rate of tissue rebiopsy.

Two case series reported on the rate of repeat biopsies performed (due to insufficient tissue for NGS, or for unspecified testing). In those with insufficient tissue for NGS, repeat biopsies were only performed in 13% to 43% of cases. The remaining cases were either tested using liquid biopsy or not biomarker tested. Li et al. (2021) reported that in cases where NGS testing had been attempted but failed due to insufficient DNA (n=71), failed library (n=3) or low-quality sequences (n=7), ARMS-PCR and IHC/FISH testing were performed, rather than repeat biopsy or liquid biopsy for a second attempt at NGS (Li, W, Li, Y, et al. 2021). No comparative information was provided, regarding whether the use of a small NGS panel(s) (DNA/RNA or DNA±RNA) would result in fewer rebiopsies than using sequential single-gene testing (Table 35).

It is unlikely that the low rate of tissue biopsy would be applicable to the Australian setting, as the current restrictions for access to PBS-listed targeted drugs requires the biomarkers to be identified on tumour tissue/cytology rather than blood. (GRADE ⊕⊖⊖⊖).

Table 35 Rate of rebiopsies in patients with insufficient tumour tissue available from initial sample

Study	Patients with insufficient tissue for testing (from initial sample)	Rebiopsy performed
(Li, W, Li, Y, et al. 2021) China	172/1184 insufficient tissue for NGS	23/173 (13.3%) repeat biopsy for tissue NGS 143/173 (82.7%) plasma NGS
(Gutierrez et al. 2017) United States	53/814 insufficient tissue for testing <i>EGFR/ALK</i> (not explicit by what method)	23/53 (43.4%) repeat biopsy 30/53 (56.6%) not tested

ALK = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; NGS = next generation sequencing

Change in treatment

In patients with sufficient tissue to test either sequential single gene testing or NGS, and in whom the two different testing methods provide concordant results, the treatment received is expected to be the same. From section 2.2.4, concordance data (adjusted with Australian prevalence data) suggested that for 1000 patients successfully tested, 733 patients would be found to be wildtype on *EGFR*, *ALK*, *ROS1* and *METex14sk* on both methods (i.e., would receive standard of care, immunotherapy and/or chemotherapy, regardless of the proposed introduction of NGS). Similarly, for every 1000 patients successfully tested, 224 patients would be found to have variants on *EGFR*, *ALK*, *ROS1* or *METex14sk* by both methods, so would be eligible for targeted drugs (assuming other criterion are met).

This section therefore focuses on the management of the 4.3% of patients with results expected to be discordant between single-gene testing and NGS. Six before-and-after case series reported on this outcome. In cases where NGS detected a variant which had been missed by single-gene testing, targeted treatment was prescribed in between 17.6% and 100% of cases (Table 36). The studies did not specify what criteria were used to determine whether particular variants were deemed 'actionable' or not (other than the presence of a targeted treatment available for variants in that particular gene). The studies are too small and heterogeneous to derive any

reliable estimates on the proportion of patients whose management is changed due to NGS. One study also reported on two cases whose management was based on peptide nucleic acid-locked nucleic acid (PNA-LNA) PCR clamp and would have missed access to a targeted drug if based on NGS alone (Sakaguchi et al. 2021). The certainty of evidence was rated as very low, due to the small number of studies and the very small numbers of discordant cases (where it can be assumed that NGS altered management compared to single-gene testing alone), with a large amount of heterogeneity low (GRADE ⊕⊖⊖⊖).

Table 36 Studies which mention treatment in discordant cases between NGS and single-gene testing

Study	Population	Intervention	Prior testing	Relevant Biomarker	Change in management
(Ali et al. 2016)	11/47 patients with NSCLC and <i>ALK</i> rearrangements, detected on NGS but not on FISH	NGS	FISH (those positive had 20 – 100% cells stained)	<i>ALK</i>	9/11 (81.8% of discordant) <i>ALK</i> + NGS/ <i>ALK</i> - FISH received crizotinib based on NGS
(de Biase et al. 2013)	14/80 patients with NSCLC with discordant results between NGS and Sanger sequencing	NGS (454 GS-Junior platform)	Sanger Sequencing	<i>EGFR</i>	14 additional cases with <i>EGFR</i> variants identified (4 additional cases of exon 19 deletions, 2 additional cases L858R, individual cases of F795S, V845M, P691T, K708N, G721W + R831H, S752F + T785I, D807G, P772S), 6/14 discordant cases treated with TKIs (42.9% of discordant) 1 case with resistance variant identified by NGS but not Sanger Sequencing (R831H) ruled out use of TKI
(DiBardino et al. 2016)	49 patients with NSCLC where extended genetic testing was specifically requested (due to having stage IV cancer, negative or mixed results on single-gene studies, non-smokers, progression of disease during chemotherapy, before enrolment in a trial, or disease recurrence)	NGS (Comprehensive genomic profiling, Foundation Medicine)	Routine testing (details not explicit)	<i>EGFR</i> , <i>ALK</i> , <i>ROS1</i> , <i>METex14</i> (and other not reported here)	5/49 had management change due to NGS 1 case given erlotinib due to <i>EGFR</i> L858R variant 2 cases discontinued erlotinib (<i>EGFR</i> E709_T710>D variant; and <i>EGFR</i> wildtype) 1 case started crizotinib (<i>METex14</i> splice site) 1 case started ceritinib for <i>ROS1</i> variant (unclear what % of discordant) Potential change in management: 1 (<i>EML4-ALK</i> fusion detected) (unclear why patient did not receive available treatment)
(Hamblin et al. 2017)	4/351 patients with NSCLC which clinicians thought would benefit from more extensive analysis, with discordant results	Ion AmpliSeq Targeted hotspot NGS cancer panel (46 genes, 189 amplicons)	Single gene testing (Cobas assay)	<i>EGFR</i>	Additional 4 cases with <i>EGFR</i> variants identified (outside the scope of cobas assay; M600T, S720C, V742I and L861Q) and received erlotinib (100% of discordant).

Study	Population	Intervention	Prior testing	Relevant Biomarker	Change in management
(Sakaguchi et al. 2021)	4/116 samples from consecutive NSCLC patients with discordant results	Oncomine Dx Target Test	PNA-LNA PCR clamp test	<i>EGFR</i>	4 discordant cases 3 exon 19 deletion detected by PNA-LNA PCA clamp but not by Oncomine Dx Target Test. 2 received osimertinib (and would not have, based on NGS)(50% of discordant). 2 had early-stage disease and underwent surgery (no change in management)
(Schrock et al. 2016)	17/77 patients with <i>EGFR</i> variants found by NGS and had previous <i>EGFR</i> test results which were discordant	Hybrid capture-based NGS	Non hybrid capture-based assay	<i>EGFR</i>	17 "false negatives" by prior testing (S752_I759del; T750_L759>NLD; T751_I759>N; 2 x T751_L760>NL; 9 x E746_A750del; 2 x L747_A750>L; L747_K754>G) 3 patients prescribed ^a <i>EGFR</i> TKI (2 afatinib, 1 erlotinib) (17.6% of discordant)

^aOne patient passed away before beginning treatment with afatinib

ALK = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; FISH = fluorescent *in situ* hybridisation; *MET* = mesenchymal-epithelial transition; NGS = next generation sequencing; NSCLC = non-small cell lung cancer; PNA-LNA PCR = peptide nucleic acid-locked nucleic acid polymerase chain reaction; *ROS1* = ROS proto-oncogene 1; TKI = tyrosine kinase inhibitor (therapy)

Timing of treatment initiation

Only one study was identified comparing the time to treatment for patients who were tested with NGS, versus being tested with other methods for biomarkers in NSCLC.

Robert et al. (2022) reported on the timing of ordering testing, testing times, and treatment in patients with metastatic NSCLC, who initiated first-line systemic therapy for metastatic disease between April 2018 and March 2020 (Robert et al. 2022). These data were from the Molecularly Informed Lung Cancer Treatment (MYLUNG) study from the United States. The methods used for testing individual biomarkers were not explicit; the article stated it included any method of testing (e.g., both IHC and ISH), although it is unclear whether NGS results were also included in the single-biomarker results. The median time from diagnosis to first-line treatment was very similar, regardless of whether single gene testing was performed, or the use of NGS (medians of 36 to 38 days).

Although the authors were able to determine the length of time between diagnosis, testing, and treatment, they were unable to determine the extent to which institutional processes had an influence (such as policies regarding reflex testing). The certainty of the evidence was rated as being very low, due to the lack of clarity regarding the applicability of the intervention and comparators, and the influence which the institutional policies would have on the applicability of the data to the Australian setting (GRADE ⊕⊖⊖⊖).

Table 37 Data from Robert et al. (2022) on time from diagnosis to testing, results, and 1L treatment initiation for single-gene testing, or for NGS (overall)

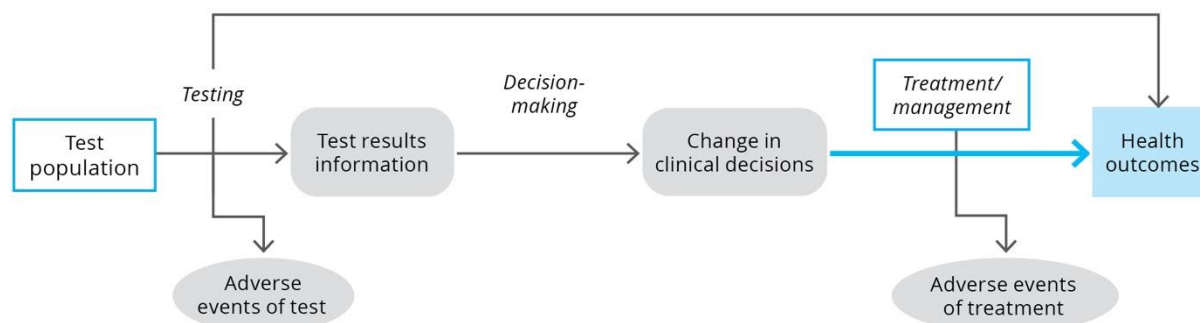
Biomarker	N	Time from diagnosis to testing order (median days, (IQR))	N	Time from diagnosis to 1L treatment (median days, (IQR))	N	Time from testing order to testing result (median days, (IQR))	N	Time from testing result to 1L treatment initiation (median days (IQR))
<i>EGFR</i>	2425	2 (1, 14)	2443	36 (23, 57)	2398	15 (10, 22)	2412	15 (4, 35)
<i>ALK</i>	2431	2 (1, 13)	2446	37 (23, 57)	2401	14 (9, 21)	2411	16 (5, 35)
<i>ROS1</i>	2331	3 (1, 14)	2348	37 (23, 57)	2298	14 (9, 22)	2309	16 (4, 34)
NGS	1276	6 (1, 19)	1288	38 (23, 57)	1268	18 (13, 27)	1278	10 (-5, 25)

1L = first-line; *ALK* = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; IQR = inter quartile range; NGS = next generation sequencing; *ROS1* = ROS proto-oncogene 1

These data, together with the test turnaround time (reported in Section 2.2), suggest that the turnaround time, and time to treatment initiation are similar between sequential single-gene testing and NGS.

The turnaround time and time to treatment initiation are likely to be influenced by institutional processes including reflex testing, and availability of combined DNA + RNA panels, versus DNA then RNA testing. It is highly uncertain how applicable these data from the United States are to the Australian Healthcare setting.

2.4 Linked evidence of health outcomes



Studies on the test performance of NGS versus single-gene testing, demonstrated that:

- NGS is more sensitive than single-gene testing at detecting variants which are potentially targetable, particularly in the *EGFR* gene.
 - A small volume of ‘change in management’ evidence suggested that some patients with uncommon variants are treated with *EGFR* TKIs.
 - A small volume of ‘change in management evidence suggested that patients identified as *ALK* positive by NGS but not by FISH do receive targeted treatments.
 - The effectiveness of targeted treatment in the incrementally diagnosed patients (those with uncommon variants in the *EGFR* gene, low variant

allele frequency, or patients with *ALK* or *ROS1* variants which were not detected by IHC/FISH) was therefore investigated.

- NGS also has a slightly lower failure rate.
 - This may lead to lower rate of rebiopsy in than single-gene testing (nb: International guidelines now recommend use of ctDNA instead of rebiopsy).
 - The safety of rebiopsies was therefore examined.
 - If rebiopsies are not performed, or are not successful, the patient may miss out on targeted treatment they could potentially have been eligible for if they had been tested.
- Results from NGS *may be* returned slightly faster than the average turnaround time for the complete testing strategy of sequential single-gene testing.
 - If so, this should lead to faster initiation of treatment.
 - The health impact of differences in the time between diagnosis and treatment for patients with NSCLC was therefore investigated.

2.4.1 Methods for undertaking the assessment

The evidence for the last step of the linked evidence approach was derived in two ways:

- Through the systematic review (for articles which included the use of NGS); and
- By targeted searches of PubMed for recent systematic reviews on NSCLC and health outcomes related to time to treatment, treatment outcomes in patients with uncommon variants, and safety of rebiopsies.

The systematic reviews were appraised using the AMSTAR 2 tool. The risk of bias from individual studies was not assessed.

2.4.2 Characteristics of the evidence base

A total of 9 studies and 3 systematic reviews met the inclusion criteria for assessing evidence on health outcomes resulting from change in management due to a small DNA/RNA NGS panel, or DNA ± RNA NGS panel compared to sequential single gene testing. Full study profiles are presented in Appendix B.

A summary of the key features of the studies providing health outcome evidence is provided in Table 38.

These studies assess the health impact of NGS (due to detection of lower allelic frequencies or rare variants or discordant results, or due to lower rate of rebiopsy, or shorter time to treatment)

Table 38 Key features of the included health outcomes evidence for assessing changes in management due to NGS

Study/SR	N	Study design Risk of bias	Population	Intervention	Comparator	Key outcome(s)	Result used in economic model
(Ali et al. 2016) United States	n=9	Retrospective cohort study Level III-2 Moderate risk of bias (QUADAS 2)	31 patients with NSCLC who were <i>ALK+</i> on NGS (and had FISH results available)	Crizotinib	NA	Response to targeted treatment	No
(Friedlaender et al. 2021) Switzerland	n=42	Cohort study Level III-2 Low risk of bias (SIGN for cohort studies)	Patients with NSCLC and <i>EGFR</i> variants Threshold for high vs low allelic frequency: 0.30	<i>EGFR</i> TKI in those with high allelic frequency	<i>EGFR</i> TKI in those with low allelic frequency	PFS, OS in high vs low allelic frequency	No
(Gieszer et al. 2021) Hungary	n=89	Cohort study Level III-2 Low risk of bias (SIGN for cohort studies)	Caucasian patients with NSCLC (adenocarcinomas), and <i>EGFR</i> variants Adjusted VAF (aVAF) = VAF/TC% x 100	<i>EGFR</i> TKI in those with high allelic frequency	<i>EGFR</i> TKI in those with low allelic frequency	PFS in high vs low allelic frequency	No
(Hall et al. 2021)	k=8 n=not stated	Systematic review of cohort studies Level III-2 AMSTAR 2: moderate risk of bias	Studies with patients with advanced NSCLC	Timely treatment	Non-timely treatment	Health outcomes (not specified)	No
(John et al. 2022)	k=13 n=2684	Systematic review of randomised and non-randomised studies Level III-2 AMSTAR 2: moderate risk of bias	Studies with over 100 patients with NSCLC and common and uncommon <i>EGFR</i> variants (excluding T790M)	<i>EGFR</i> -TKI (gefitinib, erlotinib, icotinib, afatinib, osimertinib, or a mix of different <i>EGFR</i> -TKIs)	NA	Response to <i>EGFR</i> -TKIs	No
(Kim et al. 2021)	n=247	Case series Level IV Low to moderate risk of bias (NHLBI for case series)	Patients with NSCLC (adenocarcinoma) and at least one actionable variant (<i>EGFR</i> , n=130, <i>KRAS</i> , n=48, <i>ALK</i> n=40, <i>RET</i> 6%, <i>MET</i> 3%, <i>ROS1</i> 3%, <i>BRAF</i> 2%)	Treatment with targeted therapy corresponding to actionable variant	No targeted treatment	Overall survival on targeted treatment vs no-targeted treatment	No

Study/SR	N	Study design Risk of bias	Population	Intervention	Comparator	Key outcome(s)	Result used in economic model
(Nam et al. 2021)	k=16 n=1846	Systematic review of randomised and non-randomised studies AMSTAR 2: moderate risk of bias	Patients with NSCLC who underwent PTNB biopsy (for 1L therapy) or rebiopsy (after chemo or targeted therapy)	Biopsy for the purposes of molecular analysis	NA	Complications from biopsy	No
(Sakaguchi et al. 2021) Japan	n=4	Retrospective cohort study Level III-3 Moderate risk of bias (QUADAS 2)	Patients with NSCLC and discordant results from Oncomine Dx Target Test and PNA-LNA clamp test from consecutive NSCLC patients	Osimertinib	NA	Disease progression while on targeted treatment	No
(Schrock et al. 2016) United States and Israel	n=12	Case series Level IV Moderate to high risk of bias (NHLBI for case series)	Patients with <i>EGFR</i> variants found by Hybrid-capture based NGS and missed by previous <i>EGFR</i> test (non-hybrid capture based assay)	Afatinib, erlotinib	NA	Response to targeted treatment	No
(Vollbrecht et al. 2018) Germany	n=4	Diagnostic case control study Level III-3 Moderate risk of bias (QUADAS 2)	15 patients with NSCLC and equivocal <i>ALK</i> results on IHC and FISH	<i>ALK</i> TKI	NA	Response to targeted treatment	No
(Ye et al. 2020) Australia	n=64	Cohort study Level III-2 Moderate risk of bias (SIGN for cohort studies)	Patients with NSCLC and <i>EGFR</i> variants, with stage IV disease 14 VAF <0.1% 28 VAF ≥ 0.1% 1 detectable by SS, VAF = 28.5%	Erlotinib or gefitinib in those with high allelic frequency	Erlotinib or gefitinib in those with low allelic frequency	Disease progression in high vs low allelic frequency	No
(Zeng et al. 2018) China	n=22	Case series Level IV Low risk of bias (NHLBI for case series)	Patients with NSCLC and <i>ROS1</i> rearrangements	Crizotinib as first- second- or third-line treatment	NA	Response to targeted treatment	No

ALK = anaplastic lymphoma kinase; *BRAF* = proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B; *EGFR* = epidermal growth factor receptor; FISH = fluorescent *in situ* hybridisation; IHC = immunohistochemistry; *KRAS* = Kirsten rat sarcoma; NA = not applicable (no comparator details, as case series only); NGS = next generation sequencing; NSCLC = non-small cell lung cancer; PTNB = percutaneous transthoracic needle biopsies; *RET* = rearranged during transfection; *ROS1* = ROS proto-oncogene 1; VAF = variant allelic frequency

2.4.3 Results

Safety of rebiopsy

If patients have insufficient tissue to have all their relevant biomarkers tested, then they may be rebiopsied. A systematic review on the safety of percutaneous transthoracic needle biopsies (PTNB) was identified (Nam et al. 2021). One of the main indications for PTNB in NSCLC is for the purposes of obtaining tumour tissue for molecular analysis to identify targetable variants. Two subgroups were included: those undergoing initial biopsy, and those undergoing rebiopsy after chemotherapy or targeted therapy. No mention was made of rebiopsy due to insufficient tissue from the initial biopsy. A total of 16 studies were included, reporting on the complication rate of PTNB. The overall rate was 17% (95%CI 12%, 23%), with no statistically significant difference between subgroups. The most common complication was pneumothorax (collapsed lung), with a pooled incidence of 9.2% (95%CI 4.0%, 15.7%). Six studies reported haemoptysis (coughing up blood from lungs), with rates varying between 0.5% and 21%. Major adverse events occurred in less than 1% of patients. The authors of the meta-analysis concluded that the use of PTNB is effective and safe.

The observational nature of the studies, and large heterogeneity mean that the certainty of the evidence is rated as low (GRADE ⊕⊕⊖⊖).

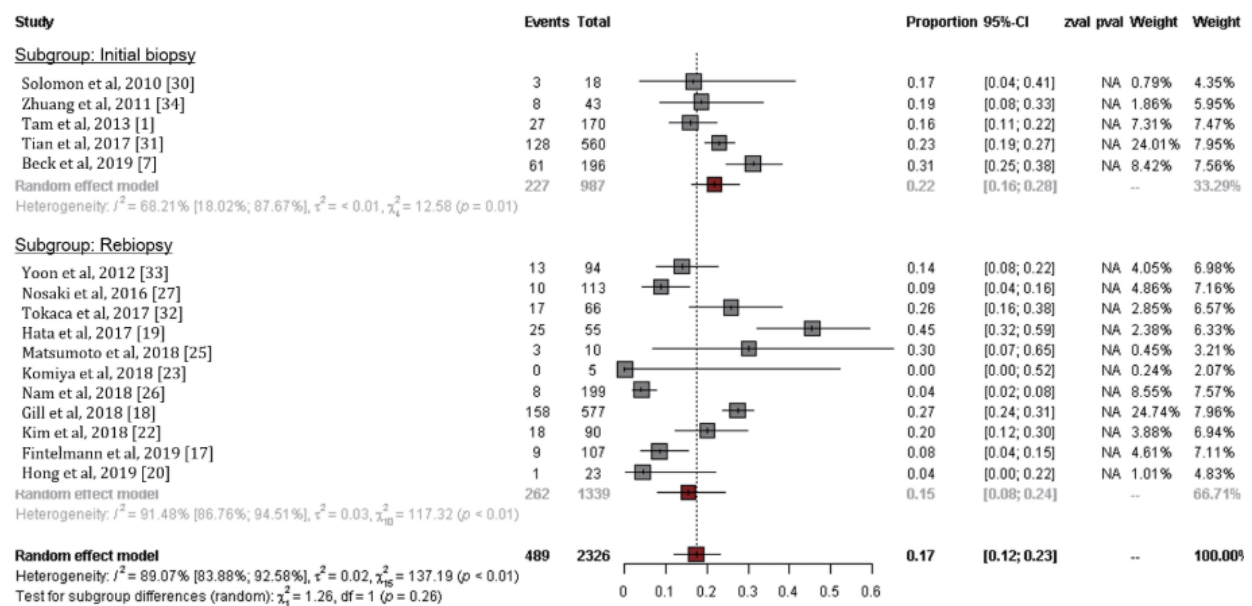


Figure 10 Forest plot of complication rates of percutaneous transthoracic needle biopsy for molecular analysis in NSCLC

Source: (Nam et al. 2021), p2089. Reproduced with permission under Creative Commons CC BY-NC 4.0.

Effectiveness of targeted treatments in incrementally diagnosed patients

Targeted therapies vs non-targeted therapies

The superiority of targeted therapies over non-targeted therapies for those with biomarkers has been demonstrated in submissions to the PBS for erlotinib, gefitinib, afatinib, osimertinib, crizotinib, ceritinib, alectinib, brigatinib, lorlatinib, entrectinib, and tepotinib. However, the population identified with biomarkers by the clinical utility standards in the key trials may differ slightly from the population identified by small NGS panels.

Kim et al. (2021) reported on a case series of patients with NSCLC who underwent NGS and did or did not receive targeted treatment. Of 247 patients with metastatic or recurrent disease who had actionable variants identified by NGS, targeted therapy was given to 159 patients (64.4%). It was reported that 88 patients (35.6%) could not receive targeted therapy, although the reasons for this were not provided. In those with actionable variants who received targeted therapy, the median overall survival was significantly longer than those with actionable variants who could not receive targeted therapy (60.5 vs 26.0 months, $p < 0.001$), although it is unknown whether this is due to confounding factors (such as patients with a poor prognosis not being eligible for targeted therapy), or due to the effectiveness of targeted treatments (Kim et al. 2021). However, in a multivariate analysis, adjusting for ECOG score, those with actionable variants treated with targeted therapy still had significantly better survival than those with (HR = 2.58, 95%CI 1.57, 4.25) or without actionable variants (HR = 3.84, 95%CI 2.44, 6.05) who did not receive targeted therapy.

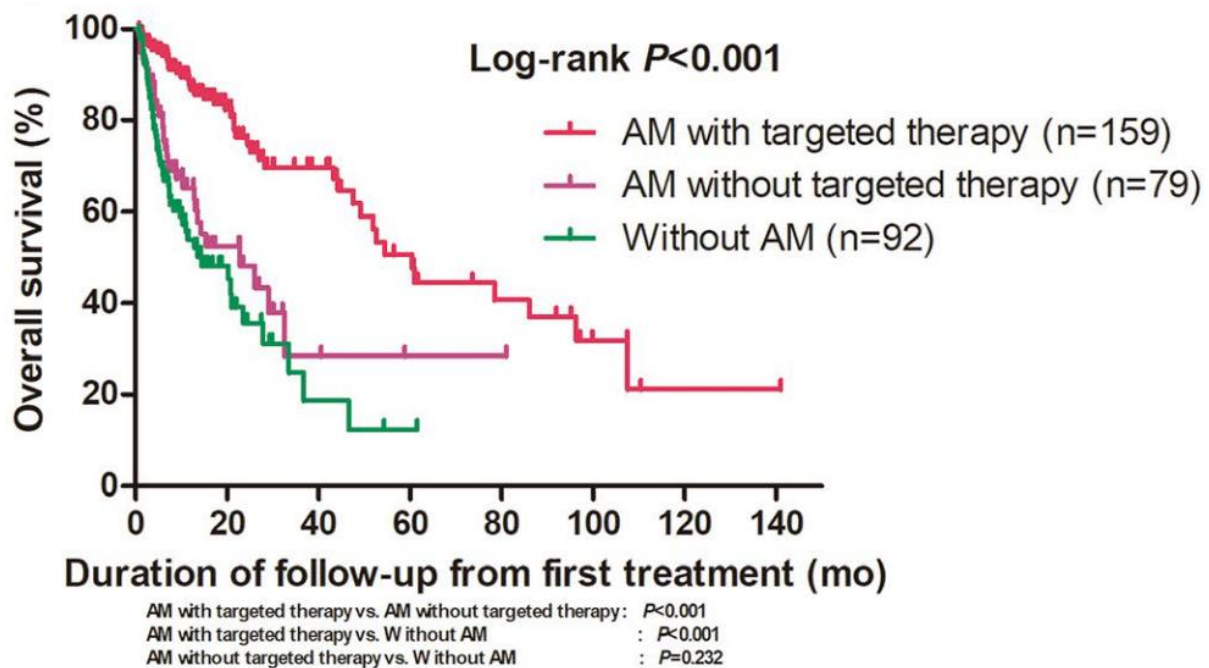


Figure 11 Kaplan-Meier plot showing survival of patients with actionable variants (AM) with and without targeted therapy and survival of patients without actionable variants identified by NGS panel

Source: Figure 3©, p 3202 (Kim et al. 2021). Reproduced under Creative Commons Attribution License
 AM = actionable mutation (variant); mo = months

Treatment of discordant EGFR results

Only two studies identified in the systematic review reported on the health outcomes of patients who had discordant *EGFR* results on NGS and non-NGS testing. The very limited evidence suggested that patients with tumours that were positive on NGS and negative on alternative testing may respond to afatinib or erlotinib, whereas a tumour which was negative on NGS and positive on single-gene testing did not respond to osimertinib. These were essentially a collection of case reports, and too limited to make any conclusions on the effectiveness of *EGFR* TKIs in patients. Further evidence was therefore sought to determine the effectiveness of *EGFR* TKIs in patients with uncommon variants (potentially missed by single-gene tests), and in those with low variant allelic frequency.

Table 39 Studies which mention treatment in discordant cases between NGS and single-gene testing for EGFR

Study	Population	Result subsets	Treatments prescribed	Health outcomes
(Sakaguchi et al. 2021)	4 patients with NSCLC and discordant results from Oncomine Dx Target Test and PNA-LNA clamp test from consecutive NSCLC patients	NGS- / PNA-LNA+	4 discordant cases 2 received osimertinib (and would not have, based on NGS)(50% of discordant). 2 had early stage disease and underwent surgery (no change in management)	1/2 had progression early after administration of EGFR-TKI 1/2 could not have their response assessed due to adverse event of pneumonitis.
(Schrock et al. 2016)	12 patients with <i>EGFR</i> non-variants found by Hybrid-capture based NGS and missed by previous <i>EGFR</i> test (non hybrid capture based assay)	NGS+ / non-hybrid-based capture test-	3/12 patients prescribed ^a EGFR TKI (2 afatinib, 1 erlotinib)	2/3 patients had partial response to afatinib or erlotinib 1/3 died before beginning treatment with afatinib

NGS = next generation sequencing; NSCLC = non-small cell lung cancer; PNA-LNA PCR = peptide nucleic acid-locked nucleic acid polymerase chain reaction

Treatment of common vs uncommon EGFR variants

A systematic review on the clinical outcomes of *EGFR* TKIs in patients whose locally advanced or metastatic NSCLC tumours had uncommon versus common *EGFR* variants was identified (John et al. 2022). Both clinical trials and observational studies were included, without restriction by geographical location. All variants except ex19del, L858R and T790M were considered uncommon. Overall response rate (ORR) and progression free survival (PFS) were considered the key endpoints, as overall survival would likely be impacted by any treatment received after the *EGFR* – TKI.

Six out of seven studies reported that ORR to first-line *EGFR*-TKIs in patients with rare *EGFR* variants was lower than the ORRs in patients with common *EGFR* variants (Figure 12). The additional patients identified with *EGFR* variants by NGS are therefore not likely to gain the same benefit on average that patients currently eligible for *EGFR* TKIs derive. In particular, it appeared that those with *EGFR* exon 20 variants did not derive much benefit from gefitinib or erlotinib. However, the size of any difference was highly uncertain, as there may have been prognostic differences associated with the different variants, and it is unknown how well these patients respond to standard of care treatment with immunotherapy ± chemotherapy.

Similar to ORR, the PFS in patients receiving first-line *EGFR* – TKIs was lower in those with rare variants than in common variants (although no meta-analysis was conducted). The lack of meta-analysis, and the large heterogeneity meant that the average efficacy of *EGFR* TKIs in patients with rare variants was unable to be determined.

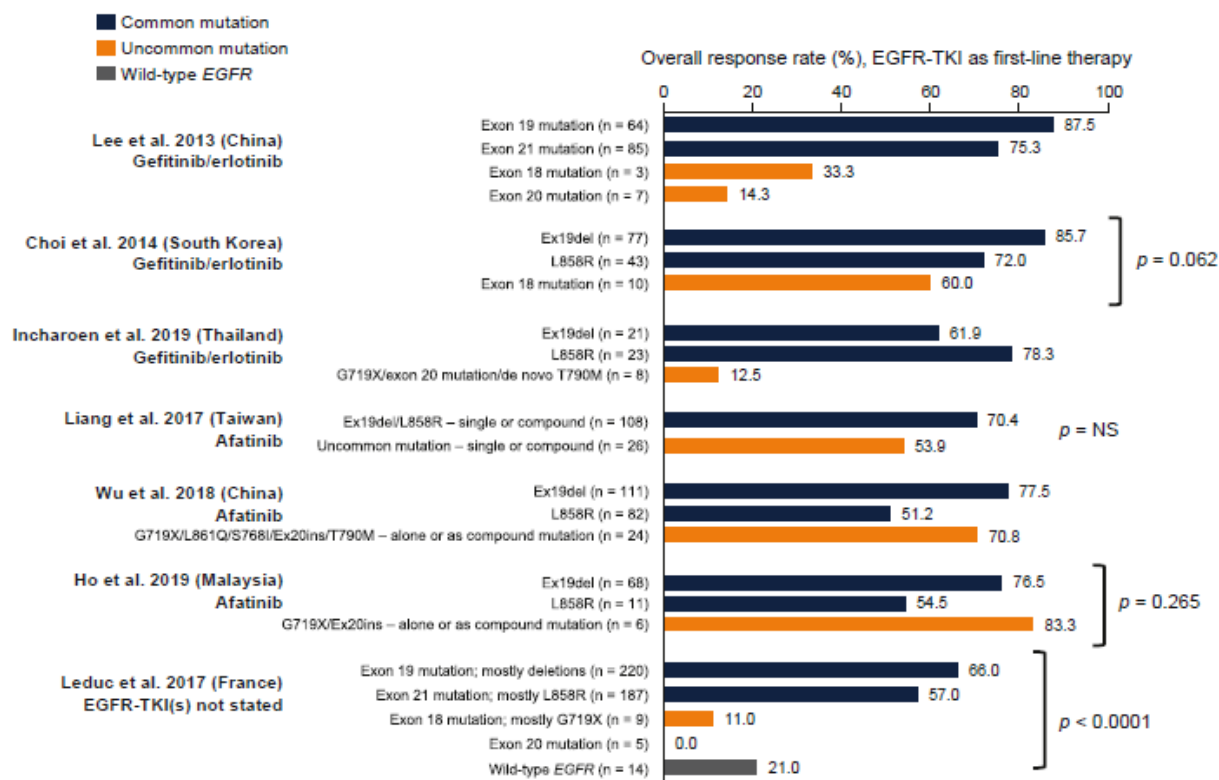


Figure 12 Overall response rate experienced by patients with common or uncommon EGFR variants receiving EGFR-TKI as first-line therapy

NB: p-values denote comparison between common and uncommon variants. Liang et al. “compound mutations” refers to two uncommon variants. Wu et al. (2018): L858R cohort includes four patients with both L858R and Ex19del. Ho et al. (2019): exact uncommon mutations are G719X/G719X + S768I/G719X + T790M/Ex19del + Ex20ins/Ex20ins. Lee et al. (2013) and Leduc et al. (2017): “Exon 20 mutations” do not include T790M.

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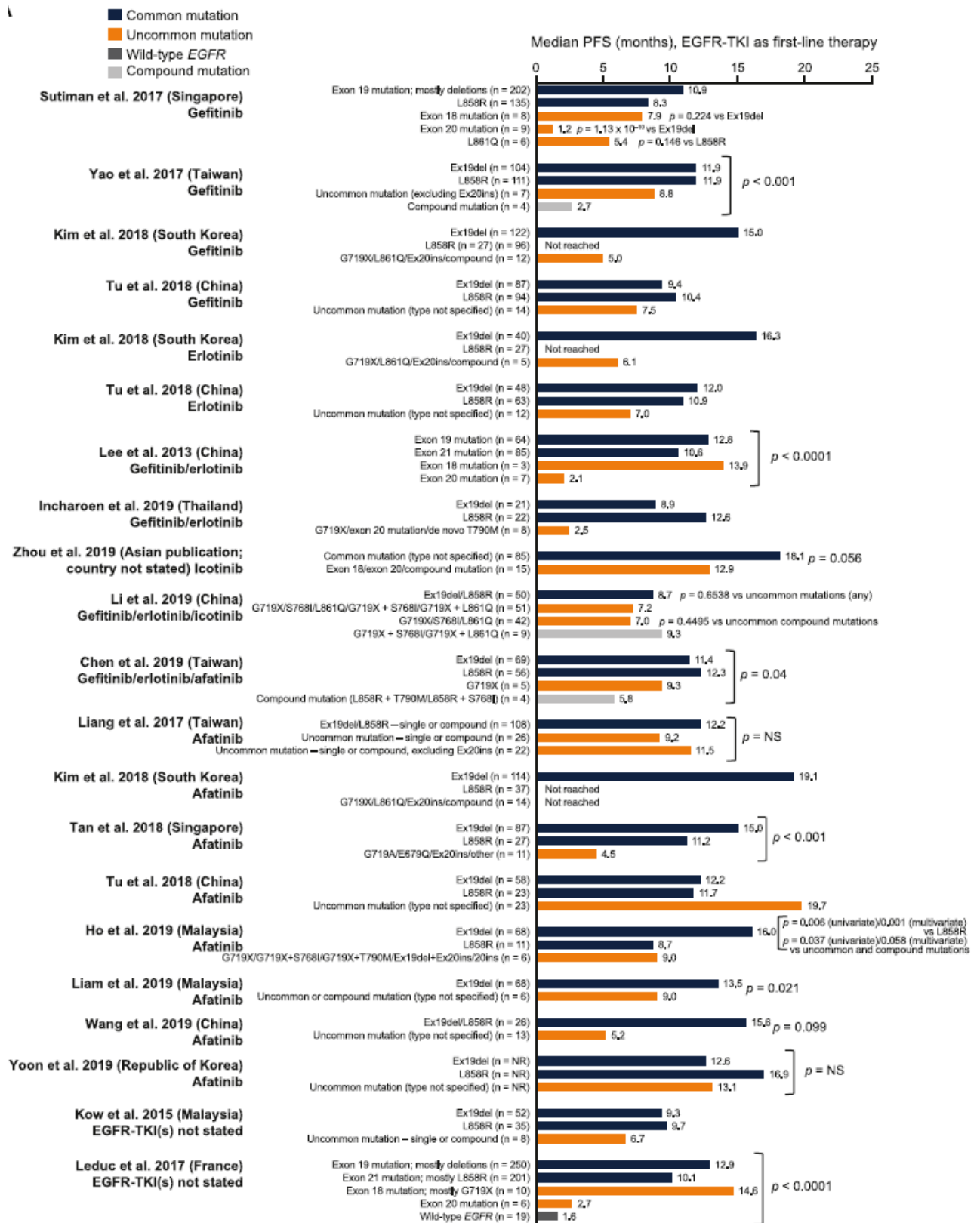


Figure 13 Progression free survival in patients with common or uncommon *EGFR* variants receiving *EGFR*-TKI as first-line therapy

NB: p-values denote comparison between common and uncommon variants.

Liang et al. "compound mutations" refers to two uncommon variants. Wu et al. (2018): L858R cohort includes four patients with both L858R and Ex19del. Ho et al. (2019): exact uncommon mutations are G719X/G719X + S768I/G719X + T790M/Ex19del + Ex20ins/Ex20ins. Lee

et al. (2013) and Leduc et al. (2017): “Exon 20 mutations” do not include T790M.
Source: (John et al. 2022) Reproduced under Creative Commons CC-BY license.

The PBS criteria for *EGFR* TKIs state that patients must have evidence of *EGFR* gene variants known to confer sensitivity to treatments with *EGFR* TKIs in tumour material. The pathogenicity of variants is documented in ClinVar, Catalogue of Somatic Mutations in Cancer (COSMIC) and oncoKB (Friedlaender et al. 2021), and is reported on pathology results provided to clinicians, as is whether the variant is likely to confer TKI sensitivity. Any additional patients receiving *EGFR* TKIs due to having rare variants identified by NGS should therefore only receive the targeted treatment if it is likely to be beneficial. Therefore, although some of the additional variants identified by a NGS panel may not respond to *EGFR* TKIs, clinicians are only likely to prescribe targeted treatment if it is appropriate. The benefit of identifying variants in *EGFR* exon 20 may change in the future if treatments for exon 20 insertions become recommended by the PBAC.

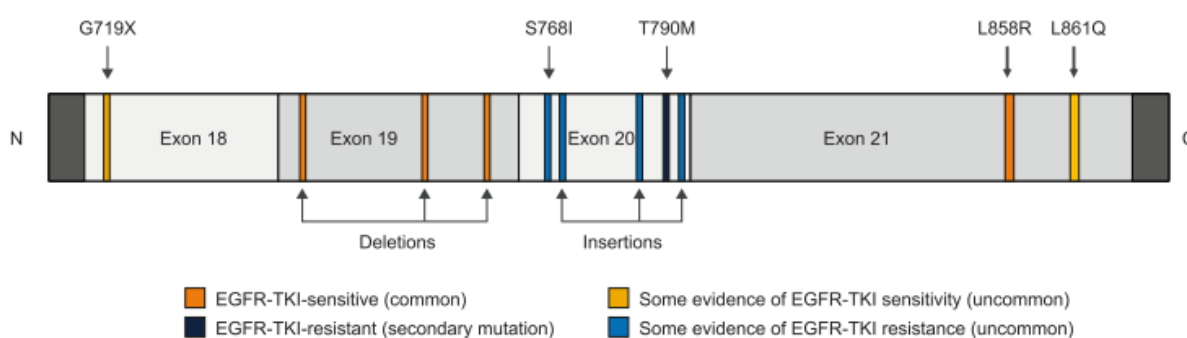


Figure 14 Common and uncommon variants in exons 18-21 of the *EGFR* gene and sensitivity to EGFR-TKIs

Source: (John et al. 2022) Reproduced under Creative Commons CC-BY license.

Treatment of low-allele frequency *EGFR* variants

In addition to NGS identifying rare variants which are outside the scope of some targeted *EGFR* assays, it may also be more sensitive than some other methods of detecting *EGFR* variants. The effectiveness of targeted treatment in those with variants only able to be detected on highly sensitive tests (with a low threshold of detection) was therefore assessed.

No systematic reviews were identified assessing the effectiveness of targeted treatment for *EGFR* variants, in those with low-allele frequency (i.e., those detected by high sensitivity methods, but missed low sensitivity methods). Three individual studies were identified, but these were not identified systematically. However, they were selected based on their topic (comparison of response to *EGFR* TKIs based on variant allelic frequency from tumour tissue), not on their results, so the process of selection is unlikely to have introduced bias.

Two studies reported that the variant allele frequency of *EGFR* sensitising variants had a significant impact on PFS in those treated with *EGFR* TKIs (Friedlaender et al. 2021; Gieszer et al. 2021). It is unknown at what threshold the variant allele frequency may be too low for the tumour to respond to *EGFR* TKIs, or whether the additional patients detected by NGS (NGS positive / single gene negative) are likely to benefit from targeted treatment, as compared to immunotherapy ± chemotherapy.

An Australian study by Ye et al. (2020) reported that a third of their sample of patients with *EGFR* variants had pretreatment T790M variants detected with allele frequency between 0.01% and 0.1%, which is below the limit of detection of standard sequencing methods and some targeted PCR methods (Ye et al. 2020). Those with T790M detected did have a faster rate of progression

while on erlotinib or gefitinib, than those without any T790M variants detected, although response to treatment and overall survival were unaffected. The clinical implications of detecting T790M variants at low frequency is unknown.

Table 40 Association between variant allele frequency and response to treatment

Study	Population	Intervention	Outcome	Results
(Friedlaender et al. 2021) Switzerland	42 patients with NSCLC and <i>EGFR</i> variants Threshold for high vs low allelic frequency: 0.30	NGS using IonAmpliseq Hotspot Panel V2 Treatment with <i>EGFR</i> TKI	PFS	High vs low: HR = 0.27 (95%CI 0.09, 0.79, p=0.017)
			OS	High vs low: HR = 0.47 (95%CI 0.17, 1.30, p=0.14)
(Gieszer et al. 2021) Hungary	89 Caucasian patients with NSCLC (adenocarcinomas), and <i>EGFR</i> variants Adjusted VAF (aVAF) = VAF/TC% x 100	Therascreen <i>EGFR</i> Pyro assay Erlotinib or gefitinib as first- or second-line treatment	PFS	Positive linear correlation between aVAF and PFS: r = 0.319, p=0.003, Spearman's correlation
			PFS	Adjusting for clinicopathological variables (age, gender, variant, treatment, treatment line), HR = 0.991 (95%CI 0.982, 0.999), p = 0.042
			OS	High vs low aVAF median 94 vs 57 weeks, p=0.011
(Ye et al. 2020) Australia	64 patients with NSCLC and <i>EGFR</i> variants, with stage IV disease 14 VAF <0.1% 28 VAF ≥ 0.1% 1 detectable by SS, VAF = 28.5%	Digital PCR Erlotinib or gefitinib	PFS	No significant difference by T790M status (log rank test p = 0.897), or T790M allele frequency (<0.1 vs ≥ 0.1%, p = 0.515)

HR = hazard ratio; NSCLC = non-small cell lung cancer; OS = overall survival; PFS = progression free survival; TC = estimated percentage of neoplastic cells; VAF = variant allele frequency, percentage of *EGFR* variant alleles determined by the assay

ALK

Direct evidence was included in section 2.1 demonstrating in a small study (n=40), that those who tested positive for *ALK* fusions by NGS, had better PFS on average, on crizotinib treatment, than those who tested positive on either IHC or FISH (Lin, C et al. 2019).

In addition to this study, a further two case series were identified which reported on what treatment some of the patients who were discordant on NGS and IHC or FISH for *ALK* variants received, and what their health outcomes were. Ali et al. (2016) reported that those who were found to have *ALK* fusions on NGS, but who were negative on FISH, had a high response rate to crizotinib (77.8%), which is unlikely to be significantly different to those who were found to have *ALK* fusions on both NGS and FISH (response rate 78.9%). For the very small number of additional cases with *ALK* fusions detected by NGS, who may be missed by IHC ± FISH, it is suggested that targeted treatment would be beneficial.

A second case series by Vollbrecht et al. (2018) reported that few clinical data were available. However, they reported four cases with ALK IHC-negative but borderline FISH who were treated with an ALK TKI. They did not receive any benefit from the treatment. It is hypothesised that earlier use of NGS would have avoided the wrong treatment in these cases. However, given their IHC-negative status, these patients would not have been eligible for FISH testing, and would therefore not have received the treatment in the Australian setting.

Table 41 Response to ALK-TKIs in patients incrementally identified by NGS (but not FISH) or vice versa

Study	Population	Comparator and intervention result subsets	Response
(Ali et al. 2016)	31 patients with NSCLC who were ALK+ on NGS (and had FISH results available)	FISH ALK- and NGS ALK+ (n=11)	9 received crizotinib 7/9 (77.8%) confirmed response to crizotinib (6 partial responses, 1 complete response) Median response 17 months (range 5 months to 28 months) 1 nonresponding patient harboured a TSC2 alteration and initially responded but progressed within 1 month 1 nonresponding patient died within 1 week, and deemed "too sick to respond" by physician
		FISH ALK+ and NGS ALK+ (n=20)	15/19 (78.9%) confirmed response to crizotinib 2/19 did not respond to crizotinib 3/19 response not known
(Vollbrecht et al. 2018)	15 patients with NSCLC and equivocal ALK results on IHC and FISH	IHC -, FISH borderline, NGS-	4/5 received ALK-TKI and did not respond

DCR = disease control rate; FISH = fluorescent *in situ* hybridisation; IHC = immunohistochemistry; NGS = next generation sequencing; ORR = overall response rate; PFS = progression free survival

NB: those ALK- on one testing method had to have discordant results and be ALK+ on at least one other method.

ROS1

NGS has high concordance with FISH and RT-PCR for detecting ROS1 fusions. ROS1 fusions also have a low prevalence in Australia. It is therefore unlikely that the use of NGS would result in any substantial increase in the number of patients eligible for ROS1- targeted therapies, or that there would be any change in the spectrum of patients identified. From the studies which provided concordance data, only a single study reported on the management of a discordant case between NGS and FISH. A single patient who was positive on NGS and negative on FISH responded to crizotinib (Mehta et al. 2020).

Zeng et al. (2018) reported on a small case series of patients who were positive for ROS1 rearrangements based on NGS testing and received crizotinib. A total of 1.5% (22/1466) of NSCLC patients had ROS1 rearrangements. Of the 22 patients with ROS1 rearrangements, 19 received crizotinib for first-, second- or third-line treatment. None of the patients had a complete response; 17 patients had partial response, one patient had stable disease, and one patient progressed while on treatment. The overall response rate was 89%, with a median progression-free survival (PFS) of 13.6 months. This is ¹⁶ than the median PFS reported in the submission for crizotinib in patients with ROS1 alterations, presented to the PBAC in July 2018

¹⁶ Redacted content is commercial in confidence

(median PFS █████ months, 95%CI █████; para 5.12, 7.13 Crizotinib, July 2018). It is unknown how similar the patient sample reported by Zeng et al. (2018) was compared to the two studies in the PBAC submission. It is therefore unknown whether the difference in PFS may be due to prognostic factors, or differences in response to crizotinib. Patients who had concomitant variants (*TP53*, *BRCA2*, *MTOR*, *PIK3CA*, *MET* amplification, *CDKN2A*, *ALK*) had significantly worse PFS than those without concomitant variants (median 8.5 months vs 14 months, $p = 0.025$) (Zeng et al. 2018). Given the absence of a comparative method of testing, it is unknown how concordant the selection of patients would have been, or if they had been selected for treatment based on IHC and FISH.

Impact of timing of treatment

A systematic review was identified which collated studies on patients in adults with NSCLC, with interventions aimed at reducing the time between primary care referral to treatment (Hall et al. 2021). Searches of Medline, EMBASE and Cochrane were performed in 2018 and updated in October 2020, as well as trial registries and hand-searching of reference lists. The systematic review identified eight primary studies in patients with advanced disease, which looked at the association between timeliness of treatment and health outcomes. Seven of these studies reported that being treated in a timely manner (with systemic therapy or palliative therapy) was deleterious, due to the “waiting time paradox”. That is, those patients who are most symptomatic (and likely to have a worse prognosis) are more likely to be treated faster than those with minimal or no symptoms. Furthermore, palliative care is likely to be delivered more rapidly than curative therapies (Hall et al. 2021). However, this systematic review did not identify any studies which assessed the relationship between timeliness of treatment and health outcomes for targeted therapies or immunotherapies. The applicability of the evidence is therefore limited. Targeted searches for this assessment did not identify any articles addressing this evidence-gap. It is hypothesised that the very small difference in timing of treatment initiation likely to occur after NGS rather than sequential single-gene testing (0 – 3 days) is unlikely to result in a measurable difference in health.

2.5 Conclusion

2.5.1 Evidence interpretation

The claim made by the RCPA, was that the use of a small DNA ± RNA NGS panel results is superior to sequential single-gene testing, due to the more efficient use of tumour tissue, resulting in a reduced need to undergo repeat biopsy. It was also claimed that NGS may have a more rapid turnaround time, which would translate into faster access to appropriate treatment. NGS may also detect concurrent variants.

These claims were assessed using a linked evidence framework, as illustrated in Figure 15.

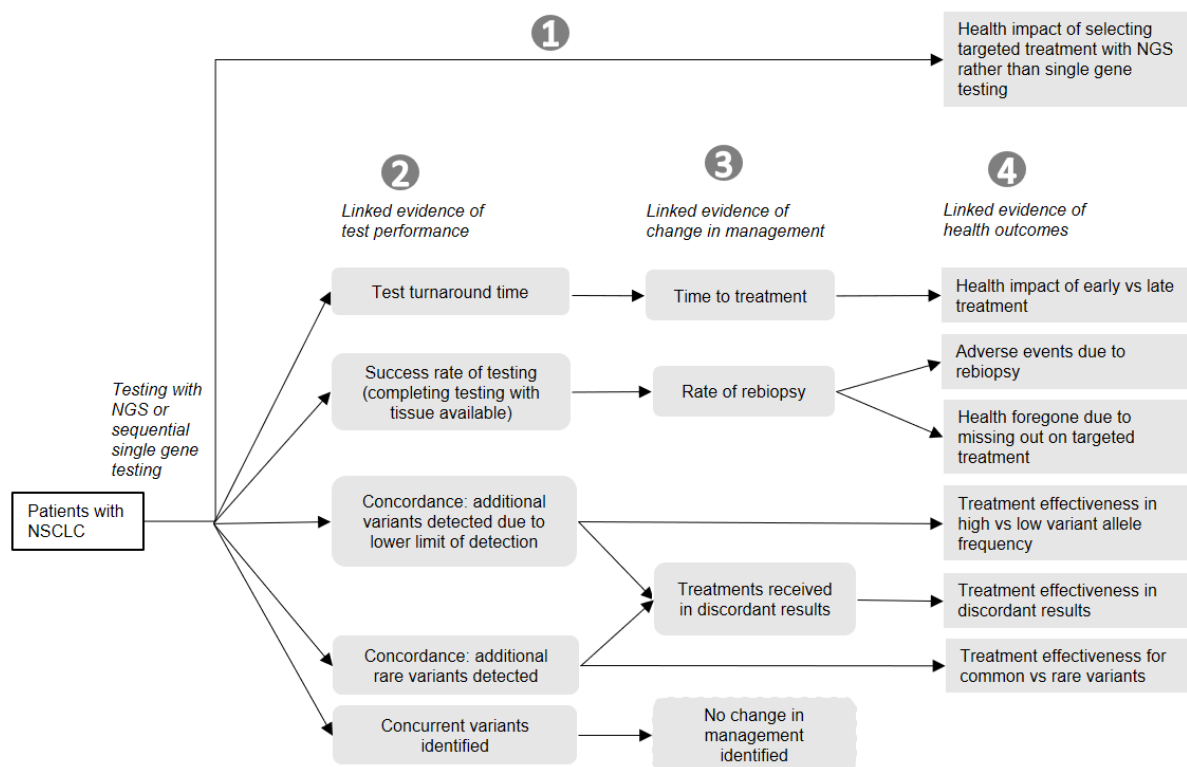


Figure 15 Assessment framework for small DNA/RNA NGS panel for NSCLC

Figure notes: 1: direct from test to health outcomes evidence; 2: test performance; 3: change in treatment/management; 4: influence of the change in management on health outcomes

In the key matters of concern from MSAC 1454 Public Summary Document, it was stated that MSAC considered that any funding should be based on a gene panel or NGS of *equivalent or better analytical performance* to sequential IHC and FISH testing. The evidence in this assessment supported NGS as being more sensitive than single-gene testing, detecting a higher proportion of cases with potentially targetable variants in the *EGFR* gene, and being as accurate as IHC and FISH for detecting *ROS1* and *ALK* variants. However, the applicant claimed NGS was superior to single-gene sequencing (not only non-inferior), so evidence on the impact of NGS testing on the management and health outcomes of patients had to be examined.

The safety of small NGS panel testing for NSCLC was established using a linked evidence approach, linking:

- the comparative success rate of NGS panel testing with sequential single gene testing (97.2% vs 94.6%),
- some evidence that a proportion of patients with insufficient tissue undergo rebiopsy (13% and 43% in the identified studies), and
- evidence that biopsies are associated with a risk of adverse events.

The effectiveness of a small NGS panel compared to sequential single gene testing was established using a linked evidence approach, linking:

- a higher success rate of NGS panel testing than sequential single gene testing, and higher rate of actionable variants being identified,

- with evidence that patients with variants identified by NGS and not by single-gene methods are frequently treated using targeted therapies,
- with evidence that those with variants identified by NGS are likely to benefit from targeted therapy.

The claim was made that NGS would be faster than sequential single gene testing, and although the evidence suggested it may be as fast, or potentially save up to 3 days, the clinical benefit of this was unknown.

Furthermore, the clinical utility of detecting concurrent variants is unclear.

A summary of the evidence identified is provided in [Table 42](#). The evidence of the high level of concordance between testing strategies was of moderate quality; the evidence on the risk of adverse events associated with rebiopsies was low quality, and all other outcomes was rated as very low quality, due to the low level of certainty from the evidence.

Table 42 Summary of findings table for safety/effectiveness of small NGS panel(s) versus sequential single gene testing

Section in report	Outcomes	Participants and studies	Results	Interpretation	Quality of evidence using GRADE
2.1 Direct from test to health outcomes evidence	Predictive of response to <i>ALK</i> -TKI	n=50 k=1 within-patient cohort study	Median PFS NGS+: 11.1m NGS-: 4.6m IHC+: 10.3m IHC-: 11.7m FISH+: 8.8m FISH-: 14.8m	NGS better able to predict response to <i>ALK</i> -TKI than FISH or IHC. Those treated with <i>ALK</i> TKI due to variant detected with NGS therefore expected to have at least non-inferior outcomes to those tested by clinical utility standard or IHC ± FISH.	⊕⊖⊖⊖
2.2 Test performance	Success rate of testing (sufficient tissue)	n=4040 k=1 between-patient cohort	NGS: 97.2% Single-gene testing: 94.6%	NGS small DNA panel ± IHC, FISH or RNA panel able to make better use of tumour tissue available than single-gene testing.	⊕⊕⊖⊖
	Concordance of NGS against single-gene testing	n=4081 k=30 within-patient cohorts	Overall concordance: 95.7% 3.5% additional actionable variants identified by NGS but not comparator 0.8% actionable variants missed by NGS (identified by comparator)	NGS highly concordant with single-gene testing, with some additional cases detected due to higher sensitivity (lower threshold of detection) and detecting rare variants.	⊕⊕⊕⊖
	Turnaround time of test results	n=5462 k=3 between or within-patient cohort studies	NGS: mean or medians of 10 to 12 days Single gene testing strategies: mean or medians of 10 to 13 days Differences: 0 to 3 days	The greatest difference in turnaround time was between a combined DNA and RNA panel and single gene testing, which reported that results were available 3 days sooner with NGS. Separate DNA then RNA panels would be expected to take longer than a combined panel.	⊕⊖⊖⊖

Section in report	Outcomes	Participants and studies	Results	Interpretation	Quality of evidence using GRADE
2.3 Change in management	Change in rate of rebiopsy	n=225 k=2 case series	Rebiopsies performed in 13.3% and 43.4% of cases with insufficient tissue	Rates of rebiopsy were low, but this may be due to the use of liquid biopsy (ctDNA being used) and may not be applicable to Australia.	⊕⊖⊖⊖
	Change in treatment received	n=99 k=6 before and after case series	In those with biomarkers detected by NGS, missed by single-gene testing, the use of targeted treatment varied (median 50%, range 17.6% to 100%)	Identification of biomarkers was only one factor in treatment decisions. Among those advanced enough to require TKIs rather than just surgery, and well enough to receive TKIs, the use of targeted treatments was high.	⊕⊖⊖⊖
	Change in timing of treatment	n=3474 k=1 retrospective cohort study	Time between diagnosis and 1L treatment: NGS: 38 days Single gene testing: 36-38 days	Those tested with NGS had treatment initiated slightly later than those tested by other methods, but the study is too confounded to be very informative.	⊕⊖⊖⊖
2.4 Therapeutic effectiveness	Safety of rebiopsy	n=2326 k=1 systematic review with 16 studies included	AEs occurred in 17% (95%CI 12%, 23%) of those who underwent biopsy Pneumothorax occurred in 9.2% (95%CI 4.0%, 15.7%)	Rebiopsies are associated with a risk of adverse events. The increased success rate of NGS should result in reduced need for rebiopsies, which reduces the risk of adverse events related to testing.	⊕⊕⊖⊖
	Effectiveness of targeted treatment in cases with incremental actionable variants	n=2921 k=2 systematic reviews of cohort studies, and 7 additional case series in discordant cases	Those with actionable variants identified by NGS show response to targeted therapies. Rare variants and low allele frequency variants are less likely to respond to <i>EGFR</i> TKIs than common variants and high allele frequency variants.	Patients with actionable variants in the <i>EGFR</i> gene identified by NGS but not on single gene testing may not respond to targeted therapies the same degree as those identified by the clinical utility standards. Treatment with targeted therapies is likely to still be superior to treatment with non-targeted therapies. Insufficient evidence in those discordant on <i>ALK</i> and <i>ROS1</i> rearrangement status (between testing methods) to determine the comparative efficacy of targeted treatments.	⊕⊖⊖⊖

Section in report	Outcomes	Participants and studies	Results	Interpretation	Quality of evidence using GRADE
	Health impact of more timely treatment	n=not stated k=1 systematic review with 8 cohort studies	Those with more symptoms (corresponding to worse prognosis) received treatment in a timelier manner and have worse health outcomes than those with delayed treatment.	Unclear whether a treatment delay of 3 days would have any impact on health outcomes, as the evidence on time to treatment and health outcomes was too confounded.	⊕⊖⊖⊖

AEs = adverse events; ALK = anaplastic lymphoma kinase; ctDNA = circulating tumour DNA (in the bloodstream); DNA = deoxyribonucleic acid; EGFR = epidermal growth factor receptor; k = number of studies; n = number patients; NGS = next generation sequencing; RNA = ribonucleic acid; ROS1 = ROS proto-oncogene 1; TKI = tyrosine kinase inhibitor (therapy); 1L = first line

⊕⊕⊕⊕ **High quality:** We are very confident that the true effect lies close to that of the estimate of effect.

⊕⊕⊕⊖ **Moderate quality:** We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

⊕⊕⊖⊖ **Low quality:** Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.

⊕⊖⊖⊖ **Very low quality:** We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

2.5.2 Conclusion of the clinical claim

There were some areas of uncertainty in the evidence base, but the conclusion from the clinical evidence is that:

The use of a small DNA ± RNA NGS panel results in marginally superior effectiveness compared with sequential single-gene testing.

The use of a small DNA ± RNA NGS panel results in superior safety compared with sequential single-gene testing.

Section 3 Cost-effectiveness analysis

In Section 2, the clinical claim of superiority was made based on:

- an improvement in the test success rate (i.e. more samples with sufficient quantity and/or quality to be able to be successfully tested for variants);
- an improvement in the yield of variants identified due to being more comprehensive (identifying “in scope” and “beyond restriction” variants) and more sensitive (detecting in-scope variants at a lower variant allelic frequency).

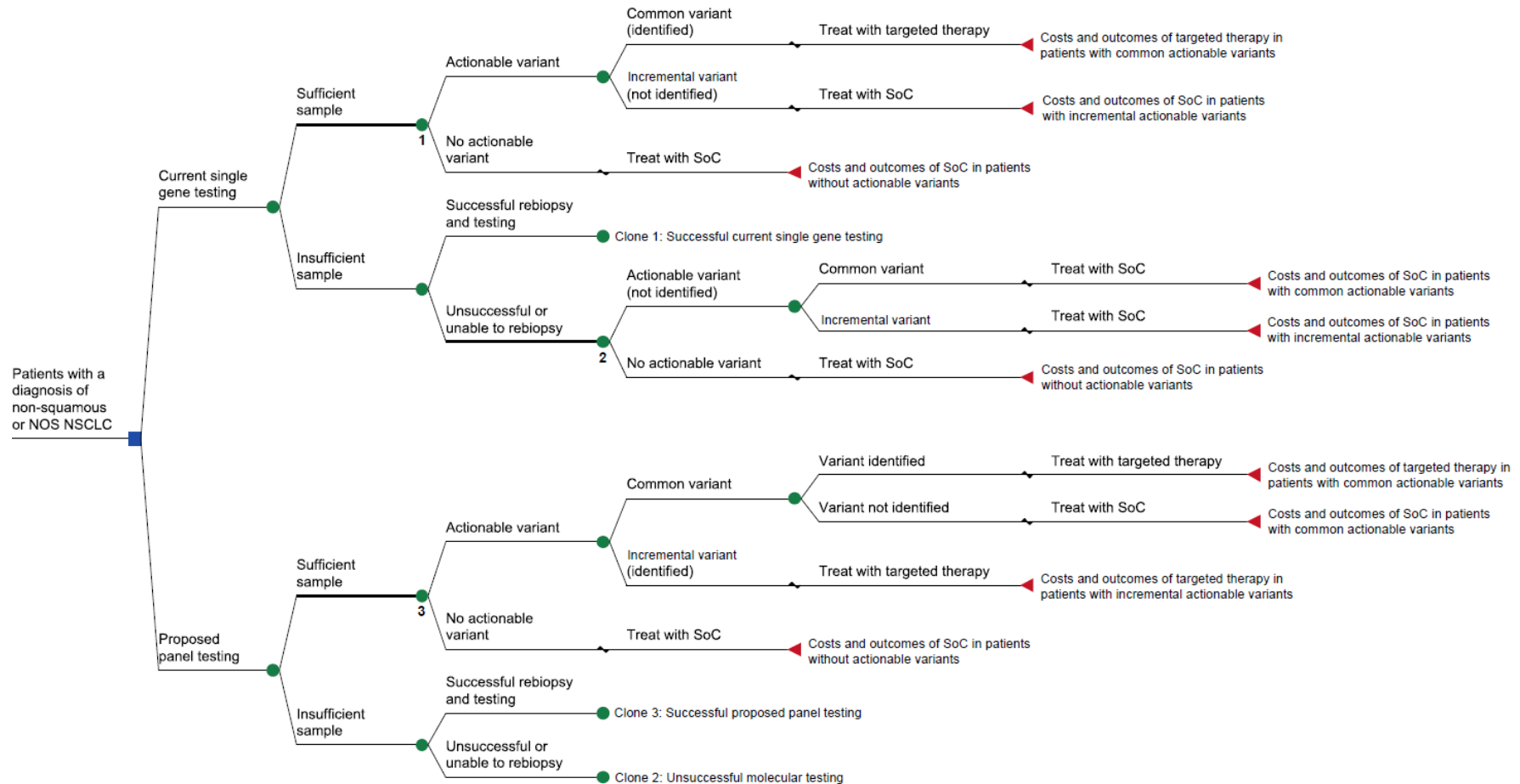
Therefore, a cost-effectiveness analysis is presented in Section 3 that is based on the results of the linked evidence approach presented in Section 2. The results of the economic analysis will be presented in a stepped manner to enable the effect of the observed benefits of small DNA and RNA panel testing to be distinguished from one another, in addition to the incorporation of relevant translations of the clinical evidence to the proposed setting.

Some evidence was identified in Section 2 to support an improvement in test turnaround time with combined DNA/RNA panels relative to a strategy of separate single-gene tests. The applicability of this to the proposed setting – where separate DNA then RNA panels are likely to be more common place – was unclear. Further, the observed difference in the time to receive test results was small and it was unclear what effect this difference would have on the subsequent effectiveness of treatment. Studies identified in Section 2 noted that patients who initiated treatment earlier tended to have poorer outcomes, though this was likely due to the initiation of treatment earlier in patients with poorer prognoses. Given these uncertainties, the economic analysis does not quantify differences in test turnaround time, nor effects of these differences, if any. The results of the analysis will however be described with these additional potential benefits in mind.

3.1 Overview and rationale of the economic evaluation

The analysis presented aims to assess the cost-effectiveness of small DNA ± RNA panel testing, relative to separate single-gene testing for actionable biomarkers, in a population with non-squamous or NOS NSCLC. A conceptual overview of the decision problem that takes into account the claimed benefits of proposed small gene panel testing is presented in Figure 16.

Figure 16 Decision problem overview



Note: Common variants are those that can be identified by either single gene tests or panel tests. Incremental variants are those identified through panel testing only. SoC in the advanced disease setting is likely to be comprised of immunotherapy and chemotherapy. In patients with Stage IV disease, in patients with no evidence of *EGFR*, *ALK* or *ROS1* variants can receive pembrolizumab preferably in combination with chemotherapy, or atezolizumab in combination with bevacizumab and platinum-doublet chemotherapy. Patients with unresectable Stage III disease, irrespective of biomarker status, can receive maintenance therapy with durvalumab following platinum-based chemoradiation therapy.

ALK = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; NOS = not otherwise specified; NSCLC = non-small cell lung cancer; RNA = ribonucleic acid; *ROS1* = ROS proto-oncogene 1; SoC = standard of care.

Based on the conceptual overview of the decision problem, differences in outcomes between the model arms would be driven by:

- The relative outcomes of targeted therapies vs standard of care (SoC) (immunotherapy and/or chemotherapy) in those with common actionable variants – due to additional variants being identified and improvement in test success rate; and
- The relative outcomes of targeted therapies vs SoC in those with uncommon actionable variants identified through small gene panel testing only.

Variants that can be identified by either current or proposed testing (referred to in the analysis as “common” variants) result in the use of targeted therapies. In patients with common variants that are missed by proposed small gene panel testing (due to discordant results or unsuccessful testing and unsuccessful rebiopsy) or those that are missed by current testing (due to IHC triage or unsuccessful testing and unsuccessful rebiopsy), patients may receive SoC in place of targeted therapies. Quantifying the foregone benefit associated with the treatment of common variants with SoC is difficult because, in many cases, SoC has evolved since the initial trials of targeted therapies. For example, the benefit of *EGFR* TKIs was established prior to the availability of immunotherapies. In most cases, immunotherapies have not recruited patients with known actionable variants, therefore indirect comparisons are likely to be confounded by prognostic differences. No evidence was identified in Section 2 to quantify the benefit of targeted therapies compared with SoC in patients with common variants.

Variants that can only be identified through small gene panel testing are referred to in the analysis as “incremental” variants, and include variants both within the current scope of eligibility to PBS-listed targeted therapies (due to detection of lower allelic frequencies), and those beyond current PBS restrictions. Best estimates from the clinical evidence base suggest that the majority of these patients would be eligible for PBS-listed targeted treatments; however, these patients may have a different spectrum of disease than those in the key trials of the targeted therapies. Therefore, treatment response and duration of treatment in patients with these incremental variants to both targeted therapy and SoC is uncertain, and so the incremental benefits and costs that may be associated with changing treatment from SoC to targeted therapy are also uncertain.

In addition to the issues regarding quantifying the differences in outcomes with proposed small gene panel testing, the modelled costs of any analyses that attempt to capture outcomes due to changes in treatment would likely be affected by existing special price arrangements for targeted therapies and immunotherapies. Analyses based on the published prices would not reflect the accepted cost-effectiveness of the included therapies, and the cost-effectiveness of proposed small gene panel testing would be influenced by confidential discounts applied to both targeted therapies and immunotherapies.

Given the considerable uncertainty associated with modelling the costs and outcomes of changes in treatment, the model presented will truncate at the point of treatment. The analysis presented therefore will be a cost-effectiveness analysis. The primary outcome of the model will be the net change in patients eligible for targeted therapy, with disaggregation by type of actionable variant identified (i.e. common or incremental).

A stepped approach is used to generate the base case analysis (Table 43). This incorporates the different aspects of the linked evidence separately to distinguish the effect of these on the results. Further, as described in Section 3.2.3, incremental yield data with proposed panel

testing have been adjusted in the economic analysis to reflect some IHC ± FISH expected in practice and to reflect comparisons to the clinical utility standard. Test success data have also been transformed to reflect implications of rebiopsies due to insufficient quantity or quality of tissue. These translations of the clinical evidence for use in the model have been added in separate steps. Other key model assumptions – RNA panel use restricted to an absence of *KRAS* and *BRAF* variants, and use of testing in patients who do not progress to advanced disease – have also been incorporated in separate steps.

Table 43 Steps used to generate the base case analysis

Step	Costs included	Outcomes modelled
Test cost only <ul style="list-style-type: none"> No difference in success or yield Patients with <i>KRAS</i> or <i>BRAF</i> activating variants eligible for RNA panel testing 	Test costs	None (no difference in outcomes)
Test cost only <ul style="list-style-type: none"> Patients with <i>KRAS</i> or <i>BRAF</i> activating variants not eligible for RNA panel testing 	Test cost, adjusted for reduced use of RNA panels in patients with activating <i>KRAS</i> or <i>BRAF</i> variants	None (no difference in outcomes)
Incorporate test success, based on Steeghs et al. (2022)	Test cost, where no cost of testing is applied where testing is not successful due to insufficient sample ^a	Actionable variant yield, which differs due to proportion of tests successful
Incorporate difference in actionable variants	Test cost, adjusted for concordance (using estimates in Table 30)	Actionable variant yield adjusted for concordance estimates in Table 30
Adjust concordance for comparison to clinical utility standard	Test cost, adjusting concordance to reflect comparisons to the clinical utility standard	Actionable variant yield adjusted for concordance to the clinical utility standard
Adjust for some IHC ± FISH use	Test cost, adjusted for IHC ± FISH use	Actionable variant yield, adjusted for IHC ± FISH use
Adjust for testing patients with early stage disease at diagnosis who do not progress	Test cost, adjusted for patients who do not experience disease progression	Patients eligible for targeted therapy
Incorporate rebiopsy due to test failure	Testing and rebiopsy costs, including additional testing in those successfully rebiopsied	Patients eligible for targeted therapy, adjusted for testing after rebiopsy Rebiopsies performed

BRAF = proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B; FISH = fluorescence *in situ* hybridisation; IHC = immunohistochemistry; *KRAS* = Kirsten rat sarcoma viral oncogene homologue; RNA = ribonucleic acid.

^a As proposed testing can only be claimed once per episode of disease and cannot be claimed in addition to single gene items, this decision rule has been applied across model arms.

3.2 Methods

3.2.1 Summary table

A summary of the key components of the economic evaluation is presented in Table 44.

Table 44 Summary of the economic evaluation

Component	Description
Perspective	Health care system perspective
Population	Patients with non-squamous or NOS NSCLC
Prior testing	Histopathology testing to confirm tumour histology
Comparator	Single gene testing (reflex <i>EGFR</i> , ALK IHC and ROS1 IHC, followed by, if relevant, reflex ALK FISH and/or <i>ROS1</i> FISH, and <i>MET</i> ex14sk testing)
Type(s) of analysis	Cost-effectiveness analysis
Outcomes	Primary: Patients eligible for targeted therapy, disaggregated by patients with common and incremental variants identified Additional: Patients with actionable (i.e. common and incremental variants) variants identified, patients with known biomarker status; changes in rebiopsies required
Time horizon	Time to first-line treatment decisions in the advanced NSCLC setting
Computational method	Decision analytic
Generation of the base case	Modelled stepped analysis, incorporating different aspects of the linked evidence, translations of the clinical evidence and other key model assumptions separately to distinguish the effect of each of these on the results.
Transition probabilities	Yield of actionable variants: Accepted estimates of variant yield as identified by the clinical utility standard ('common' variants), adjusted for additional variants identified by small gene panel testing in the same biomarker ('incremental' variants) using concordance estimates derived in Section 2. Yield estimates were adjusted to reflect some IHC ± FISH use following small DNA panel testing (in instances where tissue quantity or quality is insufficient for RNA panel testing). Success of testing was also based on estimates presented in Section 2.
Discount rate	Not applicable
Software	TreeAge Pro and Microsoft Excel

ALK = anaplastic lymphoma kinase; DNA = deoxyribose nucleic acid; *EGFR* = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; IHC = immunohistochemistry; *MET*ex14sk = MET proto-oncogene, receptor tyrosine kinase exon 14 skipping alterations; NOS = not otherwise specified; NSCLC = non-small cell lung cancer; RNA = ribonucleic acid; *ROS1* = ROS proto-oncogene 1.

3.2.2 Structure of the economic evaluation

Model structuring process

The process used to develop the model structure included a review of the literature to identify economic evaluations of similar decision analyses. Structural attributes from the existing literature were considered in the context of the clinical evidence base and the applicability to the local decision problem.

Literature review

A literature search of the Pubmed and Embase databases was conducted on 27/5/22 using the search terms presented in Table 90, Appendix F. Eight relevant studies were identified, with half conducted in the US (Table 91, Appendix F). The remaining studies were conducted in Hong Kong, Spain, Singapore and Brazil. Outcomes reported generally included time to test result (or initiation of targeted therapy), actionable (and non-actionable) variants identified, patients eligible for targeted therapy (or clinical trials), LYs or QALYs.

All studies used a decision analytic approach; hybrid decision-tree methods were often used where outcomes were reported in terms of LYs or QALYs. Modelled benefits were driven by an increase in patients eligible for targeted therapies, due either to more successful testing, more

biomarkers being tested and/or improved sensitivity of testing. Where improvements in sensitivity were modelled, and additional LYs or QALYs gained were measured, patients were assumed to respond to treatment as though they had been identified by the test used in the clinical trials of targeted therapies (i.e. the clinical utility standard).

Most studies concluded that panel testing resulted in more actionable variants being identified, and where captured, NGS was the fastest or among the faster options in terms of receiving results. Where outcomes were measured in terms of cost, actionable variants identified and time to test results, NGS was generally considered to be cost-effective. Where outcomes were extended to LYs or QALYs gained, conclusions of cost-effectiveness were less consistent and were generally considered to be more uncertain.

The results of the review of the existing economic literature suggest that the model structure should account for actionable variant status. Less consistent was the inclusion of assumptions relating to test success and subsequently, rebiopsy. All studies identified included patients with advanced disease. Where specified, models did not distinguish structurally between patients with advanced disease at diagnosis or those who had progressed from an earlier disease stage.

Model structure

The model takes the form of a decision tree. This was consistent with the existing economic literature described in Table 91, and is appropriate to measure outcomes related to the different test strategies up to the point of treatment. A depiction of the model structure is presented in Figure 17. Branches relating to actionable variant type (or biomarker status, in the instance of rebiopsies not performed or unsuccessful) have been collapsed for ease of presentation. The model structure including these details is presented in Figure 28, Appendix F.

Patients enter the model at the point of receiving testing to determine their biomarker status. The model separates between patients with advanced disease (or who progress to advanced disease) and those tested at an early disease stage who do not progress to advanced disease. This distinction has been incorporated into the model structure as patients tested early who do not progress incur the cost of testing, with no benefit in terms of being eligible for targeted therapy. Further the cost of current testing differs between these groups, as *ALK* and *ROS1* FISH and *MET*ex14sk testing are restricted to patients with advanced disease (and so would not be incurred in patients diagnosed with early disease who do not develop advanced disease).

In patients with advanced disease, those with sufficient tissue receive testing. Currently, single-gene test methods are used, whereas in the intervention arm, small gene panel testing is proposed. More detail on the circumstances of use of current and proposed testing is provided in Section 3.2.3. In patients who have an actionable *EGFR*, *ALK*, *ROS1* or *MET*ex14sk variant, this will be identified or not, based on the performance of the respective test strategies and by type of variant (common or uncommon). Uncommon variants that are identified through small gene panel testing methods alone are not assumed to be identified using current test methods. On identification of an actionable variant, patients are assumed to be eligible for targeted therapy. Where an actionable variant is not identified by testing, patients are not eligible for targeted therapy, and would receive SoC treatment.

Where patients have insufficient tissue (quantity or quality), a rebiopsy may be required. Where a rebiopsy successfully retrieves adequate tissue, testing is performed as described above. However, if rebiopsy is unsuccessful or if the patient is unable to tolerate a rebiopsy, patients would not be eligible for targeted therapy (where a target could have been identified).

This is similarly the case for patients tested at an early stage of disease who do not progress. Patients with sufficient tissue receive testing, and depending on the tests performed, performance of testing and type of actionable variant, biomarker status may or may not be revealed. However, regardless of underlying biomarker status and whether this is identified or not, as patients do not develop advanced disease, they do not require, and so are not eligible for, targeted therapy. Where these patients have insufficient tissue available, a rebiopsy is not assumed to occur (i.e. is assumed to occur on development of advanced disease, which they do not experience).

As described in Section 3.1, given the considerable uncertainty associated with modelling the costs and outcomes related to changes in treatment, the model presented will truncate at the point of treatment.

Structural assumptions

Assumptions incorporated into the structure of the model are that:

- Variants are mutually exclusive;
- Incremental variants cannot be identified by current testing;
- Patients tested at early stage of disease and who have insufficient tissue available for testing would only receive a rebiopsy on development of advanced disease. Therefore, rebiopsies are not assumed in those who do not progress to advanced disease.

Figure 17 Depiction of the model structure



NOS = not otherwise specified; NSCLC = non-small cell lung cancer.

Input data

A high level summary of the sources of data used in the analysis is presented in Table 45.

Table 45 Summary of the inputs used in the economic evaluation

Parameter	Value	Source
Proportion with advanced disease at diagnosis	65.5%	Mitchell et al. (2013)
Proportion who progress from early stage disease to advanced disease	30%	DUSC report on erlotinib and gefitinib (2017)
Test success rates	Current: 94.6% Proposed: 97.2%	Steeghs et al. (2022) identified from the systematic review conducted in Section 2
Prevalence of actionable variants using current testing	<i>EGFR</i> : 15% <i>ALK</i> : 3.0% <i>ROS1</i> : 1.61% <i>MET</i> _{ex14sk} : 3.6%	MSAC 1161 PSD (p18), November 2012 MSAC 1250.1 PSD (p5), November 2014 MSAC 1254 PSD (p12), July 2018 Tepotinib PBAC PSD (Table 11, p27), November 2021
NGS test concordance	Table 30, Section 2	Meta-analysis conducted in Section 2. However, given differences between the comparator used for <i>ALK</i> concordance in the meta-analysis, the data most aligned with the clinical utility standard were used in the base economic analysis (see Section 3.2.3 for further detail).
<i>ALK</i> IHC test performance	Sensitivity: 98.4% Specificity: 98.5%	MSAC 1250 PSD (p7), November 2013
<i>ROS1</i> IHC test performance	Sensitivity: 95.1% Specificity: 93.8%	MSAC 1254 PSD (p12), July 2018
Proportion who uptake rebiopsy, if required	100%	Assumption
Rebiopsy failure rate	20%	Kelly et al. (2019)

ALK = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; IHC = immunohistochemistry; *MET*_{ex14sk} = *MET* proto-oncogene, receptor tyrosine kinase exon 14 skipping alterations; NGS = next generation sequencing; PSD = public summary document; *ROS1* = *ROS* proto-oncogene 1.

Inputs that drive differences in outcomes across model arms were sourced from the systematic literature review conducted in Section 2. Evidence to support a difference in rebiopsies performed between proposed small gene panel testing and current single gene testing was not identified in Section 2. As rebiopsy was often included as a consequence of test failure in the existing economic literature, and the 1634 PICO noted that test failure rate should be reported as a proxy for the rebiopsy rate, a translation study is presented in Section 3.2.3 to describe the approach used to transform differences in test success to differences in rebiopsy.

3.2.3 Model population and setting

Patients with non-squamous or NOS NSCLC enter the model at the point where they receive testing to determine their biomarker status for eligibility for targeted therapy. Patients enter after the decision to receive testing as small gene panel testing is not proposed to increase uptake of testing relative to current practice.

The purpose of testing is to determine eligibility for targeted treatment in the Stage IIIB/IV setting, however the proposed and current item descriptors allow testing at the point of diagnosis (i.e. any stage). While the majority of patients (65.5%) have advanced disease at diagnosis (Mitchell et al. 2013), it is unclear what proportion of patients with early stage disease would

uptake testing. In 2016, MSAC noted that use of *EGFR* testing was significantly lower than estimated, and that this may be due to not all eligible patients being tested (1161 and 1173 PSD, November 2016 MSAC Meeting).

As described in Section 3.2.2, testing patients with early stage disease who do not experience advanced recurrence would incur the cost of testing for no benefit in terms of eligibility to targeted therapy. This applies to both the proposed small gene panel testing and to current *EGFR* testing, however current use of subsequent tests (i.e. *ALK* and *ROS1* FISH and *MET*14sk testing) is restricted to patients with advanced disease only. Therefore, small gene panel testing may be associated with an increase in (completed) testing for *ALK*, *ROS1* and *MET*14sk compared with current testing for those patients who do not progress to advanced disease.

However, given that *EGFR* testing and proposed small gene panel testing are both pathologist determinable, the base case analysis will assume that all patients receive these tests at diagnosis. Of those that are diagnosed with early stage disease (34.5%), 30% are assumed to progress to advanced disease (based on a 2017 DUSC report on erlotinib and gefitinib use), and so the total proportion of patients who enter the model with advanced disease at diagnosis, or who progress to advanced disease from an earlier stage, is 75.9%.¹⁷ The remaining 24.1% of patients are assumed to incur the cost of testing at diagnosis for no benefit in terms of eligibility to targeted therapies. A sensitivity analysis is presented assuming testing only in those with advanced disease. However, the proportion of patients diagnosed with early disease who do not progress to advanced stage may increase over time, due to the proposed lung cancer screening program (MSAC Application 1699), and shift for use of immunotherapies into the adjuvant setting.

In addition to *EGFR* testing, current testing includes *ALK* and *ROS1* IHC testing (assumed to occur at the same time as *EGFR*), *ALK* and/or *ROS1* FISH, where indicated, and *MET*14sk testing. As noted above, *ALK* and *ROS1* FISH and *MET*14sk testing are restricted to patients with advanced disease. While *EGFR*, *ALK* and *ROS1* services are all pathologist determinable, *MET*14sk testing was not recommended to be a pathologist determinable test, and so requires an additional consultation to request the service (assumed to occur after receiving the results from *EGFR* testing). Sensitivity analyses are presented varying the circumstances of *MET*14sk testing use.

Three items have been proposed for small gene panel testing, to reflect two options for use:

- Simultaneous DNA and RNA panel testing; or
- Separate items for DNA then RNA panel testing, where a documented absence of activating variants in specified biomarkers tested on the DNA panel is required for proceeding to RNA testing.

However, due to specimen requirements for RNA-based analyses, current IHC and FISH methodologies for identifying rearrangements are likely to be continued to be used in instances where tissue quantity and quality are insufficient for RNA testing (5–10% of cases, as estimated in the 1721 Application Form). Therefore, in addition to the two options for use of proposed small gene panel testing above, a third is also considered in the analysis:

¹⁷ 65.5% + 34.5% × 30%

- Small DNA panel testing, followed by IHC, and where indicated, FISH analyses to identify gene fusions in *ALK* and *ROS1*.

Information provided by the applicant during the preparation of the DCAR indicated that the majority (75–80%) of laboratories currently would use separate DNA and RNA panels. While the applicant noted that use of combined panels would likely increase over time, it would be a few years before this shift would occur. To reflect some short term increase in the use of combined DNA/RNA gene panels, the base case will assume that where RNA panels can be employed, 30% would use a combined panel approach.

Assumptions related to the circumstances of use:

- If both *ALK* IHC and *ROS1* IHC suggest FISH testing is required (e.g. one or both may be falsely positive by IHC relative to FISH), these are assumed to occur sequentially, *ALK* then *ROS1*, in line with the *ROS1* item descriptor.
- *METex14sk* testing is ordered by clinicians on receipt of negative *EGFR* result i.e. not contingent on *ALK* or *ROS1* FISH results, if required. Thus, *METex14sk* testing is assumed to occur at the same time as FISH for *ALK* and/or *ROS1*, if required (e.g. where IHC may be falsely positive relative to FISH).
- In patients who have early stage of disease at diagnosis who progress, *METex14sk* is assumed to be requested and occurs at the same time as FISH for *ALK* and/or *ROS1*, if required.

Applicability issues and translations associated with the clinical evidence

Three applicability issues associated with the clinical evidence are described:

- Applicability of the clinical data relating to comparative test success
- Applicability of the concordance data
- Applicability of the incremental yield

A further translation study is presented describing the transformation of the effect of differences in test success.

Applicability of comparative test success data

Conclusions in Section 2 for the benefit of proposed small gene panel testing in terms of improved test success rate were based on a large retrospective cohort study from the Netherlands (Steeghs et al. 2022). This study reported a high rate of success for NGS-based analyses (97.2%) compared to non-NGS based single gene testing (94.6%). It is unclear how applicable this study is to the proposed setting, where it has generally been recognised that current practice has optimised to obtain sufficient tissue for current testing. Public consultation on MSAC 1669 indicated that 2–3% of services provided by MBS item 73337 require rebiopsy. Single gene testing of other biomarkers beyond *EGFR*, *ALK*, *ROS1* and *MET* was also performed. It is unclear how the testing of these additional biomarkers affects the applicability of the success rate for single-gene testing reported.

Further, the success rate for NGS testing in Steeghs et al. (2022) was based on DNA NGS and predominantly IHC and/or FISH testing for gene fusions. As IHC and FISH methods are proposed

to remain in the instance of insufficient or inadequate quality tissue, the success rate cited may be a reasonable proxy for circumstances where RNA or IHC and/or FISH is used. However, proposed panel testing may be less successful than reported in Steeghs et al. (2022) if RNA-based methods are attempted in the instance of borderline quantity or quality tissue.

Given that these data were the best data identified to quantify differences in test success between single gene testing and small gene panels, the base case will use the success rates from Steeghs et al. (2022), as reported. Sensitivity analyses will be presented assuming no difference in the rates of success across model arms (e.g. both arms 97.2% or 94.6%). The effect on the ICER when test success data are excluded from the analyses will also be observed in the stepped generation of the base case analysis.

Applicability of concordance data

The concordance of small gene panel testing to single gene test methods for *ALK* in Section 2 was based on a comparison of NGS to FISH ± IHC. The clinical utility standard used in the trials for *ALK* targeted therapy was FISH, which was also what the prevalence estimate previously accepted in Australia was based on, and the basis for the comparison of IHC test performance previously presented to MSAC. While a subgroup analysis was also presented in Section 2 that compared concordance to FISH, in the majority of studies, the definition of *ALK* positivity was not defined or was inconsistent with the definition of positivity used for access PBS-subsidised targeted therapy (≥15% positive cells). Only one study was identified which compared small gene panel testing to *ALK* FISH and used the same definition of positivity that applies in practice (Park & Shim 2020). Data from this study is used in the base case analysis, and so common actionable variants modelled reflect those that do respond to treatment (as per the clinical utility standard), and current IHC triage may therefore miss some patients who would respond to targeted therapy. A sensitivity analysis is presented using the concordance relative to FISH ± IHC, and to the subgroup analysis of FISH only.

The concordance of small gene panel testing to single gene test methods for *ROS1* in Section 2 was based on a comparison of NGS to FISH or RT-PCR. The clinical utility standard used in the trials for *ROS1* targeted therapy included RT-PCR methods, and so the concordance data as presented in Section 2 are reasonable to use in the base case analysis. Due to the small number of studies included, subgroup analyses were unable to be performed.

Applicability of incremental yield data

Yield of actionable variants in non-squamous or NOS NSCLC can differ across countries and ethnicities. None of the studies included in Section 2 for actionable yield concordance were conducted in the Australian (or similar) setting. As estimates of yield reported in the concordance studies are not likely to be applicable, previously accepted estimates of common variant yield in Australia will be transformed in the model using the synthesised NPA values of small gene panel testing to generate total values. The transformed estimates are presented in Section 3.2.4.

As described earlier in Section 3.2.3, the model includes three options for use of proposed small gene panel testing (i.e. combined DNA/RNA panel, sequential DNA then RNA panel, or DNA then current IHC ± FISH). Estimates of actionable variant yield are assumed to be the same for combined or two-stage DNA and RNA panel testing. However, where IHC ± FISH are used, the yield of actionable variants will need to be adjusted (as, for example, IHC ± FISH are assumed not to be able to identify uncommon variants, and as different PPA values would apply). The adjusted estimates are presented in Section 3.2.4.

Transformation of test success data

Test failure reported in Steeghs et al. (2022) was generally due to insufficient quantity or quality of tissue. No comparative evidence was identified in Section 2 to determine how the difference in the proportion of tumours successfully tested translated into differences in rate of rebiopsy. In part this may be due to the use of testing ctDNA in these circumstances. However, access to targeted therapies through the PBS requires evidence of the actionable variant in tumour tissue. Further, testing may also be used to inform the use of some immunotherapies (e.g. pembrolizumab or atezolizumab) as part of the standard of care. Therefore, it may be reasonable to consider tissue rebiopsies in the model in instances of test failure.

However, given the cost of rebiopsy (and considering that it may not be successful), the inclusion of rebiopsy may not be a conservative approach. Therefore, the transformation of differences in test success into differences in rebiopsies is performed in a separate, and final, step as part of the generation of the base case analysis.

3.2.4 Model transition probabilities, variables and extrapolation

Decision tree probabilities included in the model relate to the proportion of patients tested with advanced disease (or who progress to advanced disease), the presence and identification of actionable variants and probabilities of test success and implications for subsequent rebiopsies.

Proportion of patients with advanced disease (or who progress to advanced disease)

As described in Sections 3.2.2 and Section 3.2.3, the decision tree distinguishes between patients who are tested with advanced disease (or those who progress to advanced disease following testing at an earlier stage), from those who are tested at an earlier stage of disease who do not develop advanced disease. As *EGFR* testing is currently, and small gene panel testing proposes to be, pathologist determinable, the base case analysis will assume that all patients, with non-squamous or NOS NSCLC will receive testing.

Based on a retrospective analysis of Victorian Cancer Registry data (Mitchell et al. 2013), 65.5% of patients are assumed to have Stage IIIB/IV disease at diagnosis. Of those diagnosed with Stages I–IIIA disease, 30% are assumed to experience progression to Stage IIIB/IV disease, as per a DUSC report published in 2017 on erlotinib and gefitinib use. Therefore, 75.9% of patients diagnosed with non-squamous NSCLC are modelled to have (or reach) an advanced disease stage. This approach was used in the *METex14sk* testing for access to tepotinib submission (Table 14, Tepotinib PSD, November 2021 PBAC Meeting).

An alternate approach, adopted in the entrectinib submission (Table 10, Entrectinib PSD, March 2020 PBAC Meeting), was to assume that 60% of those diagnosed with Stage IIIA disease would progress (and no progression in those diagnosed at Stage I–II). As Mitchell et al. (2013) reported that 11.8% of patients would have Stage IIIA disease at diagnosis, the total with advanced disease using the alternate approach is 72.6%. The proportion with advanced disease is tested in sensitivity analyses.

Presence of actionable variants

The yield of actionable variants is assumed to comprise of variants that can be identified by the clinical utility standard ('common' actionable variants), and those that can additionally be identified by small gene panel testing ('incremental' actionable variants). As described in Section 3.2.3, the yield of actionable variants can differ across countries and ethnicities, and so rather than using reported estimates of yield from the concordance studies identified in Section 2, the synthesised NPA values of small gene panel testing are applied to estimates of common variant yield in Australia to generate total values.

Estimates previously accepted by MSAC during the listing of the respective single gene tests are presented in Table 46, along with the synthesised concordance data and estimates of total yield applied in the model. As described in Section 3.2.3, data from only one of the included studies for *ALK* concordance were used (Park and Shim 2020), as this study presented a comparison to FISH only and reported using the $\geq 15\%$ positive cells threshold that applied in the clinical studies of crizotinib (to reflect agreement with the clinical utility standard i.e. FISH). Sensitivity analyses are presented using the concordance against a mix of IHC + FISH or FISH alone (PPA 0.92; NPA 0.99) or the subgroup analysis against FISH alone (PPA 0.93; NPA 0.97). Sensitivity analyses are also presented varying both the yield of common variants, and varying the total yield by applying the 95% CI around the NPA estimates.

Table 46 Yield of actionable variants

Biomarker	Clinical utility standard yield [A]	Small gene panel PPA (95% CI) ^a	Small gene panel NPA (95% CI) ^a [B]	Yield adjusted for additional variants identified by small gene panels ^b
<i>EGFR</i>	15.0% ^c	0.98 (0.95, 0.99)	0.97 (0.95, 0.99)	17.6%
<i>ALK</i>	3.0% ^d	1.00 (0.48, 1.00)	0.99 (0.97, 1.00)	3.8%
<i>ROS1</i>	1.6% ^e	0.86 (0.63, 0.96)	1.00 (0.99, 1.00)	1.6%
<i>METex14sk</i>	3.6% ^f	0.98 (0.89, 1.00)	1.00 (0.93, 1.00)	3.6%

ALK = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; *METex14sk* = MET proto-oncogene, receptor tyrosine kinase exon 14 skipping alterations; NPA = negative percent agreement; PPA = positive percent agreement; PSD = public summary document; *ROS1* = ROS proto-oncogene 1.

^a Table 30, Section 2. As described in Section 3.2.3, *ALK* concordance data used in the base case analysis reflected the comparison to FISH alone using a definition of *ALK* positivity of $\geq 15\%$ positive cells, rather than FISH \pm IHC (with varying definitions of *ALK* positivity), presented in Table 30.

^b $A + (1 - A) \times (1 - B)$

^c MSAC 1161 PSD (p18), November 2012

^d MSAC 1250.1 PSD (p5), November 2014

^e MSAC 1254 PSD (p12), July 2018

^f Tepotinib PBAC PSD (Table 11, p27), November 2021

Proportion of actionable variants identified

In the current testing model arm, common variants are assumed to be identified if testing is performed using the clinical utility standard (as is the case for *EGFR* and *METex14sk* testing). As an IHC triage process operates for *ALK* and *ROS1*, performance of IHC, relative to FISH, can affect the proportion of common actionable variants identified. Test performance estimates for IHC triage previously accepted by MSAC are presented in Table 47. Under current testing, 98.4% of common actionable variants in *ALK*, and 95.1% of actionable variants in *ROS1* are assumed to be identified. Incremental variants are not assumed to be identified with current testing.

Table 47 IHC test performance estimates, relative to FISH

	PPA (95% CI)	NPA (95% CI)
ALK IHC	0.984 (0.900, 0.998)	0.985 (0.972, 0.982)
ROS1 IHC	0.951 (NR)	0.938 (NR)

Source: 1250 PSD (p7), November 2013 MSAC Meeting; and 1454 PSD (p9), July 2018 MSAC Meeting.

ALK = anaplastic lymphoma kinase; NPA = negative percent agreement; NR = not reported; PPA = positive percent agreement; PSD = public summary document; ROS1 = ROS proto-oncogene 1.

With proposed small gene panel testing, the estimated PPA values are used to determine the proportion of common actionable variants identified. For *EGFR* and *MET*ex14sk, the PPA values (Table 46) are used directly in the model. However for *ALK* and *ROS1*, as variants require RNA testing, and as a proportion of samples are assumed to be tested using current IHC ± FISH methods (due to insufficient tissue quantity to perform RNA small panels), the PPA values are adjusted to reflect this use. Assuming 5% use of current IHC ± FISH methods, 99.9% of common *ALK* variants are identified, and 86.5% of common *ROS1* variants.

Likewise, all incremental variants are assumed to be identified, except where current IHC ± FISH methods are used to identify variants in *ALK* and *ROS1*.

Test success

The probability of test strategy success is derived from the results of the systematic review conducted in Section 2. The best evidence for comparative test success was a large retrospective cohort study from the Netherlands reported by Steeghs et al. that compared the success of non-NGS based single gene testing (660/698; 94.6%) to NGS-based analyses (3,248/3,342; 97.2%)(Steeghs et al. 2022).

While, as described in Section 3.2.3, some applicability concerns do exist regarding these data, better alternate estimates of the comparative success rates could not be identified. The effect on the ICER when test success data are excluded from the analyses will also be observed in the stepped generation of the base case analysis, and sensitivity analyses will be presented assuming no difference in the rates of success across model arms (e.g. both arms 97.2% or 94.6%).

Rebiopsy

As described in Section 3.2.3, test failure reported in Steeghs et al. (2022) was generally due to insufficient quantity or quality of tissue. Therefore, test failures modelled are assumed to require a rebiopsy to enable further testing of tumour tissue to be performed. Two studies were identified in Section 2 that reported repeat biopsies in 13–43% of cases; the remaining cases were either tested using liquid biopsy or not biomarker tested. It was also acknowledged in Section 2.3.3 that these data would not likely be applicable to the proposed setting as access to targeted therapies requires testing and as testing may also be used to inform use of some immunotherapies (e.g., pembrolizumab or atezolizumab) as part of the standard of care.

MSAC had previously considered that a rebiopsy rate of 63% would apply after first-line therapy (p3, 1407 PSD, November 2018 MSAC Meeting), and so a higher rate may be reasonable to assume prior to initiation of first-line treatment. Therefore in the base case analysis, this has been assumed to be 100%. Sensitivity analyses are conducted varying this estimate. Where test

failure occurs in a patient at an early stage of disease, rebiopsy is only assumed on disease recurrence.

Rebiopsies were assumed to have a 20% (12/60) failure rate, based on the number of biopsies observed in Stage IIIB/IV patients that were either obtained, but failed due to technical reasons (such as normal tissue or insufficient tumour cells) (10/60) or were not obtained due to complications (2/60) (Kelly et al. 2019). An estimate of 15% as used in the existing economic literature (Table 91) is used in a sensitivity analysis. Complications related to rebiopsy are also costed in the model. As described in Section 3.2.6, a 14% complication rate has been assumed in the base case analysis.

3.2.5 Health outcomes

As described in Section 3.1, due to the high degree of uncertainty associated with modelling the effect of changes in treatment (in terms of both outcome and cost differences), the model truncates at the point of first-line NSCLC treatment initiation decisions. Given the proposed benefits of small gene panel testing (improved test success and increased actionable variant yield), the primary outcome of the economic analysis is the net change in patients eligible for targeted therapy. Given the uncertainties associated with the effect of targeted treatment in incremental compared to common variants, outcomes reported will be disaggregated by actionable variant type (i.e. common or incremental). Knowledge of biomarker status is also considered a patient-relevant outcome, as this may be used to inform eligibility to immunotherapy in some patients.

In addition to outcomes related to eligibility to targeted therapies and knowledge of biomarker status, the change in rebiopsies performed with proposed small gene panel testing is also measured.

3.2.6 Health care resource use and costs

Current testing

Currently, all patients tested receive *EGFR* and *ALK* and *ROS1* IHC. As IHC is used as a triage test, FISH is required as a confirmatory test in those with positive IHC results. The average use of FISH testing varies by underlying *ALK* or *ROS1* biomarker status and result of IHC testing (Table 48). In addition, FISH is only used in patients with advanced disease. Where actionable variants are present, results of IHC are explicitly modelled, and so patients with a concordant result would all receive confirmatory FISH, while those that were discordant, would not.

In patients where actionable variants are not present, results from IHC testing are not explicitly modelled, and so the average FISH use in these patients is applied. The most straightforward approach to estimate the average use would be to use IHC specificity data. Patients in whom IHC returns a false positive result would receive one FISH (i.e. $1 - \text{IHC specificity}$), whereas those in whom a true negative result is returned would receive no FISH testing. Thus $1 - \text{IHC specificity}$ could be used to estimate average FISH use where actionable variants are not present.

However, as shown in Section 3.2.7, average use of *ALK* and *ROS1* FISH services per patient who enters the model and receives *EGFR* testing is lower than the observed ratio of *ALK* or *ROS1* FISH to *EGFR* services, respectively. This may be due to a number of factors – the proportion of patients with advanced disease (or who experience advanced disease) may be an underestimate, the prevalence of rearrangements may be an underestimate or the performance of IHC in terms of specificity may be an overestimate (e.g. IHC may require more unnecessary FISH). It is also

possible that some *EGFR* testing is being performed through the public hospital system (whereas subsequent FISH use may be billed to the MBS). In the absence of alternate estimates that are more consistent with the utilisation data, calibration of the estimate of FISH use in patients without actionable variants to the observed utilisation data is performed (see Appendix F). This approach is assumed to affect use and cost of FISH only (and so has no effect on the yield of actionable variants identified). Analyses are presented using the alternate approach in both Section 3.2.7 and in sensitivity analyses.

Table 48 Use of FISH testing in patients with advanced disease

	Use of <i>ALK</i> FISH	Use of <i>ROS1</i> FISH
Common variant present		
• IHC positive result	1	1
• IHC negative result	0	0
Average use where common variants are not present		
• Calibration method (base case)	0.037 ^a	0.104 ^a
• IHC specificity (sensitivity analysis)	0.015 ^b	0.062 ^c

ALK = anaplastic lymphoma kinase; FISH = fluorescence *in situ* hybridisation; IHC = immunohistochemistry; *ROS1* = ROS proto-oncogene 1.

^a see Appendix F for the derivation of the calibrated estimates used

^b 1 – *ALK* IHC specificity (1 – 98.5%)

^c 1 – *ROS1* IHC specificity (1 – 93.8%)

As *METex14sk* testing has not been recommended by MSAC to be a pathologist determinable service, an additional consultation to request this service is required. This is assumed to occur after *EGFR* and IHC results, though prior to FISH results if required, to account for the additional time required to conduct testing, including referral to central laboratories. Therefore use of *METex14sk* testing is assumed to be limited to patients that do not have actionable *EGFR* variants. A sensitivity analysis is presented assuming that *METex14sk* testing would occur at the same time as *EGFR* given that MSAC had advised that the absence of other biomarkers (*EGFR*, *ALK* and *ROS1*) need not be a pre-requisite for *METex14sk* testing (p4 MSAC 1660 PSD November 2021), and after *EGFR*, *ALK* and *ROS1*, depending on the timing of the additional consultation to request further testing.

As the FISH items and the *METex14sk* testing item are restricted to patients with advanced disease, these tests are not assumed to occur in patients tested at an early stage of disease who do not progress.

The MBS fees per service of current single gene testing are presented in Table 50. While the MSAC Guidelines state a preference for the average fees charged (and so including patient payments), MBS data provided during the preparation of the DCAR suggested high rates of bulk-billing (and so the average fee charge was lower than the MBS Fees, Table 50). It is unclear whether rates of bulk-billing would change with the proposed listing of small gene panel testing. So as not to assume asymmetrical costs across model arms, MBS schedule fees are applied in the base case, with the average fees charged applied, where available, in a sensitivity analysis.

Table 49 Schedule and average fees charged for current single gene test items

	MBS Fee	Average fee charged
<i>EGFR</i>	\$397.35	\$381.86
<i>ALK</i> FISH	\$400.00	\$384.87
<i>ROS1</i> FISH	\$400.00	\$394.62

	MBS Fee	Average fee charged
<i>METex14sk</i>	\$397.35	NA

Source: Average fee charged data were provided by the Department during the preparation of the DCAR.

ALK = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; *METex14sk* = *MET* proto-oncogene, receptor tyrosine kinase exon 14 skipping alterations; NA = not available; *ROS1* = *ROS* proto-oncogene 1.

In the MSAC 1634 Ratified PICO, PASC considered that the consequences of funding proposed comprehensive genomic profiling in NSCLC on the expected reduction in cost of IHC testing for *ALK* and *ROS1* would not be straightforward to estimate. The consequence might be billing of a cheaper MBS item for fewer IHC antibodies (i.e. without the antibodies for *ALK* or *ROS1*) or no cost-consequence if the reduction in antibodies does not change the item being charged (e.g. from use of six to four antibodies tested). The base case will adopt a conservative approach and assume no cost consequence associated with the reduction in use of *ALK* and *ROS1* IHC testing. A sensitivity analysis is presented assuming the cost difference of billing item 72847 (schedule fee \$89.40) rather than 72849 (schedule fee \$104.30) (cost difference \$14.90).

Given it is not a pathologist determinable service, *METex14sk* testing is likely to be associated with additional services, such as an additional consultation with the treating clinician (MBS item 105, \$46.15) and the retrieval of archived tissue blocks (MBS item 72860, \$85.00). These additional services included in the costing of *METex14sk* testing are assumed to cover the request and block retrieval for FISH testing, where required, following development of advanced disease.

Proposed panel testing

As described in Section 3.2.3, use of proposed small gene panel testing is likely to comprise a mix of:

- Combined small DNA and RNA panels;
- Sequential DNA then RNA panels; and
- Small DNA panel followed by *ALK* and *ROS1* IHC ± FISH.

RNA quantity and quality is likely to be insufficient in 5–10% of cases necessitating the use of current IHC ± FISH methods (1721 Application Form). A 5% rate is assumed in the base case, with estimates of 0% and 10% tested in sensitivity analyses. All patients are assumed to receive IHC testing, with the estimates of FISH use as per Table 48.

Where RNA small panels can be used, 30% are assumed to occur using the combined approach, based on Applicant feedback received during the preparation of the DCAR on the current availability of combined panels (20–25%). Therefore, 28.5%¹⁸ of tests overall are assumed to use the combined method.

Small gene DNA panel testing only would then apply in the remaining 71.5% of patients tested (comprised of 5% followed by IHC ± FISH and 66.5% followed by small RNA panels). However, use of RNA small panels in the two-stage approach is restricted to where no activating variants were identified in *EGFR*, *KRAS*, *BRAF* or *MET* exon 14 from small DNA panel testing. The rationale for this is that as activating variants are generally thought to be mutually exclusive, when non-actionable variants are identified, further testing could be avoided without missing patients who could benefit from targeted therapy. However as described in Section 2B.2.4, instances of concurrent variants may be more common than previously thought. A prospective case series from Germany (Griesinger et al. 2021) reported that of all patients with *ROS1* and *ALK* variants

¹⁸ 30% of the 95% of analyses with sufficient quantity and quality of tissue

identified, respectively, 23.7% (14/59) and 16.1% (19/118) also had variants in *BRAF* or *KRAS*. The base case will limit RNA small gene panel testing to those without variants in the biomarkers specified in the proposed item descriptor, without affecting the yield of actionable variants in *ALK* and *ROS1* as the rate of concurrent variants in the proposed setting is unknown. However a sensitivity analysis will also be presented that assumes RNA small gene panel testing would not occur only where actionable variants were identified through the small DNA gene panel (i.e. activating *EGFR* and *MET*ex14sk alterations).

KRAS variants are estimated in 28.8% (144/500) of the tested population, based on the reported yield in an Australian study of advanced non-squamous NSCLC patients (Cui et al. 2020). In the recent consideration of *KRAS* G12C testing, MSAC noted that eight studies reported the prevalence of any *KRAS* variant as being 37.5% (range 24–49%) (p4 MSAC 1669 PSD March 2022). Data on the estimated yield of *BRAF* variants in the Australian population with NSCLC could not be identified. The 1721 Application Form suggested that 1–3% of patients with NSCLC would have *BRAF* variants; 2% will be assumed in the base case analysis.

The proposed MBS fees for proposed small gene panel testing are presented in Table 50. Applicant feedback provided during the preparation of the DCAR indicated that the proposed fees were set such that additional fees should not be charged.

Table 50 Proposed fees for small gene panel testing

	Proposed MBS fee
Combined DNA/RNA	\$1,247.00
DNA panel only	\$682.35
RNA panel only	\$682.35

DNA = deoxyribose nucleic acid; FISH = fluorescence *in situ* hybridisation; RNA = ribonucleic acid.

Where IHC ± FISH methods are used, *ALK* and *ROS1* IHC are assumed to still be performed as part of the same episode as other IHC tests conducted at diagnosis (and so are assumed to be associated with no cost). The cost of FISH testing is assumed as per average fees charged in Table 49. In patients who are tested in early disease who progress, FISH testing is associated with an additional consult and block retrieval.

Cost of rebiopsy (and complications)

The cost of rebiopsy estimated in the base case analysis was based on the cost of an average bronchoscopy conducted as an admitted patient in a public hospital (NHCD 2019–2020 Cost Report for AR-DRG E42A–C). The majority of patients (86%) are assumed to have an uncomplicated rebiopsy, based on previous MSAC advice on the complication rate in this setting (14%, MSAC 1161 PSD, November 2012). An alternate complication rate of 10% was tested in a sensitivity analysis, based on the incidence of pneumothorax noted in the MSAC 1660 PSD (p10, November 2021) and reported in the source used for biopsy failure rate (Kelly et al. 2019).

Rebiopsies that are performed without complications are assumed to incur the cost of a minor complexity bronchoscopy (AR-DRG E42C \$4,086), while those with complications are assumed to incur the average cost of an intermediate or major complexity bronchoscopy (AR-DRG E42A/B, \$15,118). The weighted cost of bronchoscopies modelled in the base case analysis is \$5,630. This approach appears consistent with that used in analyses previously presented to MSAC and ESC (p13, MSAC 1407 PSD, November 2018 MSAC Meeting).

However, lung biopsies may also be performed in the outpatient setting. Steinfort et al. (2013) reported an average costs of outpatient lung biopsy of \$2,724 for endobronchial ultrasound-guided transbronchial lung biopsy; and \$2,748 for computed tomography-guided percutaneous

needle biopsy (including management of complications) in 2010/2011 Australian dollars. With updating of costs to 2021/2022 levels, based on annual health inflation estimates reported by the AIHW, the average cost of rebiopsy in the outpatient setting is estimated to be \$3,363. Sensitivity analyses are presented assuming all rebiopsy occur in the outpatient setting, and assuming a 50:50 split of use.

3.2.7 Model validation

Operational validation of the economic model

To validate the operation of the model, the proportions and cost applied at each decision tree branching point were externally estimated (see 'Model validation' tab in the included file accompanying the DCAR) (Figure 29, Appendix F). This demonstrates that the sum of the decision probabilities in each model arm equals one, with replication of the overall of the total model costs.

Other validation techniques

Ratio of FISH to EGFR use (modelled vs utilisation)

External validation was performed by presenting a comparison of the modelled estimates of FISH use per patient that enters the model to estimates of MBS service utilisation. As all patients enter the model in the comparator arm receive EGFR testing, the modelled ratio of ALK FISH use : EGFR service use (Table 51) was compared to the observed ratio of test use (Table 52).

Table 51 Modelled estimates of FISH testing, per patient tested

	EGFR use	ALK FISH use	ROS1 FISH use
FISH use calibrated to utilisation data (base case) (As a proportion of EGFR services)	0.9788	0.0447 (4.6%)	0.0744 (7.6%)
FISH use estimated from prevalence and IHC specificity (As a proportion of EGFR services)	0.9788	0.0314 (3.2%)	0.0489 (5.0%)
FISH use estimated from prevalence and IHC specificity (advanced patients only) (As a proportion of EGFR services)	0.9892	0.0414 (4.2%)	0.0645 (6.5%)

ALK = anaplastic lymphoma kinase; EGFR = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; IHC = immunohistochemistry; ROS1 = ROS proto-oncogene 1.

Table 52 Claiming of EGFR, ALK FISH and ROS1 FISH MBS items

	2016	2017	2018	2019	2020	2021
No. EGFR services	3,419	3,863	4,147	4,603	4,697	4,854
No. ALK FISH services	188	305	292	201	222	216
As a proportion of EGFR services	5.5%	7.9%	7.0%	4.4%	4.7%	4.4%
No. ROS1 FISH services				121	333	386
As a proportion of EGFR services				2.6%	7.1%	8.0%

Source: Services Australia

ALK = anaplastic lymphoma kinase; EGFR = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; ROS1 = ROS proto-oncogene 1.

ALK FISH testing was listed on the MBS in July 2015. Fluctuations in claims were observed in the first three full calendar years of listing, however claims appeared to stabilise from 2019 onwards. From 2019–2021, ALK FISH services made up approximately 4.4–4.7% of EGFR services (average 4.5%).

Claiming of ROS1 FISH testing in the first full calendar year of listing (2019) was low relative to use in 2020 and 2021. It is unclear whether claiming has at yet stabilised, though in 2020 and 2021, the number of ROS1 FISH services did reflect 7.1% and 8.0% of EGFR tests, respectively (average 7.5%).

The modelled estimates of FISH use in the base case analysis, which used the calibration approach, was very similar to the MBS utilisation data – which is unsurprising, as the estimates were calibrated to these data. As noted in Section 3.2.6, the most straightforward approach would be to use IHC specificity data. However, when this approach is used, the ratio of FISH to EGFR services is much lower than observed in the utilisation data. As described in Section 3.6.2, this may be due to a number of factors – the proportion of patients with advanced disease (or who experience advanced disease) may be an underestimate, the prevalence of rearrangements may be an underestimate or the performance of IHC in terms of specificity may be an overestimate (e.g. IHC may require more unnecessary FISH). Interestingly, when only advanced patients enter the model (i.e. no patients tested early who do not progress), estimates are closer to the utilisation data.

The choice of modelling approach has a moderate impact on the results of the analysis – a 9% increase in the base case ICER is observed when the IHC specificity data are used.

Comparison to the synthesised estimates reported in Section 2

In the conclusions reported in Section 2, the incremental yield and test success data were used to determine outcomes per 1,000 patients tested. This estimated that:

- an additional 26 patients would receive results
- small gene panel testing would identify 34 patients with uncommon variants, however would miss 2 patients with common variants (net change in 32 patients eligible for targeted therapy, assuming other criteria are met).

A direct comparison of the base case results to these estimated in Section 2 would not take into account the translations of the clinical evidence to the proposed setting performed. These included the implications of patients with early stage disease who do not progress to advanced disease (and so who do not receive complete single gene testing); the incorporation of some IHC ± FISH use with small gene panel testing; and the implications of testing following successful rebiopsy.

An analysis based on the clinical evidence before translations is presented (e.g. Step 4 in the generation of the base case). The results from this analysis are presented in Table 53, below. The results are consistent with the estimates presented in Section 2.

Table 53 Comparison of modelled estimates (Step 4) to synthesised results presented in Section 2

	Small gene panel testing	Single gene testing	Increment
Patients successfully tested	0.972	0.946	0.026
Proportion with an actionable variant identified	0.252	0.220	0.032
<ul style="list-style-type: none"> • Common variants identified • Incremental variants identified 	0.217	0.220	-0.002
	0.034	0.000	0.034

3.3 Results

3.3.1 Base-case analysis

A stepped approach is used to generate the base case analysis in order to incorporate the different aspects of the linked evidence, translations of the clinical evidence and other key model assumptions separately to distinguish the effect of each of these on the results.

Intervention costs per patient

Three items are proposed for small gene panel testing – a combined DNA/RNA panel item (schedule fee \$1,247.00), and separate items for DNA analysis only, and RNA analysis (each schedule fee \$682.35). While presently, use of the items may be limited by the availability of combined DNA/RNA panels, separate items may need to be required for instances of insufficient tissue quantity or quality to perform RNA analyses. Therefore, use of all items are included in the analysis.

The average cost of small gene panel testing in the population modelled is \$1,051.29. Based on applicant feedback provided during the preparation of the DCAR (see Section 3.2.6 for further information), approximately 71% of patients were estimated to receive the separate small DNA panel item, 28% receiving combined DNA/RNA panel testing, and 32% receiving the separate small RNA panel item.

The average cost of sequential single gene testing in the population modelled was \$689.95. The majority of patients (98%) modelled received *EGFR* and IHC testing (modelled cost of \$397.35) (assumed to occur on diagnosis of non-squamous or NOS NSCLC in those with sufficient sample for testing or who are tested following successful rebiopsy). *MET*ex14sk testing (\$397.35) was assumed on average in 64% of patients (in patients who have or progress to advanced disease, who do not have *EGFR* variants and who have sufficient sample for testing or who are tested following successful rebiopsy), with approximately 12% of patients receiving FISH testing (\$400) (in patients as for *MET*ex14sk testing, though further restricted to those with IHC positive results).

Stepped presentation of results

The results of the stepped analysis to generate the base case economic evaluation is presented in Table 54.

The steps that had the most effect on the results of the analysis included restricting RNA-only panel testing to those without *KRAS* and *BRAF* activating variants; applying an increase in actionable variant yield with panel testing, the inclusion of patients tested with early stage disease who do not progress; and including costs and outcomes related to rebiopsy.

Table 54 Stepped economic evaluation

	Small gene panel testing	Single gene testing	Increment
Step 1: Test cost difference only			
No difference in success or yield between current and proposed testing. In two-stage panel testing, patients with <i>KRAS</i> or <i>BRAF</i> variants receive RNA testing.			
Total cost	\$1,240.55	\$894.72	\$345.83
Step 2: RNA panel testing restricted to <i>KRAS</i> and <i>BRAF</i> negatives			
As per the proposed small RNA gene panel test item, where two-stage panel testing is used, patients found with <i>KRAS</i> or <i>BRAF</i> variants <u>cannot</u> receive RNA testing.			
Total cost	\$1,093.43	\$894.72	\$198.72
Step 3: Incorporate differences in test success across model arms			
Sufficient sample is available for testing in 97.2% of patients tested with small gene panels, compared to 94.6% with single gene testing, based on Steeghs et al. (2022). As proposed testing can only be claimed once per episode of disease and cannot be claimed in addition to single gene items, where testing is not successful due to insufficient sample, no cost of testing is assumed to apply in either model arm.			
Total cost	\$1,062.82	\$846.40	\$216.42
Proportion with an actionable variant identified ^a	0.2256	0.2196	0.0060
ICER per additional patient with an actionable variant identified			\$35,862
Step 4: Incorporate differences in yield across model arms			
Concordance data of small gene panel testing, relative to the respective single gene test, is incorporated. Where PPA < 1, some variants that may have otherwise been identified through single gene testing may be missed, and where NPA < 1 additional “in scope” and “beyond restriction” variants are identified. As the majority of small gene panel testing uses the two-step method, with more variants identified on the small DNA panel, fewer small RNA panels may be required (and so a reduction in small gene panel test cost is observed).			
Total cost	\$1,052.71	\$846.40	\$206.30
Proportion with an actionable variant identified ^a	0.2517	0.2196	0.0321
ICER per additional patient with an actionable variant identified			\$6,425
Step 5: Adjust <i>ALK</i> concordance for comparison to clinical utility standard			
The concordance of small gene panel testing to single gene test methods for <i>ALK</i> in Table 30 was based on a comparison of NGS to FISH ± IHC, whereas the clinical utility standard used in the trials for <i>ALK</i> targeted therapy was FISH (≥15% positive cells). Only one study that compared small gene panel testing to FISH reported using this same definition of positivity (Park & Shim 2020). PPA of <i>ALK</i> and <i>ROS1</i> IHC relative to FISH was also incorporated.			
Total cost	\$1,052.71	\$846.40	\$206.30
Proportion with an actionable variant identified ^a	0.2526	0.2184	0.0342
ICER per additional patient with an actionable variant identified			\$6,026
Step 6: Adjust for some IHC ± FISH use with proposed testing			
The applicant expected that 5–10% of tests would require current testing methods. MSAC have previously considered that small DNA panels are currently being used for <i>EGFR</i> testing (MSAC 1669 PSD, March 2022 MSAC Meeting) and so this has been assumed to apply to small RNA gene panels only, as RNA panels may have larger sampling requirements. This reduces both the cost of proposed testing and also yield (as additional “in scope” and “beyond restriction” variants would not be identified in this proportion of patients)			
Total cost	\$1,035.55	\$846.40	\$189.15
Proportion with an actionable variant identified ^a	0.2523	0.2184	0.0339
ICER per additional patient with an actionable variant identified			\$5,582

	Small gene panel testing	Single gene testing	Increment
Step 7: Incorporate patients with early disease who do not progress			
Small gene panel testing is proposed to occur on diagnosis of non-squamous or NOS NSCLC. While current <i>EGFR</i> and <i>ALK</i> and <i>ROS1</i> IHC testing also occur at diagnosis, <i>ALK</i> and <i>ROS1</i> FISH and proposed <i>MET</i> ex14sk testing do not occur until the development of advanced disease. The analysis therefore has been adjusted to reflect that not all patients who receive small gene panel testing would develop advanced disease (and so would not be eligible for targeted therapy, currently available only in the advanced setting).			
Total cost	\$1,035.54	\$743.77	\$291.76
Proportion eligible for targeted therapy	0.1913	0.1656	0.0257
ICER per additional patient eligible for targeted therapy			\$11,352
Step 8: Incorporate rebiopsies			
In those with insufficient sample for testing, rebiopsy is attempted where 20% are assumed to fail (Kelly et al. 2019).			
Total cost	\$1,173.23	\$1,004.20	\$169.02
Proportion eligible for targeted therapy	0.1957	0.1732	0.0225
ICER per additional patient eligible for targeted therapy			\$7,496

^a Incorporates variants that could be identified by either current or proposed testing, or incremental variants within the current scope of eligibility to PBS-listed targeted therapies, and those beyond current PBS restrictions.

ALK = anaplastic lymphoma kinase; *BRAF* = proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B; DNA = deoxyribonucleic acid; *EGFR* = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; IHC = immunohistochemistry; *KRAS* = Kirsten rat sarcoma viral oncogene homologue; *MET*ex14sk = *MET* proto-oncogene, receptor tyrosine kinase exon 14 skipping alterations; NGS = next-generation sequencing; NOS = not otherwise specified; NPA = negative percent agreement; NSCLC = non-small cell lung cancer; PPA = positive percent agreement; RNA = ribonucleic acid; *ROS1* = *ROS* proto-oncogene 1.

Disaggregated and aggregated base-case results

Disaggregated costs and outcomes are presented in Table 55 and Table 56, respectively.

The incremental cost is driven by costs associated with testing and, cost-offsets due to a reduction in biopsies performed. Further disaggregation of the cost of testing depicts that the majority of test costs with small gene panel testing related to use of the small gene panels (combined DNA/RNA or separate DNA and RNA panels), whereas cost offsets related primarily to the reduction in use and cost of *EGFR* and *MET*ex14sk testing.

Table 55 Disaggregated modelled costs

	Small gene panel testing	Single gene testing	Increment
Cost of testing	\$1,053.65	\$773.59	\$280.06
• Cost of <i>EGFR</i>	\$0.00	\$388.91	-\$388.91
• Cost of <i>ALK</i> FISH	\$0.91	\$17.86	-\$16.95
• Cost of <i>ROS1</i> FISH	\$1.45	\$29.76	-\$28.31
• Cost of <i>MET</i> ex14sk			
○ <i>MET</i> ex14sk test	\$0.00	\$253.41	-\$253.41
○ <i>MET</i> ex14sk block retrieval	\$0.00	\$54.21	-\$54.21
○ <i>MET</i> ex14sk consult	\$0.00	\$29.43	-\$29.43
• Cost of combined DNA/RNA panels	\$351.48	\$0.00	\$351.48
• Cost of DNA panel only	\$482.51	\$0.00	\$482.51
• Cost of RNA panel only	\$217.30	\$0.00	\$217.30

	Small gene panel testing	Single gene testing	Increment
• Cost of IHC	\$0.00	\$0.00	\$0.00
Cost of rebiopsy	\$119.58	\$230.61	-\$111.03
Total cost	\$1,173.23	\$1,004.20	\$169.02

ALK = anaplastic lymphoma kinase; DNA = deoxyribose nucleic acid; EGFR = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; IHC = immunohistochemistry; METex14sk = MET proto-oncogene, receptor tyrosine kinase exon 14 skipping alterations; RNA = ribonucleic acid; ROS1 = ROS proto-oncogene 1.

The additional patients eligible for targeted therapy was driven by an increase in patients with incremental actionable variants identified. A slight reduction in patients with common actionable variants was also observed. As described in Section 3.1, as incremental actionable variants were not identified using the same testing method as was used in the clinical trials of targeted therapy, it is unclear whether all of these patients would respond to targeted therapies to the same extent as those with common actionable variants.

Table 56 Disaggregated modelled outcomes

	Small gene panel testing	Single gene testing	Increment
Eligible for targeted therapy	0.1957	0.1732	0.0225
• Common variants	0.1706	0.1732	-0.0026
• Incremental variants	0.0251	0.0000	0.0251
Actionable variant identified	0.2556	0.2075	0.0481
• Common variants identified	0.2226	0.2075	0.0152
• Incremental variants identified	0.0330	0.0000	0.0330
Patients successfully tested	0.9890	0.9788	0.0102
Proportion with known biomarker status	0.9817	0.7583	0.2234
• Early disease	0.2322	0.0343	0.1979
• Advanced disease	0.7495	0.7240	0.0254
Proportion undergoing rebiopsy	0.0212	0.0410	-0.0197

More patients were identified with actionable variants than those considered eligible for targeted therapy (absolutely and incrementally). This was due to the inclusion of patients tested with early stage disease who do not developed advanced disease (and so are not eligible for targeted therapy). The incremental difference was also higher (and in some cases the direction of the effect changed) due to incomplete current testing performed (i.e. not FISH or METex14sk testing).

With panel testing, a slightly higher proportion of patients were identified with actionable variants than presented in Section 2 and in Section 3.2.7. This was due to the ALK concordance data used (comparison to FISH alone with positivity defined as $\geq 15\%$ positive cells from Park and Shim 2020, rather than FISH \pm IHC). As this reflects the clinical utility standard, this approach was adopted in the base case analysis (see Section 3.2.3). Sensitivity analyses are presented using the ALK concordance data compared to FISH \pm IHC.

Small gene panel testing was associated with more patients with a known biomarker status due to completion of testing in more patients (as some current tests are restricted to advanced stage only).

3.3.2 Uncertainty analysis: model inputs, structure and assumptions

Scenario analyses

Scenario analyses have been conducted assuming that current single gene testing includes other markers currently in the process of being considered by MSAC for targeted therapies in the non-squamous or NOS NSCLC population.

Table 57 Scenario analyses including additional markers under consideration

	Inc. cost	Inc. eligible for targeted therapy	ICER
Base case Targeted therapies available for actionable variants in <i>EGFR</i> , <i>ALK</i> , <i>ROS1</i> and <i>MET</i> ex14sk	\$169.02	0.0225	\$7,496
Targeted therapy additionally available for <i>KRAS</i> G12C variants (MSAC 1669) ^a	\$169.02	0.0231	\$7,330
Targeted therapy additionally available for <i>NTRK</i> actionable variants (MSAC 1602)	\$86.03	0.0227	\$3,784
Targeted therapies additionally available for both <i>KRAS</i> G12C and <i>NTRK</i>	\$86.03	0.0232	\$3,700

ALK = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; ICER = incremental cost-effectiveness ratio; IHC = immunohistochemistry; *KRAS* G12C = Kirsten rat sarcoma viral oncogene homologue with a glycine-to-cysteine substitution at codon 12; *MET*ex14sk = *MET* proto-oncogene, receptor tyrosine kinase exon 14 skipping alterations; *NTRK* = neurotrophic tropomyosin receptor kinase; *ROS1* = ROS proto-oncogene 1.

^a All assumptions used to inform this analysis were based on the 1669 PSD (April 2022 MSAC Meeting). The analysis was conducted using a 13% prevalence of *KRAS* G12C variants, assuming no additional test cost and perfect test performance.

^b All assumptions used to inform this analysis were based on the 1602.1 PSD (November 2021 MSAC Meeting). Analysis conducted using a 0.02% prevalence of *NTRK* fusions. Testing was assumed to be restricted to advanced or metastatic disease, where an IHC triage process was used. IHC was assumed to have a test performance of 88% sensitivity and 96% specificity and cost of \$74.50. For those in whom IHC returns a positive result, 50% of testing is assumed to occur by NGS (fee \$1,200) and 50% using two FISH tests (combined fee of \$533).

Sensitivity analyses

Univariate sensitivity analyses were conducted around parameters included in the model. The results of key analyses are presented in Table 58, with all analyses performed presented in Table 92, Appendix F.

Table 58 Results of the key sensitivity analyses

	Inc. cost	Inc. eligible for targeted therapy	ICER	% change
Base case	\$169.02	0.0225	\$7,496	-
Proportion of patients with advanced disease (base case: 75.9%)				
100%	\$27.97	0.0297	\$941	-87%
50%	\$320.01	0.0149	\$21,530	187%
Timing of <i>MET</i> ex14sk testing (base case: after <i>EGFR</i>)				
At the same time as <i>EGFR</i>	\$109.54	0.0225	\$4,858	-35%
After <i>EGFR</i> (excluding block retrieval and consult costs)	\$252.67	0.0225	\$11,206	49%

	Inc. cost	Inc. eligible for targeted therapy	ICER	% change
Base case	\$169.02	0.0225	\$7,496	-
Small gene panel testing strategy (base case: mixed)				
All combined DNA/RNA panel testing	\$348.65	0.0228	\$15,277	104%
All two-stage DNA then RNA panel testing	\$116.98	0.0228	\$5,126	-32%
All DNA then IHC/FISH testing	-\$162.34	0.0173	Dominant	-225%
Test success (base case: 97.2% for panels, 94.6% for single-gene testing)				
Both strategies 97.2%	\$273.97	0.0216	\$12,662	69%
97.2% for panels, 95.9% ^a for single-gene testing	\$221.50	0.0221	\$10,026	34%
97.2% for panels, 92.0% ^b for single-gene testing	\$64.07	0.0235	\$2,731	-64%
ALK small gene panel concordance (base case: vs clinical utility standard, FISH ≥15% positivity)				
ALK small gene panel concordance vs FISH ± IHC #1	\$168.99	0.0219	\$7,730	3%
ALK small gene panel concordance vs FISH	\$169.00	0.0360	\$4,697	-37%
Small panel concordance				
ALK NPA, 0.97 (base case: 0.99)	\$169.02	0.0375	\$4,509	-40%
ALK NPA, 1.00 (base case: 0.99)	\$169.02	0.0166	\$10,162	36%
ALK PPA, 0.48 (base case: 1.00)	\$168.81	0.0114	\$14,848	98%
EGFR NPA, 0.95 (base case: 0.97)	\$161.36	0.0354	\$4,562	-39%
EGFR NPA, 0.99 (base case: 0.97)	\$176.69	0.0097	\$18,168	142%
METex14sk NPA, 0.93 (base case: 1.00)	\$138.59	0.0734	\$1,887	-75%
Rebiopsy uptake rate (base case: 100%)				
30%	\$254.94	0.0248	\$10,298	37%
60%	\$218.12	0.0238	\$9,161	22%
Average fee charged for EGFR and ALK and ROS1 FISH (base case: MBS Schedule Fees)	\$185.26	0.0225	\$8,217	10%
FISH utilisation, use IHC NPA data (base case: calibrated) ^c #2	\$183.78	0.0225	\$8,151	9%
Separate RNA small panel use, allowed with KRAS or BRAF #4 (base case: not allowed)	\$307.24	0.0225	\$13,627	82%
Proportion with KRAS or BRAF activating variants, 52% (base case: 30.8%)	\$73.89	0.0225	\$3,277	-56%
Rebiopsy cost, \$3,369 [all outpatient] #3 (base case: \$5,630 [all inpatient])	\$213.63	0.0225	\$9,475	26%
Multivariate analyses				
#1 AND #2	\$183.74	0.0219	\$8,404	12%
#1, #2 AND #3	\$228.35	0.0219	\$10,444	39%
#1, #2, #3 AND #4	\$366.57	0.0219	\$16,767	124%

^a Half the difference between test strategies

^b Double the difference between test strategies

^c Estimates of FISH use in the base case was calibrated to MBS utilisation data on the ratio of EGFR:ALK or ROS1 FISH services. The sensitivity analysis uses estimates based on biomarker prevalence and IHC specificity.

ALK = anaplastic lymphoma kinase; BRAF = proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B; DNA = deoxyribose nucleic acid; EGFR = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; ICER = incremental cost-effectiveness ratio; IHC = immunohistochemistry; KRAS = Kirsten rat sarcoma viral oncogene homologue; METex14sk = MET proto-oncogene, receptor tyrosine kinase exon 14 skipping alterations; NPA = negative percent agreement; NTRK = neurotrophic tropomyosin

receptor kinase; PPA = positive percent agreement; RNA = ribonucleic acid; *ROS1* = ROS proto-oncogene 1.

The analyses were most sensitive to the proportion of patients with advanced disease (as this affects the current costs offset), the small gene panel testing strategy used (including distribution of strategies used), differences in the test success rate, concordance of small gene panel testing (particularly NPA, which is assumed to increase incremental actionable variants), and rebiopsy rate. The analysis was also sensitive to the assumption that patients found to have *KRAS* and *BRAF* variants on the DNA panel only would not receive RNA small gene panel testing (and expected yield of these non-actionable variants). As described in Section 2B.2.4, instances of concurrent variants may be more common than previously thought. A prospective case series from Germany (Griesinger et al. 2021) reported that of all patients with *ROS1* and *ALK* variants identified, respectively, 23.7% (14/59) and 16.1% (19/118) also had variants in *BRAF* or *KRAS*.

A few assumptions included in the base case analysis may not be the most conservative approach. Justification has been provided to support the use of the estimates in the base case, however multivariate analyses are performed using alternate approaches identified. The results do suggest that the analyses are sensitive to the combined effects of these changes.

3.4 Conclusions

Small gene panel testing was observed to be associated with a small incremental cost per patient receiving testing. This was driven by the cost of proposed panel testing, with offsets due to a reduction in use and cost of current tests and rebiopsies. The assumption that RNA panels cannot be used where *KRAS* and *BRAF* activating variants are identified (which do not have associated targeted therapies) and assumptions regarding the use and cost of rebiopsy are key drivers of the incremental cost.

While small gene panel testing was associated with more patients with actionable variants eligible for targeted therapy, this was driven by an increase in patients with incremental actionable variants, not found through the test used in the trials of the targeted therapies. It is unclear whether these additional cases benefit from targeted therapies relative to the standard of care (immunotherapy ± chemotherapy).

While not quantified in this analysis, small gene panel testing is also likely to identify more non-actionable variants. Knowledge of such variants may permit treatment options such as compassionate access programs or clinical trials. These may provide considerable benefit to patients whose effective treatment options are otherwise limited.

In addition to the identification of more actionable and non-actionable variants, small gene panel testing may be associated with improved test turnaround time. The extent of this was difficult to quantify in the proposed setting, however currently some patients may experience delays in testing due to restrictions related to stage of disease able to be tested, whether current testing is pathologist determinable and the extent of some current testing that is performed more centrally. Small gene panel testing as proposed may resolve some of these issues.

The analysis was sensitive to the proportion of patients tested with (or who progress to) advanced disease. This proportion may decrease over time due to the proposed lung cancer screening program (MSAC Application 1699), and shift for use of immunotherapies into the adjuvant setting.

Section 4 Use of the health technology in practice

A market-share approach is used to estimate the extent of the current market for sequential tests in non-squamous NSCLC that will be substituted with the listing of the small gene panel. The relevant funding program is the MBS. Market growth is not anticipated.

4.1 Justification of the selection of approach and data sources

A summary of the data sources used in estimating the financial impact of small gene panel testing to the MBS is presented in Table 59.

Table 59 Data sources and parameter values applied in the utilisation and financial estimates

Data	Source and value
MBS statistics for item 73337, 2015–2021	Services Australia
Relative use of combined DNA/RNA testing	Applicant feedback provided during the preparation of the DCAR
Yield of variants identified through panel testing	Section 3
Proportion of samples with sufficient sample for RNA-based testing	1721 Application Form
Proportion of services of item 73337 that receive the 75% benefit	1161 and 1173 PSD, November 2016 MSAC Meeting
Stage at diagnosis	Victorian Cancer Registry data reported by Mitchell et al. (2013)
Proportion diagnosed Stage I–IIIA who progress	DUSC report on erlotinib and gefitinib (2017)
MBS statistics for item 73341, 2016–2021	Services Australia
MBS statistics for item 73344, 2019–2021	Services Australia
Proportion of services of item 73341 and 73344 that receive the 75% benefit	MBS Data

DNA = deoxyribose nucleic acid; DCAR = Department Contracted Assessment Report; DUSC = drug utilisation subcommittee; *EGFR* = epidermal growth factor receptor; MBS = Medicare Benefits Schedule; PSD = public summary document; RNA = ribonucleic acid.

4.2 Estimation of use and financial impact of the proposed health technology

EGFR testing is currently a pathologist determinable service that occurs on diagnosis of non-squamous NSCLC. As small gene panel testing is also proposed to be pathologist determinable on diagnosis of non-squamous NSCLC, the current market for *EGFR* testing would apply to that for small gene panel testing.

No growth in the market is anticipated with small gene panel testing, however a sensitivity analysis has been performed assuming growth in the market (for example if states are currently funding *EGFR* testing, and with the listing of small gene panels, this shifts to the MBS) (see Section 4.6).

MBS item statistics on the use of *EGFR* testing (item 73337) 2015–2021 are presented in Table 60.

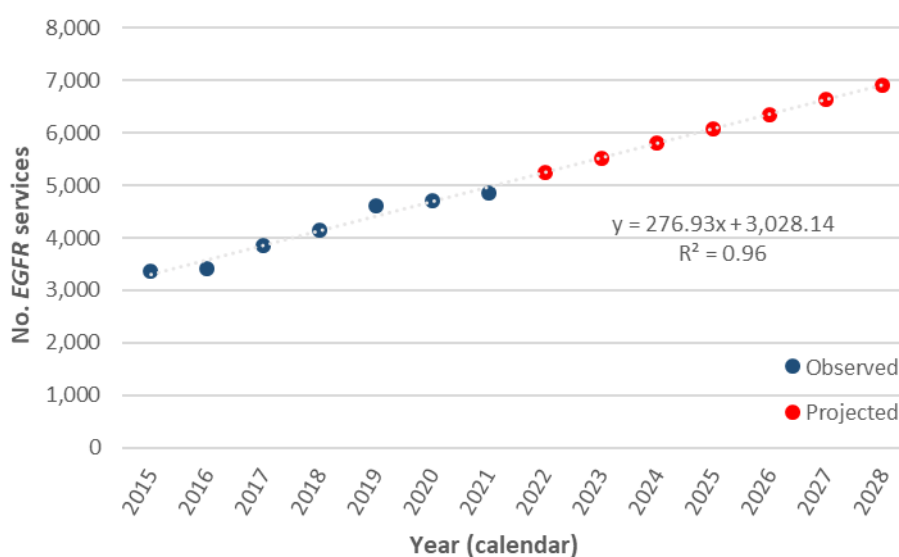
Table 60 Use of MBS item 73337, 2015–2021

	2015	2016	2017	2018	2019	2020	2021
No. services	3,368	3,419	3,863	4,147	4,603	4,697	4,854

Source: Services Australia; MBS = Medicare Benefits Schedule

Linear extrapolations of these data appear reasonable to project estimates of use 2023–2028 (Figure 18).

Figure 18 *EGFR* services, observed (2015–2021) and projected (2022–2028)



Source: 'MBS statistics' worksheet of the '1721 financial impact.xlsx' workbook accompanying the DCAR.
EGFR = epidermal growth factor receptor.

The projected number of *EGFR* services, 2023–2028 is presented in Table 61. In the base case, small gene panel testing is assumed to take 100% of the share of the market for testing. PASC noted that laboratory capacity will expand naturally with market forces over the next couple of years (and so expected that laboratories that currently perform *EGFR* testing would be able to provide small gene panel testing services).

Two funding approaches have been proposed for small gene panel testing in NSCLC:

- Combined DNA/RNA testing, where one provider can perform both DNA and RNA testing; and
- Two-step DNA, then RNA testing, should providers be unable to perform both (whether due to laboratory capacity or infrastructure, or due to quantity of sample available for RNA-based testing).

The financial impact analysis assumes that both approaches will be available, with the proportion of providers able to provide combined DNA/RNA testing based on applicant feedback. The share of services that use either the combined or two-step approach is presented in Table 61.

Table 61 Extent of use of small gene panel testing

		2023	2024	2025	2026	2027	2028
A	Projected use of item 73337	5,521	5,797	6,074	6,351	6,628	6,905
B	No. of small gene panel services [100% market share × A]	5,521	5,797	6,074	6,351	6,628	6,905
C	Proportion of services that use combined DNA/RNA testing	25.0%	25.0%	30.0%	35.0%	40.0%	50.0%
D	No. combined small panel DNA/RNA services [B × C]	1,380	1,449	1,822	2,223	2,651	3,453
F	No. services that use two-step DNA then RNA testing approach [B – D]	4,140	4,348	4,252	4,128	3,977	3,453
G	Small DNA panel services [F]	4,140	4,348	4,252	4,128	3,977	3,453
H	No. that require further testing [48.4% × G]	2,005	2,105	2,059	1,999	1,926	1,672
I	No. with sufficient sample for small RNA panel testing [95% × H]	1,905	2,000	1,956	1,899	1,829	1,588
	Total number of small gene panel services [D + G + I]	7,425	7,798	8,030	8,250	8,458	8,493

Source: 'Section 4.2' worksheet of the '1721 financial impact.xlsx' workbook accompanying the DCAR.
DNA = deoxyribose nucleic acid; RNA = ribonucleic acid.

In the two-step approach, RNA testing would only occur where no *EGFR*, *KRAS*, *BRAF* or *MET* exon 14 variants were identified through the small DNA panel. Variants are expected to be identified in 52% of patients (17.3% *EGFR*, 28.8% *KRAS*, 2.0% *BRAF* and 3.5% *MET* exon 14), and so further testing would be required in the remaining 48%. Expected yield from small gene panel testing was sourced from Section 3, including adjustments small gene panel concordance.

Due to the amount of sample required for RNA-based testing, some samples may not have sufficient tissue quantity or quality available (and so would require current IHC ± FISH tests to determine eligibility for targeted therapies). The analysis assumes that this would be the case in 5% of samples, as assumed in Section 3, based on information provided in the 1721 Application Form.

The cost of small panel testing varies depending on the approach adopted. The proposed schedule fee for the combined DNA/RNA panel is \$1,247.00. MBS data provided during the preparation of the DCAR relating to item 73337 indicate that 32% of services receive the 75% level of MBS benefit, and so this is also likely to apply for small gene panel testing. The weighted cost to the MBS per combined DNA/RNA service is \$1,087.92.¹⁹

Each component of two-step testing has a proposed schedule fee of \$682.35. The weighted cost to the MBS for either small panel DNA or small panel RNA testing is \$570.48.²⁰

The cost to the MBS of small gene panel testing is presented in Table 62.

¹⁹ 31.8% × \$935.25 [75% MBS benefit] + 68.2% × \$1,159.10 [85% MBS benefit]

²⁰ 31.8% × \$511.80 [75% MBS benefit] + 68.2% × \$594.45 [85% MBS benefit]

Table 62 Cost of small gene panel testing to the MBS

	2023	2024	2025	2026	2027	2028
No. combined DNA/RNA small panel services	1,380	1,449	1,822	2,223	2,651	3,453
Cost of combined DNA/RNA panel testing to the MBS (\$1,087.92 per service) ^a	\$1,501,460	\$1,576,778	\$1,982,517	\$2,418,382	\$2,884,375	\$3,756,107
No. small DNA panel services	4,140	4,348	4,252	4,128	3,977	3,453
Cost of DNA-based panel testing (\$568.17 per service) ^b	\$2,352,426	\$2,470,432	\$2,415,876	\$2,345,585	\$2,259,561	\$1,961,638
No. small RNA panel services	1,905	2,000	1,956	1,899	1,829	1,588
Cost of RNA-based panel testing (\$568.17 per service) ^b	\$1,082,137	\$1,136,421	\$1,111,325	\$1,078,990	\$1,039,418	\$902,371
Cost to the MBS of small gene panel testing	\$4,936,022	\$5,183,631	\$5,509,717	\$5,842,958	\$6,183,354	\$6,620,116

Source: 'Section 4.2' worksheet of the '1721 financial impact.xlsx' workbook accompanying the DCAR.

DNA = deoxyribose nucleic acid; RNA = ribonucleic acid.

^a 31.8% × \$935.25 [75% MBS benefit] +68.2% × \$1,159.10 [85% MBS benefit]

^b 31.8% × \$511.80 [75% MBS benefit] +68.2% × \$594.45 [85% MBS benefit]

4.3 Estimation of changes in use and financial impact of other health technologies

In addition to substitution of projected *EGFR* services estimated in Section 4.2, small gene panel testing will also change the use of other biomarker testing services included in the comparator, including *ALK* and *ROS1* FISH testing and *MET*ex14sk testing. While there may also be a change in the relative use of IHC testing items, PASC considered that the expected reduction in the cost of IHC testing for *ALK* and *ROS1* would not be straightforward to estimate (p10, 1634 Ratified PICO). The total number of IHC services is not likely to change with proposed small gene panel testing, and for many patients, the item claimed will not change (where the number of antibodies tested does not change the item being charged e.g. from use of ten to eight antibodies tested). A conservative approach has been adopted in the DCAR that assumes no reduction in cost of *ALK* and *ROS1* IHC testing. A sensitivity analysis is conducted that assumes all *EGFR* services would also be associated with a change in IHC item use (from 72849 [85% benefit: \$88.70] to 72847 [85% benefit: \$76.00], reduction in cost of \$12.70).

As *MET*ex14sk testing is not pathologist determinable and is restricted to use in the advanced setting, changes in use of block retrieval services have also been included in the analysis.

Change in use and cost of *EGFR* services

The number of *EGFR* services substituted by small gene panel testing was estimated in Section 4.2. The schedule fee for *EGFR* testing is \$397.35. As indicated in Section 4.2, 32% of services are expected to incur the 75% MBS benefit, and so the weighted cost of *EGFR* testing to the MBS is \$325.13.²¹

²¹ 31.8% × \$298.05 [75% MBS benefit] +68.2% × \$337.75 [85% MBS benefit]

Table 63 Reduction in use and cost of EGFR testing

	2023	2024	2025	2026	2027	2028
Projected reduction in EGFR services [Row B, Table 61]	5,521	5,797	6,074	6,351	6,628	6,905
Reduction in cost of EGFR testing to the MBS (\$325.13 per service) ^a	\$1,794,855	\$1,884,891	\$1,974,928	\$2,064,964	\$2,155,001	\$2,245,037

Source: 'Section 4.3' worksheet of the '1721 financial impact.xlsx' workbook accompanying the DCAR.

EGFR = epidermal growth factor receptor.

^a 31.8% × \$298.05 [75% MBS benefit] + 68.2% × \$337.75 [85% MBS benefit]

Change in use and cost of ALK and ROS1 FISH services

MBS statistics for the use of ALK FISH testing (item 73341), 2016–2021, are presented in Table 64.

Table 64 Use of MBS item 73341, 2016–2021

	2016	2017	2018	2019	2020	2021
No. services	188	305	292	201	222	216
As a proportion of EGFR services	5.5%	7.9%	7.0%	4.4%	4.7%	4.4%

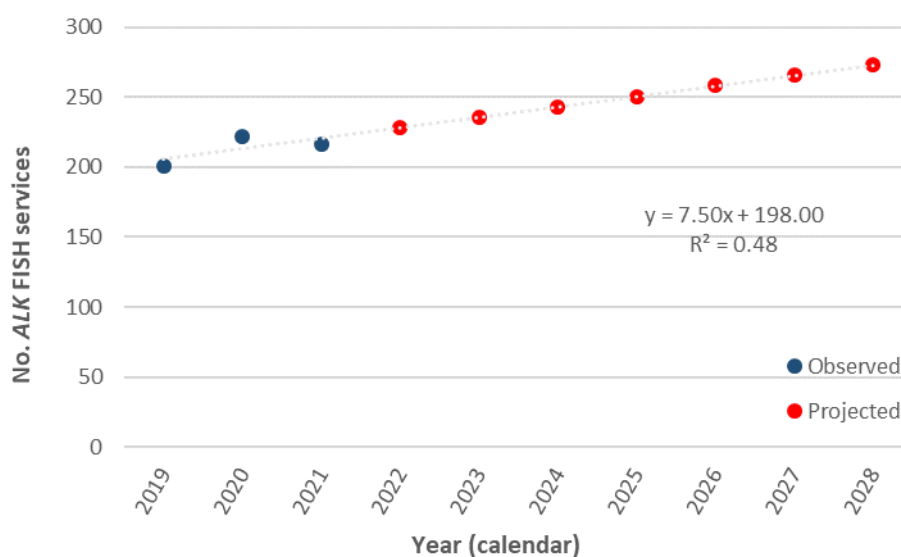
Source: 'MBS statistics' worksheet of the '1721 financial impact.xlsx' workbook accompanying the DCAR.

EGFR = epidermal growth factor receptor.

Two alternate approaches were considered for projecting ALK FISH use:

1. While fluctuations were noted in the number of ALK FISH services in the first few years of listing, these appear to have stabilised since 2019. Linear projections based on use since 2019 could be used (i.e. three calendar years). This approach projects an increase in services from 236 in 2023 to 273 in 2028 (Figure 19).

Figure 19 ALK FISH services, observed (2019–2021) and projected (2022–2028)^a [Option 1]



Source: 'MBS statistics' worksheet of the '1721 financial impact.xlsx' workbook accompanying the DCAR.

ALK = anaplastic lymphoma kinase; FISH = fluorescence *in situ* hybridisation.

^a Estimates of ALK FISH use were projected directly based on use observed in 2019–2021.

2. In addition to the number of ALK FISH services stabilising since 2019, so too has the ratio of ALK FISH to EGFR services (average 4.5%). This average estimate could be applied to the projected number of EGFR services. This approach projects that the number of ALK FISH services would increase from 249 in 2023 to 312 in 2028 (Table 65).

Table 65 ALK FISH projections, based on projected EGFR use [Option 2]

	2023	2024	2025	2026	2027	2028
Projected EGFR services	5,521	5,797	6,074	6,351	6,628	6,905
Projected ALK FISH use (4.5% of EGFR services)	249	262	274	287	299	312

Source: 'Section 4.3' worksheet of the '1721 financial impact.xlsx' workbook accompanying the DCAR.

ALK = anaplastic lymphoma kinase; EGFR = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation.

Given that a relationship between EGFR and ALK FISH use is expected and that there may be more confidence in EGFR service projections, Option 2 will be applied in the base case. The alternate approach is presented in a sensitivity analysis.

A similar approach is used to project the number of ROS1 FISH services that will be substituted by small gene panel testing. In the base case, the ratio of ROS1 FISH : EGFR services is applied to projected estimates of EGFR use. An alternate approach is tested in a sensitivity analysis applying the growth rate for ALK FISH testing estimated in Figure 19 (i.e. Option 1 for projecting ALK FISH estimates).

However not all FISH services may be able to be substituted by small gene panel testing. As described in Section 4.2, 5% of samples are assumed to have insufficient sample available for RNA-based testing, and would still require FISH. Assuming no change to the current wording of the FISH items, IHC testing would still be required prior to FISH, and so only those with a positive IHC result would require FISH. The IHC positivity rate was assumed to be 4.5% and 7.5% respectively, for ALK and ROS1, and was based on the ratio of FISH to EGFR services (as only those with positive IHC results would have had a FISH test requested). The estimated reduction in ALK and ROS1 FISH services is presented in Table 66.

The schedule fee for each FISH service is \$400.00. MBS data provided during the preparation of the DCAR indicate that 64.5% of FISH services received the 85% level of benefit. The weighted cost per FISH service to the MBS is \$325.80.²² The reduction in cost to the MBS due to changes in the use of ALK and ROS1 FISH testing are presented in Table 66.

²² $35.5\% \times \$300.00$ [75% MBS benefit] + $64.5\% \times \$340.00$ [85% MBS benefit]

Table 66 Reduction in use and cost of ALK and ROS1 FISH services

		2023	2024	2025	2026	2027	2028
J	Projected reduction in <i>EGFR</i> services [Row B, Table 61]	5,521	5,797	6,074	6,351	6,628	6,905
K	Projected estimates of <i>ALK</i> FISH use [4.5% × J]	249	262	274	287	299	312
L	No. with insufficient sample for small RNA panel testing [Row H – Row I, Table 61]	100	105	103	100	96	84
M	No. of insufficient samples that have an <i>ALK</i> IHC positive result [4.5% × L]	5	5	5	5	4	4
N	Reduction in <i>ALK</i> FISH services [K – M]	245	257	270	282	295	308
O	Reduction in cost of <i>ALK</i> FISH testing to the MBS (\$325.80 per service) ^a	\$79,720	\$83,719	\$87,826	\$91,943	\$96,070	\$100,330
P	Projected estimates of <i>ROS1</i> FISH use [7.5% × J]	415	436	457	478	499	519
Q	No. of insufficient samples that have a <i>ROS1</i> IHC positive result [7.5% × L]	8	8	8	8	7	6
R	Reduction in <i>ROS1</i> FISH services [O – P]	408	428	449	470	491	513
S	Reduction in cost of <i>ROS1</i> FISH testing to the MBS (\$325.80 per service) ^a	\$132,813	\$139,476	\$146,318	\$153,177	\$160,053	\$167,150

Source: 'Section 4.3' worksheet of the '1721 financial impact.xlsx' workbook accompanying the DCAR.

ALK = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; RNA = ribonucleic acid; *ROS1* = ROS proto-oncogene 1.

^a 35.5% × \$300.00 [75% MBS benefit] +64.5% × \$340.00 [85% MBS benefit]

Change in use and cost of *MET*ex14sk services

MET exon 14 skipping testing was recently recommended by MSAC in November 2021 and is yet to be included on the MBS. Estimates of use were redacted in the 1660 PSD. As MSAC recommended that *MET*ex14sk testing not be a pathologist determinable test, testing is assumed to occur after *EGFR* testing. Patients who are diagnosed at an earlier stage of disease would only be eligible for *MET*ex14sk testing on progression to Stage IIIB/IV disease. The distribution of disease stage at diagnosis was based on a retrospective analysis of Victorian Cancer Registry data (Mitchell et al. 2013). This study reported that 65.5% of patients had Stage IIIB/IV disease at diagnosis, with the remaining Stages I–IIIA. Of those diagnosed at an earlier stage of disease, 30% are assumed to experience progression to Stage IIIB/IV disease, as per a DUSC report published in 2017 on erlotinib and gefitinib use. For simplicity, progression is assumed to occur within the same year as lung cancer diagnosis. *MET*ex14sk testing is assumed to occur only in the proportion of patients who do not have *EGFR* variants previously identified.

The fee for the proposed *MET*ex14sk testing MBS item supported by MSAC in November 2021 was \$397.35. As testing is not proposed to be a pathologist determinable service, all services are assumed to incur the 85% benefit.

The reduction in use and cost of *MET*ex14sk testing is presented in Table 67.

Table 67 Reduction in use and cost of *METex14sk* services

		2023	2024	2025	2026	2027	2028
T	Projected reduction in <i>EGFR</i> services [Row B, Table 61]	5,521	5,797	6,074	6,351	6,628	6,905
U	No. <i>EGFR</i> tests conducted in advanced disease patients [65.5% × T]	3,616	3,797	3,979	4,160	4,341	4,523
V	No. of patients with advanced disease and who are <i>EGFR</i> -negative [85% ^a × U]	3,074	3,228	3,382	3,536	3,690	3,844
W	No. services in patients that progress to advanced disease (assumed within same year) and who do not have <i>EGFR</i> variants [34.5% × 30% × 85% × T] ^b	486	510	534	559	583	607
X	Reduction in use of <i>METex14sk</i> testing [V + W]	3,559	3,738	3,916	4,095	4,273	4,452
Y	Reduction in cost of <i>METex14sk</i> testing to the MBS (\$337.75 per service) ^c	\$1,202,121	\$1,262,424	\$1,322,727	\$1,383,030	\$1,443,333	\$1,503,635

Source: 'Section 4.3' worksheet of the '1721 financial impact.xlsx' workbook accompanying the DCAR.

EGFR = epidermal growth factor receptor; *METex14sk* = MET proto-oncogene, receptor tyrosine kinase exon 14 skipping alterations.

^a Yield of common *EGFR* variants identified through single gene testing

^b Proportion early stage at diagnosis (34.5%) × proportion that progress (30%) × proportion in whom *EGFR* variants were not identified (85%)

^c 100% × \$337.75 [85% MBS benefit]

Changes in the use and cost of other MBS services

In addition to changes in substituted biomarker testing services, a change in use of related services may also occur with the introduction of small gene panel testing. A reduction in the use of block retrieval services is expected with each *METex14sk* test, as testing is not proposed to be listed as a pathologist determinable service.

Patients who have early stage disease at diagnosis would have tissue samples archived. On progression to Stage IIIB/IV disease, a service is required to retrieve and review archived FFPE blocks for the purpose of conducting genetic testing (MBS item 72860) (including *METex14sk* testing and *ALK* or *ROS1* FISH testing, if required). This is assumed to occur in all patients who progress and in whom an *EGFR* variant had not previously been identified (i.e. Row W, Table 67).

Table 68 Reduction in use and cost of block retrieval services

	2023	2024	2025	2026	2027	2028
Estimated reduction in block retrieval services [Row X, Table 67]	3,559	3,738	3,916	4,095	4,273	4,452
Reduction in cost of block retrieval to the MBS (\$72.25 per service) ^a	\$257,153	\$270,052	\$282,952	\$295,852	\$308,751	\$321,651

Source: 'Section 4.3' worksheet of the '1721 financial impact.xlsx' workbook accompanying the DCAR.

EGFR = epidermal growth factor receptor.

^a 100% × \$72.25 [85% MBS benefit]

4.4 Net financial impact to the MBS

The estimated net financial impact to the MBS is presented in Table 69.

Table 69 Net financial implications of small gene panel testing to the MBS

Parameter	2023	2024	2025	2026	2027	2028
Estimated use and cost of the proposed health technology						
Size of the <i>EGFR</i> testing market	5,521	5,797	6,074	6,351	6,628	6,905
Share of the <i>EGFR</i> testing market (100%)	5,521	5,797	6,074	6,351	6,628	6,905
Number of services of small gene panel testing	7,425	7,798	8,030	8,250	8,458	8,493
• Combined DNA/RNA (MBS benefit: \$1,087.92) ^a	1,380	1,449	1,822	2,223	2,651	3,453
• DNA only (MBS benefit: \$568.17) ^b	4,140	4,348	4,252	4,128	3,977	3,453
• RNA only (MBS benefit: \$568.17) ^b	1,905	2,000	1,956	1,899	1,829	1,588
Cost to the MBS	\$4,936,022	\$5,183,631	\$5,509,717	\$5,842,958	\$6,183,354	\$6,620,116
Change in use and cost of other health technologies						
Reduction in use of comparator testing services						
• <i>EGFR</i> (MBS benefit: \$325.13) ^c	5,521	5,797	6,074	6,351	6,628	6,905
• <i>ALK</i> FISH (MBS benefit: \$325.80) ^d	245	257	270	282	295	308
• <i>ROS1</i> FISH (MBS benefit: \$325.80) ^d	408	428	449	470	491	513
• <i>METex14sk</i> (MBS benefit: \$337.75) ^e	3,559	3,738	3,916	4,095	4,273	4,452
Reduction in use of block retrieval services (MBS benefit: \$72.25) ^f	3,559	3,738	3,916	4,095	4,273	4,452
Net change in costs to the MBS	\$3,466,662	\$3,640,562	\$3,814,751	\$3,988,966	\$4,163,208	\$4,337,803
Net financial impact to the MBS	\$1,469,360	\$1,543,069	\$1,694,966	\$1,853,992	\$2,020,147	\$2,282,313

Source: 'Section 4.4' worksheet of the '1721 financial impact.xlsx' workbook accompanying the DCAR.

ALK = anaplastic lymphoma kinase; DNA = deoxyribose nucleic acid; *EGFR* = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; *METex14sk* = MET proto-oncogene, receptor tyrosine kinase exon 14 skipping alterations; RNA = ribonucleic acid; *ROS1* = ROS proto-oncogene 1.

^a 31.8% × \$935.25 [75% MBS benefit] + 68.2% × \$1,159.10 [85% MBS benefit]. Split of use based on MBS data for use of *EGFR* services.

^b 31.8% × \$511.80 [75% MBS benefit] + 68.2% × \$594.45 [85% MBS benefit]. Split of use based on MBS data for use of *EGFR* services.

^c 31.8% × \$298.05 [75% MBS benefit] + 68.2% × \$337.75 [85% MBS benefit]. Split of use based on MBS data for use of *EGFR* services.

^d 35.5% × \$300.00 [75% MBS benefit] + 64.5% × \$340.00 [85% MBS benefit]. Split of use based on MBS data for use of *ALK* or *ROS1* FISH services.

^e 100% × \$337.75 [85% MBS benefit]. As proposed *METex14sk* testing has not been proposed to be a pathologist determinable test, all services have been assumed to be requested in the outpatient setting.^f 100% × \$72.25 [85% MBS benefit]. Assumed for each *METex14sk* test which has been assumed to be requested in the outpatient setting.

4.5 Net financial impact to other health budgets

The net financial impact of listing small gene panel testing to the Commonwealth budget is estimated as presented in Section 4.4. While small gene panel testing may lead to an increase in use (and therefore cost) of targeted therapies attributed to the PBS, the changes in cost are difficult to quantify due to existing special pricing arrangements that exist for both targeted therapy and SoC. As described in Sections 2 and 3, it is unclear whether patients found with incremental actionable variants would benefit from targeted therapies relative to SoC, and whether these would result in a change in treatment.

An estimate of the change in patients eligible for targeted therapies is presented in Table 70. This includes both “in scope” variants, due to detection of lower variant allelic frequency, and “beyond restriction” variants. These were unable to be distinguished from one another in the analysis.

Table 70 Change in patients eligible for targeted therapies

	2023	2024	2025	2026	2027	2028
Patients tested	5,521	5,797	6,074	6,351	6,628	6,905
Proportion with advanced disease (75.9%) ^a	4,187	4,397	4,607	4,817	5,028	5,238
Patients with advanced disease identified with <i>EGFR</i>						
Proposed (<i>EGFR</i> yield: 17.3%) ^b	722	759	795	831	867	903
Current (<i>EGFR</i> yield: 15.0%) ^c	628	660	691	723	754	786
Change in patients eligible for <i>EGFR</i> TKI	94	99	104	108	113	118
Patients with advanced disease identified with <i>ALK</i>						
Proposed (<i>ALK</i> yield: 3.8%) ^b	160	168	176	184	192	200
Current (<i>ALK</i> yield: 3.0%) ^c	124	130	136	142	148	155
Change in patients eligible for <i>ALK</i> TKI	37	38	40	42	44	46
Patients with advanced disease identified with <i>ROS1</i>						
Proposed (<i>ROS1</i> yield: 1.4%) ^b	58	61	64	67	70	73
Current (<i>ROS1</i> yield: 1.5%) ^c	64	67	71	74	77	80
Change in patients eligible for <i>ROS1</i> TKI	-6	-6	-7	-7	-7	-8
Patients with advanced disease identified with <i>METex14sk</i>						
Proposed (<i>METex14sk</i> yield: 3.5%) ^b	148	155	163	170	177	185
Current (<i>METex14sk</i> yield: 3.6%) ^c	151	158	166	173	181	189
Change in patients eligible for <i>METex14sk</i> TKI	-3	-3	-3	-3	-4	-4
Change in patients eligible for targeted therapy	122	128	134	140	146	152

Source: 'Section 4.3' worksheet of the '1721 financial impact.xlsx' workbook accompanying the DCAR.

^a Based on Mitchell et al. (2013) which reported that 65.5% of patients had Stage IIIB/IV disease at diagnosis. Of the remaining 34.5% of patients, 30% are assumed to experience progression to Stage IIIB/IV disease, as per a DUSC report published in 2017 on erlotinib and gefitinib use.

^b Clinical utility standard yield × PPA of small gene panel testing + (1 – clinical utility standard yield) × (1 – NPA of small gene panel testing), where clinical utility standard yield and PPA and NPA of small gene panel testing are as used in the economic analysis (Table 46, Section 3.2.4).

^c Clinical utility standard yield as used in the economic analysis (Table 46, Section 3.2.4).

ALK = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; *METex14sk* = MET proto-oncogene, receptor tyrosine kinase exon 14 skipping alterations; NPA = negative percent agreement; PPA = positive percent agreement; *ROS1* = ROS proto-oncogene 1; TKI = tyrosine kinase inhibitor.

4.6 Identification, estimation and reduction of uncertainty in the financial estimates

Analyses that explore areas of uncertainty identified in the estimation of the net financial impact to the MBS of listing small gene panel testing is presented in Table 71.

Table 71 Sensitivity analyses around the net financial implications of small gene panel testing to the MBS

	2023	2024	2025	2026	2027	2028
Base case	\$1,469,360	\$1,543,069	\$1,694,966	\$1,853,992	\$2,020,147	\$2,282,313
Assume small panel listing increases market for <i>EGFR</i> testing by 20% (base case: no market growth)	\$2,457,351	\$2,580,621	\$2,797,716	\$3,023,367	\$3,257,573	\$3,606,992
Small gene panel testing strategy (base case: mixed)						
All combined panels	\$2,535,246	\$2,662,423	\$2,789,601	\$2,916,778	\$3,043,955	\$3,171,132
All sequential DNA then RNA	\$1,114,065	\$1,169,951	\$1,225,836	\$1,281,722	\$1,337,608	\$1,393,493
All DNA followed by IHC/FISH	-\$229,209	-\$240,707	-\$252,205	-\$263,703	-\$275,201	-\$286,699
Proportion requiring IHC/FISH, 10% (base case: 5%)	\$1,416,336	\$1,487,385	\$1,640,512	\$1,801,122	\$1,969,216	\$2,238,097
Allowing RNA panel testing in <i>KRAS</i> and <i>BRAF</i> (base case: not allowed)	\$2,160,180	\$2,268,543	\$2,404,419	\$2,542,803	\$2,683,696	\$2,858,373
<i>ALK</i> projection approach (base case: relative use of <i>EGFR:ALK</i> FISH)	\$1,473,829	\$1,549,167	\$1,702,693	\$1,863,348	\$2,031,133	\$2,294,929
<i>ROS1</i> projections assuming <i>ALK</i> growth rate (base case: relative use of <i>EGFR:ROS1</i> FISH)	\$1,467,518	\$1,543,646	\$1,697,961	\$1,859,406	\$2,027,980	\$2,292,565
Exclude block retrieval with <i>METex14sk</i> testing (base case: included with each service)	\$1,726,513	\$1,813,121	\$1,977,918	\$2,149,843	\$2,328,898	\$2,603,964
<i>METex14sk</i> testing at the same time as <i>EGFR</i> (base case: after in advanced patients only)	\$665,229	\$698,600	\$810,158	\$928,846	\$1,054,663	\$1,276,491
Assume IHC item changed from 72849 to 72847 in all patients, cost to MBS difference \$12.70 (base case: no change)	\$1,399,250	\$1,469,442	\$1,617,821	\$1,773,330	\$1,935,968	\$2,194,618

Source: 'Section 4.4' worksheet of the '1721 financial impact.xlsx' workbook accompanying the DCAR.

ALK = anaplastic lymphoma kinase; *BRAF* = proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B; DNA = deoxyribose nucleic acid; *EGFR* = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; IHC = immunohistochemistry; *KRAS* = Kirsten rat sarcoma viral oncogene homologue; *METex14sk* = *MET* proto-oncogene, receptor tyrosine kinase exon 14 skipping alterations; RNA = ribonucleic acid; *ROS1* = *ROS* proto-oncogene 1.

^a 31.8% × \$935.25 [75% MBS benefit] +68.2% × \$1,159.10 [85% MBS benefit]

^b 31.8% × \$511.80 [75% MBS benefit] +68.2% × \$594.45 [85% MBS benefit]

The net financial impact estimates were most sensitive to the distribution of use of combined or sequential small gene panels and whether separate RNA panels are allowed in those found to have *KRAS* or *BRAF* activating variants. If there is substantial growth in the market due to the

listing of small gene panels (e.g. if some testing currently is being funded through the state system, and this shifts to the MBS), then the net impact to the MBS may be higher. However the extent of this shift is unknown.

Section 5 Other relevant information

Redacted.

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Appendix A Systematic review methods

Method of assessment and research questions

The assessment framework used to link the test population through to health outcomes is shown in Figure 20. The assessment identified a very small amount of predictive evidence which linked testing through to health outcomes (component 1 in the figure below). The assessment also examined the claims regarding superiority of NGS based on turnaround time, the efficient use of tumour tissue, and the detection of concurrent variants.

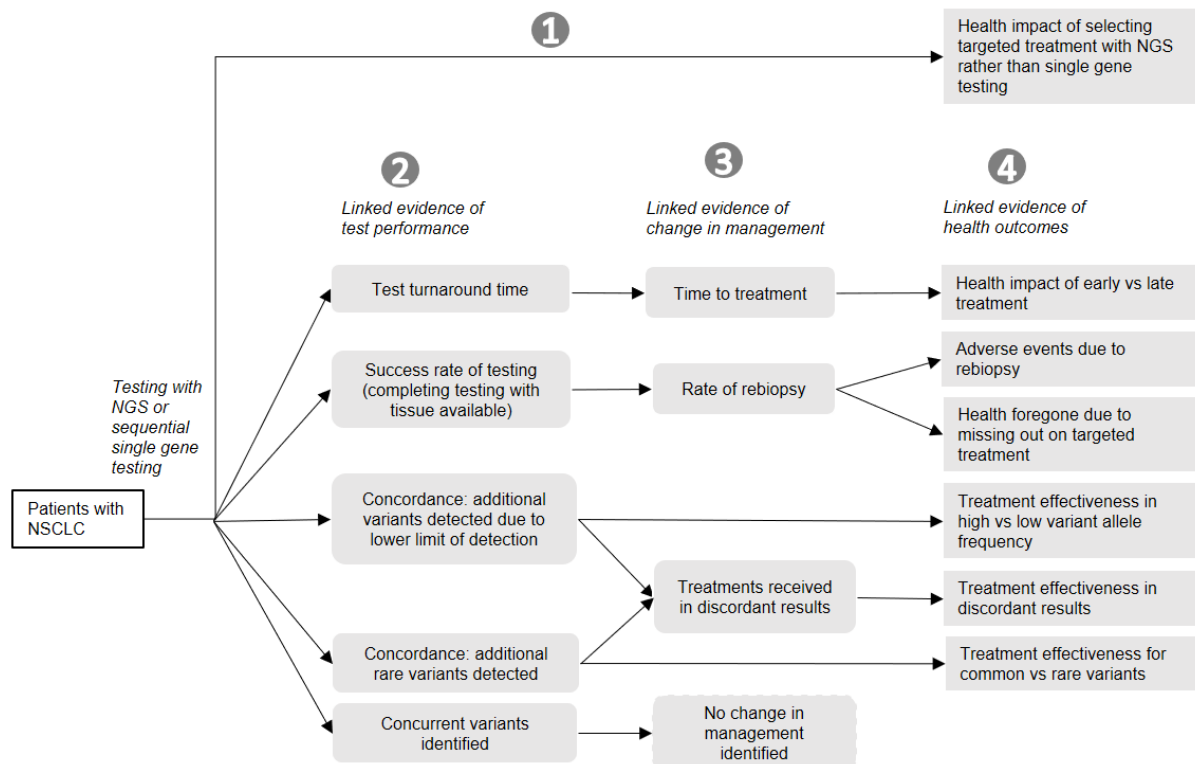


Figure 20 Assessment framework for small DNA/RNA NGS panel for NSCLC

Figure notes: 1: direct from test to health outcomes evidence; 2: test performance; 3: change in treatment/management; 4: influence of the change in management on health outcomes

Systematic review questions

DIRECT FROM TEST TO HEALTH OUTCOMES EVIDENCE

1. Does the use of a small NGS DNA/RNA panel in place of sequential single gene testing result in the claimed superior health outcomes?
2. What are the adverse events associated with the small NGS DNA/RNA panel(s) and the sequential single gene testing?

LINKED EVIDENCE

3. How does the information from the NGS DNA/RNA panel differ from that of sequential single gene testing? What is the concordance of the findings from NGS DNA/RNA panel relative to sequential single gene testing (in particular, the clinical utility standard)?
 - a. If there are multiple tests in clinical practice likely to be able to utilise the same funding arrangements, are these tests concordant with the proposed test and/or clinical utility standard?
4. How does use of small NGS DNA/RNA panel(s) alter the management of patients compared to sequential single gene testing?
5. Do the differences in the management derived from small NGS DNA/RNA panel(s), relative to sequential single gene testing (e.g. differences in rate of biopsies, type of treatment received, or timing of treatment), result in the claimed health outcomes?
 - a. If NGS DNA/RNA panel results in targeted treatment being provided to a broader population than the clinical utility standard identifies, what are the health outcomes associated with this treatment? Is it biologically plausible that the size of effect from the targeted treatment will be as effective in this population as those identified from the clinical utility standard?
6. What are the adverse events associated with rebiopsies?

Development of a research protocol

Prior to the start of the systematic review, a research protocol was developed, based on the PICO confirmation ratified by the PICO Advisory Sub-Committee of MSAC for MSAC assessment 1634 (Comprehensive genomic profiling in NSCLC) and MSAC assessment 1495 (Somatic tumour panel testing in NSCLC). The research protocol was registered with the international prospective register of systematic reviews (PROSPERO) with the registration number CRD42022334620.

PICO criteria

The Prior tests, Population, Investigation/Index test, Comparator and Outcomes (PPICO) that were prespecified to guide the systematic literature review for direct evidence are presented in Table 72.

Table 72 PPICO criteria for assessing small DNA/RNA panel(s) for NSCLC (direct from test to health outcomes evidence)

Component	Description
Patients	Patients with non-squamous (or histology not otherwise specified) non-small cell lung cancer (NSCLC)
Prior tests	Disease staging and histology workup. This is part of routine management and there would be no change between the intervention and comparator
Intervention	1. Small NGS panel to simultaneously test DNA/RNA for relevant point variants/small indels or fusions in the following genes: <i>EGFR</i> , <i>ALK</i> , <i>ROS1</i> , and <i>METex14sk</i> ; or 2. NGS DNA panel for relevant point variants/small indels in the following genes: <i>EGFR</i> and <i>METex14sk</i> . If negative, then an RNA panel for fusions in the following genes: <i>ALK</i> and <i>ROS1</i> . Testing performed on tumour tissue or cytology (not liquid biopsy).
Comparator	Sequential single gene testing for activating mutations in the <i>EGFR</i> gene, <i>METex14sk</i> , <i>ALK</i> IHC and <i>ROS1</i> IHC, with subsequent <i>ALK</i> FISH and/or <i>ROS1</i> FISH as appropriate Testing performed on tumour tissue or cytology (not liquid biopsy).
Outcomes	Safety outcomes

Component	Description
	Any adverse events related to treatment, repeated biopsies, adverse effects of delayed treatment due to time taken to test Effectiveness outcomes Disease-free and/or overall survival, disease-related or and/or all-cause mortality, disease progression, tumour control (regression/remission), incidence of metastases, tumour recurrence, quality of life, and other patient-relevant outcomes Healthcare resources Cost Cost-effectiveness Net Australian Government healthcare costs
Study design	Randomised or non-randomised controlled trials, comparative studies with or without concurrent controls, or systematic reviews of these study designs
Language	Studies in languages other than English will only be translated if they represent a higher level of evidence than that available in the English language evidence-base
Search period	Database inception – 9/5/22
Systematic review question: What is the safety, effectiveness and cost-effectiveness of small NGS DNA/RNA panel(s) compared to sequential single gene testing in patients with non-squamous (or histology not otherwise specified) non-small cell lung cancer?	

ALK = anaplastic lymphoma kinase; DNA = deoxyribonucleic acid; EGFR = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; IHC = immunohistochemistry; KRAS = Kirsten rat sarcoma; MET = mesenchymal-epithelial transition; NGS = next generation sequencing; NTRK = neurotrophic tyrosine receptor kinase; RET = rearranged during transfection; RNA = ribonucleic acid; ROS1 = ROS proto-oncogene 1

The Population, Prior tests, Investigation/Index test, Comparator and Outcomes (PPICO) that were prespecified to guide the systematic literature review for a linked evidence approach are presented in Table 73 and Table 74

Table 73 PPICO criteria for assessing small DNA/RNA panel(s) versus sequential testing in patients with NSCLC (linked evidence for test performance and change in management)

Component	Description
Patients	Patients with non-squamous (or histology not otherwise specified) non-small cell lung cancer (NSCLC)
Prior tests	Disease staging and histology workup. This is part of routine management and there would be no change between the intervention and comparator
Intervention	1. Small NGS panel to simultaneously test DNA/RNA for relevant point variants/small indels or fusions in the following genes: EGFR, ALK, ROS1, and METex14sk; or 2. NGS DNA panel for relevant point variants/small indels in the following genes: EGFR and METex14sk. If negative, then an RNA panel for fusions in the following genes: ALK and ROS1. Testing performed on tumour tissue or cytology (not liquid biopsy).
Comparator	Sequential single gene testing for activating mutations in the EGFR gene, METex14sk, ALK IHC and ROS1 IHC, with subsequent ALK FISH and/or ROS1 FISH as appropriate Testing performed on tumour tissue or cytology (not liquid biopsy).
Clinical utility standards	EGFR cobas® real time PCR test ALK FISH ROS1 FISH METex14sk by RNA or DNA testing on plasma or tumour tissue
Outcomes	Test performance Positive percent agreement and negative percent agreement of DNA/RNA assays against the clinical utility standards

Component	Description
	Positive predictive value and negative predictive value of DNA/RNA assays against the clinical utility standards Concordance between DNA/RNA assays and comparator biomarker assays Test turnaround time Test failure rate / inadequate sample rate (e.g. from an inadequate cytological specimen) Change in management Any changes in management between small DNA/RNA panel(s) and sequential testing (e.g. rebiopsy rate, timing of treatment initiation, different treatments received due to FP, FN)

Systematic review questions:

How does the information from the small NGS DNA/RNA panels(s) differ from that of sequential single gene testing for patients with non-squamous (or histology not otherwise specified) NSCLC? What is the concordance of findings from NGS DNA/RNA panel relative to the clinical utility standards?

How does use of small NGS DNA/RNA panel(s) alter the management of patients compared to sequential single gene testing for patients with non-squamous (or histology not otherwise specified) NSCLC?

ALK = anaplastic lymphoma kinase; *DNA* = deoxyribonucleic acid; *EGFR* = epidermal growth factor receptor; *FISH* = fluorescence *in situ* hybridisation; *FN* = false negative (missed on NGS but detected on clinical utility standard); *FP* = false positive (identified by NGS but missed by clinical utility standard); *IHC* = immunohistochemistry; *KRAS* = Kirsten rat sarcoma; *MET* = mesenchymal-epithelial transition; *NGS* = next generation sequencing; *NTRK* = neurotrophic tyrosine receptor kinase; *RET* = rearranged during transfection; *RNA* = ribonucleic acid; *ROS1* = ROS proto-oncogene 1

The changes in management expected to be identified due to the patients undergoing testing with a small NGS panel rather than single gene testing, is a reduction in the rate of rebiopsies, and slightly earlier treatment initiation.

For results which are discordant between the NGS panel and the clinical utility standards:

- Those who are “false negatives” (i.e., biomarker identified by the clinical utility standard and not detected by NGS), these patients are assumed to benefit from targeted treatment, as established by co-dependent MSAC-PBAC submissions.
- Those who are “false positives” (i.e., biomarker not identified by the clinical utility standard, but is detected by NGS panel), the effectiveness of targeted treatment in this population will be sought. If no information is available on this population on the effectiveness of targeted versus non-targeted treatment, studies will be sought that compare health outcomes for those with variants/fusions identified by the clinical utility standard, and those with variants/fusions only identified by NGS.

Table 74 PICO criteria for assessing the health impact of changes in management associated with small DNA/RNA panel(s)

Component	Description		
Population	Patients with non-squamous (or histology not otherwise specified) non-small cell lung cancer (NSCLC) <i>and</i> with biomarkers identified by NGS but not by clinical utility standard	Patients with non-squamous (or histology not otherwise specified) non-small cell lung cancer (NSCLC)	
Intervention	Targeted treatment	Early treatment	Reduced rate of rebiopsy
Comparator	Non-targeted treatment	Late treatment	Increased rate of rebiopsy
Outcomes	Health outcomes:	Health outcomes:	Safety

Component	Description		
	Health outcome changes based on increase in number of patients eligible for PBS-listed targeted therapies	Health outcome changes based on earlier commencement of treatment	Harms (physical and/or psychological) due to rebiopsy
	Safety Adverse events from changes in management		
Systematic review questions:			
What impact do the changes in management from small NGS DNA/RNA panel have on health outcomes?			
Do the differences in the management derived from small NGS DNA/RNA panel(s), relative to sequential single gene testing (e.g. differences in rate of biopsies, type of treatment received, or timing of treatment), result in the claimed health outcomes?			
If NGS DNA/RNA panel results in targeted treatment being provided to a broader population than the clinical utility standard identifies, what are the health outcomes associated with this treatment? Is it biologically plausible that the size of effect from the targeted treatment will be as effective in this population as those identified from the clinical utility standard?			
What are the adverse events associated with rebiopsies?			

DNA = deoxyribonucleic acid; NGS = next generation sequencing; NSCLC = non-small cell lung cancer; RNA = ribonucleic acid

Literature sources and search strategies

The medical literature was searched on 9th May 2022 to identify relevant studies and systematic reviews published during the period database inception to May 2022. Searches were conducted of the databases and sources described in Table 76. Search terms are described in Table 75. The search strategy was tested using the SearchRefinery tool (Scells & Zuccon 2018), using relevant articles from DCAR 1495 and application 1721 as the seed citations.

Table 75 Search terms used PubMed platform

Category	Description	Search terms
Study design (if justified)	not restricted	-
Population	Non-squamous NSCLC	(NSCLC OR "non-small cell lung" OR "non small cell lung cancer" OR "non small cell lung carcinoma") AND
Intervention	Small RNA/DNA panels using NGS	((("next generation sequencing" OR NGS OR (gene* OR molecular OR DNA OR RNA OR comprehensive) AND (panel OR profile OR profiling OR sequencing OR test) OR transcriptome) AND
Comparator		-
Outcomes		("treatment selection" or actionable OR targets OR targeted OR personalised OR personalized OR precision OR biomarker OR companion OR tailored OR "therapeutic options" OR clinical utility OR comparative OR comparison OR cost OR yield OR time)
Limits		Humans; written in English unless the English written abstract indicates the article may be of higher level than other evidence identified.

DNA = deoxyribonucleic acid; NGS = next generation sequencing; NSCLC = non-small cell lung cancer; RNA = ribonucleic acid

Table 76 Record of search strategies

Source	Date span of search
MEDLINE (via PubMed)	database inception – 9 th May 2022
EMBASE (e.g. Embase.com)	database inception – 9 th May 2022
Cochrane Library ^a	database inception – 9 th May 2022
ClinicalTrials.gov	1 st June 2022
International Clinical Trials Registry Platform ^b	1 st June 2022
Australian Clinical Trials Registry	1 st June 2022
INAHTA HTA database	1 st June 2022
Prospective Register of Systematic Reviews (PROSPERO)	20 th May 2022
Backward and forward citation chasing	1 st June 2022

^a Includes the Cochrane Database of Systematic Reviews, the Cochrane Central Register of Controlled Trials

^b [International Clinical Trials Registry Platform](https://www.who.int/clinical-trials-registry-platform)²³

Citation Chaser was used to perform backwards and forwards citations (pearling and snowballing of reference lists) (Haddaway, Grainger & Gray 2021).

Study selection

Studies were selected by two reviewers. A single reviewer assessed every citation using Endnote, and a second reviewer assessed the most relevant 50% of citations, using Rayyan (relevance determined using Rayyan's algorithms). Citations reviewed as potentially relevant by either reviewer, based on title and/or abstract, were retrieved for full text assessment.

A single reviewer assessed full text articles.

Studies that may have met the inclusion criteria but contained insufficient or inadequate data for inclusion (such as conference abstracts) are listed as excluded studies in Appendix C. All other studies that met the inclusion criteria are listed in Appendix B.

Appraisal of the evidence

Appraisal of the evidence was conducted in 4 stages:

Stage 1: Appraisal of the risk of bias within individual studies (or systematic reviews) included in the review. <Some risk of bias items were assessed for the study as a whole, while others were assessed at the outcome level>.

- Systematic reviews were appraised using the AMSTAR-2 tool.
- Studies reporting concordance were appraised using the QUADAS-2 tool.
- Had any randomised trial been available, they would have been appraised using the Cochrane Risk of Bias 2.0 tool.
- Cohort studies comparing outcomes between testing strategies (i.e. comparing rate of rebiopsy or turnaround time) were evaluated using the SIGN methodology checklist for cohort studies.
- Studies comparing health outcomes between subgroups receiving targeted treatment due to being identified with the biomarker with the comparator/clinical utility standard, vs

²³ www.who.int/clinical-trials-registry-platform

those receiving targeted treatment due to being identified with the biomarker due to NGS were evaluated using the QUIPS tool for prognosis.

Stage 2: Appraisal of the precision, size of effect and clinical importance of the results reported in the evidence base as they relate to the prespecified primary outcomes for this assessment <and determining the assumed baseline risk>.

Stage 3: Rating the overall quality of the evidence per outcome, across studies, based on the study limitations (risk of bias), imprecision, inconsistency of results, indirectness of evidence and the likelihood of publication bias (Appendix E Evidence profile tables).

Stage 4: Integration of this evidence (across outcomes) for conclusions about the net clinical benefit of the test and associated interventions in the context of Australian clinical practice. (Section 2A.5 or 2.5 in the assessment report).

Appendix B Studies included in the systematic review

PRISMA flowchart of included studies

A PRISMA flowchart (Figure 21) provides a graphic depiction of the results of the literature search and the application of the study selection criteria (listed in Table 21) (Liberati et al., 2009).

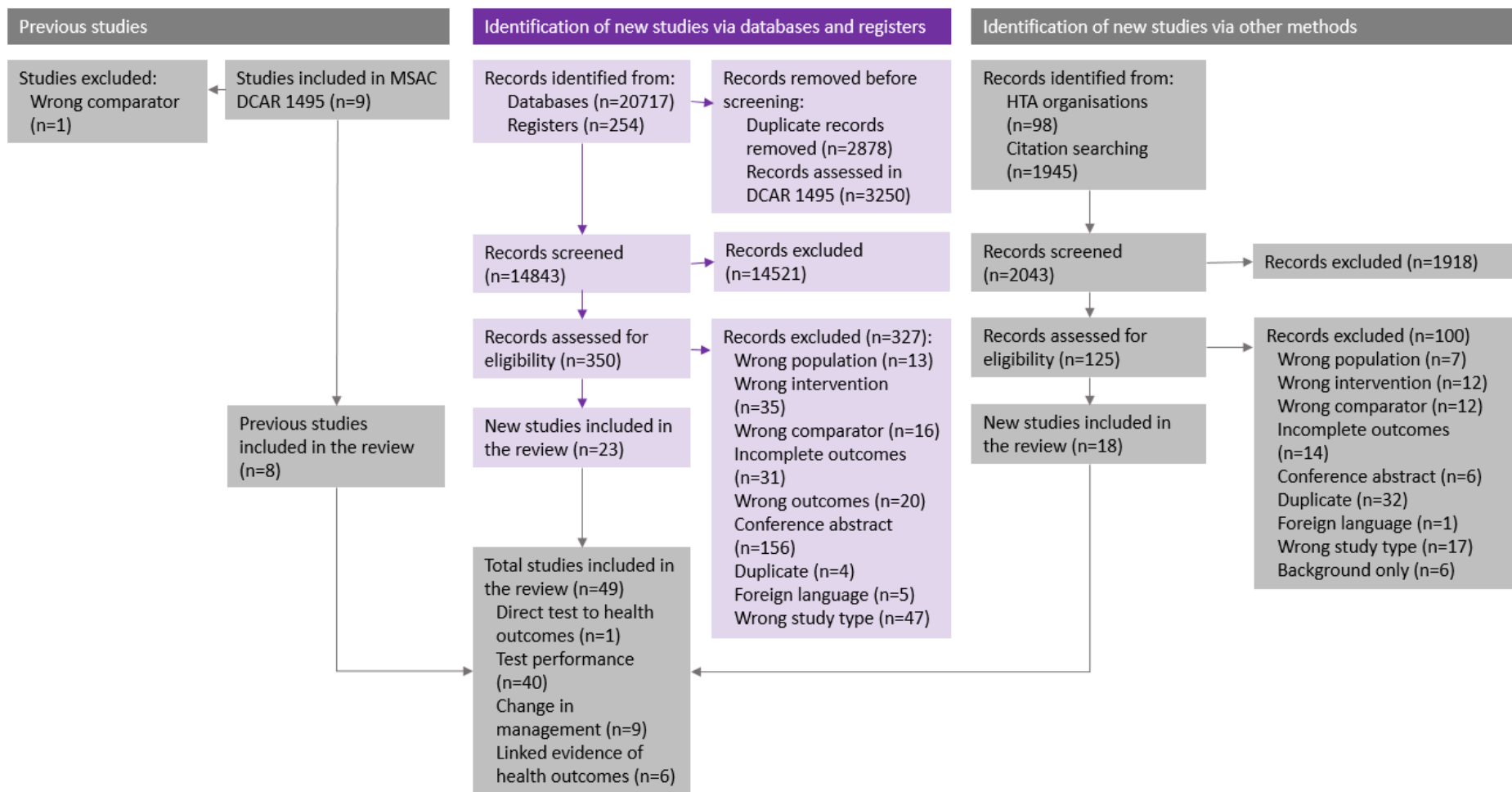


Figure 21 PRISMA flowchart for studies included in DCAR 1721

DCAR = Department Contracted Assessment Report; HTA = health technology assessment; MSAC = Medical Services Advisory Committee

Study profiles of included studies

Table 77 Study profiles for studies included in the systematic review

Authors Publication Year	Study type Level of evidence ^a risk of bias assessment ^b	Location Setting Length of follow-up	Study population characteristics	Description of Intervention	Description of Comparator	Relevant outcomes assessed
(Ali et al. 2016)	Retrospective cohort study Level III-2 Moderate risk of bias (QUADAS 2)	USA Samples submitted for comprehensive genetic profiling at multiple institutions Follow up time unclear: response reported up to 28 months	N=1070, n=45 with concordance data Patients with advanced lung carcinoma who were assayed during course of clinical care 724 adenocarcinoma 12 Adenosquamous carcinoma 3 LCC 23 Mucoepidermoid 146 Non-small cell carcinoma NOS	Hybrid-capture based CGP using NGS, 236 cancer related genes, plus 47 introns from 19 genes frequently rearranged in cancer	ALK FISH testing	Concordance Treatment received in discordant cases
(Ariyasu et al. 2021)	Retrospective cohort study Level III-2 Low to moderate risk of bias (QUADAS 2)	Japan Samples submitted for NGS at a single hospital No follow up	N=167 Consecutive patients with advanced NSCLC Samples obtained through biopsy, surgical resection or other	NGS based test for four driver genes and 42 other mutations for research use	ALK IHC Cobas <i>EGFR</i>	Concordance Turnaround time Success rate

			113 adenocarcinoma 39 squamous 15 other			
(Batra et al. 2021)	Prospective cohort study Level III-2 <u>Concordance:</u> Moderate to high risk of bias (QUADAS 2) Health outcomes: Moderate to high risk of bias (QUIPS)	India Samples submitted for NGS at a single centre 12.5 months (median) for health outcomes	N=58 (evaluated in 3 modalities) Consecutive patients with biopsy proven NSCLC and positive for ALK on IHC; samples also had to be evaluable by FISH and HGS Patients with adequate tumour blocks included (FFPE). Tissue source not reported Health outcomes: n=28, excluding patients did not receive treatment or did not have first response evaluation 68 adenocarcinoma 2 squamous cell carcinoma 1 sarcomatoid carcinoma	NGS using a custom assay of 71 ALK variants and 12 assay expression controls	ALK IHC ALK FISH	Concordance Success rate Health outcomes (PFS and OS)
(Canterbury et al. 2021)	Retrospective cohort study Level III-2 Low to moderate risk of bias (QUADAS 2)	USA Review of cases of lung carcinomas that had previously undergone testing for ALK No follow-up	N=90 patients with lung adenocarcinomas who underwent testing for an ALK gene rearrangement with ≥ 1 testing modality Specimens from resections, biopsies and cytology (pleural effusion and fine-needle aspiration)	RNA NGS, a custom NGS panel using AMP technology (Archer Dx) which targets 17 genes	ALK FISH using Vysis ALK break-apart probe kit (Abbott Molecular) <u>ALK IHC using ALK (D5F3) rabbit monoclonal primary antibody (Ventana)</u>	Concordance

					RNA ISH using RNAScope 2.5 LS Probe-Hs-ALK (Advanced Cell Diagnostics)	
(Chang, Kim & Shin 2020)	Diagnostic case-control study Level III-3 High risk of bias (QUADAS 2)	Korea Case series of patients at a single centre Cross sectional	N=10 Specimens of patients with NSCLC (all adenocarcinoma) in a tissue bank; with and without <i>ALK</i> on FISH Sourced from biopsy and surgically resected tumour	NGS sequencing of 80 genes Also RNA panel	<i>ALK</i> FISH	Concordance
(Choi et al. 2022)	Retrospective cohort study Level III-2 High risk of bias (QUADAS 2)	Korea Patients enrolled into a precision medicine study; testing done at a single site but patients allocated to trials at different sites depending on results Cross sectional	N=109 <i>EGFR</i> N=95 <i>ALK</i> N=42 <i>ROS1</i> Specimens of patients with refractory NSCLC who had been assessed for <i>ALK</i> , <i>EGFR</i> and <i>ROS1</i> Note study included other cancer types	NGS using one of two cancer panels: one with exons of 183 genes and one with whole exomes of 409 cancer related genes. Note study included other cancer types so panels not NSCLC specific	<i>EGFR</i> pyrosequencing or PCR <i>ALK</i> IHC or FISH <i>ROS1</i> PCR	Concordance
(D'Haene et al. 2015)	Retrospective cohort study Level III-2 Moderate risk of bias (QUADAS 2)	Belgium Tumour samples from patients at a single centre Cross sectional	N=39 Specimens from NSCLC patients already tested for <i>EGFR</i> Samples from biopsy, resection and cell blocks	NGS colon and lung panel of 22 genes	<i>EGFR</i> PCR	Concordance Success rate
(Dall'Olio et al. 2020)	Retrospective cohort study Level III-2 Low risk of bias (QUADAS 2)	Italy Samples from patients at a single centre Cross sectional	N=537 Consecutive NSCLC (adenocarcinoma) patients Histology and cytology samples	NGS panel detecting hotspot mutations in 35 genes, 19 amplifications and 23 rearrangements	Sequential testing beginning with <i>EGFR</i> and <i>KRAS</i> Pyrosequencing for <i>KRAS</i> and <i>BRAF</i> PCR for <i>EGT</i>	Prevalence of mutations using each method Turnaround time

					FISH and IHC for <i>ALK</i> FISH for <i>ROS1</i> , <i>MET</i> and <i>RET</i> ISH and SISH for <i>HER2</i>	
(de Biase et al. 2013)	Cohort study Level III-2 Low risk of bias (QUADAS 2)	Italy Multiple pathological laboratories	N=80 NSCLC samples randomly selected from patients underwent diagnostic workup Cytology and FFPE biopsy specimens 52 adenocarcinoma 28 NSCLC NOS	NGS, targeting <i>EGFR</i> exon 18-21, using a 454 GS-Junior Next Generation sequencer (Roche Diagnostics)	Sanger sequencing carried out using the GenomeLab DTCS Kit (Beckman Coulter, U.S.A.) and a CEQ2000 XL automatic DNA sequencer (Beckman Coulter) and the BigDye Terminator kit (version 3.1; Life Technologies)	Concordance Predicted change in management
(DiBardino et al. 2017)	Retrospective cohort study Level III-2 Low to moderate risk of bias (QUADAS 2)	USA Samples submitted for STGPT at a single centre 2 years	N=22 Consecutive NSCLC patients, 20 (91%) stage IV FNA, surgical biopsy and other	NGS using customised NSCLC panel of 467 genes	SS for <i>EGFR</i> , FISH for <i>ALK</i>	Concordance
(DiBardino et al. 2016)	Retrospective cohort study Level III-2 Low to moderate risk of bias (QUADAS 2)	USA Samples submitted for STGPT at a single centre 12 months	N=49 NSCLC patient case series, 30 (61%) metastatic Surgical lung, lymph node and metastatic tumour samples, cytological samples 100% adenocarcinoma	Hybridisation capture and sequencing of exons of 236 genes and 19 rearrangement	Single gene assay for <i>EGFR</i> (n=25), FISH for <i>ALK</i> (n=20)	Test failure rate Inadequate sample rate Concordance
(Fernandes et al. 2019)	Retrospective cohort study Level III-2	Portugal Samples from patients at a single centre	N=117 Patients with advanced lung adenocarcinoma,	NGS colon and lung cancer research panel, 22 genes	Sequential testing using SS and FISH for <i>EGFR</i> and <i>ALK</i>	Concordance Success rate

	Low to moderate risk of bias (QUADAS 2)	Follow up time not specified	previously tested for <i>EGFR</i> and <i>ALK</i> Biopsy and cytology specimens			
(Griesinger et al. 2021)	Prospective cohort Level III-2 Low to moderate risk of bias (SIGN for cohorts)	Germany Data from a registry from 150 sites No follow-up (for turnaround time)	N=3,717 patients with advanced NSCLC, recruited into the CRISP registry at start of systemic therapy 78.6% non-squamous 21.4% squamous	NGS, no detailed information provided	IHC, FISH and other sequencing, no detailed information provided	Turnaround time
(Gutierrez et al. 2017)	Retrospective cohort study Level III-2 Low risk of bias (SIGN for cohorts)	USA Review of medical records of patients treated within a regional cancer care network 3 years	N=814 Patients identified on the COTA database with non-squamous NSCLC in 2013 to 2015 89% adenocarcinoma 2% LCC 7% non-small cell, NOS 3% other NSCLC	Full panel NGS testing for 7 genes	Partial testing: <i>EGFR</i> and <i>ALK</i>	Rebiopsy rate Test failure rate Sample failure rate
(Hamblin et al. 2017)	Prospective cohort Level III-2 Low risk of bias (SIGN for cohorts)	UK A single pathological laboratory (samples submitted to a diagnostic centre) Follow-up not reported	N=108 NSCLC FFPE samples from small diagnostic cancer biopsies, from patients who treating clinicians thought might benefit from more extensive genetic analysis	NGS using 46-gene Ion ampliSeq Cancer Hotspot Panel (Thermo Fisher Scientific)	Roche cobas <i>EGFR/KRAS/BRAF</i> (for NSCLC samples)	Success rate Change in management
(Hinrichs et al. 2015)	Diagnostic case-control Level III-3	The Netherlands A single pathological laboratory (samples	N=25 FFPE NSCLC primary tumour or metastasis samples selected, with known	2 NGS platforms based on clonally amplified templates through emulsion PCR:	High-resolution melting prescreening in combination with Sanger sequencing (detecting clinical hotspot	Concordance

	Moderate risk of bias (QUADAS 2)	selected from a biobank No follow-up	<i>KRAS</i> and <i>EGFR</i> mutations. Biopsy, surgical and fine-needle aspiration cytology specimens	454 Genome Sequencer junior (NGS-454, Roche Diagnostics), used 454 FLX amplicon chemistry for analysis of the mutational hotspot regions of <i>KRAS</i> (exons 2 and 3) and <i>EGFR</i> (exons 19, 20, and 21). Ion Torrent Personal Genome Machine (NGS-IonT), used Ion AmpliSeq Cancer Panel detecting mutational hotspots in 46 oncogenes	mutations in exons 2 and 3 of the <i>KRAS</i> gene and exons 19, 20, and 21 of the <i>EGFR</i> gene) 2 mutation-specific analysis platforms based on real-time PCR technology: cobas z 480, cobas <i>KRAS</i> assay detects 19 <i>KRAS</i> mutations in codons 12, 13 and 61; <i>EGFR</i> assay detects 41 mutation in exons 18, 19, 20 and 21 of the <i>EGFR</i> gene. Rotor-Gene Q, <i>KRAS</i> assay detects 7 <i>KRAS</i> mutations in codons 12 and 13, and the <i>EGFR</i> assay detects 29 mutations in exons 18, 19, 20, and 21 of the <i>EGFR</i> gene.	
(Ilie et al. 2022)	Cohort study (retrospective or prospective unknown) Level III-2 Low risk of bias (QUADAS 2)	France A single pathological laboratory (in-house samples and samples from outside centres) No follow-up	N=259/345 consecutive patients diagnosed with non-squamous NSCLC Biopsy, surgical, pleural effusion (cellblock) and endobronchial ultrasound specimens 97% adenocarcinoma 3% LCC	Ion Torrent Genexus Sequencer, DNA- and RNA-based NGS, panel used being Oncomine Precision Assay GX, which includes 50 genes	Idylla <i>EGFR</i> mutation test Idylla <i>KRAS</i> mutation test ALK IHC and/or <i>ALK</i> FISH <i>ROS1</i> IHC and/or FISH <i>BRAFV600E</i> IHC S5 system (Thermo Fisher Scientific) using the DNA Ion AmpliSeq™ Cancer Hotspot Panel RNA Oncomine Focus Assay	Concordance

(Ji et al. 2019)	<p>Cohort study (retrospective or prospective unknown)</p> <p>Level III-2</p> <p>Low to moderate risk of bias (QUADAS 2)</p>	<p>China</p> <p>A single pathological laboratory (tissues from patients in two centres)</p> <p>No follow-up</p>	<p>N=199 NSCLC patients with adequate tumour cells in their donated tissue samples</p> <p>FFPE surgical specimens</p> <p>86.9% adenocarcinoma</p> <p>11.1% SCC</p> <p>1.5% LCC</p> <p>0.5% adenosquamous carcinoma</p>	<p>10-gene, 32-mutation detection NGS</p>	<p>Sanger sequencing of <i>EGFR</i> (DNA samples) and <i>ALK</i> (RNA samples)</p> <p>Amplification Refractory Mutation System (AMRS) PCR (for some inconsistent samples only)</p>	Concordance
(Jiang et al. 2020)	<p>Retrospective cohort study</p> <p>Level III-2</p> <p>Moderate to high risk of bias (QUADAS 2)</p>	<p>China</p> <p>Multiple pathological laboratories (samples from patients in multiple centres)</p> <p>No follow-up</p>	<p>N=253 NSCLC samples (a subset of 452 total cases)</p> <p>FFPE specimens</p>	<p>NGS panel OncoAim (Singlera Genomics) covering mutational hotspots of 59 genes</p>	<p>Amplified Refractory Mutation System (ARMS)-PCR test for <i>EGFR</i> mutation status</p>	Concordance
(Jing et al. 2018)	<p>Cohort study (retrospective or prospective unknown)</p> <p>Level III-2</p> <p>Low risk of bias (NGS vs. Sanger sequencing) (QUADAS 2)</p> <p>Moderate risk of bias (NGS vs. ddPCR) (QUADAS 2)</p>	<p>China</p> <p>A single pathological laboratory (samples from patients in a single centre)</p> <p>No follow-up</p>	<p>N=112 samples from NSCLC patients in a single centre</p> <p>FFPE specimens, fresh resection specimens, fine needle aspiration specimens, and pleural effusion specimens</p> <p>217 adenocarcinoma</p> <p>4 adenosquamous carcinoma</p> <p>1 LCC</p> <p>56 SCC</p>	<p>NGS lung panel including 7 genes (including <i>BRAF</i>, <i>EGFR</i>, <i>KRAS</i>, <i>NRAS</i>, <i>PIK3CA</i>, <i>Her-2</i> and <i>TP53</i>) using the IonTorrent personal genome machine (PGM)</p>	<p>Sanger sequencing for detecting, primers used for exon 18-21 of <i>EGFR</i></p> <p>Droplet digital PCR for detecting <i>EGFR</i> mutations, genotypes with L858R, exon 19 deletion, T790M or G719S</p>	Concordance

			144 unknown			
(Jurmeister et al. 2021)	Prospective cohort Level III-2 Low risk of bias (SIGN for cohorts)	Germany and Switzerland Multiple pathologic laboratories (samples from the archives of multiple centres) No follow-up	N=57 participants assessing 10 pretested NSCLC specimens with known ALK status	RNA/DNA NGS	ISH ALK testing (either FISH or CISH) IHC ALK testing	Interrater reliability
(Kato et al. 2021)	Cohort study (retrospective or prospective unknown) Level III-2 Moderate risk of bias (QUADAS 2)	Japan Pathologic laboratory(ies) No follow-up	N=150 (for EGFR) N=733 (for ALK) N=109 (for ROS1) N=99 (for MET) FFPE NSCLC samples	NGS panel, consists of 2 DNA modules (for EGFR, BRAF, KRAS Her2, and MET mutations/variants) and 2 RNA modules (for ALK, MET, ROS1 and RET mutations/variants)	EGFR: Cobas® EGFR Mutation Test v2 ALK: Histofine ALK iAEP® kit and Vysis® ALK Break Apart FISH ROS1: OncoGuide® AmyouDx® ROS1 MET: Archer®MET	Concordance
(Kim et al. 2021)	<u>Concordance:</u> Retrospective cohort study Level III-2 Moderate risk of bias (QUADAS 2) <u>Health outcome:</u> Case series Level IV Low to moderate risk of bias	Korea Data review of NSCLC patients treated in a single centre 16.8 months	N=391 patients with lung adenocarcinoma who underwent NGS FFPE archival biopsy or surgical specimens <u>Concordance:</u> n=320/391 <u>Health outcome:</u> N=330/391	Targeted NGS using the MiSeq platform (Illumina) with OncoPanel AMC version 3, targeting a total of 382 genes	Conventional single-targeting PCR for EGFR mutations	Concordance Health outcome (OS)

	(NHLBI for case series)					
(Lassalle et al. 2020)	Prospective case series Level IV Low to moderate risk of bias (NHLBI for case series)	France A single pathological laboratory centre (tissues from patients hospitalised) No follow-up	N=83 patients with non-squamous lung cancer, <i>EGFR</i> wild-type determined by Idylla assay Biopsy specimens 88.2% adenocarcinoma 10.1% NSCLC NOS 1.7% LCC	Hotspot NGS panel	Not applicable	Turnaround time Change in management
(Legras et al. 2018)	Prospective cohort Level III-2 Low to moderate risk of bias (QUADAS 2)	France A single pathological laboratory (samples addressed to the laboratory for molecular diagnosis) No follow-up	N=1,343 NSCLC samples Commercial FFPE samples and genomic DNAs with validated allelic ratio for various mutations 59% adenocarcinoma 4% SCC 0.7% sarcomatoid 0.5% LCC 0.3% small cell 13% undifferentiated 22% unknown	Dedicated NGS panel of 92 amplicons (Ion AmpliSeq Colon-Lung Cancer Research Panel version 2), covering >500 hotspot mutations in <i>KRAS</i> , <i>EGFR</i> , <i>BRAF</i> , <i>ALK</i> etc.	Competitive allele-specific TaqMan technology using TaqMan mutation assays for <i>EGFR</i> and TaqMan probes for <i>KRAS</i> (Thermo Fisher Scientific).	Concordance per variant (not per patient)
(Li, T et al. 2021)	Retrospective case series Level IV High risk of bias (NHLBI for case series)	China A single hospital Up to 4 years	N=6/42 patients with Stage IIIB/IV lung adenocarcinoma bearing uncommon <i>EGFR</i> mutations treated with afatinib	NGS, with no detailed information provided	Not applicable	Test to health outcomes (ORR, time to treatment failure)

(Li, W, Li, Y, et al. 2021)	Cohort study (retrospective or prospective unknown) Level III-2 Low to moderate risk of bias (QUADAS 2)	China A single pathological laboratory (tissue from NSCLC patients who requested molecular testing) No follow-up	N=1,392 newly diagnose, treatment-naïve metastatic NSCLC patients with limited tissue sample FFPE samples from core biopsy, fine-needle aspiration, bronchoscopic biopsy, pleural effusion (cytology specimen), and excisional biopsy <u>Turnaround time:</u> n=884 <u>Concordance:</u> n=109-572 100% adenocarcinoma	Tissue NGS panel designed against 56 cancer-related genes (Burning Rock Biotech, China), sequenced on the NextSeq N500 platform (Illumina)	Amplification refractory mutation system (ARMS)-PCR for <i>EGFR/KRAS/BRAF</i> , using human <i>EGFR/KRAS/BRAF</i> Gene Mutation Detection Kit (ACCB, China) IHC: Ventana Benchmark XT stainer (Ventana Medical Systems) for <i>ALK</i> FISH: Vysis LSI Dual Color and breakapart rearrangement probes specific to the <i>ROS1</i> and <i>RET</i> genes (Abbott Molecular) for <i>ROS1</i> and <i>RET</i>	Concordance Turnaround time Success rate
(Lin, C et al. 2019)	Retrospective cohort study Level III-2 <u>Concordance:</u> Low to moderate risk of bias (QUADAS 2) <u>Health outcomes:</u> Moderate to high risk of bias (QUIPS)	China A single laboratory (tissues from a hospital tissue bank) and review of medical record No follow-up for concordance and up to 58 months for health outcomes	N=55 <i>ALK</i> positive NSCLC patients at a hospital FFPE tumour tissues <u>Concordances:</u> n=34-55 Health outcomes: n=40 <i>ALK</i> + patients who received crizotinib 91% adenocarcinoma 0% SCC 9% NSCLC NOS	NGS panel targeting 416 cancer specific genes designed and carried out by Geneseeq Technology (China), KAPA Hyper Prep Kit (Kapa Biosystems, USA) utilised for DNA library preparation	<i>ALK</i> FISH using the Vysis <i>ALK</i> Break Apart <i>FISH</i> kit (Abbott Molecular) <i>ALK</i> IHC using VENTANA <i>ALK</i> (Clone D5F3)CDx Kit and benchmark Ultra Immunostainer (Ventana Medical Systems)	Concordance Success rate Health outcomes (PFS, ORR and DCR)
(Lin, HM et al. 2022)	Retrospective cohort study Level III-3	USA Data from the flatiron de-identified	N=67,281 advanced NSCLC patients with at least 2 clinic visits who	Any NGS platform used by 280 cancer clinics across the USA	<i>EGFR</i> specific PCR platforms.	Turnaround time

	High risk of bias (QUADAS 2)	electronic health database No follow up	were tested for <i>EGFR</i> variants. 81.6% non-squamous 14% SCC 4.4% NSCLC NOS			
(Mehrad et al. 2018)	Retrospective cohort study Level III-2 (QUADAS 2)	USA Comparison of patients care pathways in a single centre 3 years	N=225 Patients with metastatic lung cancer (n= 46 cases ADC and NSCLC, NOS) 29 (63%) samples from metastatic tumours	NGS 50 gene pane	8 gene non-NGS panel including SS for <i>EGFR</i> , FISH for <i>ALK</i> and <i>ROS1</i>	Concordance
(Mehta et al. 2020)	Prospective cohort study Level III-2 Low risk of bias (QUADAS 2)	India Patients diagnosed at a single centre. Follow up of 20 months.	N=100 patients newly diagnosed with advanced NSCLC underwent predictive biomarker testing with NGS and single gene testing. 98% adenocarcinoma 2% SCC	Ampliseq Cancer Hotspot panel Oncomine solid tumor DNA and Oncomine Fusion transcript kit	<i>EGFR</i> testing by RT-PCT, <i>ALK</i> testing with IHC, and <i>ROS1</i> testing with FISH	Concordance
(Miller et al. 2018)	Prospective cohort study Level III-3 moderate risk of bias	USA Patients diagnosed at a single centre were screened using NGS Clinical up not detailed	N=302 advanced adenocarcinoma patients. After validation of the assay the turnaround time for the first 302 patients was recorded.	Oncomine Focus Assay targeting 52 genes relevant to solid tumors	<i>EGFR</i> theascreen FoundationOne testing	Turnaround time
(Park & Shim 2020)	Retrospective cohort study Level III-2	South Korea Patients treated at university severance hospital	N=241 Lung cancer patients who underwent NGS testing 83.4% adenocarcinoma	Trusight tumor 170 (illumina) which targets 170 cancer genes.	Rt-qPCR for <i>EGFR</i> IHC then FISH for <i>ALK</i> and <i>ROS1</i>	Concordance

	Moderate to high risk of bias (QUADAS 2)	No follow up	2.9% SCC 1.2% adenosquamous 1.7% sarcomatoid 2.5% invasive mucinous adenocarcinoma 4.6% NSCLC NOS 2.5% small cell carcinoma 1.2% carcinoid tumour	Customised cancer panel which targets 46 cancer genes		
(Pisapia et al. 2022)	Retrospective cohort study Level III-2 High risk of bias (SIGN for cohorts)	Italy Semi-structured surveys of relevant professional in multiple referral Italian institutions No follow-up	N=1,461 advanced stage NSCLC patients undergoing first-line treatment and tested on tissue specimens	NGS platforms	Standard/conventional single-test platforms	Retesting rate due to failure
(Robert et al. 2022)	Retrospective cohort study Level III-2 Low risk of bias	USA Patients treated within the US oncology network from practises using iKnowmed EHR. Minimum of 30 days follow up	N=3474 patients with mNSCLC that initiated 1 st line treatment and had not received diagnosis or treatment for another cancer 81.2% nonsquamous 17.2% SCC	Time from testing order to testing result for NGS	Time from testing order to testing result for single biomarker testing of <i>EGFR</i> , <i>ALK</i> , <i>ROS1</i> , <i>BRAF</i> , and PD-L1	Turnaround time
(Sakaguchi et al. 2021)	Retrospective cohort study Level III-3 Moderate risk of bias (QUADAS 2)	Japan Review of patients from a single centre No follow up.	N=116 NSCLC samples that underwent NGS and conventional screening for <i>EGFR</i> variants simultaneously. 64% adenocarcinoma 29% SCC	Oncomine Dx target test	PNA-LNA PCR (Rt-qPCR) clamp test for <i>EGFR</i> variants.	Concordance Health outcomes

			6% non-squamous non-adenocarcinoma 1% NSCLC NOS			
(Schrock et al. 2016)	Case series Level IV Moderate to high risk of bias (NHLBI for case series)	USA and Israel Review NSCLC cases assayed with comprehensive genomic profiling (CGP) in the course of clinical care	N=400 consecutive NSCLC cases with <i>EGFR</i> exon 19 deletions identified by CGP	Hybrid capture-based CGP using NGS	Not applicable	Test to health outcomes evidence
(Simarro et al. 2019)	Cohort study (retrospective or prospective unknown) Level III-1 Moderate risk of bias (QUADAS 2)	Spain Patients diagnosed at a single centre (university hospital La Fe, Valencia) No follow up	N=106 advanced NSCLC patients diagnosed between 2015 and 2017 87% adenocarcinoma 3% SCC 10% NOS	ThermoFisher OncoPrint solid tumour NGS	IHC and FISH for <i>ALK</i>	Concordance
(Steeghs et al. 2022)	Retrospective cohort study (between patient) Level III-2 Low to moderate risk of bias (QUADAS 2)	Netherlands Patient data collected from the Netherlands cancer registry and the Dutch pathology registry. No follow up	Stage IV NSCLC patients. 3343 NGS patients, 698 non-NGS patients 100% adenocarcinoma (SCC and NSCLC NOS also included in article, but results extracted separately for adenocarcinoma)	NGS (on DNA, with gene fusions testing with either IHC, FISH or RNA-NGS)	Various non-NGS single gene testing such as IHC and FISH used throughout clinical practice in the Netherlands	Success rate Comparative yield Turnaround time
(Tachon et al. 2019)	Retrospective cohort study Level III-2	France Patients selected based on routine molecular testing	N=37 NSCLC samples that underwent routine molecular testing	Archer fusionplex and Qiagen human lung cancer panel	IHC and FISH for <i>ALK</i> and <i>ROS1</i>	Concordance

	Low to Moderate risk of bias (QUADAS 2)	performed at one site. no follow up				
(Tan et al. 2020)	Retrospective cohort study Level III-2 Low risk of bias (QUADAS 2)	Singapore Patients that underwent routine molecular testing at a single centre. Patients that had insufficient sample for NGS were excluded No follow up.	N=174 patients with newly diagnosed NSCLC that underwent routine molecular testing. 95% adenocarcinoma 5% other	NGS DNA panel for 29 selected genes and a RNA fusion panel for <i>ALK</i> , <i>ROS1</i> , and <i>RET</i> .	Standard molecular testing of RT-PCR for <i>EGFR</i> and FISH for <i>ALK</i> , <i>ROS1</i> , <i>MET</i> , and <i>RET</i>	Concordance Turnaround time
(Vendrell et al. 2017)	Retrospective cohort study Level III-2 Low risk of bias (QUADAS 2)	Montpellier, France. Samples that had been submitted to a single centre No follow up.	N=1128 samples submitted for detection of <i>ALK</i> mutations, n=37 were randomly chosen (15 <i>ALK</i> positive, 22 <i>ALK</i> negative) to undergo NGS screening	Ampliseq and Archer fusionsplex NGS assays targeted at 70 known mutations across the <i>ALK</i> , <i>RET</i> , <i>ROS1</i> , and <i>NTRK1</i> genes.	<i>ALK</i> IHC and <i>ALK</i> FISH	Concordance
(Vollbrecht et al. 2018)	Diagnostic case control study Level III-3 Moderate risk of bias (QUADAS 2)	Germany Pathological laboratory(ies) No follow-up	N=33 NSCLC samples, already by <i>ALK</i> IHC and FISH FFPE operative or biopsy specimens 32 adenocarcinoma 1 adenosquamous carcinoma	RNA-based analysis using a targeted multiplex-PCR panel followed by IonTorrent sequencing and by direct transcript counting using a digital probe-based assay (NanoString)	<i>ALK</i> IHC (VENTANA <i>ALK</i> (D5F3) CDx Assay, Ventana Medical Systems) and <i>ALK</i> FISH (Vysis LSI <i>ALK</i> Dual Color, Abbott Molecular)	Concordance Health outcomes
(Wei et al. 2021)	<u>Concordance:</u> Diagnostic case-control study Level III-3	Netherlands Data review of diagnostic samples	N=1,729 NSCLC samples from 1,566 patients analysed by NGS <u>Concordance:</u>	Amplicon-based NGS, 2 custom-designed AmpliSeq™ panels (amplicons for 11 genes and 36 genes), resulting	Multiplex ligation-dependent probe amplification, using the SALSA MLPA P105 Glioma-2 probe mix (MRC Holland)	Concordance Health outcomes (PFS and OS)

	<p>Moderate to high risk of bias (QUADAS 2)</p> <p><u>Health outcomes:</u></p> <p>Retrospective case series</p> <p>Level IV</p> <p>Low risk of bias (NHLBI for case series)</p>	<p>that were subjected to NGS analysis</p> <p>No follow-up for concordance, follow-up NR for health outcome</p>	<p>n=49 samples</p> <p><u>Health outcomes:</u></p> <p>n=57+3 patients treated with first-line <i>EGFR</i>-TKI</p> <p>100% adenocarcinoma</p>	<p>libraries generated and processed for sequencing on the IonTorrent PGM sequencing system (Life Technologies)</p>		
(Xie et al. 2019)	<p><u>Concordance:</u></p> <p>Prospective cohort study</p> <p>Low to moderate risk of bias (QUADAS 2)</p> <p>Level III-2</p> <p><u>Health outcome:</u></p> <p>Prospective case series</p> <p>Level IV</p> <p>Low to moderate risk of bias (NHLBI for case series)</p>	<p>China</p> <p>A single hospital</p> <p>Up to 27 months</p>	<p>N=85 patients with advanced non-squamous NSCLC</p> <p>FFPE samples from endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA)</p> <p><u>Concordance:</u></p> <p>n=77, excluding 7 patients ineligible for comparator tests or having insufficient DNA for NGS</p> <p><u>Health outcome:</u></p> <p>n=33 patients with driver mutations (<i>EGFR</i>, <i>ALK</i> or <i>ROS1</i>) who received targeted therapy</p> <p>83.1% adenocarcinoma</p> <p>16.9% NSCLC NOS</p>	<p>Capture-base targeted sequencing was performed with the Lung core 56 Gene Panel (Burning Rock Dx, China), indexed samples sequenced on Nextseq500 sequencer (Illumina)</p>	<p>Routine testing, amplification refractory mutation system (ARMS) PCR for <i>EGFR</i>, using <i>EGFR</i> 21 Mutation Detection Kit (Amoy Diagnostics, China)</p> <p>IHC for <i>ALK</i> with the use of VENTANA <i>ALK</i> (D5F3) assay (F. Hoffmann-La Roche, AZ), confirmed by FISH with the use of Vysis <i>ALK</i> Break Apart FISH Probe Kit (Abbott Molecular)</p> <p>Quantitative reverse transcription PCR for <i>ROS1</i> with use of <i>ROS1</i> Gene Fusions Detection Kit (Amoy Diagnostics)</p>	<p>Concordance</p> <p>Success rate</p> <p>Health outcome (PFS)</p>

(Xu, X. et al. 2016)	Prospective cohort study Level II Low to moderate risk of bias (QUADAS 2)	China A clinical laboratory in a hospital (samples from patients treated in this hospital) No follow-up	N=188 consecutive samples from patients with NSCLC who underwent radical surgical resection of primary lung cancer FFPE specimens or fresh tissues 79.3% adenocarcinoma 13.8% SCC 0.5% adenosquamous carcinoma 6.4% other	NextDaySeq Lung Panel on Ion Torrent™ System (Beijing ACCB Biotech), targeting 4 gene (<i>EGFR</i> , <i>KRAS</i> , <i>BRAF</i> and <i>PIK3CA</i>), library pool sequenced using Ion Torrent PGM system (Thermo Fisher Scientific)	Quantitative Real-Time PCR(qPCR), mutation status of <i>EGFR</i> , <i>KRAS</i> , <i>PIK3CA</i> and <i>BRAF</i> examined using the Human <i>EGFR</i> Gene Mutations Detection Kit, Human <i>KRAS</i> Gene Mutations Detection Kit, Human <i>PIK3CA</i> Gene Mutations Detection Kit, and Human <i>BRAF</i> Gene Mutations Detection Kit ACCB Biotech, China)	Concordance
(Yu et al. 2019)	Comparative study without concurrent controls Level III-3 Moderate risk of bias (SIGN for cohorts)	USA Analysis of the records from a commercial laboratory No follow-up	N=169 investigational Oncomine Dx Target Tests on archived FFPE advanced NSCLC tissue samples (index test) N=3,659 single-gene tests across 1,402 clinician-submitted samples (comparator tests)	Oncomine Dx Target Tests for 23 genes (Ion Torrent PGM Dx Sequencer, Thermo Fisher Scientific)	Therascreen <i>EGFR</i> RGQ PCR Kit (QIAGEN Manchester) Vysis IntelliFISH for <i>ALK</i> (Abbott Laboratory) cobas 4800 <i>BRAF</i> V600 Mutation Test (Roche) Laboratory-developed tests (LDTs) for <i>BRAF</i> , <i>KRAS</i> , <i>MET</i> amplification, <i>RET</i> , <i>ERBB2</i> , <i>FGFR1</i> , and <i>ROS1</i> . <i>BRAF</i> and <i>KRAS</i> LDTs used real-time, or quantitative, PCR. All other LDTs used FISH.	Test success (failure) rate, tissue stewardship (number of slides per testing)
(Zeng et al. 2018)	Retrospective case series Level IV Moderate risk of bias (NHLBI for case series)	China A single hospital (samples submitted for targeted NGS testing at a single centre)	N=1,466 patients with NSCLC who received targeted NGS detection <u>Health outcome:</u> n=19 patients tested positive for <i>ROS1</i>	DNA profiled using a commercially available capture-based targeted sequencing panel (Burning Rock Biotech, Guangzhou, China), targeting 56 or 168	Not applicable	Diagnostic Yield Health outcomes (ORR, PFS, and AEs)

		16 months (mean)	rearrangement and receiving crizotinib 95.5% adenocarcinoma 0% SCC 4.5% adenosquamous carcinoma	genes, sequenced on a Nextseq (Illumina)		
(Zugazagoitia et al. 2018)	Retrospective cohort study Level III-2	Spain A single hospital (samples submitted for targeted NGS testing at a single centre) 18 months	N=109 consecutive advanced stage (mostly stage IV) NSCLC patients reviewed in a single centre 75.2% adenocarcinoma 18.4% SCC 6.4% LCC	NGS DNA analysis for hot spots in 22 genes, RNA analysis for 72 fusion variants	Cobas for <i>EGFR</i> , IHC for ALK and <i>ROS1</i>	Rebiopsy rate Test failure rate Inadequate sample exclusion rate Concordance

Appendix C Excluded studies

Studies which may have met the inclusion criteria, but were excluded for other reasons, are listed below.

Conference abstract

Aggarwal, A, Sabnis, N, Mishra, A, Kumar, V, Mohanty, SS, Kini, L, Sharma, S & Mohanty, S 2021, 'Relevance of next-generation sequencing in non-small cell lung cancer; molecular epidemiology study in Indian patients', *Laboratory Investigation*, vol. 101(SUPPL 1), March, pp. 1087-1088.

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Appendix D Additional details for Section 2

In order for the main body of the report to be more succinct, further details are provided here on the studies included in the linked evidence assessment of small gene panel testing for NSCLC.

Linked evidence of test performance

Characteristics of the evidence base

Table 78 Key features of the included evidence test performance of NGS vs sequential *EGFR*, *ALK* and *ROS1* testing

Study	N	Design Risk of bias	Patient population	Intervention	Comparator	Key outcome(s)	Result used in economic model
(Ali et al. 2016) United States	45	Retrospective cohort study Level III-2 Moderate risk of bias	Patients with advanced lung carcinoma who were assayed during course of clinical care	Hybrid-capture based CGP using NGS, 236 cancer related genes, plus 47 introns from 19 genes frequently rearranged in cancer	<i>ALK</i> FISH testing Definition of positivity unclear, although stated those positive had pools representing fractions ranging from 20% to 100% of fusions.	Concordance for <i>ALK</i>	Yes
(Ariyasu et al. 2021) Japan	167	Retrospective cohort study Level III-2 Low to moderate risk of bias	Consecutive patients with advanced NSCLC Samples obtained through biopsy, surgical resection or other	NGS based test for four driver genes and 42 other mutations for research use	<i>ALK</i> IHC Cobas <i>EGFR</i>	Concordance for <i>EGFR</i> Turnaround time	Yes
(Batra et al. 2021) India	58	Prospective cohort study	Consecutive patients with biopsy proven NSCLC and positive for <i>ALK</i> on IHC;	NGS using a custom assay of 71 <i>ALK</i> variants and 12 assay expression controls	<i>ALK</i> IHC <i>ALK</i> FISH	Concordance for <i>ALK</i>	Yes

		Level III-2 Moderate to high risk of bias	samples also had to be evaluable by FISH and HGS Patients with adequate tumour blocks included (FFPE). Tissue source not reported		FISH positivity defined as those exhibiting split signals with the separation being more than two signal diameters apart		
(Canterbury et al. 2021) United States	90	Retrospective cohort study Level III-2 Low to moderate risk of bias	Patients with lung adenocarcinomas who underwent testing for an <i>ALK</i> gene rearrangement with ≥ 1 testing modality Specimens from resections, biopsies and cytology (pleural effusion and fine-needle aspiration)	RNA NGS, a custom NGS panel using AMP technology (Archer Dx) which targets 17 genes	<i>ALK</i> FISH using Vysis <i>ALK</i> break-apart probe kit (Abbott Molecular) FISH positivity defined as staining ≥10% of tumour cells <i>ALK</i> IHC using <i>ALK</i> (D5F3) rabbit monoclonal primary antibody (Ventana) RNA ISH using RNAScope 2.5 LS Probe-Hs- <i>ALK</i> (Advanced Cell Diagnostics)	Concordance for <i>ALK</i>	Yes
(Chang, Kim & Shin 2020) Italy	10	Diagnostic case-control study Level III-3 High risk of bias	Specimens of patients with NSCLC in a tissue bank; with and without <i>ALK</i> on FISH Sourced from biopsy and surgically resected tumour	NGS sequencing of 80 genes Also RNA panel	<i>ALK</i> FISH Threshold for positivity not defined	Concordance for <i>ALK</i>	Yes
(Choi et al. 2022) Korea	109	Retrospective cohort study Level III-2 High risk of bias	Specimens of patients with refractory NSCLC who had been assessed for <i>ALK</i> , <i>EGFR</i> and <i>ROS1</i>	NGS using one of two cancer panels: one with exons of 183 genes and one with whole exomes of 409 cancer related genes. Note study included other cancer types so panels not NSCLC specific	<i>EGFR</i> pyrosequencing or PCR <i>ALK</i> IHC or FISH <i>ROS1</i> PCR	Concordance for <i>EGFR</i>	Yes
(Dall'Olio et al. 2020) Italy	1221	Retrospective cohort study Level III-2 Low risk of bias	Consecutive NSCLC (adenocarcinoma) patients Histology and cytology samples	NGS panel detecting hotspot mutations in 35 genes, 19 amplifications and 23 rearrangements	Sequential testing beginning with <i>EGFR</i> and <i>KRAS</i> Pyrosequencing for <i>KRAS</i> and <i>BRAF</i> FISH and IHC for <i>ALK</i>	Volume of tissue required Turnaround time	No

					FISH for <i>ROS1</i> , <i>MET</i> and <i>RET</i> ISH and SISH for <i>HER2</i>		
(de Biase et al. 2013) Italy	80	Cohort study Level III-2 Low risk of bias	NSCLC samples randomly selected from patients underwent diagnostic workup Cytology and FFPE biopsy specimens	NGS, targeting <i>EGFR</i> exon 18-21, using a 454 GS-Junior Next Generation sequencer (Roche Diagnostics)	Sanger sequencing carried out using the GenomeLab DTCS Kit (Beckman Coulter, U.S.A.) and a CEQ2000 XL automatic DNA sequencer (Beckman Coulter) and the BigDye Terminator kit (version 3.1; Life Technologies)	Concordance for <i>EGFR</i>	Yes
(D'Haene et al. 2015) Belgium	39	Retrospective cohort study Level III-2 Moderate risk of bias	Specimens from NSCLC patients already tested for <i>EGFR</i> Samples from biopsy, resection and cell blocks	NGS colon and lung panel of 22 genes	<i>EFGR</i> PCR	Concordance for <i>EGFR</i>	Yes
(DiBardino et al. 2017) United States	22	Retrospective cohort study Level III-2 Low to moderate risk of bias	NSCLC samples randomly selected from patients underwent diagnostic workup Cytology and FFPE biopsy specimens	NGS, targeting <i>EGFR</i> exon 18-21, using a 454 GS-Junior Next Generation sequencer (Roche Diagnostics)	Sanger sequencing carried out using the GenomeLab DTCS Kit (Beckman Coulter, U.S.A.) and a CEQ2000 XL automatic DNA sequencer (Beckman Coulter) and the BigDye Terminator kit (version 3.1; Life Technologies)	Concordance for <i>EGFR</i> Rate of concurrent variants	Yes
(Fernandes et al. 2019) Portugal	117	Retrospective cohort study Level III-2 Low to moderate risk of bias	Patients with advanced lung adenocarcinoma, previously tested for <i>EGFR</i> and <i>ALK</i> Biopsy and cytology specimens	NGS colon and lung cancer research panel, 22 genes	Sequential testing using SS and FISH for <i>EFGR</i> and <i>ALK</i>	Concordance for <i>EGFR</i>	Yes
(Griesinger et al. 2021) Germany	3,717	Prospective cohort Level III-2 Low to moderate risk of bias	Patients with advanced NSCLC, recruited into the CRISP registry at start of systemic therapy	NGS, no detailed information provided	IHC, FISH and other sequencing, no detailed information provided	Rate of concurrent variants Turnaround time	No
(Hinrichs et al. 2015) The Netherlands	25	Diagnostic case-control Level III-3	FFPE NSCLC primary tumour or metastasis samples selected, with	2 NGS platforms based on clonally amplified templates through emulsion PCR:	High-resolution melting prescreening in combination with Sanger sequencing (detecting clinical hotspot mutations in exons	Concordance for <i>EGFR</i>	Yes

		Moderate risk of bias	known <i>KRAS</i> and <i>EGFR</i> mutations. Biopsy, surgical and fine-needle aspiration cytology specimens	454 Genome Sequencer junior, and Ion Torrent Personal Genome Machine	2 and 3 of the <i>KRAS</i> gene and exons 19, 20, and 21 of the <i>EGFR</i> gene) 2 mutation-specific analysis platforms based on real-time PCR technology: cobas z 480, cobas <i>KRAS</i> assay detects 19 <i>KRAS</i> mutations in codons 12, 13 and 61; <i>EGFR</i> assay detects 41 mutation in exons 18, 19, 20 and 21 of the <i>EGFR</i> gene. Rotor-Gene Q, <i>KRAS</i> assay detects 7 <i>KRAS</i> mutations in codons 12 and 13, and the <i>EGFR</i> assay detects 29 mutations in exons 18, 19, 20, and 21 of the <i>EGFR</i> gene.		
(Ilie et al. 2022) France	259	Cohort study (retrospective or prospective unknown) Level III-2 Low risk of bias	Consecutive patients diagnosed with non-squamous NSCLC Biopsy, surgical, pleural effusion (cellblock) and endobronchial ultrasound specimens	Ion Torrent Genexus Sequencer, DNA- and RNA-based NGS, panel used being Oncomine Precision Assay GX, which includes 50 genes	Idylla <i>EGFR</i> mutation test Idylla <i>KRAS</i> mutation test <i>ALK</i> IHC and/or <i>ALK</i> FISH <i>ROS1</i> IHC and/or FISH <i>BRAFV600E</i> IHC S5 system (Thermo Fisher Scientific) using the DNA Ion AmpliSeq™ Cancer Hotspot Panel RNA Oncomine Focus Assay	Concordance for <i>EGFR</i>	Yes
(Ji et al. 2019) China	199	Cohort study (retrospective or prospective unknown) Level III-2 Low to moderate risk of bias	NSCLC patients with adequate tumour cells in their donated tissue samples FFPE surgical specimens	10-gene, 32-mutation detection NGS	Sanger sequencing of <i>EGFR</i> (DNA samples) and <i>ALK</i> (RNA samples) Amplification Refractory Mutation System (AMRS) PCR (for some inconsistent samples only)	Concordance for <i>EGFR</i> Rate of concurrent variants	Yes

(Jiang et al. 2020) China	253	Retrospective cohort study Level III-2 Moderate to high risk of bias	NSCLC samples (a subset of 452 total cases) FFPE specimens	NGS panel OncoAim (Singlera Genomics) covering mutational hotspots of 59 genes	Amplified Refractory Mutation System (ARMS)-PCR test for <i>EGFR</i> mutation status	Concordance for <i>EGFR</i>	Yes
(Jing et al. 2018) China	112	Cohort study (retrospective or prospective unknown) Level III-2 Low risk of bias (NGS vs. Sanger sequencing) Moderate risk of bias (NGS vs. ddPCR)	Samples from NSCLC patients in a single centre FFPE specimens, fresh resection specimens, fine needle aspiration specimens, and pleural effusion specimens	NGS lung panel including 7 genes (including <i>BRAF</i> , <i>EGFR</i> , <i>KRAS</i> , <i>NRAS</i> , <i>PIK3CA</i> , <i>Her-2</i> and <i>TP53</i>) using the IonTorrent personal genome machine (PGM)	Sanger sequencing for detecting, primers used for exon 18-21 of <i>EGFR</i> Droplet digital PCR for detecting <i>EGFR</i> mutations, genotypes with L858R, exon 19 deletion, T790M or G719S	Concordance for <i>EGFR</i> Rate of concurrent variants	Yes
(Jurmeister et al. 2021) Germany and Switzerland	57	Prospective cohort Level III-2 Low risk of bias	N=57 participants assessing 10 pretested NSCLC specimens with known <i>ALK</i> status	RNA/DNA NGS	ISH <i>ALK</i> testing (either FISH or CISH) IHC <i>ALK</i> testing	Interrater reliability	No
(Kato et al. 2021) Japan	150	Cohort study (retrospective or prospective unknown) Level III-2 Moderate risk of bias	N=150 (for <i>EGFR</i>) N=733 (for <i>ALK</i>) N=109 (for <i>ROS1</i>) N=99 (for <i>MET</i>) FFPE NSCLC samples	NGS panel, consists of 2 DNA modules (for <i>EGFR</i> , <i>BRAF</i> , <i>KRAS</i> <i>Her2</i> , and <i>MET</i> mutations/variants) and 2 RNA modules (for <i>ALK</i> , <i>MET</i> , <i>ROS1</i> and <i>RET</i> mutations/variants)	<i>EGFR</i> : Cobas® <i>EGFR</i> Mutation Test v2 <i>ALK</i> : Histofine <i>ALK</i> iAEP® kit and Vysis® <i>ALK</i> Break Apart FISH Threshold for positivity on FISH not defined <i>ROS1</i> : OncoGuide® AmyuDx® <i>ROS1</i> <i>MET</i> : Archer® <i>MET</i>	Concordance for <i>ALK</i> , <i>ROS1</i> , <i>MET</i> ex14sk	Yes
(Kim et al. 2021) Korea	391	Retrospective cohort study Level III-2	Patients with lung adenocarcinoma who underwent NGS	Targeted NGS using the MiSeq platform (Illumina) with OncoPanel AMC version 3, targeting a total of 382 genes	Conventional single-targeting PCR for <i>EGFR</i> mutations	Concordance for <i>EGFR</i>	Yes

		Moderate risk of bias	FFPE archival biopsy or surgical specimens				
(Lassalle et al. 2020) France	83	Prospective case series Level IV Low to moderate risk of bias	Patients with non-squamous lung cancer, <i>EGFR</i> wild-type determined by Idylla assay Biopsy specimens	Hotspot NGS panel	Rapid <i>EGFR</i> -specific PCR assay (Idylla)	Turnaround time	No
(Legras et al. 2018) France	1343	Prospective cohort Level III-2 Low to moderate risk of bias	NSCLC samples Commercial FFPE samples and genomic DNAs with validated allelic ratio for various mutations	Dedicated NGS panel of 92 amplicons (Ion AmpliSeq Colon-Lung Cancer Research Panel version 2), covering >500 hotspot mutations in <i>KRAS</i> , <i>EGFR</i> , <i>BRAF</i> , <i>ALK</i> etc.	Competitive allele-specific TaqMan technology using TaqMan mutation assays for <i>EGFR</i> and TaqMan probes for <i>KRAS</i> (Thermo Fisher Scientific).	Concordance per variant (not per patient)	No
(Li, W, Guo, L, et al. 2021) China	109-572	Cohort study (retrospective or prospective unknown) Level III-2 Low to moderate risk of bias	Newly diagnose, treatment-naïve metastatic NSCLC patients with limited tissue sample FFPE samples from core biopsy, fine-needle aspiration, bronchoscopic biopsy, pleural effusion (cytology specimen), and excisional biopsy	Tissue NGS panel designed against 56 cancer-related genes (Burning Rock Biotech, China), sequenced on the NextSeq N500 platform (Illumina)	Amplification refractory mutation system (ARMS)-PCR for <i>EGFR/KRAS/BRAF</i> , using human <i>EGFR/KRAS/BRAF</i> Gene Mutation Detection Kit (ACCB, China) IHC: Ventana Benchmark XT stainer (Ventana Medical Systems) for <i>ALK</i> FISH: Vysis LSI Dual Color and breakapart rearrangement probes specific to the <i>ROS1</i> and <i>RET</i> genes (Abbott Molecular) for <i>ROS1</i> and <i>RET</i> Samples with more than 15% of tumour cells with splitting of one or both 5' and 3' probe signals or isolated 3' probe signals were deemed positive.	Concordance for <i>EGFR</i> , <i>ROS1</i> Rate of concurrent variants Turnaround time	Yes

(Lin, HM et al. 2022) United States	67,281	Retrospective cohort study Level III-3 High risk of bias	Advanced NSCLC patients with at least 2 clinic visits who were tested for EGFR mutations.	Any NGS platform used by 280 cancer clinics across the USA	EGFR specific PCR platforms.	Turnaround time	No
(Mehrad et al. 2018) United States	225	Retrospective cohort study Level III-2	Patients with metastatic lung cancer (n= 46 cases ADC and NSCLC, NOS) 29 (63%) samples from metastatic tumours	NGS 50 gene pane	8 gene non-NGS panel including SS for <i>EGFR</i> , FISH for <i>ALK</i> and <i>ROS1</i>	Concordance for <i>EGFR</i> Rate of concurrent variants	Yes
(Mehta et al. 2020) India	100	Prospective cohort study Level III-2 Low risk of bias	Patients newly diagnosed with advanced NSCLC underwent predictive biomarker testing with NGS and single gene testing.	Ampliseq Cancer Hotspot panel Oncomine solid tumor DNA and Oncomine Fusion transcript kit	<i>EGFR</i> testing by RT-PCT, <i>ALK</i> testing with IHC, and <i>ROS1</i> testing with FISH Average of ≥15% signals considered positive	Concordance for <i>ROS1</i>	Yes
(Miller et al. 2018) United States	302	Prospective cohort study Level III-2 Low risk of bias	Advanced adenocarcinoma patients. After validation of the assay the turnaround time for the first 302 patients was recorded.	Oncomine Focus Assay targeting 52 genes relevant to solid tumors	<i>EGFR</i> theascreen FoundationOne testing	Turnaround time	No
(Park & Shim 2020) South Korea	241	Retrospective cohort study Level III-2 Moderate to high risk of bias	Lung cancer patients who underwent NGS testing	Trusight tumor 170 (illumina) which targets 170 cancer genes. Customised cancer panel which targets 46 cancer genes	RT-qPCR for <i>EGFR</i> IHC then FISH for <i>ALK</i> and <i>ROS1</i> Threshold for positivity on FISH average ≥15% signals	Concordance for <i>EGFR</i> , <i>ALK</i> , <i>ROS1</i>	Yes
(Robert et al. 2022) United States	3474	Retrospective cohort study Level III-2 Low risk of bias	Patients with mNSCLC that initiated 1 st line treatment and had not received diagnosis or treatment for another cancer	Time from testing order to testing result for NGS	Time from testing order to testing result for single biomarker testing of <i>EGFR</i> , <i>ALK</i> , <i>ROS1</i> , <i>BRAF</i> , and PD-L1	Turnaround time	No
(Sakaguchi et al. 2021) Japan	116	Retrospective cohort study	NSCLC samples that underwent NGS and conventional screening for	Oncomine Dx target test	PNA-LNA PCR (Rt-qPCR) clamp test for <i>EGFR</i> mutations.	Concordance for <i>EGFR</i>	Yes

		Level III-3 Moderate risk of bias	<i>EGFR</i> mutations simultaneously.				
(Simarro et al. 2019) Spain	106	Cohort study (retrospective or prospective unknown) Level III-1 Moderate risk of bias	Advanced NSCLC patients diagnosed between 2015 and 2017	ThermoFisher OncoPrint solid tumour NGS	ddPCR IHC and FISH for <i>ALK</i>	Concordance for <i>EGFR</i> Rate of concurrent variants	Yes
(Steehgs et al. 2022) The Netherlands	4,040	Retrospective cohort study (between patient) Level III-2 Low to moderate risk of bias	Stage IV NSCLC patients, success rates provided for adenocarcinoma patients	NGS gene panels	Predominantly traditional single gene tests (e.g. Sanger sequencing). High sensitive single-gene tests (i.e. ddPCR) only used in <0.5% of analyses)	Test success rate Comparative yield Turnaround time	Yes
(Tachon et al. 2019) France	37	Retrospective cohort study Level III-2 Low to Moderate risk of bias	NSCLC samples that underwent routine molecular testing	Archer fusionplex and Qiagen human lung cancer panel	IHC and FISH for <i>ALK</i> and <i>ROS1</i> <i>ALK</i> positivity defined as ≥15% of tumor cells showing signal or split red and green signals	Concordance for <i>ALK</i>	Yes
(Tan et al. 2020) Singapore	174	Retrospective cohort study Level III-2 Low risk of bias	Patients with newly diagnosed NSCLC that underwent routine molecular testing.	NGS DNA panel for 29 selected genes and a RNA fusion panel for <i>ALK</i> , <i>ROS1</i> , and <i>RET</i> .	Standard molecular testing of RT-PCR for <i>EGFR</i> and FISH for <i>ALK</i> , <i>ROS1</i> , <i>MET</i> , and <i>RET</i> Threshold for positivity on FISH ≥15%	Concordance for <i>ALK</i> , <i>ROS1</i> Rate of concurrent variants Turnaround time	Yes
(Vendrell et al. 2017) France	37	Retrospective cohort study	Samples submitted for detection of <i>ALK</i> mutations,	Ampliseq and Archer fusionplex NGS assays	<i>ALK</i> IHC and <i>ALK</i> FISH	Concordance for <i>ALK</i>	Yes

		Level III-2 Low risk of bias	n=37 were randomly chosen (15 ALK positive, 22 ALK negative) to undergo NGS screening	targeted at 70 known mutations across the <i>ALK</i> , <i>RET</i> , <i>ROS1</i> , and <i>NTRK1</i> genes.	Threshold for positivity on FISH ≥15%		
(Vollbrecht et al. 2018) Germany	33	Diagnostic case control study Level III-3 Moderate risk of bias	NSCLC samples, already by ALK IHC and FISH FFPE operative or biopsy specimens	RNA-based analysis using a targeted multiplex-PCR panel followed by IonTorrent sequencing and by direct transcript counting using a digital probe-based assay (NanoString)	ALK IHC (VENTANA ALK (D5F3) CDx Assay, Ventana Medical Systems) and ALK FISH (Vysis LSI ALK Dual Color, Abbott Molecular) Threshold for positivity ≥15% on FISH	Concordance for ALK	Yes
(Wei et al. 2021) The Netherlands	49	Diagnostic case-control study Level III-3 Moderate to high risk of bias	729 NSCLC samples from	Amplicon-based NGS, 2 custom-designed AmpliSeq™ panels (amplicons for 11 genes and 36 genes), resulting libraries generated and processed for sequencing on the IonTorrent PGM sequencing system (Life Technologies)	Multiplex ligation-dependent probe amplification, using the SALSA MLPA P105 Glioma-2 probe mix (MRC Holland) Threshold for positivity on FISH not stated	Concordance for <i>EGFR</i> and <i>ROS1</i>	Yes
(Xie et al. 2019) China	77	Prospective cohort study Low to moderate risk of bias Level III-2	Patients with advanced non-squamous NSCLC FFPE samples from endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA)	Capture-base targeted sequencing was performed with the Lung core 56 Gene Panel (Burning Rock Dx, China), indexed samples sequenced on Nextseq500 sequencer (Illumina)	Routine testing, amplification refractory mutation system (ARMS) PCR for <i>EGFR</i> , using <i>EGFR</i> 21 Mutation Detection Kit (Amoy Diagnostics, China) IHC for <i>ALK</i> with the use of VENTANA ALK (D5F3) assay (F. Hoffmann-La Roche, AZ), confirmed by FISH with the use of Vysis ALK Break Apart FISH Probe Kit (Abbott Molecular) Threshold for positivity on FISH not stated Quantitative reverse transcription PCR for <i>ROS1</i> with use of <i>ROS1</i> Gene Fusions Detection Kit (Amoy Diagnostics)	Test success rate Concordance for <i>EGFR</i> and <i>ALK</i> Rate of concurrent variants	Yes

(Xu, X. et al. 2016) China	188	Prospective cohort study Level II Low to moderate risk of bias	Consecutive samples from patients with NSCLC who underwent radical surgical resection of primary lung cancer FFPE specimens or fresh tissues	NextDaySeq Lung Panel on Ion Torrent™ System (Beijing ACCB Biotech), targeting 4 gene (<i>EGFR</i> , <i>KRAS</i> , <i>BRAF</i> and <i>PIK3CA</i>), library pool sequenced using Ion Torrent PGM system (Thermo Fisher Scientific)	Quantitative Real-Time PCR(qPCR), mutation status of <i>EGFR</i> , <i>KRAS</i> , <i>PIK3CA</i> and <i>BRAF</i> examined using the Human <i>EGFR</i> Gene Mutations Detection Kit, Human <i>KRAS</i> Gene Mutations Detection Kit, Human <i>PIK3CA</i> Gene Mutations Detection Kit, and Human <i>BRAF</i> Gene Mutations Detection Kit ACCB Biotech, China)	Concordance for <i>EGFR</i> Rate of concurrent variants	Yes
(Yu et al. 2019) United States	1,571	Comparative study without concurrent controls Level III-3 Moderate risk of bias	Lung tumour samples from patients with advanced NSCLC submitted for sequential single gene testing in a single centre	Oncomine Dx Target multigene panel test (n=169) (intervention)	Therascreen <i>EGFR</i> RGQ PCR Kit (QIAGEN Manchester) Vysis IntelliFISH for <i>ALK</i> (Abbott Laboratory) cobas 4800 <i>BRAF</i> V600 Mutation Test (Roche) Laboratory-developed tests (LDTs) for <i>BRAF</i> , <i>KRAS</i> , <i>MET</i> amplification, <i>RET</i> , <i>ERBB2</i> , <i>FGFR1</i> , and <i>ROS1</i> . <i>BRAF</i> and <i>KRAS</i> LDTs used real-time, or quantitative, PCR. All other LDTs used FISH.	Test success rate Volume of tissue required	No
(Zugazagoitia et al. 2018) Spain	109	Retrospective cohort study Level III-2	Consecutive advanced stage NSCLC patients reviewed in a single centre	NGS DNA analysis for hot spots in 22 genes, RNA analysis for 72 fusion variants	Cobas for <i>EGFR</i> , IHC for <i>ALK</i> and <i>ROS1</i>	Test success rate	No

ALK = anaplastic lymphoma kinase; aNSCLC = advanced non-small cell lung cancer; ARMS-PCR = amplification-refractory mutation system polymerase chain reaction; *BRAF* = proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B; DNA = deoxyribonucleic acid; *EGFR* = epidermal growth factor receptor; *ERBB2* = erb-b2 receptor tyrosine kinase 2; FISH = fluorescent in situ hybridisation; IHC = immunohistochemistry; ISH = in situ hybridisation; *KRAS* = Kirsten rat sarcoma; LDTs = laboratory developed tests; MLPA = Multiplex ligation-dependent probe amplification; *MET* = mesenchymal-epithelial transition; NGS = next generation sequencing; NOS = not otherwise specified; NSCLC = non-small cell lung cancer; *RET* = rearranged during transfection; RNA = ribonucleic acid; *ROS1* = ROS proto-oncogene 1; RT-PCR = real time polymerase chain reaction; SISH = silver in situ hybridisation; SS = Sanger sequencing;

Table 79 Risk of bias of test accuracy studies (using QUADAS 2 checklist)

Study	RISK OF BIAS																APPLICABILITY CONCERNS		
	Patient selection				Index test			Reference standard			Flow and timing						Patient selection	Index test	Reference standard
	1	2	3	4	1	2	3	1	2	3	1	2	3	4	5	6	1	1	1
Study																	-	-	-
		☹️/☹️/?			☹️/☹️/?			☹️/☹️/?					☹️/☹️/?				☹️/☹️/?	☹️/☹️/?	☹️/☹️/?
(Canterbury et al. 2021)	N	N	U	U	U	Y	Low	Y	U	Low	N	N	U	Y	Y	U	U	Low	Low
		?			☺️				☺️				?				?	☺️	☺️
(de Biase et al. 2013)	Y	Y	U	Low	U	Y	Low	Y	U	Low	NA	N	U	Y	Y	Y	U	Low	Low
		☺️			☺️				☺️				☺️				?	☺️	☺️
(DiBardino et al. 2017)	Y	Y	U	Low	U	Y	Low	Y	U	Low	N	N	U	N	Y	N	U	U	Low
		☺️			☺️				☺️				?				?	?	☺️
(DiBardino et al. 2016)	Y	Y	U	Low	U	Y	Low	Y	U	Low	N	Y	Y	N	Y	N	U	Low	Low
		☺️			☺️				☺️				?				?	☺️	☺️
(Hinrichs et al. 2015)	N	N	U	High	N	NA	Low	Y	Y	Low	N	N	U	Y	Y	Y	Ltd info	Low	Low
		☹️			☺️				☺️				?				?	☺️	☺️
(Ilie et al. 2022)	Y	Y	N	Low	U	NA	Low	Y	U	Low	N	N	U	N	N	N	Low	Low	Low
		☺️			☺️				☺️				☹️				☺️	☺️	☺️
(Ji et al. 2019)	N	Y	U	U	U	NA	Low	Y	U	Low	Y	N	U	Y	Y	Y	SQ NSCLC included	Unclear	Unclear
		?			☺️				☺️				☺️				?	?	☺️
(Jiang et al. 2020)	N	Y	U	U	U	NA	Low	U	U	Ltd info	N	N	U	N	Y	N	Ltd info	Unclear	Low
		?			☺️				?				☹️				?	?	☺️
(Jing et al. 2018)	N	Y	U	U	U	NA	Low	Y	U/N	Low/U	Y	N	U	Y/N	Y	Y/N	SQ NSCLC included	Low	Low

	?				☺			☺ (for Sanger)/ (? for ddPCR)			☺ (for Sanger)/ (? for ddPCR)						?	☺	☺
(Kato et al. 2021)	N	Y	U	U	Y	NA	Low	Y	Y	Low	N	Y	Y	N	N	N	Ltd info	Unclear	Low
	?				☺			☺			☹						?	?	☺
(Kim et al. 2021)	N	Y	U	U	U	NA	U	U	Y	Low	N	N	U	N	Y	N	Unclear	Low	Low
	?				?			☺			☹						?	☺	☺
(Legras et al. 2018)	Y	Y	U	Low	U	Y	Low	Y	U	Low	N	N	U	N	Y	N	Ltd info	Low	Low
	☺				☺			☺			☹						?	☺	☺
(Li, W, Guo, L, et al. 2021)	N	Y	U	U	U	NA	Low	Y	U	Low	N	N	U	N	N	N	Low	Low	Unclear
	?				☺			☺			☹						☺	☺	?
(Lin, C et al. 2019)	N	Y	U	U	Y	NA	Low	Y	Y	Low	Y	Y	Y	Y	Y	N	U	U	Low
	?				☺			☺			☺						?	?	☺
(Vollbrecht et al. 2018)	N	N	U	High	Y	Y	U	Y	Y	Low	N	N	U	N	Y	N	U	Y	Y
	☹				?			☺			☹						?	☺	☺
(Wei et al. 2021)	N	N	U	High	Y	N	U	Y	Y	Low	N	N	U	N	Y	N	U	Y	U
	☹				?			☺			☹						?	☺	?
(Xie et al. 2019)	N	Y	N	Low	U	NA	Low	Y	U	Low	Y	N	U	N	Y	N	Low	U	Low
	☺				☺			☺			?						☺	?	?
(Xu, X. et al. 2016)	Y	Y	Y	Low	Y	NA	Low	Y	Y	Low	Y	N	U	Y	Y	N	SQ NSCLC included	U	U
	☺				☺			☺			☺						?	?	?

☺ Low risk ☹ High risk ? Unclear risk

Results

Comparative rate of successful testing

One of the key benefits of NGS is claimed to be the ability to make efficient use of tumour tissue to test multiple genes at once, whereas the success of sequential single-gene testing can decrease, the more genes are analysed. Three within-patient studies provided the overall success rates of small gene panel testing with NGS, versus the overall success of sequential single gene testing. These are shown in Table 80.

Table 80 Success rates of sequential single-gene testing and NGS (within patient comparisons)

Study	Population	Success of sequential single-gene analyses				Success of NGS
		<i>EGFR</i>	<i>ALK</i>	<i>ROS1</i>	Overall	
(Xie et al. 2019) China	85 patients with advanced non-squamous NSCLC FFPE samples from endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA)	ARMS-PCR using 10-15 FFPE sections 5 µm thick	IHC performed on 71/85 (83.5%)	Quantitative reverse transcription PCR. Total RNA extracted from 10-15 FFPE sections 5 µm thick	80/85 (94.1%)	77/80 (96.2%) of those successfully tested single-gene tests
(Yu et al. 2019) United States	N=169 investigational Oncomine Dx Target Tests on archived FFPE NSCLC tissue samples (index test) N=3,659 single-gene tests across 1,402 clinician-submitted samples (comparator tests)	~83%,	FISH: 89.1%	76.6%	88.4% for ≥1 biomarkers 76.6% for ≥4 biomarkers	98.9% in surgical resection samples, 75.4% in CNB and 69.2% in FNA
(Zugazagoitia et al. 2018) Spain	N=109 consecutive advanced stage (mostly stage IV) NSCLC patients reviewed in a single centre	87/92 (94.5%) 5 non-informative results	85/92 (92.3%) 7 insufficient tissue for IHC (including 5 cytologies)	82/92 (89.1) 10 insufficient tissue for IHC (including 2 cytologies)	75/92 (81.5%)	95/109 (87.1%) 7 unsuccessful due to insufficient tissue, 4 had poor DNA quality, 3 due to analytical reasons

ALK = anaplastic lymphoma kinase; CNB = core needle biopsy; *EGFR* = epidermal growth factor receptor; FFPE = formalin-fixed paraffin embedded; FISH = fluorescent in situ hybridisation; FNA = fine needle aspirate; IHC = immunohistochemistry; NGS = next generation sequencing; NSCLC = non-small cell lung cancer; RNA = ribonucleic acid; *ROS1* = ROS proto-oncogene 1

Studies which reported the success of NGS versus a single-gene test for individual genes are shown in Table 81. The success of *EGFR* testing alone was better performed by single-gene testing than NGS in nearly every study. However, if patients have wildtype *EGFR*, they would be

indicated for further testing, so the success rates of *EGFR* alone by single-gene testing do not represent the overall success. Two studies which reported on the success rates of *ALK* testing favoured NGS rather than single-gene testing using FISH.

For the comparative success of the whole testing strategy (*EGFR* ± *ALK* ± *ROS1*) by sequential single-gene testing versus NGS, see 'Success rate of testing/rate of sufficient tissue', pg 58 in the main body.

Table 81 Success rates of NGS and single-gene testing for individual genes

Study	Intervention	Comparator	Intervention success (NGS)	Comparator success (single-gene testing)	Difference
EGFR					
Ariyasu, 2021	Oncomine Dx Target test	cobas for <i>EGFR</i>	110/134 (82.1%)	137/142 (96.5%)	-14.4%
D'Haene, 2015	Ion Ampliseq Colon and Lung cancer panel	quantitative PCR for <i>EGFR</i>	38/39 (97.4%)	35/39 (89.7%)	7.7%
Fernandes, 2019	NGS	SS	117/121 (96.7%) 4 insufficient samples	122/125 (97.6%) 3 insufficient samples	-0.9%
Hamblin, 2017	NGS	cobas	342/351 (97.4%)	275/278 (98.9%)	-1.5%
Hinrichs, 2015	NGS 454	cobas for <i>EGFR</i>	20/25 (80.0%)	25/25 (100%)	-20%
(Ji et al. 2019)	NGS	Sanger sequencing	194/199 (97.4%) 4 excluded due to low amount of DNA sample, 1 due to low DNA quality	191/194 (98.4%) 3 insufficient samples after NGS	-1.0%
Lassalle, 2020	NGS	Idylla <i>EGFR</i> test panel	66/83 (79.5%)	889/901 (98.7%) (Note: only included 901/1368 which had more than 10% tumour cell content)	-19.2%
Legras, 2018	NGS	TaqMan real-time qPCR	1268/1343 (94.4%)	1207/1274 (94.7%)	-0.3%
Li, 2021	Tissue NGS	ARMS-PCR for <i>EGFR</i>	930/1184 (78.5%) 73 had insufficient tissue, 71 had insufficient DNA, 3 with failed library, 7 with low-quality sequences	78/81 (96.3%) in NGS-failed samples	-17.8%
Sakaguchi, 2021	Oncomine Dx Target Test	PNA-LNA PCR clamp for <i>EGFR</i>	82/85 (96.5%) 2 ineligible for NGS due to insufficient tumour content, 1 had insufficient DNA	80/85 (94.1%)	-10.3%
		Overall success of <i>EGFR</i> testing	3189/3595 (88.7%)	3075/3175 (96.9%)	-8.1%

Study	Intervention	Comparator	Intervention success (NGS)	Comparator success (single-gene testing)	Difference
ALK					
(Batra et al. 2021)	NGS	ALK FISH	58/58 (100%)	58/71 (81.7%) 13 FISH unevaluable due to depleted tissue/signal problems	18.3%
(Lin, C et al. 2019)	NGS	ALK FISH	55/55 (100%)	34/45 (75.6%) 11 did not show appropriate hybridisation signals	24.4%
		Overall success of ALK testing	113/113 (100%)	92/116 (79.3%)	20.7%

ALK = anaplastic lymphoma kinase; DNA = deoxyribonucleic acid; EGFR = epidermal growth factor receptor; FISH = fluorescent in situ hybridisation; IHC = immunohistochemistry; IQR = inter quartile range; FNA = fine needle aspirate; ISH = in situ hybridisation; KRAS = Kirsten rat NGS = next generation sequencing; PNA-LNA = peptide nucleic acid, locked nucleic acid polymerase chain reaction; qPCR = quantitative polymerase chain reaction

The likelihood of successful testing is related to the amount of tissue required for testing, and tissue availability. Yu et al. (2019) reported that on average, the EGFR therascreen used 2.7 slides per test (n=1107), ALK FISH required an average of 1.1 slides per test (n=979), MET amplification required 1.3 slides per test (n=614). The tests which required the largest number of slides was BRAF testing (mean of 7.1 slides per test, n=20), and KRAS (mean 6.8 slides per test, n=190). The more single-gene tests ordered, the lower the probability of success across all biomarkers ordered. The success rate for ≥ 1 test was 88.4%, whereas for samples where ≥ 4 tests were ordered, the success rate for all the biomarkers was 76.6% (Yu et al. 2019). In this study, the use of the Oncomine Dx Target Test was artificially restricted to a single slide based on the investigational nature of the test. In the real-world setting, the number of slides is limited by the sample retrieved, and likely to be much higher than a single slide. The success rates of the Oncomine Dx Target Test was 98.9% in surgical resection samples, 75.4% in core needle biopsy (CNB) samples and 69.2% in fine needle aspirate (FNA) samples (Yu et al. 2019). These success rates of the 23 gene Oncomine Dx test were similar to single-gene testing of 1 to 2 biomarkers on tumour tissue, 4 to 5 biomarkers on CNB samples, and 3 to 4 biomarkers on FNA samples.

(Dall'Olio et al. 2020) reported that for extraction of DNA and RNA for NGS, 25 μm of biopsied tumour tissue, or 10 μm of surgical sample was required. The total quantity of extracted DNA would not be sufficient for all PCR genes if testing in a sequential method. Each FISH and IHC analysis required another 3 μm . Using a sequential step-wise algorithm, they estimated that ~33 μm thick tumour cell sections were required to complete a panel of predictive biomarkers (PCR tests for EGFR, KRAS, BRAF and MET, and IHC for ALK, ROS1, RET and HER2). This did not include FISH for ALK and ROS1, so would have underestimated the amount of tissue required had sequential testing followed the Australian algorithm.

Concordance of NGS compared to sequential single-gene testing

The forest plots for the concordance of small gene panel testing (using NGS) against single gene testing are shown in Figure 22 to Figure 26.

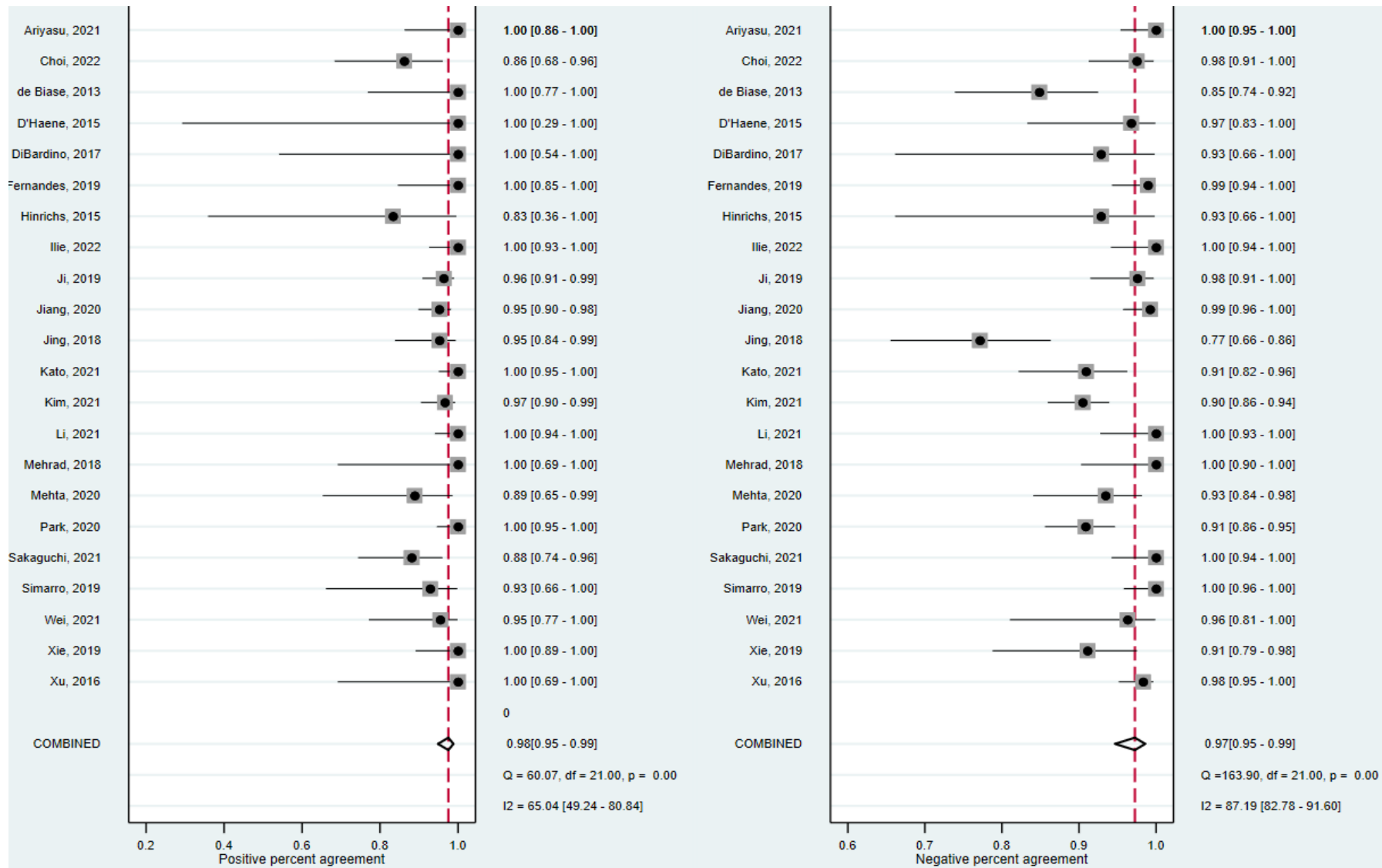


Figure 22 PPA and NPA of NGS compared to single-gene testing for EGFR variants

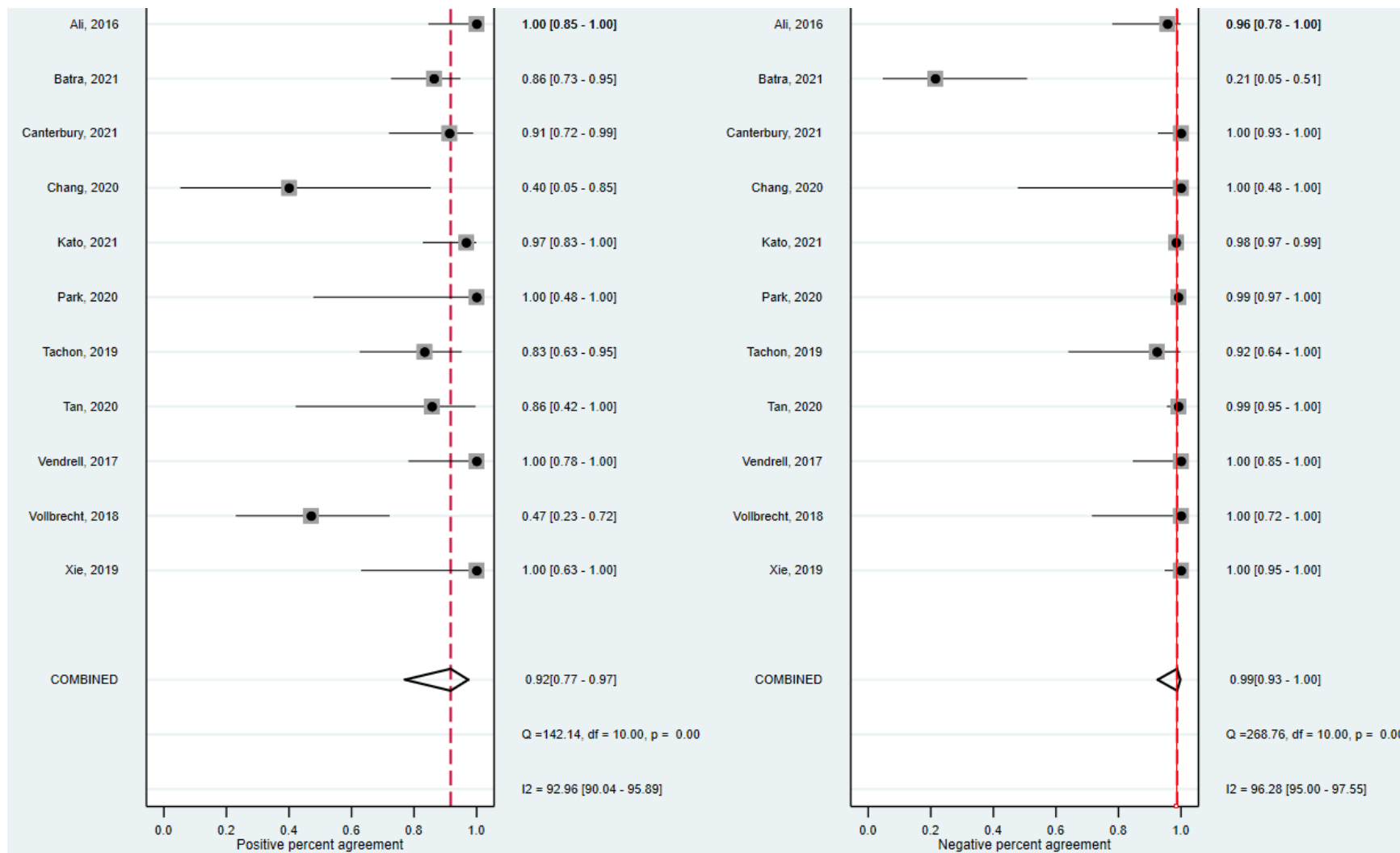


Figure 23 PPA and NPA of NGS compared to single-gene testing for ALK fusions (comparator a mix of IHC + FISH or FISH alone)

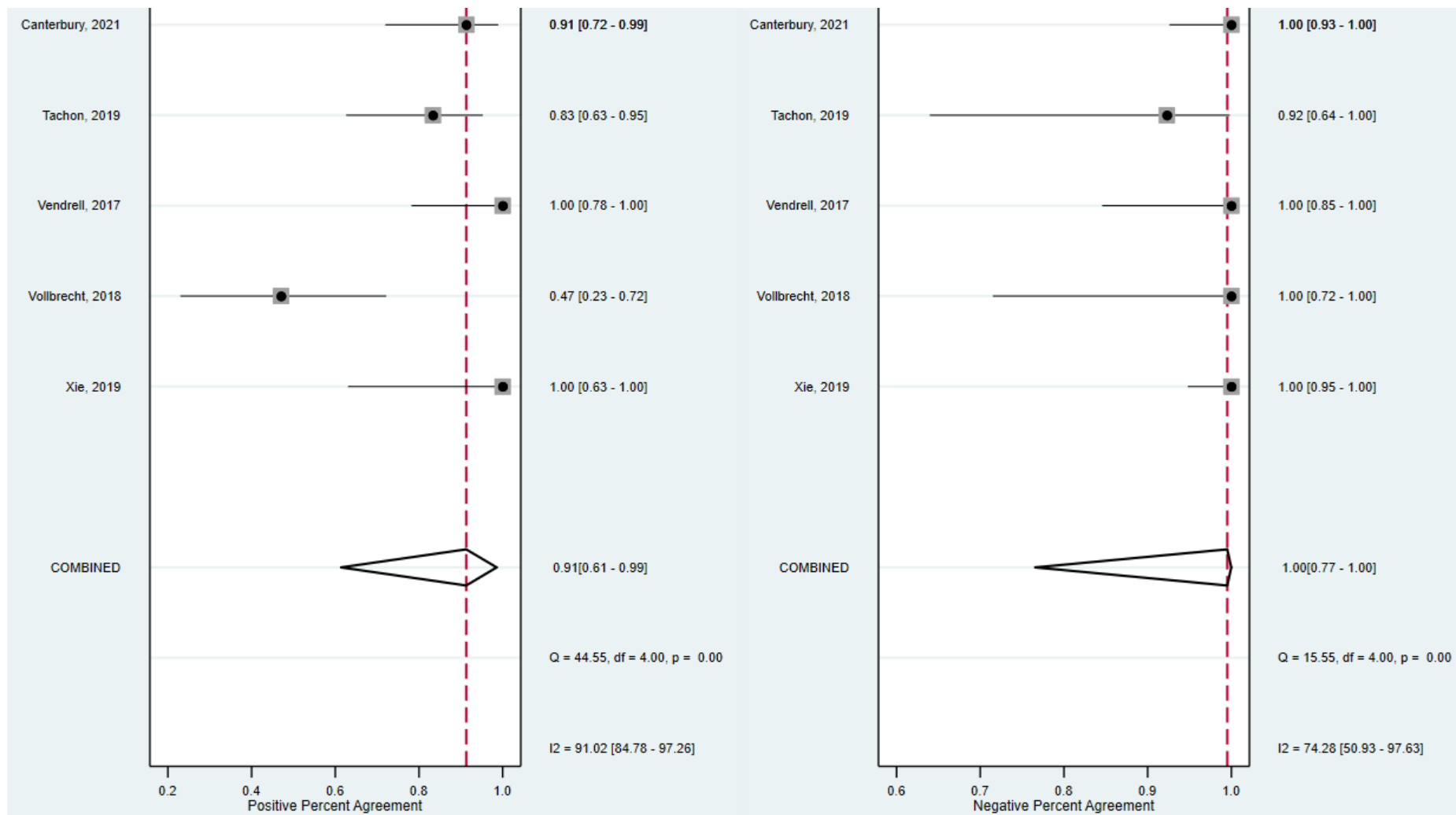


Figure 24 PPA and NPA of NGS compared to single-gene testing for ALK fusions (comparator IHC + FISH)

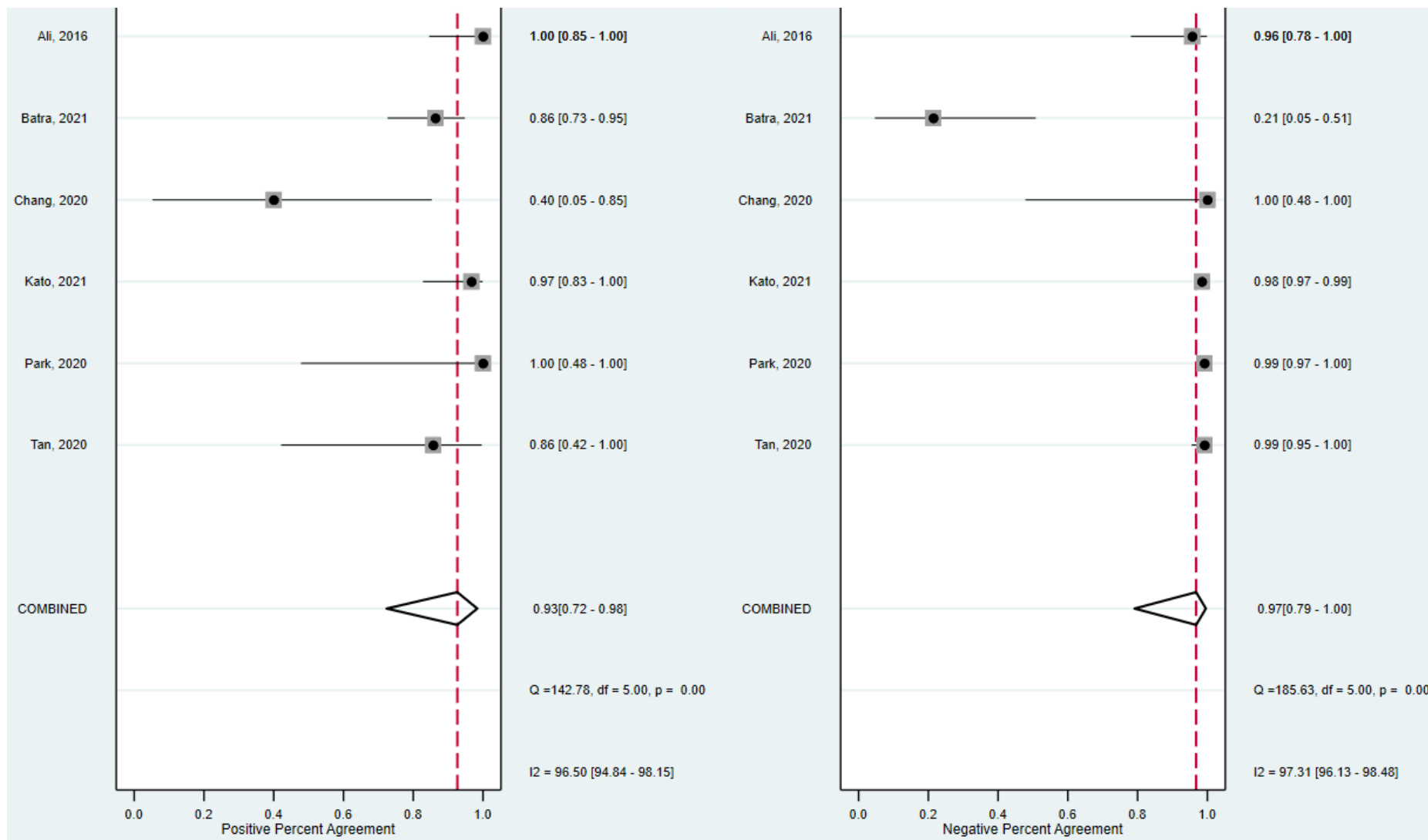


Figure 25 PPA and NPA of NGS compared to single-gene testing for ALK fusions (comparator FISH)

There was a higher rate of “false positives” detected by NGS when compared to the clinical utility standard of FISH, than when compared to the Australian comparator, IHC ± FISH.

Vollbrecht et al. (2018) explored the concordance of massive parallel sequencing (MPS; IonTorrent) with IHC and FISH, including in patients with discordant IHC and FISH results. The clinical utility standard for drugs targeting ALK fusion is FISH, whereas in Australia, IHC triage testing occurs prior to FISH. Vollbrecht reported that although there were cases of discordance between FISH and MPS, there was complete concordance when both IHC and FISH were positive (Vollbrecht et al. 2018).

It is hypothesised that cases who are positive on IHC and borderline on FISH might have a higher chance of being considered to have the ALK fusion, than those considered borderline on FISH, without the additional information. This may explain the differences in results found in the subgroup analyses in Figure 24 and Figure 25.

Table 82 Concordance of massive parallel sequencing NGS with IHC and FISH for detecting ALK fusions

Study	Standard test results	NGS +	NGS -	NGS not analysable
(Vollbrecht et al. 2018)	IHC- / FISH-	0	11	1
	IHC+ / FISH+	6	0	0
	IHC uncertain/ FISH borderline +	0	1	0
	IHC + / FISH borderline +	2	0	0
	IHC - / FISH borderline +	0	5	0
	IHC - / FISH borderline -	0	4	0
	IHC - / FISH +	0	3	0

ALK = anaplastic lymphoma kinase; IHC = immunohistochemistry; FISH = fluorescent in situ hybridisation; NGS = next generation sequencing;

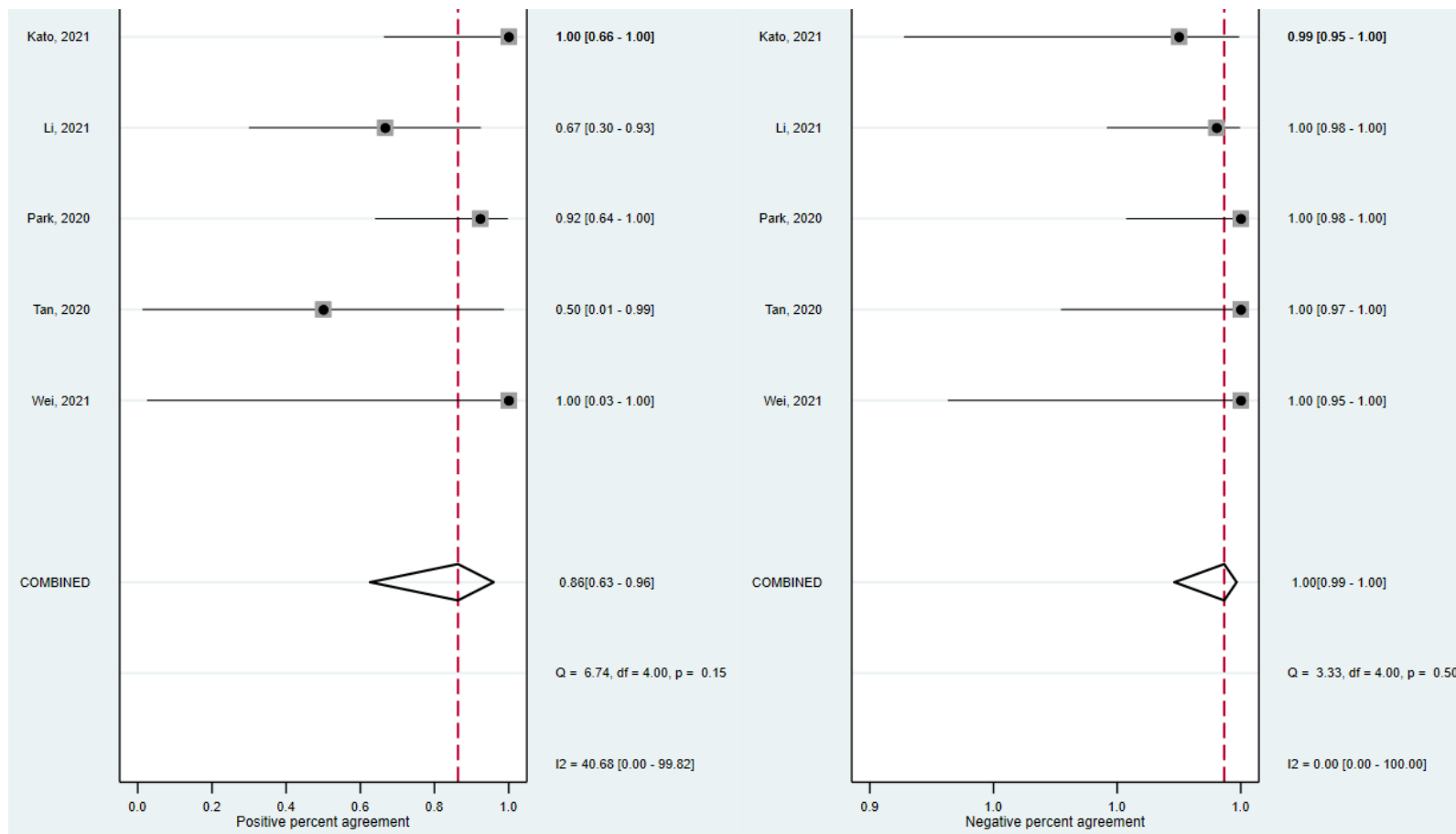


Figure 26 PPA and NPA of NGS compared to single-gene testing for *ROS1* fusions

Note: Wei et al. (2021) and Kato et al. (2021) used reverse transcription or real time PCR for single-gene testing rather than FISH. These studies were retained as STATA did not allow meta-analysis of fewer than 4 studies. A small additional study 'Mehta et al. 2021' was also included, but had no true positives, and caused errors.

Table 83 Comments on discordance

Gene	Reference	Intervention (NGS)	Comparator (SG)	Reasons for NGS+/SG-	Reasons for NGS-/SG+
	(de Biase et al. 2013)	NGS (454 NGS)	Sanger sequencing	Variant reads were below the analytical sensitivity threshold of Sanger sequencing.	
EGFR	(DiBardino et al. 2017)	NGS	Single-gene PCR	Single-gene testing missed a second-variant in one patient (T790M) and patient was put on erlotinib. This underlying variant was present at a variant frequency below the sensitivity of the single-gene PCR.	-
	(Ji et al. 2019)	NGS	Sanger Sequencing and/or ARMS-PCR	SS and/or ARMS-PCR missed 2 resistance-conferring variants	4 cases with a low abundance of EGFR were missed by NGS
	(Jiang et al. 2020)	OncoAim	ARMS-PCR	NGS detected a variant missed by ARMS-PCR (variant confirmed on SS)	NGS missed cases with low allele frequency (ARMS-PCR could detect 1%, whereas NGS could detect >5%)
	(Jing et al. 2018)	Lung panel on IonTorrent personal genome machine (Thermo Fisher) (7 genes)	Sanger Sequencing	SS had sensitivity of 10% whereas NGS had sensitivity of 0.1% (NGS picked up low frequency variants)	
	(Kato et al. 2021)	Compact NGS panel	cobas EGFR mutation test v2	7 "false positive" variants by NGS confirmed by digital PCR	
	(Legras et al. 2018)	NGS	TaqMan real-time qPCR	40 rare variants not included in TaqMan real-time PCR	
	(Mehta et al. 2020)	Ion Ampliseq Cancer Hotspot Panel V2	Qiagen EGFR Therascreen PCR		
	(Park & Shim 2020)	TruSight Tumour 170 (DNA and RNA sequencing)	PCR	8 hotspot variants missed by PCR, as well as 8 rare variants not able to be detected by PCR	
	(Sakaguchi et al. 2021)	Oncomine Dx Target Test	PNA-LNA PCR clamp		PNA-LNA PCR clamp able to detect a lower concentration than NGS

Gene	Reference	Intervention (NGS)	Comparator (SG)	Reasons for NGS+/SG-	Reasons for NGS-/SG+
	(Simarro et al. 2019)	DNA NGS	RT-PCR		One T790M variant not detected by NGS
	(Xie et al. 2019)	NGS (Lung core 56 gene panel)	ARMS-PCR with EGFR 21 Mutations Detection Kit	NGS detected variants not covered by ARMS-PCR	
ALK	(Park & Shim 2020)	TruSight Tumour 170 (DNA and RNA sequencing)	FISH	2 cases positive on IHC but not on FISH were positive on NGS	
	Kato, 2021	Compact NGS panel	FISH		1 rare type of fusion not covered on compact NGS panel
ROS1	(Park & Shim 2020)	TruSight Tumour 170 (DNA and RNA sequencing)	FISH		ROS1 IHC returned diffuse moderate protein expression, whereas NGS identified non-functional ROS1 fusion

ARMS-PCR = amplification-refractory mutation system polymerase chain reaction; DNA = deoxyribonucleic acid; IHC = immunohistochemistry; FISH = fluorescence *in situ* hybridisation; NGS = next generation sequencing; PCR = polymerase chain reaction; PNA-LNA = peptide nucleic acid, locked nucleic acid polymerase chain reaction; RNA = ribonucleic acid; RT-PCR = real time polymerase chain reaction; SG = single-gene testing; SS = Sanger Sequencing

Concordance per variant (rather than per gene/patient)

Two studies reported on the concordance of NGS against single-gene testing (Sanger sequencing, cobas real time-PCR or TaqMan real-time PCR) separately for different variants (rather than per patient) (Legras et al. 2018; Tan et al. 2020). Tan et al. (2020) reported that in a sample of 173 patients from Singapore, NGS identified an additional 12 variants which single-gene testing did not, including 4 targetable *EGFR* sensitising variants, 3 T790M variants, and 5 'other' (unknown if the variants would confer sensitivity or resistance to TKIs). In this study, the addition of NGS would therefore have influenced the treatment of 7/173 patients (4.0%).

Legras et al. (2018) reported on a case series of 1274 patients with NSCLC from France, with tumours tested with both NGS and single-gene testing for *EGFR* variants. When focusing only on sensitising *EGFR* variants, NGS identified an additional 44/1175 (3.7%) sensitising *EGFR* variants (exon 19 deletions or L858A variants), and an additional 2 cases of T790M variants. It is unknown how cases were selected for testing of T790M variants, or how representative they were of the whole sample.

Table 84 Per variant analyses of concordance

Study	Intervention (NGS)	Comparator (SG)	EGFR Variant	NGS+ /SG+	NGS+ /SG-	NGS- / SG+	NGS- /SG-
Tan et al. 2020	NGS		Exon 19 deletion	42	3	3	122

Study	Intervention (NGS)	Comparator (SG)	EGFR Variant	NGS+ /SG+	NGS+ /SG-	NGS- / SG+	NGS- /SG-
			L858R mutation	34	1	1	138
			T790M mutation	0	3	0	171
		SS or Cobas® RT-PCR	other mutations	10	5	1	158
Legras et al. 2018	NGS (Massive parallel sequencing of 92 amplicons, Ion AmpliSeq Colon-Lung Cancer Research Panel)	TaqMan real-time qPCR	EGFR L858A and Exon 19 deletion	182	44	0	949
			T790M mutation	11	2	3	4

EGFR = epidermal growth factor receptor; NGS = next generation sequencing; RT-PCR = real time polymerase chain reaction; SG = single-gene testing

Interrater reliability

One study was identified which reported on how reliable RNA/DNA sequencing using NGS was, compared to in situ hybridisation (ISH) and IHC, for detection *ALK* alterations in tumour sections from NSCLC (Jurmeister et al. 2021). A total of 57 participants analysed tissue from 10 different specimens. The highest interrater agreement (Fleiss' kappa) was reported for RNA/DNA sequencing (0.975), followed by ISH (0.898) and IHC (0.888).

Identification of concurrent variants

In addition to more patients having been identified with pathological variants by NGS than single-gene testing, there is also the benefit that NGS may identify concurrent variants in multiple genes at once. Studies reported additional variants in combination with *EGFR* and reported worse health outcomes on average than those with only *EGFR* variants. However, it is unclear whether this information would alter management, although the prognostic implications may be value for the individuals to know.

One of the claimed benefits of NGS is the ability to detect multiple variants at once. Conversely, with sequential testing, a limited range of genes are tested, and further single-gene tests would not be performed if a causative variant is identified, even if a targeted treatment is not available for that variant. No studies were identified which compared the rate of concurrent variants identified by NGS and sequential single-gene testing strategies. Therefore, single-arm data (from NGS) are presented. Further single-arm studies were not sought.

Nine cohort studies provided information on the rate of concurrent variants identified by NGS, although it was not always explicit whether the multiple variants were within the same or different genes, and therefore whether they would have been detected by single-gene testing or not. When genes such as TP53 were included in the analysis, the rate of concurrent variants was high. However, when limited to the genes proposed to be listed as the minimum variants for the

NGS panel(s) (i.e., *EGFR*, *BRAF*, *KRAS*, *MET* exon 14, *ALK*, *ROS1*, *RET* and *NTRK*), the rate of concurrent variants between genes was low in all except one study. In the eight studies shown in Table 85 which provided details on the rate of concurrent variants in different genes, from a total of 962 patients, there were 50 patients with concurrent variants in the genes specifically listed (5.2%). Three patients (from three separate studies) had variants in both *EGFR* and *ALK* genes (0.3% of patients), and one patient had variants in both *ALK* and *ROS1* (0.1%). It is unclear how the additional concurrent variants identified by NGS would impact on the clinical management of the patient.

Table 85 Rate of concurrent variants identified by NGS

Study	Population	Intervention	Overall rate of concurrent variants identified	Rate of concurrent variants
(DiBardino et al. 2017)	20 patients with NSCLC	Targeted NGS of tumour DNA (Colombia Combined Cancer Panel)	1/20 (5%) had multiple variants	1 case had 2 <i>EGFR</i> variants (L858R and T790M)
(Ji et al. 2019)	199 patients with NSCLC	NGS assessing 10 genes, DNA and RNA	5/199 (2.5%) double variants in <i>EGFR</i> gene 4/199 (2.0%) had 2 genes with variants	1 case with <i>EGFR</i> -T790M + <i>ALK</i> M1 1 case <i>KRAS</i> G12A + <i>EGFR</i> G719A 1 case <i>KRAS</i> G12C + <i>ALK</i> M2 1 case <i>KRAS</i> G12V + <i>PIK3CA</i> H1047R
(Jing et al. 2018)	112 patients with NSCLC	NGS: The Lung Panel including <i>BRAF</i> , <i>EGFR</i> , <i>KRAS</i> , <i>NRAS</i> , <i>PIK3CA</i> , <i>Her-2</i> and <i>TP53</i> from DNA	24/112 (21.4%) with variants in 2 or more genes 2 with variants in 3 genes	13 cases of <i>EGFR</i> + <i>TP53</i> 3 cases <i>KRAS</i> + <i>TP53</i> 2 cases <i>TP53</i> + <i>PIK3CA</i> 1 case <i>EGFR</i> + <i>PIK3CA</i> 1 case <i>EGFR</i> + <i>KRAS</i> 1 case <i>EGFR</i> + <i>NRAS</i> 1 case <i>EGFR</i> + <i>Her-2</i> 1 case <i>BRAF</i> + <i>TP53</i> 1 case <i>BRAF</i> + <i>PIK3CA</i>
(Li, W, Li, Y, et al. 2021)	166 patients with NSCLC	NGS of DNA of <i>EGFR</i> , <i>ALK</i> , <i>ROS1</i> , <i>KRAS</i> , <i>BRAF</i> V600E, <i>HER2</i> , <i>RET</i> , <i>MET</i> ex14sk, <i>MET</i> amplification, and <i>NTRK</i>	1/166 (0.6%)	1 case of <i>EGFR</i> + <i>MET</i> amplification
(Mehta et al. 2020)	100 patients with NSCLC	NGS of 28 different genes including fusions for <i>ALK</i> , <i>ROS1</i> , <i>RET</i> and <i>NTRK</i> (in 70/100 tumours)	27/100 (27%) variants in 2 genes (most common gene <i>TP53</i>) 10 variants in 3 genes 5 variants in >3 genes	8 cases <i>EGFR</i> + <i>TP53</i> 2 cases <i>EGFR</i> + <i>KRAS</i> 1 case <i>EGFR</i> + <i>BRAF</i> 2 cases <i>EGFR</i> + <i>PIK3CA</i> 1 case <i>EGFR</i> + <i>PTEN</i> Unclear what concurrent variants did not occur with <i>EGFR</i>

Study	Population	Intervention	Overall rate of concurrent variants identified	Rate of concurrent variants
(Simarro et al. 2019)	106 patients with NSCLC	Oncomine Solid Tumor kit (DNA) and Oncomine Solid Tumor Fusion Transcript kit (RNA)	35/106 (33%) with concurrent variants	see Figure 27
(Tan et al. 2020)	174 patients with NSCLC	NGS on separate DNA and RNA		1 <i>EGFR</i> L858R + <i>ALK</i> fusion 1 <i>EGFR</i> ex20ins + <i>RET</i> fusion 4 cases <i>EGFR</i> + <i>KRAS</i> variants 1 <i>KRAS</i> + <i>MET</i> variants
(Xie et al. 2019)	85 patients with non-squamous NSCLC	Capture-based NGS of 56 genes on samples collected from EBUS-TBNA	5/85 (5.9%) with concurrent variants in genes of interest	1 case <i>EGFR</i> + <i>ROS1</i> 1 case <i>ALK</i> + <i>ROS1</i> 1 case <i>EGFR</i> + <i>KRAS</i> 1 case <i>ALK</i> + <i>KRAS</i> 1 case <i>EGFR</i> + <i>MET</i>
(Xu, Xinnan et al. 2016)	188 patients with NSCLC	NextDaySeq Lung panel on Ion Torrent™ system	10/188 with concurrent variants (unclear if in same or different gene)	Not stated

ALK = anaplastic lymphoma kinase; *DNA* = deoxyribonucleic acid; *EBUS-TBNA* = endobronchial ultrasound-guided transbronchial needle aspiration; *EGFR* = epidermal growth factor receptor; *NGS* = next generation sequencing; *NSCLC* = non-small cell lung cancer; *NTRK* = neurotrophic tyrosine receptor kinase; *RET* = rearranged during transfection; *ROS1* = ROS proto-oncogene 1

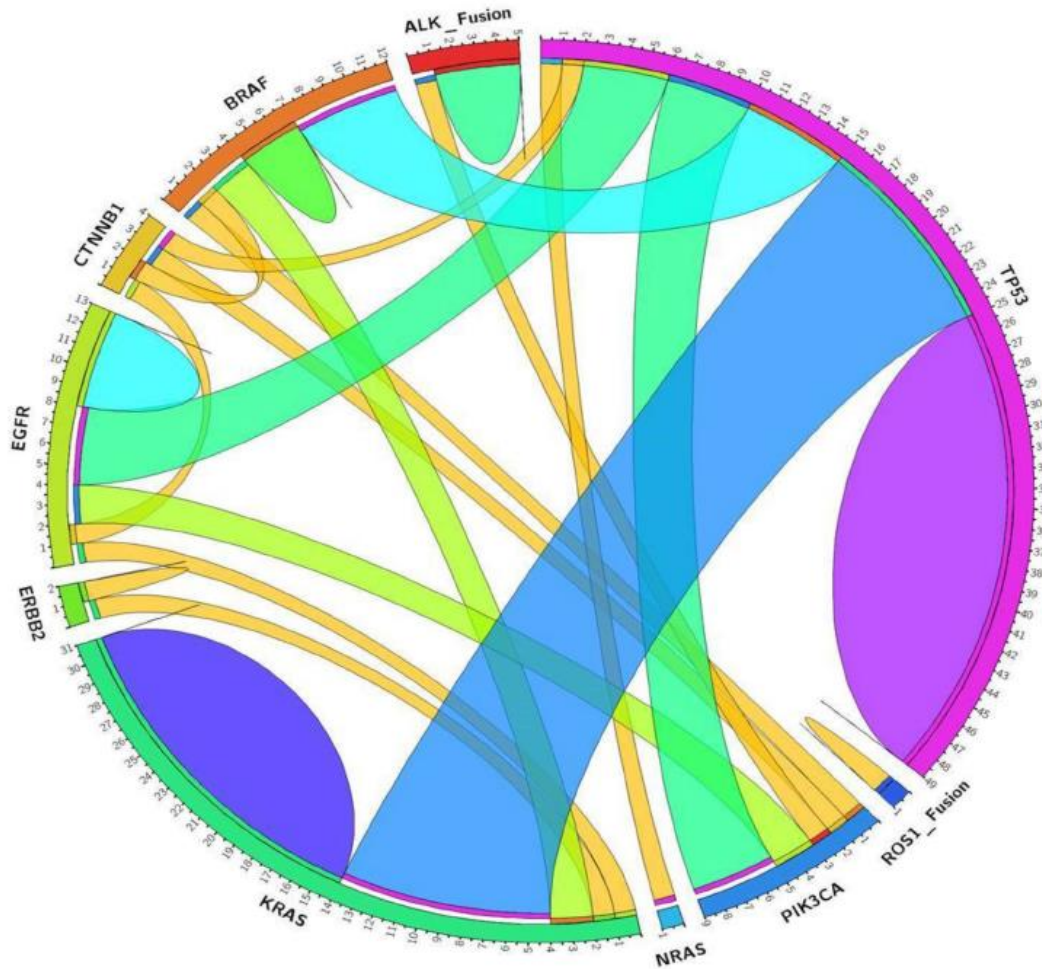


Figure 27 Circos diagram showing associations of the most prevalent concurrent variants in NSCLC tumours

Source: (Simarro et al. 2019) Used under Creative Commons Attribution License.

ALK = anaplastic lymphoma kinase; *BRAF* = proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B; *CTNNB1* = Catenin Beta 1; *EGFR* = epidermal growth factor receptor; *ERBB2* = erb-b2 receptor tyrosine kinase 2; *KRAS* = Kirsten rat sarcoma; *NRAS* = Neuroblastoma RAS viral oncogene homolog; *NSCLC* = non-small cell lung cancer; *PIK3CA* = Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha; *ROS1* = ROS proto-oncogene 1; *TP53* = tumour protein P53

One study reported on the proportion of patients with concurrent variants identified by any method. Although this study was less relevant than those reporting only on NGS, it was included due to size of the study (larger than all the other studies combined), and level of detail it provided. Griesinger et al. (2021) reported on a prospective case series of patients from Germany with non-squamous NSCLC. Testing was performed through a variety of methods, including NGS, non-NGS-sequencing, IHC and FISH. The level of concurrent variants identified was much higher than reported in the studies in Table 85, but the prevalence of variants in total was high in this population (EGFR variants in 28.2%, almost twice the 15% expected in Australia; ROS1 was 5.2%, approximately three times higher than the 1.61% expected in Australia, and ALK was 10.4%, over three times higher than the 3% prevalence expected in Australia). The applicability of these German data to the Australian setting is therefore limited, as the results likely overestimate the frequency of concurrent variants.

If patients with *EGFR* variants were not tested for *ALK* or *ROS1* variants (as per sequential single-gene testing), 25 patients who had concurrent EGFR and ALK or ROS1 variants would have been missed (2.2% of the total tested population).

If patients with variants identified on *EGFR*, *BRAF*, *C-MET* or *KRAS* were ineligible for testing for *ROS1*, *ALK* or *RET*, a total of 78/1131 (6.9%) cases with rearrangements in these genes would have been missed. This equates to 47.4% of those with *ROS1* rearrangements, and 29.7% of those with *ALK* rearrangements (or 2.5% and 3.1% of the total NSCLC population) who would not have these rearrangements detected due to the RNA panel not being performed.

Table 86 Concurrent alterations in 1,131 patients with non-squamous NSCLC (detected by any method)

Alteration	<i>EGFR</i> (n=320; 28.2%)	<i>BRAF</i> (n=65; 5.7%)	<i>C-MET</i> (n=96; 8.5%)	<i>KRAS</i> (n=504; 44.6%)	<i>ROS1</i> (n=59; 5.2%)	<i>ALK</i> (n=118; 10.4%)	<i>RET</i> (n=16; 1.4%)
<i>EGFR</i>	x	4 (25.0%)	7 (10.8%)	8 (8.3%)	12 (20.3%)	13 (11.0%)	4 (25.0%)
<i>BRAF</i>	7 (2.2%)	x	6 (6.3%)	13 (2.6%)	5 (8.5%)	5 (4.2%)	2 (12.5%)
<i>C-MET</i>	8 (2.5%)	6 (9.2%)	x	34 (6.7%)	2 (3.4%)	3 (2.5%)	2 (12.5%)
<i>KRAS</i>	16 (5.0%)	13 (20.0%)	34 (35.4%)	x	9 (15.3%)	14 (11.9%)	7 (43.8%)
<i>ROS1</i>	12 (3.8%)	5 (7.7%)	2 (2.1%)	9 (1.8%)	x	11 (9.3%)	1 (6.3%)
<i>ALK</i>	13 (4.1%)	5 (7.7%)	3 (3.1%)	14 (2.8%)	11 (18.6%)	x	2 (12.5%)
<i>RET</i>	4 (1.3%)	2 (3.1%)	2 (2.1%)	7 (1.4%)	1 (1.7%)	2 (1.7%)	x

Source: (Griesinger et al. 2021)

Green highlighting are the concurrent variants likely missed if an RNA panel not used due to a positive finding on a DNA panel

ALK = anaplastic lymphoma kinase; *BRAF* = proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B; *C-MET* = receptor tyrosine kinase belonging to MET gene; *EGFR* = epidermal growth factor receptor; *KRAS* = Kirsten rat sarcoma; *NSCLC* = non-small cell lung cancer; *ROS1* = ROS proto-oncogene 1

Turnaround time (for NGS vs individual gene testing)

Testing of individual biomarkers using single-gene methods, took between a mean or median of 1 to 12 days for *EGFR*, 1 to 6 days for IHC, and 5 to 11 days for FISH. NGS took a mean or median of 4.8 days to 28 days. The shortest times were reported by Miller et al. In this study the study institution implemented reflex testing from pathology for stage III and IV tumours in order to reduce delays in follow-up testing and to help ensure all samples received appropriate testing.

Table 87 Turnaround time for NGS vs single-gene tests

Study	Population	Intervention (NGS)	Comparator (SG)	Turnaround time for NGS	Turnaround time for comparator
(Ariyasu et al. 2021)	N=167 Consecutive patients with advanced NSCLC Samples obtained through biopsy, surgical resection or other	Oncomine Dx	cobas <i>EGFR</i>	Median 13 (range 9-29)	Median 6 days (range 5-18)
			ALK IHC		median 1 (range 1-3)
(Dall'Olio et al. 2020)	N=537 Consecutive NSCLC (adenocarcinoma) patients Histology and cytology samples	Oncomine Focus Assay	Single gene PCR	~10 days	~5 days
			IHC		~2 days
			FISH		~5 days
(Griesinger et al. 2021)	N=3,717 patients with advanced NSCLC,	NGS	Non-NGS sequencing	Median 13 (IQR 9 - 18)	Median 8 days (IQR 5 - 13)

Study	Population	Intervention (NGS)	Comparator (SG)	Turnaround time for NGS	Turnaround time for comparator
	recruited into the CRISP registry at start of systemic therapy		IHC		Median 6 days (IQR 3-11)
			FISH		Median 11 days (IQR 7-16)
(Lassalle et al. 2020)	N=83 patients with non-squamous lung cancer, <i>EGFR</i> wild-type determined by Idylla assay Biopsy specimens	NGS Hotspot panel	Idylla <i>EGFR</i> mutation test	Mean 8 days (range 4 - 16)	Mean 2 days (range 1 - 3)
(Lin, HM et al. 2022)	N=67,281 advanced NSCLC patients with at least 2 clinic visits who were tested for <i>EGFR</i> mutations.	NGS	PCR (<i>EGFR</i>)	<i>From diagnosis to result</i> : median 28 days (IQR 20 - 63)	<i>From diagnosis to result</i> : median 12 days (IQR 11 - 39 days)
				<i>From receipt of sample to result</i> : median 11 days (IQR 8-14)	<i>From receipt of sample to result</i> : median 8 days (IQR 5-13)
(Miller et al. 2018)	N=302 advanced adenocarcinoma patients. After validation of the assay the turnaround time for the first 302 patients was recorded.	Oncomine Focus Assay	<i>EGFR</i> theascreen only	Mean 4.8 ± 2.1 days	3.6 days
		FoundationOne		8.5 days	
(Robert et al. 2022)	N=3474 patients with mNSCLC that initiated 1 st line treatment and had not received diagnosis or treatment for another cancer	NGS	Individual biomarker	Median 6 (IQR 1, 19)	Median 1 day (IQR 1, 11) to 4 days (IQR 1, 15)
(Tan et al. 2020)	N=174 patients with newly diagnosed NSCLC that underwent routine molecular testing.	Targeted NGS panel	<i>EGFR</i> PCR	Median 15 days: 5 (range 3-28) for assay, 10 (range 6-30) for report	10 days
			Quad FISH		3 – 10 days
			Repeat biopsy		7 days

DNA = deoxyribonucleic acid; *EGFR* = epidermal growth factor receptor; FISH = fluorescent in situ hybridisation; IHC = immunohistochemistry; IQR = inter quartile range; *KRAS* = Kirsten rat sarcoma; mNSCLC = metastatic NSCLC; NGS = next generation sequencing; NSCLC = non-small cell lung cancer; PCR = polymerase chain reaction; RNA = ribonucleic acid; SG = single-gene testing

Linked evidence of change in management

Pisapia et al. (2022) reported on a multicentre study in Italy, surveying five institutions with high expertise in molecular predictive analysis for advanced stage NSCLC (Pisapia et al. 2022). They reported on the average retesting rates for standard testing (predominantly RT-PCR, FISH and IHC) and for maximised NGS (i.e., where NGS is used for all genes of interest, but not for PD-L1). The reliability of these results is unclear, as the data were sought by survey, rather than by prospectively collating data, or by retrospectively analysing records. The method individual centres used to complete the survey is unclear. Two centres provided details of what tests were most likely to be used in the event of retesting due to failure of standard testing methods: one centre reported using standard techniques again (predominantly RT-PCR for *EGFR*, and IHC for *ALK* and *ROS1*, and Sanger sequencing for *MET* exon 14 skipping alterations); the other centre always used NGS. No details about what tests were used in the event of testing due to failure of NGS.

Table 88 Retesting rates due to failure

Gene	% failure retest	
	Standard	NGS
<i>EGFR</i>	8%	3.4%
<i>ALK</i>	9%	0.2%
<i>ROS1</i>	9%	0.2%
<i>MET</i> ex14 skipping	15%	0.3%

ALK = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; *MET* = mesenchymal-epithelial transition exon 14 skipping alteration; NGS = next generation sequencing; *ROS1* = ROS proto-oncogene 1
Source: (Pisapia et al. 2022)

Appendix E Evidence profile tables

Table 89 Summary of findings table for important outcomes comparing small NGS panels with sequential single gene testing

<p>Question: What is the safety and effectiveness of small NGS DNA/RNA panel(s) compared to sequential single gene testing in patients with non-squamous (or histology not otherwise specified) non-small cell lung cancer?</p> <p>Patient or population: Patients with non-squamous (or histology not otherwise specified) non-small cell lung cancer</p> <p>Intervention: Small NGS DNA/RNA panel(s)</p> <p>Comparator: Sequential single gene testing</p>						
Section in report	Aim/outcomes	Participants and studies	Quality of evidence	Results	Interpretation	GRADE
2.1 Direct from test to health outcomes evidence	Predictive of response to ALK-TKI	n=50 k=1 within-patient cohort study	Risk of bias: -1 Inconsistency: NA Indirectness: -1 Imprecision: -1 Publication bias: NA Other: 0	Median PFS NGS+: 11.1m NGS-: 4.6m IHC+: 10.3m IHC-: 11.7m FISH+: 8.8m FISH-: 14.8m	NGS better able to predict response to ALK-TKI than FISH or IHC. Those treated with ALK TKI due to variant detected with NGS therefore expected to have at least non-inferior outcomes to those tested by clinical utility standard or IHC ± FISH.	⊕⊖⊖⊖ Very high uncertainty as single study, observational evidence, moderate to high risk of bias, indirectness as population does not match target population (only ALK positive by ≥1 test), and small study.
2.2 Test performance	Success rate of testing (sufficient tissue)	n=4040 k=1 between-patient cohort	Risk of bias: 0 Inconsistency: NA Indirectness: -1 Imprecision: 0 Publication bias: 0 Other: 0	NGS: 97.2% Single-gene testing: 94.6%	NGS small DNA panel ± IHC, FISH or RNA panel able to make better use of tumour tissue available than single-gene testing.	⊕⊕⊖⊖ Moderate uncertainty as single observational study in a healthcare setting likely similar to Australia, low to moderate risk of bias, but many more genes were tested for than proposed in Australia (in intervention and comparator).
2.2 Test performance (continued)	Concordance of NGS against single-gene testing	n=4081 k=30 within-patient cohorts	Risk of bias: -1 Inconsistency: 0 Indirectness: 0 Imprecision: 0	Overall concordance: 95.7% 3.5% additional actionable	NGS highly concordant with single-gene testing, with some additional cases detected due to higher sensitivity (lower threshold of detection), and detecting rare variants.	⊕⊕⊕⊖ Low uncertainty due to the volume of studies, and good consistency between studies.

<p>Question: What is the safety and effectiveness of small NGS DNA/RNA panel(s) compared to sequential single gene testing in patients with non-squamous (or histology not otherwise specified) non-small cell lung cancer?</p> <p>Patient or population: Patients with non-squamous (or histology not otherwise specified) non-small cell lung cancer</p> <p>Intervention: Small NGS DNA/RNA panel(s)</p> <p>Comparator: Sequential single gene testing</p>						
Section in report	Aim/outcomes	Participants and studies	Quality of evidence	Results	Interpretation	GRADE
			Publication bias: 0 Other: 0	variants identified by NGS but not comparator 0.8% actionable variants missed by NGS (identified by comparator)		
	Turnaround time of test results	n=5462 k=3 between or within-patient cohort studies	Risk of bias: -1 Inconsistency: -1 Indirectness: -1 Imprecision: 0 Publication bias: 0 Other: 0	NGS: mean or medians of 10 to 12 days Single gene testing strategies: mean or medians of 10 to 13 days Differences: 0 to 3 days	The greatest difference in turnaround time was between a combined DNA and RNA panel and single gene testing, which reported that results were available 3 days sooner with NGS. Separate DNA then RNA panels would be expected to take longer than a combined panel.	⊕⊖⊖⊖ High level of uncertainty, as none of the studies used the two-step DNA then RNA testing expected to be most common in Australia in the near future. There was heterogeneity between studies in whether NGS was time saving or not.
2.3 Change in management	Change in rate of rebiopsy	n=225 k=2 case series	Risk of bias: -1 Inconsistency: -1 Indirectness: -1 Imprecision: -1 Publication bias: 0 Other: 0	Rebiopsies performed in 13.3% and 43.4% of cases with insufficient tissue	Rates of rebiopsy were low, but this may be due to the use of liquid biopsy (ctDNA being used), and may not be applicable to Australia.	⊕⊖⊖⊖ High level of uncertainty as unclear how applicable the data are to Australia. There was no comparative data on rebiopsy rates in NGS strategy vs sequential single-gene testing strategies.

Question: What is the safety and effectiveness of small NGS DNA/RNA panel(s) compared to sequential single gene testing in patients with non-squamous (or histology not otherwise specified) non-small cell lung cancer?

Patient or population: Patients with non-squamous (or histology not otherwise specified) non-small cell lung cancer

Intervention: Small NGS DNA/RNA panel(s)

Comparator: Sequential single gene testing

Section in report	Aim/outcomes	Participants and studies	Quality of evidence	Results	Interpretation	GRADE
	Change in treatment received	n=99 k=6 before and after case series	Risk of bias: -1 Inconsistency: -1 Indirectness: 0 Imprecision: -1 Publication bias: 0 Other: 0	In those with biomarkers detected by NGS, missed by single-gene testing, the use of targeted treatment varied (median 50%, range 17.6% to 100%)	Identification of biomarkers was only one factor in treatment decisions. Among those advanced enough to require TKIs rather than just surgery, and well enough to receive TKIs, the use of targeted treatments was high.	⊕⊖⊖⊖ High level of uncertainty due to the small number of cases, and the high level of heterogeneity between studies.
	Change in timing of treatment	n=3474 k=1 retrospective cohort study	Risk of bias: 0 Inconsistency: NA Indirectness: -2 Imprecision: 0 Publication bias: 0 Other: 0	Time between diagnosis and 1L treatment: NGS: 38 days Single gene testing: 36-38 days	Those tested with NGS had treatment initiated slightly later than those tested by other methods, but the study is too confounded to be very informative.	⊕⊖⊖⊖ High level of uncertainty as observational study, and unclear what organisational policies would influence timing of testing and treatment.
2.4 Therapeutic effectiveness	Safety of rebiopsy	n=2326 k=1 systematic review with 16 studies included	Risk of bias: 0 Inconsistency: -1 Indirectness: -1 Imprecision: 0 Publication bias: 0 Other: 0	AEs occurred in 17% (95%CI 12%, 23%) of those who underwent biopsy Pneumothorax occurred in 9.2% (95%CI 4.0%, 15.7%)	Rebiopsies are associated with a risk of adverse events. The increased success rate of NGS should result in reduced need for rebiopsies, which reduces the risk of adverse events related to testing.	⊕⊕⊖⊖ Moderate level of uncertainty, as the frequency of adverse events was heterogeneous, and the population was indirect, as none of the rebiopsies were performed due to insufficient tissue at diagnosis (all rebiopsies were due to disease progression).

<p>Question: What is the safety and effectiveness of small NGS DNA/RNA panel(s) compared to sequential single gene testing in patients with non-squamous (or histology not otherwise specified) non-small cell lung cancer?</p> <p>Patient or population: Patients with non-squamous (or histology not otherwise specified) non-small cell lung cancer</p> <p>Intervention: Small NGS DNA/RNA panel(s)</p> <p>Comparator: Sequential single gene testing</p>						
Section in report	Aim/outcomes	Participants and studies	Quality of evidence	Results	Interpretation	GRADE
	Effectiveness of targeted treatment in cases with incremental actionable variants	n=2921 k=2 systematic reviews of cohort studies, and 7 additional case series in discordant cases	Risk of bias: -1 Inconsistency: -1 Indirectness: 0 Imprecision: -1 Publication bias: 0 Other: 0	Those with actionable variants identified by NGS show response to targeted therapies. Rare variants and low allele frequency variants are less likely to respond to <i>EGFR</i> TKIs than common variants and high allele frequency variants.	Patients with actionable variants in the <i>EGFR</i> gene identified by NGS but not on single gene testing may not respond to targeted therapies the same degree as those identified by the clinical utility standards. Treatment with targeted therapies is likely to still be superior to treatment with non-targeted therapies. Insufficient evidence in those discordant on <i>ALK</i> and <i>ROS1</i> rearrangement status (between testing methods) to determine the comparative efficacy of targeted treatments.	⊕⊖⊖⊖ High level of uncertainty as a very small number of patients had discordant results and therefore different treatment due to NGS. Unclear what proportion of incremental cases are found to due rare variants or higher sensitivity, so unclear how applicable these studies are.
2.4 Therapeutic effectiveness (continued)	Health impact of more timely treatment	n=not stated k=1 systematic review with 8 cohort studies	Risk of bias: -2 Inconsistency: 0 Indirectness: 0 Imprecision: 0 Publication bias: 0 Other: 0	Those with more symptoms (corresponding to worse prognosis) received treatment in a timelier manner, and have worse health outcomes than those with	Unclear whether a treatment delay of 3 days would have any impact on health outcomes, as the evidence on time to treatment and health outcomes was too confounded.	⊕⊖⊖⊖ High level of uncertainty as evidence was observational studies with a very high level of confounding in the studies that would likely influence the results.

Question: What is the safety and effectiveness of small NGS DNA/RNA panel(s) compared to sequential single gene testing in patients with non-squamous (or histology not otherwise specified) non-small cell lung cancer?
Patient or population: Patients with non-squamous (or histology not otherwise specified) non-small cell lung cancer
Intervention: Small NGS DNA/RNA panel(s)
Comparator: Sequential single gene testing

Section in report	Aim/outcomes	Participants and studies	Quality of evidence	Results	Interpretation	GRADE
				delayed treatment.		

AEs = adverse events; ALK = anaplastic lymphoma kinase; ctDNA = circulating tumour DNA (in the bloodstream); DNA = deoxyribonucleic acid; EGFR = epidermal growth factor receptor; k = number of studies; n = number patients; NGS = next generation sequencing; RNA = ribonucleic acid; ROS1 = ROS proto-oncogene 1; TKI = tyrosine kinase inhibitor (therapy)

⊕⊕⊕⊕ **High quality:** We are very confident that the true effect lies close to that of the estimate of effect.

⊕⊕⊕⊖ **Moderate quality:** We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

⊕⊕⊖⊖ **Low quality:** Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.

⊕⊖⊖⊖ **Very low quality:** We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

Appendix F Economic evaluation supporting evidence and additional analyses

Structure of the economic evaluation

Table 90 Economic literature review

Search	Query	Results
Pubmed		
#1	NSCLC OR nonsmall cell lung OR non small cell lung cancer OR non small cell lung carcinoma OR carcinoma, non-small-cell lung[MeSH Terms]	96,934
#2	next generation sequencing OR NGS OR (gene* OR molecular OR DNA OR RNA OR comprehensive) AND (panel OR profile OR profiling OR sequencing OR test) OR high-throughput nucleotide sequencing[MeSH Terms] OR transcriptome	3,307,391
#3	"economics"[MeSH Terms] OR "costs and cost analysis"[MeSH Terms] OR "cost allocation"[MeSH Terms] OR "cost benefit analysis"[MeSH Terms] OR "cost control"[MeSH Terms] OR "cost savings"[MeSH Terms] OR "cost of illness"[MeSH Terms] OR "health care costs"[MeSH Terms] OR "drug costs"[MeSH Terms] OR "health expenditures"[MeSH Terms] OR "economics, medical"[MeSH Terms] OR "economics, pharmaceutical"[MeSH Terms] OR "fees and charges"[MeSH Terms] OR "budgets"[MeSH Terms] OR "cost"[All fields] OR "high cost"[All Fields] OR "low cost"[All Fields] OR "cost utility"[All Fields] OR "economics"[All Fields] OR "financial"[All Fields] OR "finance"[All Fields] OR "healthcare cost"[All Fields] OR "health care cost"[All Fields] OR "cost estimate"[All Fields] OR "unit cost"[All Fields] OR "economics, pharmaceutical"[MeSH Terms] OR ("economics"[All Fields] AND "pharmaceutical"[All Fields]) OR "pharmaceutical economics"[All Fields] OR "pharmacoeconomic"[All Fields] OR "commerce"[MeSH Terms] OR "commerce"[All Fields] OR "price"[All Fields] OR (("costs"[All Fields] OR "cost"[All Fields]) AND "analysis"[All Fields]) OR "costs and cost analysis"[All Fields] OR "pricing"[All Fields] OR "cost-effectiveness"[All Fields] OR "cost effectiveness"[All Fields] OR "economic evaluation"[All Fields]	1,796,377
#4	#1 AND #2 AND #3	1,147
Embase		
1	lung non small cell cancer/ or non small cell lung cancer/ or NSCLC.mp. or nonsmall cell lung.mp. or non small cell lung cancer.mp. or non small cell lung carcinoma.mp.	167,872
2	next generation sequencing.mp. or high throughput sequencing/ or NGS.mp. or ((gene* or molecular or DNA or RNA or comprehensive) and (panel or profile or profiling or sequencing or test)).mp. or transcriptome/ or transcriptome.mp.	2,071,722
3	health economics/ or cost/ or cost effectiveness analysis/ or health care cost/ or drug cost/ or pharmacoeconomics/ or budget/ or cost utility analysis/ or cost.mp. or cost effectiveness analysis.mp. or cost utility analysis.mp. or health economics.mp.	1,043,896
4	1 and 2 and 3	937
Combined results		
	Total, after duplicates were excluded	1,820
	Studies identified from pearling	0
	Total studies included	8

Search conducted 27/5/22

Table 91 Summary of existing economic literature

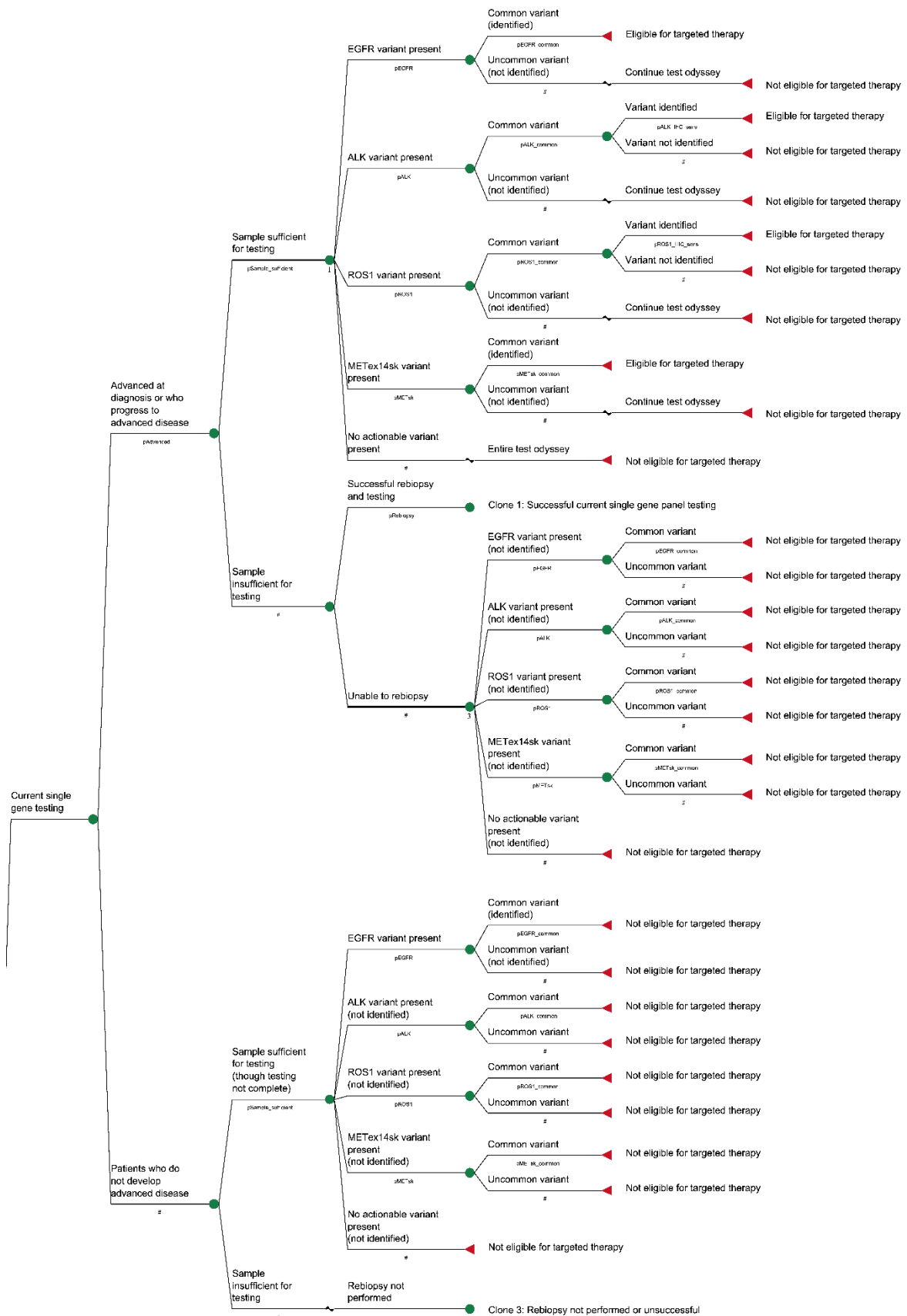
Study	Summary
Vanderpoel et al. (2022)	<p>US budget impact analysis that compared simultaneous panel testing (NGS) vs different scenarios of sequential testing in patients with metastatic NSCLC. A decision-tree analytic was used that measured cost, number of actionable variants and time to initiation of targeted therapy. Test costs and costs related to testing were included, such as specialist and outpatient visits, and costs of rebiopsies and rebiopsy-related complications.</p> <p>NGS found more patients with actionable variants, was associated with the shortest time-to-result and lowest cost per patient. The increase in actionable variants was due to an improvement in the test success rates, and differences in rebiopsy uptake.</p>
Loong et al. (2022)	<p>Hong Kong cost-consequence analysis that compared upfront NGS testing vs different scenarios of sequential testing in patients with metastatic NSCLC. A decision-tree analytic was used that measured cost, number of actionable variants and time to initiation of targeted therapy. The analysis included only genomic testing-related costs.</p> <p>NGS was the most expensive, however was associated with 100% of actionable and non-actionable variants identified and the average time to appropriate treatment was 2 weeks (equal second). The increase in actionable variants was due to an improvement in test success rates. With sequential testing, 92.6% of actionable variants and 49.8% of non-actionable variants were identified. This approach was associated with the longest time to appropriate treatment (5.2 weeks).</p>
Dong et al. (2022)	<p>US cost-utility analysis that compared comprehensive genomic profiling (including <i>ALK</i>, <i>BRAF</i>, <i>EGFR</i>, <i>ERBB2</i>, <i>MET</i>, <i>NTRK1/2/3</i>, <i>RET</i>, and <i>ROS1</i>) vs targeted gene panel testing (<i>ALK</i>, <i>BRAF</i>, <i>EGFR</i>, and <i>ROS1</i> by targeted sequencing that may include FISH) in patients with metastatic adenocarcinoma NSCLC. A decision-tree analytic was used that assigned first-line treatment based on test results. Second-, third- and fourth-line treatment options were also modelled. Outcomes from each line of treatment (PFS, AEs and death prior to progression) were derived from the respective clinical trials. The sum of the respective PFS estimates for each line of therapy was used to estimate overall life expectancy. Costs included those related to testing, treatment and treatment-related costs.</p> <p>Comprehensive genomic profiling was associated with an additional 8% actionable variants identified. The ICER per additional QALY gained compared to targeted gene panel testing was \$445,545. Additional actionable variants were identified due to additional genes being included on the comprehensive panel.</p>
de Alava et al. (2022)	<p>Spanish cost-effectiveness (outcome of additional patients eligible for targeted therapy) and exploratory cost-utility analysis that compared NGS vs sequential single-gene testing in patients with advanced or metastatic NSCLC. A hybrid decision-tree partitioned survival model was used, where the decision tree component assigned treatment and the partitioned model measured long term outcomes from assigned treatment. The partitioned model was based on PFS and OS data from the respective clinical trials of treatments, with extrapolation using exponential models. Costs included were the cost of testing, treatment and treatment-related and disease management costs.</p> <p>NGS was associated with an ICER per additional patient eligible for targeted therapy of €617. The increase in actionable variants identified was due to additional genes being tested through NGS and due to the reduction in biopsies required. The ICER per additional QALY gained in the cost-utility analysis, which included costs and outcomes related to treatment, was €9,084. NGS was faster sequential single-gene testing (9 vs 16.7 days)</p>
Tan et al. (2020)	<p>Singaporean cost-effectiveness analysis that compared different scenarios of NGS testing vs current testing (concurrent <i>EGFR</i> sequencing and <i>ALK</i>, <i>MET</i>, <i>RET</i> and <i>ROS1</i> FISH) in patients with Stage IV adenocarcinoma NSCLC. A decision-tree analytic was used that measured additional patients eligible for targeted therapy and was based on real world local data from a cohort of 104 patients.</p> <p>Current testing was dominated by the scenario of upfront NGS testing (cost saving of SGD645.2, with 5% increase in patients on targeted therapy), though NGS may be associated with a longer time to result (10 days vs 10–15 days). NGS was associated with additional actionable variants due to improved sensitivity for variants in currently tested markers and due to additional markers being tested. While the model structure allowed insufficient tissue to be considered, the extent to which this had on the additional variants identified was not clear.</p>

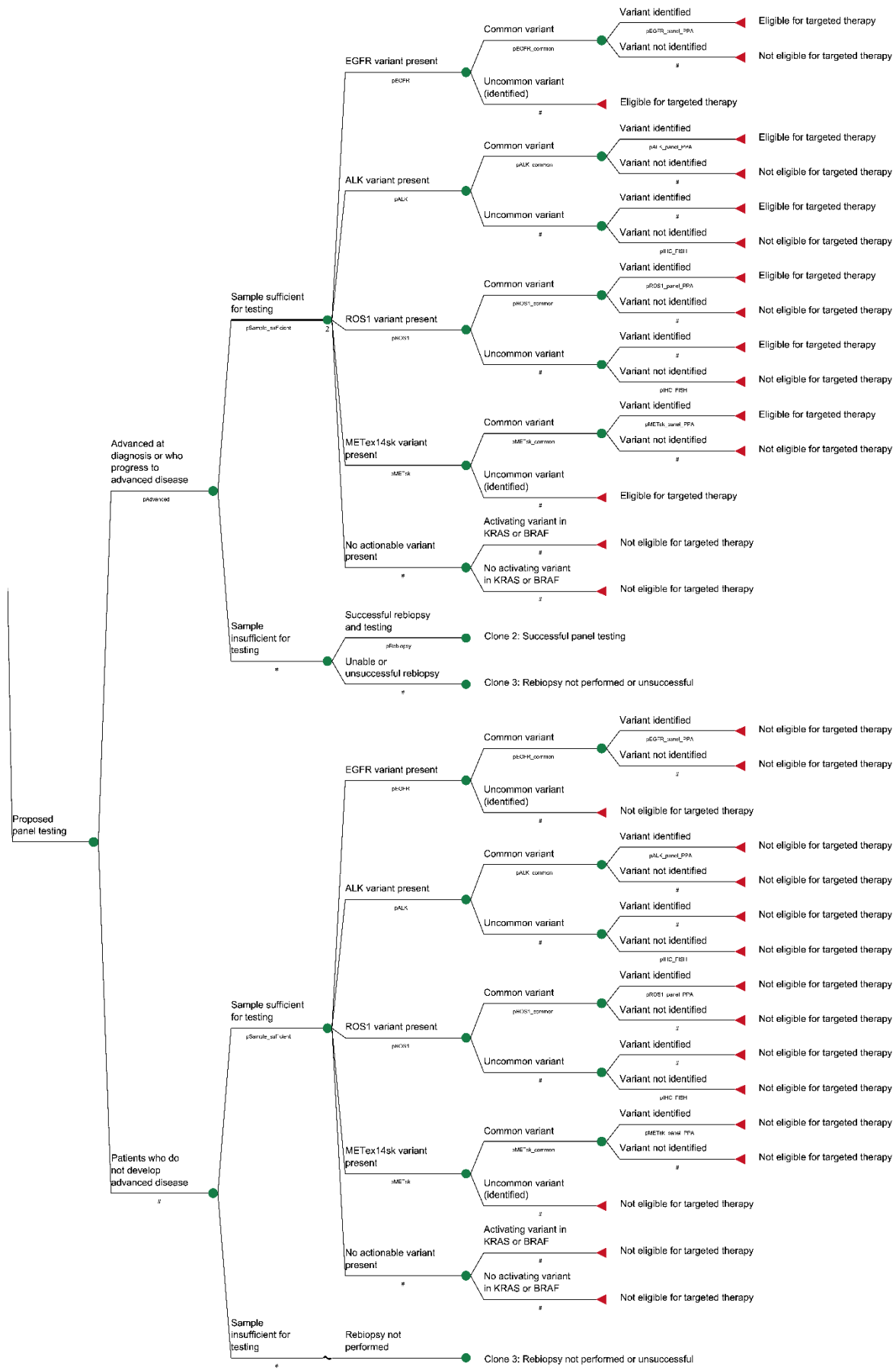
Study	Summary
Schluckebier et al. (2020)	<p>Brazilian cost-effectiveness (outcome correct diagnoses) and cost-utility analysis that compared NGS panel testing of <i>EGFR</i>, <i>ALK</i> and <i>ROS1</i> vs scenarios of sequential RT-PCR <i>EGFR</i> testing and <i>ALK</i> and <i>ROS1</i> FISH in patients with Stage IV adenocarcinoma NSCLC. A hybrid decision-tree microsimulation model was used, where outcomes related to testing were captured in the decision tree component, and outcomes of treatment from the microsimulation. PFS estimates from clinical trials of targeted therapies and chemotherapies were used to inform life years with up to three lines of treatment. Costs included in the model were those related to testing and treatment.</p> <p>NGS yielded the most correct diagnoses due to improved sensitivity for <i>EGFR</i> variants and due to a lower rate of unknown test results. NGS compared to <i>EGFR</i> then <i>ALK</i> and <i>ROS1</i> FISH was associated with an ICER of USD3,479.11 per additional correct case detected, and USD214,000 per additional QALY gained.</p>
Steuten et al. (2019)	<p>US cost-effectiveness analysis (outcome life years) that compared multigene panel sequencing (≥ 30 or more genes) vs single-marker genetic testing (<i>EGFR</i>, <i>ALK</i> and <i>ROS1</i>) in patients with Stage III/IV non-squamous NSCLC (including those who progress from incident early stage disease). A decision-tree model was used where terminal nodes consisted of targeted therapy, immunotherapy, chemotherapy, BSC and clinical trials. OS, by treatment type, was derived from survival observed in advanced NSCLC patients in the Flatiron Health database or from published data. However outcomes from chemotherapy and immunotherapy appeared to have been combined. Costs included test costs; drug treatment and administration cost; and cost for BSC, hospitalisations, and management of serious adverse events.</p> <p>Multigene panel testing was associated with more actionable variants identified (30.1% vs 23.3%) and treated (21.4% vs 18.7%), however was more costly (average test costs \$1,948 v \$467). Additional actionable variants was driven by yield in markers not included in single gene testing. When the costs and effects of changes in treatment were modelled, the ICER per LY gained was \$148,478.</p>
Pennell et al. (2019)	<p>US budget impact analysis that compared upfront NGS testing vs different scenarios of sequential testing in patients with metastatic NSCLC. A decision-tree analytic was used that measured the time to test results and proportion of patients with actionable variants identified (with or without FDA-approved therapies). Costs modelled included those related to testing and rebiopsy.</p> <p>NGS was assumed to identify all variants with and without FDA-approved therapies (100.0% vs 97.7% with sequential testing for alterations with approved therapies, and 100.0% vs 56.3% for alterations without FDA-approved therapies). Additional actionable variants were identified due the need for rebiopsy with sequential testing. NGS was also the cheapest option and equal fastest (with hotspot panel testing)</p>

AE = adverse event; *ALK* = anaplastic lymphoma kinase; *BRAF* = proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B; BSC = best supportive care; DNA = deoxyribose nucleic acid; *EGFR* = epidermal growth factor receptor; *ERBB2* = erb-b2 receptor tyrosine kinase 2; FDA = (US) Food and Drug Administration; FISH = fluorescence in situ hybridisation; ICER = incremental cost-effectiveness ratio; IHC = immunohistochemistry; LY = life year; *MET* = mesenchymal-epithelial transition; *METex14sk* = MET proto-oncogene, receptor tyrosine kinase exon 14 skipping alterations; NGS = next generation sequencing; NOS = not otherwise specified; NSCLC = non-small cell lung cancer; NTRK = neurotrophic tyrosine receptor kinase; OS = overall survival; PFS = progression-free survival; QALY = quality-adjusted life year; *RET* = rearranged during transfection; RNA = ribonucleic acid; *ROS1* = ROS proto-oncogene 1; RT-PCR = real-time polymerase chain reaction.

Figure 28

Model structure





Note: Common variants are those that can be identified by either single gene tests or panel tests. Uncommon variants are those identified

through panel testing only.

ALK = anaplastic lymphoma kinase; *BRAF* = proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B; *EGFR* = epidermal growth factor receptor; *KRAS* = Kirsten rat sarcoma viral oncogene homologue; *MET*_{ex14sk} = MET proto-oncogene, receptor tyrosine kinase exon 14 skipping alterations; NOS = not otherwise specified; NSCLC = non-small cell lung cancer; *ROS1* = ROS proto-oncogene 1.

Health care resource use and costs

Calibration of ALK FISH use

MBS data on the number of *EGFR*, *ALK* and *ROS1* services was extracted (Table 52). While fluctuations were noted in the number of *ALK* FISH services in the first few years of listing, these – and the ratio of *ALK* FISH services to *EGFR* services – has appeared to stabilise since 2019. The average over 2019–2021 was estimated to be 4.51%.

As *EGFR* can be used in patients diagnosed at early stages who do not progress to advanced disease, this estimate of average *ALK* FISH use therefore too reflects use across all disease stages.

As 75.9% of patients modelled experience advanced disease, average *ALK* FISH use in patients with advanced disease can be estimated as:

$$[1] \quad \frac{4.51\%}{75.9\%} = 5.95\%$$

This estimate includes *ALK* FISH in those that do have an actionable variant correctly identified by IHC, and those who do not have actionable variant incorrectly identified by IHC.

Those that do have an actionable variant correctly identified by IHC is estimated by:

$$[2] \quad Prevalence_{ALK} \times IHC\ sensitivity_{ALK} = 3.0\% \times 98.4\% = 2.95\%$$

Therefore, use of *ALK* FISH in those who do not have actionable variants can be derived from:

$$[3] \quad (1) - (2) = 5.95\% - 2.95\% = 3.00\%$$

This estimate is applied across all patients with advanced disease – and so includes patients with previously identified *EGFR* variants, and also those with actionable *ALK* variants identified in [2]. Therefore, the estimate reported in [3] is adjusted to reflect use in those without actionable *ALK* variants, eligible for *ALK* FISH.

$$[4] \quad \frac{3.00\%}{1 - Prevalence_{ALK} - Yield_{EGFR}} = \frac{3.00\%}{82.00\%} = 3.66\%$$

Calibration of ROS1 FISH

A similar approach is used to calibrate *ROS1* FISH use in patients that do not have *ROS1* actionable variants. Given the more recent listing of *ROS1* FISH, the average over 2020–2021 was used (7.52%).

Average *ROS1* FISH use in patients with advanced disease can be estimated as:

$$[5] \quad \frac{7.52\%}{75.9\%} = 9.92\%$$

This estimate includes *ROS1* FISH in those that do have an actionable variant correctly identified by IHC, and those who do not have actionable variant incorrectly identified by IHC.

Those that do have an actionable variant correctly identified by IHC is estimated by:

$$[6] \quad \text{Prevalence}_{ROS1} \times \text{IHC sensitivity}_{ROS1} = 1.61\% \times 95.1\% = 1.53\%$$

Therefore, use of *ROS1* FISH in those who do not have actionable variants can be derived from:

$$[7] \quad (1) - (2) = 9.92\% - 1.53\% = 8.38\%$$

Following adjustment to reflect use in those without actionable *ROS1* variants, eligible for *ROS1* FISH (i.e. those without actionable *EGFR* or *ALK* variants).

$$[8] \quad \frac{8.38\%}{1 - \text{Prevalence}_{ROS1} - \text{Yield}_{EGFR} - \text{Yield}_{ALK}} = \frac{8.38\%}{80.44\%} = 10.42\%$$

Model validation

Figure 29 Model validation

			Incorporating test success and early disease who do not progress													
Current single gene testing			Advanced							Early				Total		
			Test success		Successful rebiopsy		Failed rebiopsy		Unable to rebiopsy			Test success				No rebiopsy
<i>EGFR</i>	common		10.76%	\$397.35	0.49%	\$6,027.62	0.12%	\$5,630.27	0.00%	\$0.00	3.43%	\$397.35	0.20%	\$0.00		
	uncommon		1.83%	\$982.18	0.08%	\$6,612.44	0.02%	\$5,630.27	0.00%	\$0.00	0.58%	\$397.35	0.03%	\$0.00		
<i>ALK</i>	common		2.12%	\$1,325.85	0.10%	\$6,956.12	0.02%	\$5,630.27	0.00%	\$0.00	0.67%	\$397.35	0.04%	\$0.00		
	IHC FN		0.03%	\$967.54	0.00%	\$6,597.81	0.00%	\$5,630.27	0.00%	\$0.00	0.01%	\$397.35	0.00%	\$0.00		
	uncommon		0.59%	\$982.18	0.03%	\$6,612.44	0.01%	\$5,630.27	0.00%	\$0.00	0.19%	\$397.35	0.01%	\$0.00		
<i>ROS1</i>	common		1.10%	\$1,340.48	0.05%	\$6,970.75	0.01%	\$5,630.27	0.00%	\$0.00	0.35%	\$397.35	0.02%	\$0.00		
	IHC FN		0.06%	\$940.48	0.00%	\$6,570.75	0.00%	\$5,630.27	0.00%	\$0.00	0.02%	\$397.35	0.00%	\$0.00		
	uncommon		0.00%	\$982.18	0.00%	\$6,612.44	0.00%	\$5,630.27	0.00%	\$0.00	0.00%	\$397.35	0.00%	\$0.00		
<i>MET</i> ex14sk	common		2.58%	\$982.18	0.12%	\$6,612.44	0.03%	\$5,630.27	0.00%	\$0.00	0.82%	\$397.35	0.05%	\$0.00		
	uncommon		0.00%	\$982.18	0.00%	\$6,612.44	0.00%	\$5,630.27	0.00%	\$0.00	0.00%	\$397.35	0.00%	\$0.00		
None			52.68%	\$982.18	2.41%	\$6,612.44	0.60%	\$5,630.27	0.00%	\$0.00	16.77%	\$397.35	0.96%	\$0.00		
Average use				\$652.99		\$214.31		\$46.12		\$0.00		\$90.78		\$0.00		
Proposed small gene panel testing			Advanced							Early				Total		
			Test success		Successful rebiopsy		Failed rebiopsy		Unable to rebiopsy			Test success		No rebiopsy		
<i>EGFR</i>	Common	identified	10.84%	\$843.28	0.25%	\$6,473.54	0.06%	\$5,630.27	0.00%	\$0.00	3.45%	\$843.28	0.10%	\$0.00		
		not identified	0.22%	\$1,299.98	0.01%	\$6,930.25	0.00%	\$5,630.27	0.00%	\$0.00	0.07%	\$1,297.04	0.00%	\$0.00		
	Uncommon	identified	1.88%	\$843.28	0.04%	\$6,473.54	0.01%	\$5,630.27	0.00%	\$0.00	0.60%	\$843.28	0.02%	\$0.00		
<i>ALK</i>	Common	identified	2.21%	\$1,317.93	0.05%	\$6,948.20	0.01%	\$5,630.27	0.00%	\$0.00	0.70%	\$1,297.04	0.02%	\$0.00		
		not identified	0.00%	\$1,299.98	0.00%	\$6,929.48	0.00%	\$5,630.27	0.00%	\$0.00	0.00%	\$1,297.04	0.00%	\$0.00		
		identified	0.58%	\$1,299.98	0.01%	\$6,930.25	0.00%	\$5,630.27	0.00%	\$0.00	0.18%	\$1,297.04	0.01%	\$0.00		
	Uncommon	not identified	0.03%	\$1,299.98	0.00%	\$6,930.25	0.00%	\$5,630.27	0.00%	\$0.00	0.01%	\$1,297.04	0.00%	\$0.00		
<i>ROS1</i>		Common	identified	1.03%	\$1,318.70	0.02%	\$6,948.96	0.01%	\$5,630.27	0.00%	\$0.00	0.33%	\$1,297.04	0.01%		
	not identified		0.16%	\$1,297.80	0.00%	\$6,928.07	0.00%	\$5,630.27	0.00%	\$0.00	0.05%	\$1,297.04	0.00%	\$0.00		
	identified		0.00%	\$1,299.98	0.00%	\$6,930.25	0.00%	\$5,630.27	0.00%	\$0.00	0.00%	\$1,297.04	0.00%	\$0.00		
	Uncommon	not identified	0.00%	\$1,299.98	0.00%	\$6,930.25	0.00%	\$5,630.27	0.00%	\$0.00	0.00%	\$1,297.04	0.00%	\$0.00		
<i>MET</i> ex14sk		Common	identified	2.60%	\$843.28	0.06%	\$6,473.54	0.01%	\$5,630.27	0.00%	\$0.00	0.83%	\$843.28	0.02%		
	not identified		0.05%	\$1,299.98	0.00%	\$6,930.25	0.00%	\$5,630.27	0.00%	\$0.00	0.02%	\$1,297.04	0.00%	\$0.00		
	identified		0.00%	\$843.28	0.00%	\$6,473.54	0.00%	\$5,630.27	0.00%	\$0.00	0.00%	\$843.28	0.00%	\$0.00		
None	BR <i>AF</i> , or <i>KRAS</i> identified		22.71%	\$846.22	0.52%	\$6,476.48	0.13%	\$5,630.27	0.00%	\$0.00	7.23%	\$843.28	0.21%	\$0.00		
		BR <i>AF</i> or <i>KRAS</i> not identified	31.42%	\$1,299.98	0.72%	\$6,930.25	0.18%	\$5,630.27	0.00%	\$0.00	10.00%	\$1,297.04	0.29%	\$0.00		
				\$786.01		\$113.77		\$23.92		\$0.00		\$249.53		\$0.00	\$1,173.23	100.00%

ALK = anaplastic lymphoma kinase; *BRAF* = proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B; *EGFR* = epidermal growth factor receptor; *KRAS* = Kirsten rat sarcoma viral oncogene homologue; *MET*ex14sk = *MET* proto-oncogene, receptor tyrosine kinase exon 14 skipping alterations; *ROS1* = *ROS* proto-oncogene 1.

Uncertainty analysis: model inputs, structure and assumptions

Table 92 Results of the sensitivity analysis

	Inc. cost	Inc. eligible for targeted therapy	ICER	% change
Base case	\$169.02	0.0225	\$7,496	-
Proportion of patients with advanced disease (base case: 75.9%)				
100.0%	\$27.97	0.0297	\$941	-87%
72.6%	\$188.12	0.0216	\$8,719	16%
50%	\$320.01	0.0149	\$21,530	187%
Timing of <i>MET</i> ex14sk testing (base case: after <i>EGFR</i>)				
At the same time as <i>EGFR</i>	\$109.54	0.0225	\$4,858	-35%
After <i>EGFR</i> (excluding block retrieval and consult costs)	\$252.67	0.0225	\$11,206	49%
After <i>EGFR</i> , <i>ALK</i> and <i>ROS1</i>	\$186.80	0.0225	\$8,285	11%
Proportion of IHC ± FISH use with small gene panel testing (base case: 5%)				
0%	\$186.48	0.0228	\$8,171	9%
10%	\$151.57	0.0223	\$6,805	-9%
Small gene panel testing strategy (base case: mixed)				
All combined DNA/RNA panel testing	\$348.65	0.0228	\$15,277	104%
All two-stage DNA then RNA panel testing	\$116.98	0.0228	\$5,126	-32%
All DNA then IHC/FISH testing	-\$162.34	0.0173	Dominant	-225%
5% DNA panel and IHC/FISH, 95% combined DNA/RNA panels	\$323.08	0.0225	\$14,329	91%
5% DNA panel and IHC/FISH, equal split of combined and separate DNA and RNA panels	\$213.04	0.0225	\$9,449	26%
5% RNA by IHC/FISH, 95% separate DNA and RNA panels	\$103.00	0.0225	\$4,568	-39%
Test success (base case: 97.2% for panels, 94.6% for single-gene testing)				
Both strategies 100%	\$279.14	0.0218	\$12,829	71%
Both strategies 97.2%	\$273.97	0.0216	\$12,662	69%
Both strategies 94.6%	\$269.18	0.0215	\$12,506	67%
97.2% for panels, 95.9% ^a for single-gene testing	\$221.50	0.0221	\$10,026	34%
95.9% for panels, 94.6% ^a for single-gene testing	\$219.10	0.0220	\$9,943	33%
97.2% for panels, 92.0% ^b for single-gene testing	\$64.07	0.0235	\$2,731	-64%
Common variant yield				
<i>EGFR</i> , 17.9% (base case: 15.0%)	\$168.16	0.0216	\$7,797	4%
<i>ALK</i> , 3.3% (base case: 3.0%)	\$169.03	0.0226	\$7,487	-0%
<i>ALK</i> small gene panel concordance (base case: vs clinical utility standard, FISH ≥15% positivity)				
<i>ALK</i> small gene panel concordance vs FISH ± IHC	\$168.99	0.0219	\$7,730	3%
<i>ALK</i> small gene panel concordance vs FISH	\$169.00	0.0360	\$4,697	-37%
Small panel concordance				
<i>ALK</i> NPA, 0.97 (base case: 0.99)	\$169.02	0.0375	\$4,509	-40%

	Inc. cost	Inc. eligible for targeted therapy	ICER	% change
Base case	\$169.02	0.0225	\$7,496	-
ALK NPA, 1.00 (base case: 0.99)	\$169.02	0.0166	\$10,162	36%
ALK PPA, 0.48 (base case: 1.00)	\$168.81	0.0114	\$14,848	98%
EGFR NPA, 0.95 (base case: 0.97)	\$161.36	0.0354	\$4,562	-39%
EGFR NPA, 0.99 (base case: 0.97)	\$176.69	0.0097	\$18,168	142%
EGFR PPA, 0.95 (base case: 0.98)	\$171.05	0.0192	\$8,931	19%
EGFR PPA, 0.99 (base case: 0.98)	\$168.35	0.0237	\$7,110	-5%
METex14sk NPA, 0.93 (base case: 1.00)	\$138.59	0.0734	\$1,887	-75%
METex14sk PPA, 0.89 (base case: 0.98)	\$170.49	0.0201	\$8,480	13%
METex14sk PPA, 1.00 (base case: 0.98)	\$168.70	0.0231	\$7,306	-3%
ROS1 NPA, 0.99 (base case: 1.00)	\$169.02	0.0296	\$5,711	-24%
ROS1 PPA, 0.63 (base case: 0.86)	\$168.97	0.0199	\$8,493	13%
ROS1 PPA, 0.96 (base case: 0.86)	\$169.05	0.0237	\$7,133	-5%
ALK IHC PPA (base case 0.984)				
0.900	\$169.06	0.0243	\$6,945	-7%
0.998	\$169.02	0.0222	\$7,597	1%
Rebiopsy uptake rate (base case: 100%)				
13%	\$275.81	0.0253	\$10,905	45%
30%	\$254.94	0.0248	\$10,298	37%
43%	\$238.99	0.0243	\$9,816	31%
60%	\$218.12	0.0238	\$9,161	22%
Rebiopsy failure rate, 15% (base case: 20%)	\$168.29	0.0224	\$7,530	0%
Average fee charged for EGFR and ALK and ROS1 FISH (base case: MBS Schedule Fees)	\$185.26	0.0225	\$8,217	10%
FISH utilisation, use IHC NPA data (base case: calibrated)	\$183.78	0.0225	\$8,151	9%
IHC test cost, \$14.90 (base case: \$0)	\$154.44	0.0225	\$6,850	-9%
Separate RNA small panel use, allowed with <i>KRAS</i> or <i>BRAF</i> (base case: not allowed)	\$307.24	0.0225	\$13,627	82%
Proportion with <i>KRAS</i> or <i>BRAF</i> activating variants (base case: 30.8%)				
25%	\$195.05	0.0225	\$8,651	15%
52%	\$73.89	0.0225	\$3,277	-56%
Rebiopsy cost (base case: \$5,630 [all inpatient])				
\$3,369 [all outpatient]	\$213.63	0.0225	\$9,475	26%
\$4,499 [50% inpatient, 50% outpatient]	\$191.33	0.0225	\$8,485	13%
Rebiopsy complication rate (base case: 14%)				
10%	\$177.73	0.0225	\$7,882	5%
17%	\$162.50	0.0225	\$7,207	-4%

^a Half the difference between test strategies

^b Double the difference between test strategies

ALK = anaplastic lymphoma kinase; BRAF = proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B; DNA = deoxyribose nucleic acid; EGFR = epidermal growth factor receptor; FISH = fluorescence in situ hybridisation; ICER = incremental cost-effectiveness ratio; IHC = immunohistochemistry; KRAS = Kirsten rat sarcoma viral oncogene homologue; METex14sk = MET proto-

oncogene, receptor tyrosine kinase exon 14 skipping alterations; NPA = negative percent agreement; *NTRK* = neurotrophic tropomyosin receptor kinase; PPA = positive percent agreement; RNA = ribonucleic acid; *ROS1* = ROS proto-oncogene 1.