National Institute for Health and Clinical Excellence

Diagnostics Assessment Programme

Evidence overview

Epidermal growth factor receptor tyrosine kinase (EGFR-TK) mutation testing in adults with locally advanced or metastatic non-small-cell lung cancer

This overview summarises the key issues for the Diagnostics Advisory Committee's consideration. It includes a brief description of the topic, a description of the analytical structure and model, a discussion of the analytical difficulties, and a brief summary of the results. It is not a complete summary of the diagnostics assessment report, and it is assumed that the reader is familiar with that document. This overview contains sections from the original scope and the diagnostics assessment report, as well as referring to specific sections of these documents.

1 Background

1.1 Introduction

Epidermal growth factor receptor tyrosine kinase (EGFR-TK) mutation testing in adults with locally advanced or metastatic non-small-cell lung cancer (NSCLC) was referred by the Medical Technologies Advisory Committee for recommendations on use in the NHS. Multiple technologies and methodologies are available for EGFR-TK mutation testing and those identified during the scoping phase and included in the assessment are described in section 2.

The purpose of this assessment is to evaluate the clinical and cost effectiveness of the different technologies and methodologies for EGFR-TK mutation testing. Provisional recommendations will be formulated by the Diagnostics Advisory Committee at the meeting on the 6 March 2013.

It should be noted that NICE technology appraisal guidance 192 shows the EGFR tyrosine kinase inhibitor, gefitinib, to be cost effective for the first line treatment of locally advanced or metastatic EGFR-TK positive NSCLC. Similarly, NICE technology appraisal guidance 258 shows the EGFR tyrosine kinase inhibitor, erlotinib, to be cost effective for the first line treatment of locally advanced or metastatic EGFR-TK positive NSCLC. The assessment does not re-evaluate the cost effectiveness of gefitinib or erlotinib.

1.2 The condition

Lung cancer is the most commonly diagnosed cancer in the world and the most common cause of cancer related death. It is the second most common cancer in the UK accounting for 1 in 7 new cancer cases. The prognosis for people with lung cancer is poor, with the likelihood of surviving 1 year after diagnosis around 30% and the likelihood of surviving 5 years after diagnosis less than 10%. NSCLC is the most common type of lung cancer in England and Wales, accounting for around 72% of all lung cancer cases. NSCLC can be further categorised by histological subtype; the three main types being squamous cell carcinoma, adenocarcinoma and large cell carcinoma.

The presence of EGFR-TK mutations in the tumours of patients with NSCLC can affect the response of the tumours to treatment. For patients with EGFR-TK mutation positive tumours, treatment with EGFR tyrosine kinase inhibitors leads to improved response compared with standard chemotherapy treatment. For patients with EGFR-TK mutation negative tumours, treatment with EGFR tyrosine kinase inhibitors results in a worse response compared with standard chemotherapy treatment. The prevalence of EGFR-TK mutations in NSCLC varies widely with population ethnicity, with reported prevalence ranging from 10.4% in an Italian study (Marchetti et al., 2005) to 50% in a Japanese study (Kosaka et al., 2004). The estimated proportion of EGFR-TK mutations in NSCLC in England and Wales is 16.6% (Rosell et al., 2009).

1.3 Diagnostic and care pathways

Diagnosis and staging of lung cancer

The NICE clinical guideline on the diagnosis and treatment of lung cancer was updated in 2011. It recommends that patients with suspected lung cancer should be urgently referred for a chest x-ray. If the results are suggestive of lung cancer a contrast-enhanced computed tomography (CT) scan of the chest, upper abdomen and lower neck is performed. Further investigations to confirm a diagnosis and to provide information on the stage of the disease are then carried out. These investigations generally include a biopsy for histological confirmation and subtyping, but may also include PET-CT, endobronchial ultrasound (EBUS)-guided transbronchial needle aspiration (TBNA), endoscopic ultrasound (EUS)-guided fine needle aspiration (FNA), or non-ultrasound-guided TBNA. Where biopsy is successfully undertaken, DNA extraction and mutation analysis may be carried out on the biopsy tissue, generally stored as formalin-fixed paraffin-embedded tissue, to determine whether the tumour is EGFR-TK mutation positive or negative. If biopsy tissue is not available, DNA extracted from cytology samples can be used for mutation analysis.

In 2009, participants at a European multidisciplinary workshop “EGFR testing in NSCLC: from biology to clinical practice” emphasised the importance of standardisation and validation of EGFR-TK mutation tests and recommended that testing should only be undertaken in a quality assured, accredited setting. However, there was no consensus on which laboratory test should be used for clinical decision making. Participants also agreed that the decision to request EGFR-TK mutation testing should be made by the treating physician and that results should be reported within seven working days of request. Conversely, guidelines from the Royal College of Pathologists recommend that to minimise turnaround time molecular diagnostic tests should be ordered by the pathologist reporting on the histology of the tumour.

First line treatment of NSCLC

NICE clinical guideline 121 recommends that once NSCLC has been confirmed, chemotherapy should be offered to people with stage III or IV NSCLC and a good performance status (WHO 0, 1 or Karnofsky score 80-100) with the aim of improving survival, disease control and quality of life. Treatment with curative intent is not possible for these patients. First line chemotherapy should be a combination of a single third-generation drug (docetaxel, gemcitabine, paclitaxel or vinorelbine) and a platinum drug (carboplatin or cisplatin). People who are unable to tolerate a platinum combination may be offered single-agent chemotherapy with a third generation drug. NICE technology appraisal guidance 181 recommends pemetrexed in combination with cisplatin as a first line treatment for patients with locally advanced or metastatic NSCLC, if the histology of the tumour has been confirmed as adenocarcinoma or large cell tumour. NICE technology appraisal guidance 192 recommends the EGFR tyrosine kinase inhibitor gefitinib as an option for the first line treatment of people with locally advanced or metastatic NSCLC, whose tumour tests positive for EGFR-TK mutation. NICE technology appraisal guidance 258 recommends erlotinib as an option for the first line treatment of people with locally advanced or metastatic NSCLC if their tumour tests positive for an EGFR-TK mutation.

2 The technologies

Multiple technologies are available for EGFR-TK mutation testing and they can be divided into two subgroups: mutation screening and targeted mutation detection. The former technologies screen samples for all EGFR-TK mutations, known and novel variants. The latter technologies analyse samples for specific known EGFR-TK mutations only.

2.1 Technologies under evaluation

Therascreen EGFR RGQ PCR Kit (Qiagen)

The Therascreen EGFR RGQ PCR Kit is a CE-marked real-time PCR assay for the targeted detection of 29 EGFR-TK mutations, as listed in Table 1. The

DNA is first isolated from a specimen of formalin-fixed paraffin-embedded tissue using the QIAamp DNA FFPE Tissue Kit to adhere to the CE-marking. The total amount of DNA in the sample is assessed by a control assay. The Therascreen EGFR RGQ PCR Kit then uses two technologies for the detection of mutations: ARMS (Amplification Refractory Mutation System) for mutation specific DNA amplification and Scorpions for detection of amplified regions. Scorpions are bi-functional molecules containing a PCR primer covalently linked to a fluorescently labelled probe. A real-time PCR instrument (Rotor-Gene Q 5-Plex HRM for consistency with CE-marking) is used to perform the amplification and to measure fluorescence.

The limits of detection (the percent mutant DNA present in a background of wild-type DNA at which ≥95% of replicates were determined positive) reported by the manufacturer for the different mutations designed to be detected by the EGFR RGQ PCR Kit are presented in Table 1.

Table 1: Limits of detection for each of the EGFR-TK mutation assays

Mutation	Percentage mutation detectable
T790M in exon 20	7.02
19 deletions in exon 19*	1.64
L858R in exon 21	1.26
L861Q in exon 21	0.50
G719X (G719S/G719A/G719C)* in exon 18	5.43
S768I in exon 20	1.37
3 insertions in exon 20*	2.03

* The test detects the presence of these mutations but does not distinguish between them.

It is important to note than an older version of the kit exists – the Therascreen EGFR PCR Kit which was inherited from Qiagen's acquisition of DxS Ltd. This older version of the kit uses the same methods as the newer Therascreen EGFR RGQ PCR Kit, and detects 28 of the same mutations, but is not designed to detect the resistance mutation T790M. The limit of detection claimed by the manufacturers for the Therascreen EGFR PCR Kit is 1% mutant DNA in a background of wild type DNA. This version is no longer being actively marketed by Qiagen, was not used in any of the studies included in this review and has been superseded by the Therascreen EGFR RGQ PCR

Kit. Further, an earlier version of the Therascreen EGFR PCR Kit, which did include an assay for T790M, was used to analyse all samples in the IPASS trial. This version is no longer available, but is considered equivalent to the Therascreen EGFR RGQ PCR Kit for the purpose of this assessment.

Cobas EGFR Mutation Test (Roche Molecular Systems)

The cobas EGFR Mutation Test is a CE-marked real-time PCR test for the targeted detection of 41 EGFR-TK mutations, as listed in Table 2. The first step is to process the tumour tissue using the cobas DNA Sample Preparation Kit. The second step is PCR amplification and detection of EGFR-TK mutations using complementary primer pairs and fluorescently labelled probes. The PCR is run using the cobas z 480 analyser which automates amplification and detection. Cobas 4800 software provides automated test result reporting.

The limits of detection (lowest amount of DNA [ng] per reaction well to achieve ≥95% 'mutation detected' rate) as reported by the manufacturer for the different mutations detected by the cobas EGFR Mutation Test are presented in Table 2.

Table 2: Limits of detection for each of the EGFR-TK mutation assays

Mutation	Lowest amount of DNA (ng) detectable
T790M in exon 20	3.13
29 deletions and complex mutations* in exon 19	0.78
L858R in exon 21	0.78
G719X (G719S/G719A/G719C)* in exon 18	3.13
S768I in exon 20	0.78
5 insertions in exon 20*	3.13

* The test detects the presence of these mutations but does not distinguish between them.

Sanger sequencing of samples with >30% tumour cells and Therascreen EGFR RGQ PCR Kit for samples with <30% tumour cells

In this test strategy, Sanger sequencing of exons 18 to 21 (described in section 2.2) is used to detect EGFR-TK mutations in test samples which have

>30% tumour cells, and the Therascreen EGFR RGQ PCR Kit (described above) is used to detect EGFR-TK mutations in samples which have <30% tumour cells.

Sanger sequencing of samples with >30% tumour cells and cobas EGFR Mutation Test for samples with <30% tumour cells

In this test strategy, Sanger sequencing of exons 18 to 21 (described in section 2.2) is used to detect EGFR-TK mutations in test samples which have >30% tumour cells, and the cobas EGFR Mutation Test (described above) is used to detect EGFR-TK mutations in samples which have <30% tumour cells.

Sanger sequencing followed by fragment length analysis and PCR of negative samples

This test strategy is a screening method of mutation detection. Sanger sequencing of exons 18 to 21 is used as an initial test. Fragment length analysis to detect exon 19 deletions and real-time PCR to detect the exon 21 mutation L858R are then used on samples identified as having insufficient tumour cells or samples which produce a negative result using Sanger sequencing.

Pyrosequencing and fragment length analysis

This test strategy is a screening method of mutation detection and combines in-house methods of pyrosequencing (to detect mutations T790M, L858R, L861Q, G719X and S768I) with in-house methods of fragment length analysis (to detect exon 19 deletions and exon 20 insertions) for EGFR-TK mutation detection.

Pyrosequencing involves extracting DNA from the sample and amplifying it using PCR. The pyrosequencing reaction involves the sequential addition of nucleotides to the amplified PCR product. A series of enzymes incorporate nucleotides into the complementary DNA strand, generate light proportional to

the number of nucleotides added and degrade unincorporated nucleotides. The DNA sequence is determined from the resulting pyrogram trace.

In fragment length analysis, DNA is first extracted from the sample then it is amplified and labelled with fluorescent dye using PCR. Amplified DNA is mixed with size standards and is analysed using capillary electrophoresis. The fluorescence intensity is monitored as a function of time and analysis software can determine the size of the fragments. The presence or absence of a deletion/insertion can then be reported.

Therascreen EGFR Pyro Kit (Qiagen)

The Therascreen EGFR Pyro Kit is a CE-marked pyrosequencing kit. This kit is a targeted method of mutation detection and is designed to detect and distinguish between:

- G719S, G719A and G719C in exon 18
- The 20 most common deletions in exon 19
- S768I and T790M in exon 20
- L858R and L861Q in exon 21.

The kit provides all primers, controls, buffers and reagents necessary to perform the assay. Samples are analysed on the PyroMark Q24 System and a Plug-in report tool is available which simplifies analysis of the pyrogram trace.

Single strand conformation polymorphism analysis

Single strand conformation polymorphism analysis is a screening method of mutation detection. The DNA is first extracted from the sample and amplified using PCR. The PCR product is then prepared for analysis by heat denature and analysed using capillary electrophoresis under non-denaturing conditions. Sequence variations (single-point mutations and other small changes) are detected through electrophoretic mobility differences.

High resolution melt analysis

High resolution melt analysis is a screening method of mutation detection. The DNA is first extracted from the sample and amplified using PCR. The PCR

product is then precisely warmed so that the two strands of DNA ‘melt’ apart. Fluorescent dye which only binds to double stranded DNA is used to monitor the process. A region of DNA with a mutation will ‘melt’ at a different temperature to the same region of DNA without a mutation. These changes are documented as melt curves and the presence or absence of a mutation can be reported.

Next generation sequencing

Next generation sequencing is a screening method of mutation detection. The concept is similar to Sanger sequencing (described in section 2.2), however the sample DNA is first fragmented into a library of small segments that can be sequenced in parallel reactions.

2.2 Comparator

Sanger sequencing

Sanger sequencing (also called direct sequencing) is a screening method of mutation detection. Sanger sequencing is a commonly used method; however, there is much variation in the detail of how the method is carried out. In general, after DNA is extracted from the sample it is amplified using PCR. The PCR product is then cleaned up and sequenced in both forward and reverse directions. The sequencing reaction uses dideoxynucleotides labelled with coloured dyes which randomly terminate DNA synthesis creating DNA fragments of various lengths. The sequencing reaction product is then cleaned up and analysed using capillary electrophoresis. The raw data are analysed using analysis software to generate the DNA sequence. All steps are performed at least in duplicate to increase confidence that an identified mutation is real. It should be noted that sequencing only works well when viable tumour cells constitute at least 25% or more of the sample.

3 The evidence

3.1 Clinical effectiveness

The External Assessment Group conducted a systematic review to summarise the evidence on the clinical effectiveness of the different EGFR-TK mutation testing options for the identification of previously un-treated adults with locally advanced or metastatic NSCLC who may benefit from first line treatment with EGFR tyrosine kinase inhibitors (gefitinib or erlotinib). The methods used to perform the systematic review are described starting on page 29 of the diagnostics assessment report, with the results reported on pages 35 to 62. In addition to the systematic review, a web-based survey was conducted to gather data on the technical performance characteristics of EGFR-TK mutation tests in use in NHS laboratories participating in the United Kingdom National External Quality Assessment Service (UK NEQAS) pilot scheme for EGFR-TK mutation testing. Results of this survey are reported starting on page 38 of the diagnostics assessment report.

The objectives of the systematic review and the web-based survey were to provide answers to the following questions:

1. What is the technical performance of the different EGFR-TK mutation tests?
2. What is the accuracy of EGFR-TK mutation testing, using any test, for predicting response to treatment with EGFR tyrosine kinase inhibitors?
3. How do clinical outcomes from treatment with EGFR tyrosine kinase inhibitors vary according to which test is used to select patients for treatment?

Technical performance of EGFR-TK mutation tests

One study was identified from the systematic review which evaluated the technical performance of EGFR-TK mutation tests. The study was conducted in the Department of Molecular Diagnostics at the Royal Marsden Hospital and the Institute of Cancer Research. The study reported data for 2 years of

EGFR-TK mutation testing from January 2009 to January 2011. During year 1 of the testing the Therascreen EGFR PCR Kit was used. During year 2 a combination of the Therascreen EGFR PCR Kit, fragment analysis (for exon 19 deletions and exon 20 insertions) and Sanger sequencing (for the rarer exon 19 or exon 21 mutations) were used. A total of 121 patients were tested during year 1 and 755 during year 2. The mean turnaround time for the Therascreen EGFR PCR test alone during year 1 was 4.9 business days (95% confidence interval [CI]: 4.5 to 5.5 days). However, the actual time from the test request to the result was 17.8 days (95% CI: 16.4 to 19.4 days). The test failure rate was 19% but this improved over time from 33% during the first 3 months to 13% during the last 3 months of year 1 testing. The failure rate was lower in year 2 at only 5%.

There were 24 UK laboratories participating in the 2012-2013 UK NEQAS pilot scheme for EGFR-TK mutation testing. Of these, 14 provided information to NICE during the scoping phase of the assessment and were invited to participate in the survey. Thirteen of the 14 laboratories completed the web-based survey. The Therascreen EGFR PCR Kit was the most commonly used EGFR-TK mutation test with 6 laboratories using this test. A combination of fragment length analysis and pyrosequencing was used in 2 laboratories. Sanger sequencing was used by 2 laboratories, however, one of these laboratories also use the cobas EGFR Mutation Test for verification of mutations, or where sample contains insufficient tumour cells for Sanger sequencing (<30%); the second of these laboratories also use fragment length analysis/TaqMan/real-time PCR for verification of mutations, or where sample contains insufficient tumour cells for Sanger sequencing (<30%). The following methods were used in single laboratories: single strand conformation analysis, high resolution melt analysis, pyrosequencing. One laboratory also provided information on a next generation sequencing method that they are in the process of developing and validating.

The survey results show that there were no clear differences between tests in terms of batch size, turnaround time, number of failed samples or test cost

(summarised in Table 3). This suggests that test logistics, technical performance and costs may depend on individual laboratories rather than on the test method. It was also noted by UK NEQAS that error rates seen in the quality assurance scheme are not always method related, and may be due to processing and reporting problems. As laboratories do not always provide information relating to reasons for errors, it is not reasonable to relate error rates to test method. However, UK NEQAS note that there has been no correlation between any method used for EGFR-TK mutation testing and errors since the scheme was started in 2010.

Table 3: Summary of the web-based survey results

EGFR-TK mutation test	Number of labs using method	Test logistics			Technical performance			Test costs	
		Samples per week	Batch size	Time to result	Minimum % tumour cells required	Estimate of total failed samples	Estimate of failures due to insufficient tumour cells	Cost of test	Price charged for test
Therascreen EGFR PCR Kit	6	≤5 to >20	5 to 7	24-48 hours to 8-10 days	≤1% to 6-10%	0 to 10%	0 to 5%	£120 to £190	£120 to £190
Fragment length analysis **and** pyrosequencing	2	6 to 10	5	6-7 days	1 to 5%	5%	2%	£150 to £175	£175 to £200
Sanger sequencing **and/or** fragment length analysis/TaqMan/ real-time PCR	1	≤5	1 to 3	6-7 days	Sequencing: >30%; other methods: not reported	0	0	Not reported	£140
Sanger sequencing **and/or** cobas EGFR Mutation Test	1	16 to 20	6	Sequencing: 6-7 days; Cobas: 3-5 days	Sequencing: >30%; Cobas: 6 to 10%	Sequencing: 4%; Cobas: 5%	Sequencing: 3%; Cobas: 4%	Not reported	Sequencing: £120; Cobas: £140
High resolution melt analysis	1	11 to 15	7	3-5 days	6 to 10%	0.2%	0.2%	£140	£150
Next generation sequencing	1	≤5	5	3-5 days	1 to 5%	Not reported	Not reported	Not reported	Not reported
Pyrosequencing	1	16 to 20	6 to 8	6-7 days	1 to 5%	5%	2%	£175	£175
Single strand conformation analysis	1	>20	10	3-5 days	1 to 5%	10%	2%	£110	£140

Accuracy of EGFR-TK mutation testing

There is no 'gold standard' test for identifying EGFR-TK mutations. Further, there is uncertainty around the clinical significance of individual EGFR-TK mutations and the level of mutation. Therefore it was necessary for the External Assessment Group to use an alternative approach in order to calculate the accuracy of EGFR-TK mutation tests. Studies used to provide information on the accuracy of EGFR-TK mutation testing were those that provided clinical response data on both mutation positive and mutation negative tumours treated with an EGFR tyrosine kinase inhibitor. Accuracy was calculated using clinical response to treatment as the reference standard. It was assumed that the tumour response to treatment with an EGFR tyrosine kinase inhibitor was an indication of the true EGFR-TK mutation status, rather than there being any other reasons for a tumour response (or lack of tumour response). The following definitions were used for the test accuracy statistics:

True positives	Patients with tumours identified as having an EGFR-TK mutation that have a positive response to EGFR tyrosine kinase inhibitor treatment
False positives	Patients with tumours identified as having an EGFR-TK mutation that do not respond to EGFR tyrosine kinase inhibitor treatment
False negatives	Patients with tumours identified as not having an EGFR-TK mutation that have a positive response to EGFR tyrosine kinase inhibitor treatment
True negatives	Patients with tumours identified as not having an EGFR-TK mutation that do not respond to EGFR tyrosine kinase inhibitor treatment

Two types of response were used as reference standards to calculate accuracy; objective response (OR) and disease control (DC), which are based on the RECIST criteria and are summarised in Table 4.

Table 4: Definitions of response based on the RECIST criteria

	Reference standard	
	Objective response (OR)	**Disease control (DC)**
Positive response to EGFR tyrosine kinase inhibitor treatment	Best observed response was complete response or partial response	Best observed response was complete response, partial response or stable disease
No response to EGFR tyrosine kinase inhibitor treatment	Best observed response was stable disease or disease progression	Best observed response was disease progression

Best observed response is defined as the best response recorded from the start of treatment to disease progression.

Complete response is defined as the disappearance of all target lesions and no new lesions.

Partial response is defined as at least 30% decrease in the sum of the longest diameter of target lesions, taking the sum of the baseline diameters as the reference, and no new lesions.

Stable disease is defined as neither sufficient shrinkage to be classified as partial response or sufficient increase to be classified as progressive disease, taking the smallest sum of the longest diameters recorded since treatment started as the reference, and no new lesions.

Progressive disease is defined as at least a 20% increase in the sum of the longest diameter of target lesions, taking the smallest sum of the longest diameters recorded since treatment started as the reference, or appearance of one or more new lesions.

It should be noted that studies were not excluded based on the method used for EGFR-TK mutation testing, therefore the diagnostics assessment report contains results relating to test methods included in the scope and on some methods not included in the scope.

Six studies, two randomised controlled trials and four cohort studies, provided data on the accuracy of EGFR-TK mutation testing for predicting the response to treatment with EGFR tyrosine kinase inhibitors in patients with advanced or metastatic NSCLC. Three studies were conducted in patients treated with gefitinib and 3 were conducted in patients treated with erlotinib. Patient characteristics varied across studies which is important to keep in mind given the assumptions made when comparing accuracy between studies. One study included mainly Caucasian patients and 1 study included mainly East Asian patients (4 studies did not report on ethnicity of patients). All studies reported

a high proportion of patients with metastatic disease. Most patients had a histological diagnosis of adenocarcinoma (45% to 100%), but 2 studies included some patients with squamous cell carcinoma (9% and 15%). Four studies included mainly or only patients who had never smoked, whereas 2 studies included mainly current and former smokers. Full details of patients are reported in Appendix 2 of the diagnostics assessment report.

Five studies evaluated Sanger sequencing methods for the identification of any EGFR-TK mutation; 3 assessed exons 18 to 21, 1 assessed exons 19 to 21, and 1 assessed exons 18 to 24 (Sanger sequencing or WAVE-HS for inadequate samples [<50% tumour cells]). One study assessed the Therascreen EGFR PCR Kit (the version designed to detect 29 mutations, including T790M). Test accuracy results are presented in full starting on page 47 of the diagnostics assessment report and are summarised in Table 5.

The Therascreen EGFR PCR Kit appears to have the best overall performance for discriminating between patients who are likely to benefit from EGFR tyrosine kinase inhibitor treatment and those who are not. The sensitivity and specificity estimates using objective response as the reference standard were 99% (95% CI: 94% to 100%) and 69% (95% CI: 60% to 77%) respectively. Of the 5 studies which used Sanger sequencing methods to identify EGFR-TK mutations 4 reported high estimates of specificity (>80%) and sensitivities ranged from 60% to 80% when objective response was used as the reference standard. The remaining Sanger sequencing study reported low specificity (61%) with high sensitivity (84%) for objective response as the reference standard. When disease control was used as the reference standard, specificities were higher and sensitivities were lower as disease control represents a lower threshold for response to treatment.

All Sanger sequencing studies had small sample sizes, reflected in the wide confidence intervals around sensitivity and specificity estimates. It is possible that the lower specificity values observed in two studies (IPASS and Yang 2008) may be partially explained by the classification of resistance mutations as a positive result for EGFR-TK mutation testing. The 4 Sanger sequencing

studies which reported high specificity estimates either stated that patients whose tumours showed resistance or non-sensitising mutations were classified as EGFR-TK mutation negative, or did not identify any patients whose tumours showed these types of mutation. Data relating best response to individual mutations appeared to indicate that there may be a less favourable response to EGFR tyrosine kinase inhibitors in tumours with T790M or other exon 20 mutations.

Table 5: Accuracy of EGFR mutation testing for the prediction of response to treatment with EGFR tyrosine kinase inhibitors

Study	EGFR-TK mutation test	Disease control		Objective response	
		Sensitivity % (95% CI)	Specificity % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)
IPASS (Fukuoka 2011)	Therascreen EGFR PCR Kit	77 (70 to 83)	83 (70 to 91)	99 (94 to 100)	69 (60 to 77)
Giaccone 2006	Sanger sequencing of exons 18-21	29 (10 to 56)	100 (74 to 100)	80 (28 to 100)	96 (79 to 100)
First-SIGNAL (Han 2012)	Sanger sequencing of exons 19-21	NR	NR	76 (57 to 90)	83 (63 to 95)
Jackman 2007	Sanger sequencing or WAVE-HS for inadequate samples of exons 18-24	35 (15 to 56)	100 (72 to 100)	60 (15 to 95)	81 (64 to 93)
Pallis 2012	Sanger sequencing of exons 18-21	33 (16 to 55)	92 (62 to 100)	60 (26 to 88)	89 (70 to 98)
Yang 2008	Sanger sequencing of exons 18-21	66 (54 to 71)	50 (19 to 81)	84 (71 to 94)	61 (44 to 77)

Clinical effectiveness of EGFR tyrosine kinase inhibitors according to EGFR-TK mutation testing

This section of the assessment aimed to address how the clinical effectiveness of EGFR tyrosine kinase inhibitors for treatment of patients with advanced or metastatic NSCLC whose tumours tested positive for an EGFR-

TK mutation varied according to how these patients were selected (which EGFR-TK mutation test was used). Studies used to provide this information were those that provided data on patients with tumour identified as EGFR-TK mutation positive who were treated with either an EGFR tyrosine kinase inhibitor or standard chemotherapy.

Five randomised controlled trials provided data on the clinical effectiveness of EGFR tyrosine kinase inhibitors compared with standard chemotherapy in patients with advanced or metastatic NSCLC whose tumours tested positive for EGFR-TK mutations. One additional study reported data for a subgroup of patients from the EURTAC trial whose samples had been re-analysed using a different EGFR-TK mutation testing method. Three of the trials included only patients with EGFR-TK mutation positive tumours, and the remaining 2 trials (IPASS and First-SIGNAL) included all patients regardless of EGFR-TK mutation status, but also reported a subgroup analysis for patients whose tumours tested positive for EGFR-TK mutations. The trials compared the EGFR tyrosine kinase inhibitors gefitinib or erlotinib with various single agent or combination standard chemotherapy regimens. It should be noted that the IPASS and the First-SIGNAL trials also provided data on the accuracy of EGFR-TK mutation tests.

Patient characteristics varied across studies. Four studies were conducted in East Asia and 1 was conducted in Western Europe. One study included patients who had never smoked, 1 study included mainly patients who had never smoked (94%) and the rest included between 62% and 71% of patients who had never smoked. One study included only patients with a diagnosis of adenocarcinoma, while in the remaining studies approximately 90% had a diagnosis of adenocarcinoma. The majority of patients (>75%) in all studies had metastatic disease. Full details of patients are reported in Appendix 2 of the diagnostics assessment report.

Two studies used Sanger sequencing methods to assess EGFR-TK mutation status, however, both limited the definition of positive EGFR-TK mutation status to the presence of an ‘activating mutation’ (exon 19 deletions or exon

21 mutation L858R). The remaining studies used EGFR-TK mutation tests which targeted a wider range of mutations. One study reported the results of a re-analysis of samples from the EURTAC trial using the cobas EGFR Mutation Test. One study (IPASS) used the Therascreen EGFR PCR Kit (version designed to detect 29 mutations, including T790M). The North East Japan Study Group (NEJSG) trial used fragment length analysis, targeting exon 19 deletions, exon 21 point mutations (L858R, L861Q), exon 18 point mutations (G719A, G719C, G719S), and exon 20 point mutation (T790M). The First-SIGNAL trial used Sanger sequencing of exons 19 to 21.

Clinical effectiveness results are presented in full starting on page 55 of the diagnostics assessment report and are summarised in Table 6. All studies reported improvements in objective response and improvements or trends towards improvement in progression free survival for patients with EGFR-TK mutation positive tumours who were treated with EGFR tyrosine kinase inhibitors compared with those given standard chemotherapy. Three studies reported overall survival but none found a significant difference between patients treated with EGFR tyrosine kinase inhibitors and those given standard chemotherapy.

The results from the IPASS trial showed that progression free survival in patients with EGFR-TK mutation negative tumours was significantly shorter when treated with EGFR tyrosine kinase inhibitors compared with standard chemotherapy. A similar trend for patients with EGFR-TK mutation negative tumours, although not statistically significant, was observed in the First-SIGNAL trial.

Table 6: Clinical outcomes in patients with EGFR-TK positive tumours who were treated with EGFR tyrosine kinase inhibitors compared with those treated with standard chemotherapy

Study	EGFR test	Progression free survival	Objective response	Disease control
		Hazard ratio* (95% CI)	Relative risk** (95% CI)	Relative risk** (95% CI)
EURTAC (Benlloch 2012)	Cobas EGFR Mutation Test	0.35 (0.21 to 0.58)	NR	NR
IPASS (Fukuoka 2011)	Therascreen EGFR PCR Kit	0.48 (0.36 to 0.64)	1.15 (1.23 to 1.88)	1.05 (0.96 to 1.15)
First-SIGNAL (Han 2012)	Sanger sequencing of exons 19-21	0.54 (0.27 to 1.10)	2.26 (1.31 to 4.65)	NR
NEJSG (Maemondo 2010)	Fragment length analysis (exon 19 deletions; exon 21: L858R, L861Q; exon 18:G719A, G719C, G719S; exon 20: T790M)	0.30 (0.22 to 0.41)	2.40 (1.81 to 3.26)	1.12 (1.00 to 1.47)
EURTAC (Rosell 2012)	Sanger sequencing (exon 19 deletions and exon 21 mutation L858R)	0.37 (0.25 to 0.54)	3.89 (2.34 to 6.68)	1.21 (1.00 to 1.47)
OPTIMAL (Zhou 2011)	Sanger sequencing (exon 19 deletions and exon 21 mutation L858R)	0.16 (0.10 to 0.26)	2.30 (1.70 to 3.23)	1.18 (1.06 to 1.35)

* Hazard ratios for progression free survival: ratios less than 1 indicate that treatment with EGFR tyrosine kinase inhibitors is associated with a reduced risk of progression compared with treatment with chemotherapy.
** Relative risks for objective response and disease control: values greater than 1 favour EGFR tyrosine kinase inhibitors compared with chemotherapy.

3.2 Cost effectiveness

The External Assessment Group developed a decision analytic model to assess the cost effectiveness of different methods for EGFR-TK mutation testing to decide between treatment with standard chemotherapy and EGFR tyrosine kinase inhibitors in patients with locally advanced or metastatic

NSCLC. The analysis was not limited to EGFR-TK test methods included in the scope, but also included additional methods for which data were available. Results relating to EGFR-TK test methods not included in the scope are presented in italics in the results tables (Tables 26 to 29) in the diagnostics assessment report. Three different analytic approaches, described below, were used to calculate cost effectiveness, each using different levels of evidence.

1. ‘Evidence on comparative effectiveness available’ analysis

   This analysis used data on the comparative effectiveness (progression free survival and overall survival) of EGFR tyrosine kinase inhibitors and standard chemotherapy in patients with EGFR-TK mutation positive, EGFR-TK mutation negative and EGFR-TK mutation unknown tumours. The only tests with this type of data were the Therascreen EGFR PCR Kit and Sanger sequencing of all exon 19 to 21 mutations. A major assumption underlying the use of these data is that the difference in comparative effectiveness is solely due to the use of different mutation tests.

2. ‘Linked evidence’ analysis

   This analysis is the same as the ‘evidence on comparative effectiveness available’ analysis, except that it allowed the inclusion of EGFR-TK mutation tests which have data on the accuracy of the test for the prediction of response to EGFR tyrosine kinase inhibitors, but have no data on comparative effectiveness (progression free survival and overall survival in patients with EGFR-TK mutation positive, EGFR-TK mutation negative and EGFR-TK mutation unknown tumours). This type of data were available for two tests: Sanger sequencing of all exon 18 to 21 mutations and Sanger sequencing or WAVE-HS for inadequate samples (<50% tumour cells) of all exon 18 to 24 mutations. Therefore this analysis included four EGFR-TK mutation test strategies. In addition to the assumption made for the ‘evidence on

comparative effectiveness available' analysis, the 'linked evidence' analysis assumed that for the Sanger sequencing methods without comparative effectiveness data, the relative progression free survival and overall survival for EGFR-TK mutation positive and EGFR-TK mutation negative tumours correlate perfectly with relative progression free survival and overall survival as observed for Sanger sequencing of all exon 19 to 21 mutations.

3. 'Assumption of equal prognostic value' analysis

   For the remaining EGFR-TK mutation tests included in the scope, no data were available on either the comparative effectiveness or the accuracy of the test for the prediction of response to EGFR tyrosine kinase inhibitors. Therefore, for these tests it was only possible to make a comparison based on differences in technical performance and test costs retrieved from the web-based survey, whilst assuming equal prognostic value across tests. This assumption was not based on evidence of equality, but rather absence of any reliable evidence to model a difference in prognostic value for these tests. The equal prognostic value assigned was based on data for the Therascreen EGFR PCR Kit.

In order to ensure consistency between the modelling approach used in NICE technology appraisal guidance 192 and the assessment of the cost-effectiveness of different methods for EGFR-TK mutation testing, the External Assessment Group received the health economic model submitted by Astra Zeneca for NICE technology appraisal guidance 192. The External Assessment Group also took into account amendments made by the Evidence Review Group (the academic group that assessed the Astra Zeneca health economic model on NICE's behalf) during the appraisal of gefitinib. This model calculates the expected cost effectiveness of gefitinib compared with standard chemotherapy for the first-line treatment of locally advanced or metastatic NSCLC patients with a positive EGFR-TK mutation test based on the Therascreen EGFR PCR Kit. This model was used to inform the

development of a de novo model in which the long term consequences of using different EGFR-TK mutation tests were assessed not only in patients with a positive EGFR-TK mutation test, but also in patients with a negative test result, or an unknown test result.

Model structure

A decision tree and a Markov model were developed to analyse the long-term consequences of technical performance and accuracy of the different EGFR-TK mutation tests and test combinations followed by treatment with either standard chemotherapy or an EGFR tyrosine kinase inhibitor in patients with NSCLC. The decision tree was used to model the test result (positive, negative or unknown) and the treatment decision. Patients with a positive test result receive an EGFR tyrosine kinase inhibitor. Patients with a negative test result or an unknown EGFR mutation status receive standard chemotherapy (pemetrexed and cisplatin). The decision tree is shown in Figure 1.

The Markov model was used to estimate the long-term consequences in terms of costs and QALYs. The model has a cycle time of 21 days (resembling the duration of one cycle of chemotherapy), and a time horizon of 6 years. Health states in the Markov model are: progression free (subdivided into ‘response’ and ‘stable disease’ based on the objective response rate), disease progression and death. In the progression free state, patients are on treatment (either EGFR tyrosine kinase inhibitor or standard chemotherapy). The Markov model is shown in Figure 2.

Figure 1: Decision tree structure

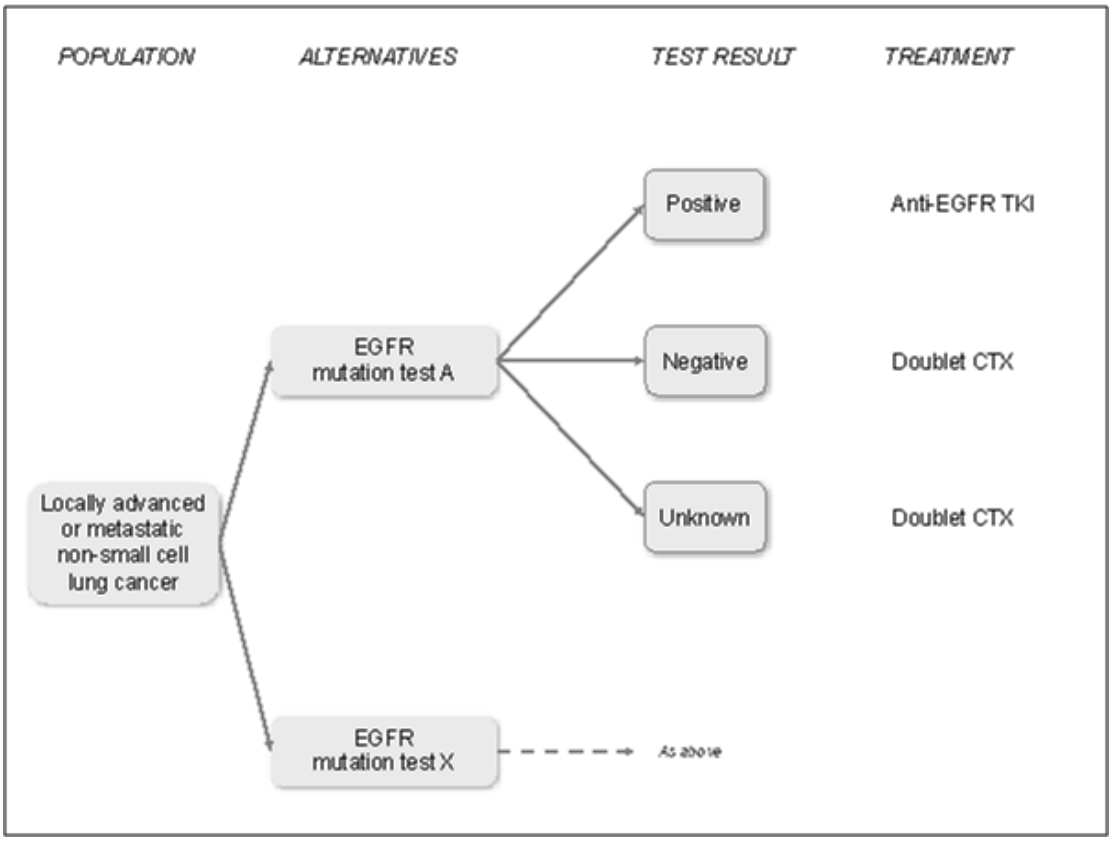
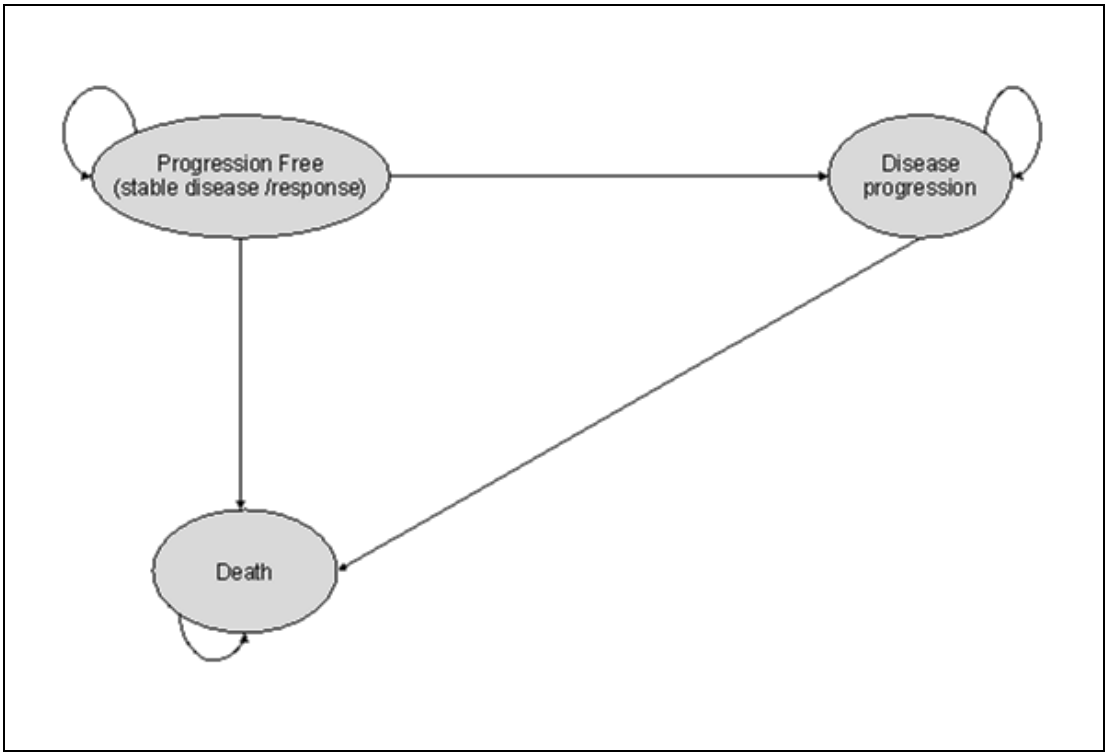


Figure 2: Markov model structure



Model inputs

Estimates for model input parameters were retrieved from NICE technology appraisal guidance 192, the assessment of the clinical effectiveness of different EGFR mutation tests, the web-based survey of NHS laboratories in England and Wales, and the Personal Social Services Research Unit. The input parameters for the model are described in the diagnostics assessment report starting on page 75.

Test results

The proportions of positive and negative EGFR-TK mutation test results (Table 7) were based on the estimated proportions of NSCLC patients with EGFR-TK mutation positive tumours in England and Wales (16.6%, standard error: 0.8%), the test accuracy (sensitivity and specificity with objective response to EGFR tyrosine kinase inhibitor as reference standard, Table 5) and the proportion of patients with an unknown test result. The proportions of patients with an unknown test result were based on data from published studies (IPASS and Jackman 2007), calculated from the proportions of patients without an EGFR-TK mutation status relative to the number of patients for whom a tissue sample was available. As these clinical trials do not represent clinical practice, this might be an overestimation of the proportion of patients with an unknown test result in clinical practice. One possible reason for this is that in the trials the tissue samples were not generally taken for the purpose of EGFR-TK mutation testing, and may therefore have been inadequate more often than would be the case in current clinical practice. In contrast, the results of the web-based survey are likely to provide an underestimation of the total proportion of patients with an unknown test result, as laboratories are not likely to have insight into the total proportion of pre-test failures (samples considered inadequate by the pathologist and not sent to the laboratory).

Table 7: Probability of a positive, unknown and negative test result

Mutation test	Probability (se) of test result		
	Positive	Unknown	Negative
Therascreen EGFR PCR Kit	32.8% (2.9%)	22.7% (1.8%)	44.6% (3.0%)
Sanger sequencing of exons 19-21	16.5% (4.2%)	37.7% (5.2%)	45.8% (5.6%)
Sanger sequencing of exons 18-21	29.0% (4.6%)	37.7% (4.2%)	33.4% (4.9%)
Sanger sequencing or WAVE-HS for inadequate samples	16.0% (4.4%)	37.7% (5.7%)	46.4% (5.9%)

Response to treatment

Patients who are in the progression-free state are subdivided over the 'stable disease' and 'response' states based on the objective response rate, as presented in Table 8. Patients with a positive EGFR-TK mutation status were assumed to receive an EGFR tyrosine kinase inhibitor, whereas those with an unknown or negative EGFR-TK mutation status were assumed to receive standard chemotherapy treatment. As various regimens of standard chemotherapy were used across trials, objective response rates for unknown and negative EGFR-TK status were adjusted to correspond to treatment with pemetrexed and cisplatin. The ratios used to make the adjustments were taken from the updated mixed treatment comparison used in NICE technology appraisal guidance 192. The unadjusted objective response rates are presented on page 79 of the diagnostics assessment report, and the adjusted objective response rates are presented in Table 8.

Table 8: Objective response rates

EGFR-TK mutation test	Objective response rate (se)			Source study
	Positive EGFR-TK status	Unknown EGFR-TK status	Negative EGFR-TK status	
Therascreen EGFR PCR Kit	0.712 (0.039)	0.403	0.335	IPASS
Sanger sequencing of exons 19-21	0.846 (0.069)	As for Therascreen EGFR PCR Kit	0.604	First-SIGNAL
Sanger sequencing of exons 18-21	0.731 (0.061)	As for Therascreen EGFR PCR Kit	As Sanger sequencing of exons 19-21	Yang 2008
Sanger sequencing or WAVE-HS for inadequate samples	0.333 (0.149)	As for Therascreen EGFR PCR Kit	As Sanger sequencing of exons 19-21	Jackman 2007

Survival

For testing using the Therascreen EGFR PCR Kit, progression free survival and overall survival were modelled using Weibull regression models based on the IPASS study and a hazard ratio favouring treatment with EGFR tyrosine kinase inhibitor (HR 0.43, 95% CI: 0.34 to 0.53) (based on a meta-analysis and mixed treatment comparison used in NICE technology appraisal guidance 192). Details of this model are given on pages 80 and 81 of the diagnostic assessment report. For testing using Sanger sequencing of exon 19 to 21 mutations, progression free survival and overall survival for patients with EGFR-TK mutation positive or negative tumours were modelled using Kaplan-Meier curves extracted from the First-SIGNAL trial. Progression free survival and overall survival for patients with tumours of unknown EGFR-TK mutation status were based on the IPASS Weibull model for unknown mutations, since these were not reported in the First-SIGNAL trial.

Progression free survival and overall survival estimates for patients with EGFR-TK mutation unknown and mutation negative status were adjusted to correspond to treatment with pemetrexed and cisplatin (as for objective response rate).

Adverse events

The occurrence of adverse events was assumed to be dependent on treatment and independent of EGFR-TK mutation status, that is, adverse events for patients with EGFR-TK mutation negative and mutation unknown tumours were assumed to be equal. The source of data was NICE technology appraisal guidance 192, and probabilities of adverse events were adjusted (as for objective response rate) to correspond to standard chemotherapy treatment with pemetrexed and cisplatin rather than paclitaxel and carboplatin. The unadjusted probabilities of adverse events are presented on page 85 of the diagnostics assessment report, and the adjusted probabilities of adverse events are presented in Table 9.

Table 9: Adverse events associated with EGFR tyrosine kinase inhibitors and standard chemotherapy

Adverse event	Probability with EGFR tyrosine kinase inhibitor	Probability with pemetrexed and cisplatin
Neutropenia	0.0%	18.7%
Febrile Neutropenia	0.0%	0.8%
Fatigue	0.0%	5.8%
Nausea &/or vomiting	0.0%	35.0%
Diarrhoea	5.3%	0.8%
Hair Loss (grade 2)	1.2%	31.6%
Rash	2.3%	0.0%
Anaemia	1.5%	14.2%

Health state utilities

Utility values were in line with those used in NICE technology appraisal guidance 192 and are presented on page 86 of the diagnostics assessment report.

Resource use and costs

Resource use and costs were taken from NICE technology appraisal guidance 192, with the exception of the EGFR-TK mutation test costs. The test costs were based on the charged prices from the web-based survey of NHS laboratories in England and Wales (presented on page 88 of the diagnostics assessment report). In the case of an unknown EGFR-TK mutation status due

to a pre-laboratory clinical failure, no test costs were taken into account. In the case of an unknown EGFR-TK mutation status due to a technical failure within the laboratory full test costs were taken into account. The proportion of pre-laboratory failures and within laboratory failures was calculated from the proportion of patients with an unknown EGFR-TK mutation status and the total proportion of technical failures as reported in the web-based survey (presented on page 88 of the diagnostics assessment report). Other costs used in analyses are presented in the diagnostics assessment report on page 91.

Sensitivity analyses

Two sensitivity analyses were performed:

1. Treatment costs and adverse event costs were updated to 2012 costs (with the exception of EGFR tyrosine kinase inhibitor costs). The updated costs used in this sensitivity analysis are presented on page 93 of the diagnostics assessment report. This sensitivity analysis was performed on the 'evidence of comparative effectiveness available' analysis and the 'linked evidence' analysis.

2. The proportion of patients with unknown mutation status was based on the results from the web-based survey rather than information from published trials (Table 10). This sensitivity analysis was performed for all three analytical approaches.

Table 10: Probability of positive test result, unknown test result and negative test result based on results from the web-based survey

EGFR-TK mutation test	Probability of test result		
	Positive	Unknown	Negative
'Evidence on comparative effectiveness available' and 'linked evidence' analyses			
Therascreen EGFR PCR Kit	40.8%	3.8%	55.4%
Sanger sequencing of exons 19-21	25.3%	4.5%	70.2%
Sanger sequencing of exons 18-21	44.4%	4.5%	51.1%
Sanger sequencing or WAVE-HS for inadequate samples	24.5%	4.5%	71.0%
'Assumption of equal prognostic value' analysis			
Therascreen EGFR PCR Kit	40.8%	3.8%	55.4%
Sanger sequencing of exons 19-21	40.5%	4.5%	55.0%
Sanger sequencing of exons 18-21	40.5%	4.5%	55.0%
Sanger sequencing or WAVE-HS for inadequate samples	40.5%	4.5%	55.0%
Sanger sequencing or Therascreen EGFR PCR Kit for samples with insufficient tumour cells	40.7%	3.9%	55.4%
Fragment length analysis combined with pyrosequencing	40.3%	5.0%	54.7%
Sanger sequencing or cobas EGFR Mutation Test for samples with insufficient tumour cells	40.5%	4.5%	55.0%
Sanger sequencing and fragment length analysis/real-time PCR	42.3%	0.1%	57.6%
High resolution melt analysis	42.3%	0.2%	57.5%
Cobas EGFR Mutation Test	40.3%	5.0%	54.7%
Single strand conformation analysis	38.1%	10.0%	51.9%

Results

Much of the incremental cost effectiveness analysis resulted in a comparison where the intervention tests are less effective but cheaper than the comparator test (Sanger sequencing). In situations where an ICER is derived from decreased effectiveness and decreased costs, the commonly assumed decision rule of accepting ICERs below a given threshold is reversed, and so the higher the ICER, the more cost effective the intervention test becomes.

Two intervention tests, next generation sequencing and the Therascreen EGFR Pyro Kit, were not included in the cost effectiveness analysis. This was due to a lack of evidence on the test failure rate and test cost, as no

laboratories completing the web-based survey used these test methods in clinical practice and therefore no data were available.

'Evidence on comparative effectiveness available' analysis

Due to a lack of comparative effectiveness data for Sanger sequencing of exons 18 to 21, in this analysis Sanger sequencing of exons 19 to 21 was used as the comparator. The Therascreen EGFR PCR Kit was both less effective and less costly compared with Sanger sequencing of all exons 19-21 at an ICER of £32,167 saved per QALY lost (Table 11). The lower costs and QALYs for the Therascreen EGFR PCR Kit can be explained by the fact that patients whose tumours are EGFR-TK mutation negative have shorter overall survival in the IPASS trial (Therascreen EGFR PCR Kit) than in the First-SIGNAL trial (Sanger sequencing of exons 19 to 21), whereas for patients whose tumours are EGFR-TK mutation positive the outcome is comparable. For patients whose tumours are EGFR-TK mutation unknown, overall survival is the same by assumption. Therefore, on average, with the Therascreen EGFR PCR Kit patients have shorter overall survival, and therefore fewer QALYs compared with Sanger sequencing of exons 19 to 21. The apparent shorter survival also reduces costs.

Sensitivity analyses had little impact on the base case results, with the Therascreen EGFR PCR Kit always less effective and less expensive than Sanger sequencing of all exon 19 to 21 mutations.

It should be noted that this analysis is based on a number of assumptions, of which the following 2 are particularly problematic:

- The proportion of patients with a positive or negative EGFR-TK mutation test result after the use of these tests in the NHS population was estimated based on the proportion of NSCLC patients with EGFR-TK mutation positive tumour in England and Wales, the proportion of patients with an unknown test result, and test accuracy for the prediction of treatment response derived from two separate trials.

- The differences in relative treatment response, progression free survival and overall survival between the results of First-SIGNAL trial (Sanger sequencing of exons 19 to 21) and the results of the IPASS trial (Therascreen EGFR PCR Kit), are solely due to the different EGFR-TK mutation tests used to distinguish between patients whose tumours are EGFR-TK mutation positive (and receive EGFR tyrosine kinase inhibitor treatment) and patients whose tumours are EGFR-TK mutation negative (and receive standard chemotherapy).

Table 11: Probabilistic results of 'Evidence on comparative effectiveness available' analysis: base case and sensitivity analyses

Test strategy	Cost	QALY	Compared with Sanger sequencing (exons 19-21)		
			Incremental cost	Incremental QALY	Cost saving/ QALY lost
Base case					
Therascreen EGFR PCR Kit	[redacted]	0.902	-£6,660	-0.207	£32,167*
Sanger sequencing of exons 19-21	[redacted]	1.109			
Sensitivity analysis: updated costs					
Therascreen EGFR PCR Kit	[redacted]	0.874	-£9,194	-0.286	£32,196*
Sanger sequencing of exons 19-21	[redacted]	1.160			
Sensitivity analysis: unknowns from survey					
Therascreen EGFR PCR Kit	[redacted]	0.905	-£7,130	-0.206	£34,555*
Sanger sequencing of exons 19-21	[redacted]	1.111			

* Note: ICERs represent cost savings per QALY lost compared with Sanger sequencing of exons 19 to 21.

'Linked evidence' analysis

In the base case analysis, compared with Sanger sequencing of all exon 18 to 21 mutations, the Therascreen EGFR PCR Kit was less costly and less effective at an ICER of £31,849 saved per QALY lost (Table 12). Sanger sequencing of all exon 19 to 21 mutations and Sanger sequencing or WAVE-HS for inadequate samples were both more expensive and more effective

than Sanger sequencing of all exon 18 to 21 mutations (shown in italics - these test strategies are not included as interventions in the scope). The explanation for the lower costs and fewer QALY for the Therascreen EGFR PCR Kit compared with Sanger sequencing is the same as given for the 'evidence on comparative effectiveness available' analysis (page 31 of the overview).

Sensitivity analyses had little impact on the base case results, with the Therascreen EGFR PCR Kit always being less expensive and less effective than Sanger sequencing of all exon 18 to 21 mutations. Results of sensitivity analyses are presented in Appendix 7 on pages 212 to 216 of the diagnostic assessment report.

Table 12: Probabilistic results of 'linked evidence' analysis: base case

Test strategy	Cost	QALY	Compared with Sanger sequencing (exons 18 to 21)		
			Incremental cost	Incremental QALY	Cost/QALY
Therascreen EGFR PCR Kit	[redacted]	0.902	-£6,040	-0.190	£31,849 (saving per QALY lost)*
Sanger sequencing of exons 18 to 21	[redacted]	1.092			
Sanger sequencing of exons 19 to 21	[redacted]	*1.109*	*£619*	*0.017*	*£35,634*
Sanger sequencing or WAVE-HS for inadequate samples	[redacted]	*1.109*	*£658*	*0.017*	*£38,251*

* Note: The ICER for the Therascreen EGFR PCR Kit represents cost savings per QALY lost compared with Sanger sequencing of exons 18 to 21.

'Assumption of equal prognostic value' analysis

In this analysis, the comparative effectiveness, test accuracy and proportion of patients with unknown mutation status for each test strategy was assumed equal to those of the Therascreen EGFR PCR Kit. Therefore the test strategies only differ with respect to costs. As shown in Table 13, the test strategy of Sanger sequencing or the cobas EGFR Mutation Test for samples with insufficient tumour cells is the least expensive and fragment length

analysis combined with pyrosequencing is the most expensive strategy. However, the difference between the costs of these strategies amounts to only £47. Results for Sanger sequencing of all exon 19 to 21 mutations and Sanger sequencing or WAVE-HS for inadequate samples are presented for interest only as they are not included as intervention tests in the scope.

In a sensitivity analysis the proportion of patients with tumours of unknown EGFR-TK mutation status were taken from the web-based survey of NHS laboratories in England and Wales instead of based on the literature. As a result, in this sensitivity analysis a difference in QALYs is modelled. This has some impact on the results, as single stand conformation analysis becomes the most costly and most effective test strategy, with Sanger sequencing and fragment length analysis / real-time PCR of negative samples becoming the least costly least effective test strategy. However, the difference in costs between the strategies is only £490 and the difference in QALYs is 0.015. Results of the sensitivity analysis are presented on pages 99 to 100 of the diagnostics assessment report.

Table 13: Probabilistic results for 'assumption of equal prognostic value' analysis: base case

EGFR-TK mutation test strategy	Costs (95% CI)	Incremental costs compared with Sanger sequencing (exons 18 to 21)
Sanger Sequencing or the cobas EGFR Mutation Test for samples with insufficient tumour cells	[redacted]	-£15
Sanger sequencing and fragment length analysis/real-time PCR of negative samples	[redacted]	-£11
Sanger sequencing or the Therascreen EGFR PCR Kit for samples with insufficient tumour cells	[redacted]	-£9
Cobas EGFR Mutation Test	[redacted]	-£9
High Resolution Melt analysis	[redacted]	-£3
Sanger sequencing of exons 19-21	[redacted]	*£0*
Sanger sequencing of exons 18-21	[redacted]	
Single strand conformation analysis	[redacted]	£1
Sanger sequencing or WAVE-HS	[redacted]	*£1*
Therascreen EGFR PCR Kit	[redacted]	£5
Fragment length analysis combined with pyrosequencing	[redacted]	£33

4 Issues for consideration

One study was identified from the systematic review which provided information on the technical performance characteristics of EGFR-TK mutation tests. Results of this study show that failure rates decreased over time, which suggests that the level of experience a laboratory has in using a particular test method impacts on the failure rate. In addition, when a combination of test methods was used, failure levels decreased further. The results from the web-based survey indicated that the Therascreen EGFR PCR

Kit is the most widely used method of performing EGFR-TK mutation testing. The survey results show that there were no clear differences between tests in terms of batch size, turnaround time, number of failed samples or test cost, which suggests that test logistics, technical performance and costs may depend on individual laboratories rather than on the test method. In addition, UK NEQAS has indicated that no correlation has been observed between any of the methods used for EGFR-TK mutation testing and the error rates. This may indicate that errors are not only test-related, but may be affected by processing and reporting issues. However, the Therascreen EGFR PCR Kit may be associated with potentially shorter turnaround times, as the only laboratory to report a turnaround time of less than 3 days (24 to 48 hours) used this method.

There is some concern over whether the costs of the tests (including purchase cost, personnel, material and overheads) have been correctly estimated by the individuals completing the web-based survey. Several laboratories completing the survey did not provide a cost for the test used, and therefore the price charged for EGFR-TK mutation tests was used in the economic modelling. Although the price charged may not always reflect the real cost to the laboratory, it does reflect the true cost charged to the NHS.

A key difference between the EGFR-TK mutation tests is the variation in the limit of detection (that is, the minimum percentage of mutation in tumour cells required to produce a positive result). A lower limit of detection can enhance the ability of laboratories to produce results from poor quality samples; however the clinical consequences of a low proportion of tumour cells on prognosis are not well studied. Another difference between the EGFR-TK mutation tests is that they are designed to detect different mutations. Although over 90% of patients treated with EGFR tyrosine kinase inhibitors have exon 19 deletions or the exon 21 mutation L858R, other rarer mutations exist. The additional clinical value of using tests which target a wider range of mutations remains uncertain, since the relative low frequency of other mutations means

that the effect of EGFR tyrosine kinase inhibitors on these tumours is not well studied.

Results of the review of test accuracy suggest that the Therascreen EGFR PCR Kit may be more accurate than Sanger sequencing for predicting response to treatment with EGFR tyrosine kinase inhibitors. Due to the absence of a 'gold standard' test method, and because of uncertainty around the clinical significance of individual EGFR-TK mutations and level of mutation, the accuracy of different test methods was calculated using response to treatment with EGFR tyrosine kinase inhibitors as the reference standard. Tested patients were categorised as described on page 14 of the overview. In this analysis false positives were classified as patients with tumours identified as having an EGFR-TK mutation that did not respond to treatment with an EGFR tyrosine kinase inhibitor. However, not all tumours with an EGFR-TK mutation will respond to treatment with an EGFR tyrosine kinase inhibitor. In reality, treatment response is dependent upon multiple factors, and estimates of test accuracy are likely to vary according to the characteristics of the population in which the test is assessed. This makes between study comparisons of the performance of different tests particularly problematic.

A further complication relating to the accuracy of EGFR-TK mutation tests is that specificity estimates may be affected by the way EGFR-TK resistance mutations were classified in the included studies. Four studies of Sanger sequencing either stated that patients whose tumours showed EGFR-TK resistance or non-sensitising mutations were classified as EGFR-TK mutation negative, or did not identify any patients whose tumours showed these types of mutation. Two other studies, 1 of Sanger sequencing and 1 of the Therascreen EGFR PCR Kit classified EGFR-TK resistance mutations as a positive result.

The robustness of the economic model is an important consideration in this assessment. The results of the cost effectiveness analysis show that the Therascreen EGFR PCR Kit is less costly and less effective than Sanger

sequencing for deciding between EGFR tyrosine kinase inhibitors and standard chemotherapy for the first line treatment of patients with NSCLC. The lower number of QALYs for the Therascreen EGFR PCR Kit compared with Sanger sequencing seems counterintuitive given that Therascreen EGFR PCR Kit appears to be more accurate than Sanger sequencing (that is, better at discriminating between patients who are likely to benefit from EGFR tyrosine kinase inhibitor treatment and those who are not). The lower costs and QALYs can be explained by the fact that on average, patients tested with the Therascreen EGFR PCR Kit have a shorter overall survival compared to patients tested with Sanger sequencing, as explained on page 31 of the overview. However, as stated by the External Assessment Group, these results should be interpreted with extreme caution due to the substantial assumptions made in order to perform the economic analysis. The most problematic of these is that it was assumed that the differences in relative treatment response, progression free survival and overall survival between the results of the IPASS trial and the results of the First-SIGNAL trial were solely due to the different mutation tests used (Therascreen EGFR PCR Kit and Sanger sequencing of all exon 19 to 21 respectively) to distinguish between patients whose tumours are EGFR-TK mutation positive and patients whose tumours are EGFR-TK mutation negative. It is questionable whether this assumption would hold as it ignores all other factors which can explain variations in outcomes between studies.

The third analysis strategy which made the assumption of equal prognostic value between all EGFR-TK mutation tests was conducted in order to give an indication of the cost effectiveness of EGFR-TK mutation tests where no comparative effectiveness or test accuracy data were available. Results of this analysis show that there is very little difference in overall costs and QALYs between the different test strategies. Two intervention tests, next generation sequencing and the Therascreen EGFR Pyro Kit, were not included in the cost effectiveness analysis due to a lack of data on test cost and failure rate.

5 Equality considerations

The frequency of EGFR-TK mutations is highest in Asian women who have never smoked and have tumours with adenocarcinoma histology. However, NICE technology appraisal guidance 192 (Gefitinib for the first line treatment of locally advanced or metastatic non-small-cell lung cancer) recommends that testing should be carried out on all eligible patients irrespective of gender, ethnicity and smoking status, to ensure that all eligible patients who could benefit from treatment with gefitinib would be identified.

This overview was prepared by:

Frances Nixon
Topic Lead

Pall Jonsson
Technical Adviser

January 2013

Appendix A: Sources of evidence considered in the preparation of the overview

A. The diagnostics assessment report for this assessment was prepared by Kleijnen Systematic Reviews Ltd in collaboration with Erasmus University Rotterdam and Maastricht University:

- Westwood ME, Joore MA, Whiting P, van Asselt T, Ramaekers B, Armstrong N, Misso K, Severens J, Kleijnen J. Epidermal growth factor receptor tyrosine kinase (EGFR-TK) mutation testing in adults with locally advanced or metastatic non-small-cell lung cancer: a systematic review and cost-effectiveness analysis. A Diagnostic Assessment Report. Kleijnen Systematic Reviews Ltd, 2012.

B. The following organisations and/or their members accepted the invitation to participate in this assessment as stakeholders. They were invited to attend the scoping workshop and to comment on the diagnostics assessment report:

I. Manufacturers/sponsors:

- Roche Molecular Systems, Inc.
- Qiagen Ltd.

II. Professional/specialist and patient/carer groups:

- All Wales Molecular Genetics Lab
- AstraZeneca
- Boehringer Ingelheim Limited
- Bristol Genetics Laboratory
- British Thoracic Oncology Group (BTOG)
- Cancer Research UK

- Coventry and Warwickshire Pathology Services
- Department of Molecular Haematology, Oxford University Hospitals Trust
- Edinburgh Cancer Centre
- European Molecular Genetics Quality Network
- Guy's and St. Thomas' NHS Foundation Trust
- Leeds Teaching Hospital
- The Lothian University Hospitals
- NCRI Clinical Studies Group/Royal College of Physicians/Royal College of Radiologists/Joint Collegiate Council on Oncology/Association of Cancer Physicians
- New Gene Ltd
- NHS Grampian
- NHS Greater Glasgow and Clyde
- Royal Devon and Exeter NHS Trust
- Sheffield Diagnostics Genetics Service
- St James's Hospital
- St Mary's Hospital
- UCL Advanced Diagnostics
- UK NEQAS (Edinburgh)
- United Lincolnshire Hospitals NHS Trust
- University College London Hospital & MRC Clinical Trials Unit
- University Hospitals Birmingham NHS