

*Canadian Agency for
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RAPID RESPONSE REPORT: Peer-Reviewed Summary with Critical Appraisal

CADTH

Epidermal Growth Factor Receptor
Mutation Analysis in Advanced Non-Small
Cell Lung Cancer: A Review of the Clinical
Effectiveness and Guidelines

AUGUST 2010

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Cite as: Mujoomdar M, Moulton K, Spry C. *Epidermal Growth Factor Receptor Mutation Analysis in Advanced Non-Small Cell Lung Cancer: A Review of the Clinical Effectiveness and Guidelines*. Ottawa: Canadian Agency for Drugs and Technologies in Health; 2010.

Production of this report is made possible by financial contributions from Health Canada and the governments of Alberta, British Columbia, Manitoba, New Brunswick, Newfoundland and Labrador, Northwest Territories, Nova Scotia, Nunavut, Prince Edward Island, Saskatchewan, and Yukon. The Canadian Agency for Drugs and Technologies in Health takes sole responsibility for the final form and content of this report. The views expressed herein do not necessarily represent the views of Health Canada, or any provincial or territorial government.

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CADTH is funded by Canadian federal, provincial, and territorial governments.

Legal Deposit – 2010
Library and Archives Canada
ISSN: 1922-8139 (print)
ISSN: 1922-8147 (online)
M0017 – August 2010

PUBLICATIONS MAIL AGREEMENT NO. 40026386
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Canadian Agency for Drugs and Technologies in Health

**Epidermal Growth Factor Receptor Mutation
Analysis in Advanced Non-Small Cell Lung Cancer:
A Review of the Clinical Effectiveness and Guidelines**

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August 2010

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Health technology assessment (HTA) agencies face the challenge of providing quality assessments of medical technologies in a timely manner to support decision-making. Ideally, all important deliberations would be supported by comprehensive health technology assessment reports, but the urgency of some decisions often requires a more immediate response.

The Health Technology Inquiry Service (HTIS) provides Canadian health care decision-makers with health technology assessment information, based on the best available evidence, in a quick and efficient manner. Inquiries related to the assessment of health care technologies (drugs, devices, diagnostic tests, and surgical procedures) are accepted by the service. Information provided by the HTIS is tailored to meet the needs of decision-makers, taking into account the urgency, importance, and potential impact of the request.

Consultations with the requestor of this HTIS assessment indicated that a review of the literature would be beneficial. The research question and selection criteria were developed in consultation with the requestor. The literature search was carried out by an information specialist using a standardized search strategy. The review of evidence was conducted by one internal HTIS reviewer. The draft report was internally reviewed and externally peer-reviewed by two or more peer reviewers. All comments were reviewed internally to ensure that they were addressed appropriately.

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CADTH takes sole responsibility for the final form and content of this report. The statements and conclusions in this report are those of CADTH and not of reviewers.

This document is prepared by the Health Technology Inquiry Service (HTIS), an information service of the Canadian Agency for Drugs and Technologies in Health. The service is provided to those involved in planning and providing health care in Canada. HTIS responses are based on a comprehensive and systematic search of literature available to CADTH at the time of preparation. The intent is to provide a summary and critical appraisal of the best evidence on the topic that CADTH could identify using all reasonable efforts within the time allowed. This response has been peer-reviewed by clinical experts. The information in this document is intended to help Canadian health care decision-makers make well-informed decisions and thereby improve the quality of health care services. HTIS responses should be considered along with other types of information and health care considerations. It should not be used as a substitute for the application of clinical judgment in respect of the care of a particular patient or other professional judgment in any decision-making process, or as a substitute for professional medical advice. Readers are also cautioned that a lack of good-quality evidence does not necessarily mean a lack of effectiveness, particularly in the case of new and emerging health technologies for which little information can be found, but which may in future prove to be effective. While CADTH has taken care in the preparation of the document to ensure that its contents are accurate, complete, and up to date as of the date of publication, CADTH does not make any guarantee to that effect. CADTH does not guarantee and is not responsible for the quality, currency, propriety, accuracy, or reasonableness of any statements, information, or conclusions contained in the source documentation. CADTH is not responsible for any errors or omissions or injury, loss, or damage arising from or relating to the use (or misuse) of any information, statements, or conclusions contained in or implied by the information in this document or in any of the source documentation.

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Conflicts of Interest: Dr. Diana Ionescu is a consultant for Eli Lilly Canada Inc. and AstraZeneca Canada.

ABBREVIATIONS

| | |
|----------------|--|
| AGREE | Appraisal of Guidelines for Research & Evaluation |
| ARMS | amplification refractory mutation system |
| bp | base pairs |
| DNA | deoxyribonucleic acid |
| EGFR | epidermal growth factor receptor |
| FISH | fluorescent in situ hybridization |
| HRM | high resolution melting |
| IHC | immunohistochemistry |
| KRAS | Kirsten rat sarcoma viral oncogene homolog |
| ME-PCR | mutant-enriched polymerase chain reaction |
| MS-PCR | mutation-specific polymerase chain reaction |
| NE-PCR | nonenriched polymerase chain reaction |
| NSCLC | non-small cell lung cancer |
| PCR | polymerase chain reaction |
| RNA | ribonucleic acid |
| SCLC | small cell lung cancer |
| Scorpions-ARMS | Scorpions-amplification refractory mutation system |
| SMAP | SMart Amplification Process |
| SSCP | single-strand conformation polymorphism |
| TKI | tyrosine kinase inhibitor |

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TITLE: Epidermal Growth Factor Receptor Mutation Analysis in Advanced Non-Small Cell Lung Cancer: A Review of the Clinical Effectiveness and Guidelines

DATE: July 2010

EXECUTIVE SUMMARY

Context and Policy Issues

Lung cancer is the second most common cancer in Canada and the leading cause of cancer-related deaths. Non-small cell lung cancer (NSCLC) accounts for more than 80% of lung cancer cases. Some cases of NSCLC are associated with an over-expression of the protein epidermal growth factor receptor (EGFR). The over-expression of EGFR is linked to a more aggressive disease and a poor prognosis. The treatment protocols for NSCLC are evolving to include emerging therapies such as EGFR tyrosine kinase inhibitors (TKIs). Mutations in the EGFR gene have been identified and proposed to be associated with a high responsiveness to TKI treatment.

Many testing methods are used to investigate the mutational status of the EGFR gene. This review will focus on the clinical effectiveness and diagnostic performance of polymerase chain reaction (PCR)-based methods that are used to detect the presence of EGFR mutations in patients with advanced NSCLC. A quality assessment of the guidelines on testing for EGFR mutations in patients with advanced NSCLC will also be presented.

Research Questions

1. What is the clinical effectiveness of epidermal growth factor receptor mutation analysis using polymerase chain reaction for the identification of patients with advanced non-small cell lung cancer who are likely to respond to treatment with tyrosine kinase inhibitors?
2. What is the diagnostic performance of polymerase chain reaction-based methods that are used to evaluate mutations in the epidermal growth factor receptor in patients with advanced non-small cell lung cancer?
3. What is the quality of the guidelines regarding testing for epidermal growth factor receptor mutations in patients with non-small cell lung cancer?

Methods

A literature search was conducted on key health technology assessment resources, including MEDLINE, Embase, BIOSIS Previews, The Cochrane Library (Issue 2, 2010), University of York Centre for Reviews and Dissemination databases, ECRI (Health Devices Gold), EuroScan, international health technology agencies, and a focused Internet search. The search was limited to English language articles that were published between January 1, 2005 and March 16, 2010. Regular alerts were current to May 10, 2010. Filters were applied to limit the retrieval to health technology assessments, systematic reviews, meta-analyses, randomized controlled trials, controlled clinical trials, observational studies, and guidelines. Two independent reviewers screened articles using predefined criteria. Any disagreements were resolved through discussion until consensus was achieved.

Summary of Findings

Eleven observational studies and one evidence-based guideline were included. Overall, PCR-based approaches were found to be effective and reliable in identifying EGFR gene mutations when compared with direct sequencing. Several studies concluded that PCR-based tests can be performed without large tissue samples and on various clinical specimens including formalin-fixed, paraffin-embedded tissue. Two of the 11 studies reported the clinical outcomes of patients with EGFR mutation-positive status and who were treated with the TKI gefitinib. Findings from the two studies suggested that PCR-based mutational analysis was predictive of a clinical response. One Canadian consensus statement was identified for inclusion. The guideline was

published in 2009 and did not recommend the use of molecular markers to select patients who may respond to treatment with a TKI. Biomarker testing in patients with NSCLC was reviewed at a consensus meeting of Canadian medical oncologists and pathologists in March 2010. The group recommended that clinical samples from patients with NSCLC be tested for the presence of EGFR mutations.

Conclusions and Implications for Decision- or Policy-Making

Overall, the evidence from observational studies suggests that PCR-based approaches can be used to identify mutations in the EGFR gene with a

similar sensitivity to that of direct sequencing. Therefore, PCR-based tests are likely useful for identifying patients with NSCLC who are likely to respond to treatment with a TKI. In several studies, genetic material was microdissected from tumour-rich areas. This may require additional expertise and incur cost. In most of the studies, a commercially available reagent kit was not used. Commercially available kits may have quality-control advantages over “in-house” developed assays regarding quality control in a clinical laboratory setting. In December 2009, Health Canada approved the TKI gefitinib as a first-line treatment for patients with locally-advanced or metastatic NSCLC who also have activating mutations in the EGFR gene.

1 CONTEXT AND POLICY ISSUES

In 2010, there will be more than 24,000 estimated new diagnoses of lung cancer — the second most common cancer in Canada. Lung cancer is the leading cause of cancer-related death, with a five-year survival rate of approximately 15%.¹ The two most common types of lung cancer are small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), with NSCLC comprising more than 80% of lung cancer diagnoses.¹

The treatment options for patients with NSCLC, which depend on the patient's stage of disease and health status, include surgery, radiation, and chemotherapy.² For advanced (stage IIIB/IV), the treatment protocols for conventional chemotherapy typically involve first-line, platinum-based doublet therapy, followed by second-line treatment (docetaxel or pemetrexed) for patients who fail on platinum therapy.³ The treatment protocols for NSCLC may differ across Canada and are evolving to include combination-based treatment approaches and emerging therapies such as epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs). EGFR is a protein expressed on the surface of normal cells and tumour cells that plays a role in cellular processes such as cell growth, cell differentiation, and cell motility.^{4,5} EGFR is over-expressed on a number of solid tumours including NSCLC and is often associated with a more aggressive disease and a poor prognosis.⁵

Gefitinib (Iressa) and erlotinib (Tarceva) are two TKIs that are approved for use in Canada.⁶ Both drugs act by competing for a binding site on the EGFR and prevent activation of the receptor and subsequent downstream events including cell proliferation and cell motility.⁷ Despite the positive expectations about these drugs in the treatment of patients with NSCLC, the clinical response to TKIs has been variable.² Studies report that the responder population is often East Asian and female, with adenocarcinoma and a history of non-smoking.⁸ In 2004, activating mutations in the EGFR gene were identified in the TKI responder population and in other patients with NSCLC. These mutations were associated with a high responsiveness to TKIs.⁸

There are numerous methods to test the mutational status of the EGFR gene and include direct sequencing of genomic deoxyribonucleic acid (DNA) and polymerase chain reaction (PCR).⁹ Adding to the complexity, gene copy number and extent of protein expression have also been evaluated using fluorescent in situ hybridization (FISH) and immunohistochemistry (IHC) respectively, based on an observed positive correlation between high gene copy number or protein over-expression, and the presence of activating mutations.⁹

Although these methods may be routine in research and preclinical laboratories, clinical laboratories consider additional factors including tissue sample preparation (fresh, frozen, or paraffin-embedded), technical expertise, reliability, costs, turnaround time, and presence of a quality assurance program. This review will focus on the clinical effectiveness and diagnostic performance of PCR-based laboratory methods that are used to examine the presence of EGFR-activating mutations, with the goal of identifying patients with advanced NSCLC who are likely to respond to treatment with TKIs. A quality assessment of the guidelines regarding testing for EGFR mutations in patients with advanced NSCLC will also be presented.

2 RESEARCH QUESTIONS

1. What is the clinical effectiveness of epidermal growth factor receptor mutation analysis using polymerase chain reaction for the identification of patients with advanced non-small cell lung cancer who are likely to respond to treatment with tyrosine kinase inhibitors?
2. What is the diagnostic performance of polymerase chain reaction-based methods that are used to evaluate mutations in the epidermal growth factor receptor in patients with advanced non-small cell lung cancer?
3. What is the quality of the guidelines regarding testing for epidermal growth factor receptor mutations in patients with non-small cell lung cancer?

3 METHODS

3.1 Literature Search

Peer-reviewed literature searches were conducted to obtain published literature for this review. All search strategies were developed by an Information Specialist with input from the project team.

The following bibliographic databases were searched through the Ovid interface: MEDLINE, Medline In-Process & Other Non-Indexed Citations, Embase and BIOSIS Previews. Parallel searches were run in PubMed and The Cochrane Library (Issue 2, 2010). The search strategy comprised controlled vocabulary, such as the National Library of Medicine's MeSH (Medical Subject Headings), and keywords. Methodological filters were applied to limit the retrieval to health technology assessments, systematic reviews, meta-analyses, randomized controlled trials, controlled clinical trials, observational studies, and guidelines. Appendix 1 shows the detailed search strategy.

The search was restricted to English language clinical articles that were published between January 1, 2005 and March 16, 2010. Regular alerts were established on MEDLINE, Embase, and Biosis, and information that was retrieved via alerts was current to May 10, 2010.

Grey literature (literature that is not commercially published) was identified by searching the websites of health technology assessment and related agencies, professional associations, and other specialized databases. Google and other Internet search engines were used to search for additional web-based materials and information. These searches were supplemented by hand-searching the bibliographies and abstracts of key papers, and through contacts with appropriate experts and agencies.

HTIS reports are organized so that the higher-quality evidence is presented first. Therefore, health technology assessment reports, systematic reviews, and meta-analyses are presented first.

These are followed by randomized controlled trials, controlled clinical trials, observational studies, and evidence-based guidelines.

3.2 Article Selection

Two independent reviewers (MM and KM) screened the titles and abstracts of the retrieved citations. The same two reviewers independently evaluated the full-text publications for final article selection. Studies were considered for inclusion if they were of any of the following: health technology assessments, systematic reviews, meta-analyses, randomized controlled trials, controlled clinical trials, observational studies, or evidence-based guidelines. Primary studies involving patients with advanced NSCLC (stages IIIB, IV, metastatic, or recurrent) were considered for inclusion. Some studies may have involved a mixed population of patients including those with stage I to II disease. The interventions were PCR-based methods of detecting mutations in the EGFR kinase domain or other rapid methods. The direct sequencing of genomic DNA was used as a comparator. The outcomes included sensitivity, reliability, ease of performance, and ability to predict TKI responders.

Primary studies and secondary studies (health technology assessments, systematic reviews, and meta-analyses) were excluded if they did not report adequate methodological details to allow for evaluation of the technique that was used for mutational analysis; if a separate analysis for each technique was not performed; and if the study's primary objective was to evaluate clinical outcomes after treatment with TKIs, instead of an assessment of the methods that were used to select the patient population to be treated. Secondary studies were excluded if the reported method did not seem to be systematic (it did not include a search of more than one database or it did not involve multiple reviewers during literature selection). Differences among the two reviewers in the selection of articles for inclusion were resolved by consensus. A formal quality assessment of the studies that met the inclusion criteria for this report was not performed. The Appraisal of Guidelines for Research & Evaluation (AGREE) instrument¹⁰

was used by two independent reviewers (MM and KM) to evaluate the quality of the guideline that was included in this report. Standardized domain scores were calculated using the scores that were assigned by the two reviewers.

4 SUMMARY OF FINDINGS

Of the 869 citations that were identified in the literature search, 806 were excluded after a screening of titles and abstracts, and 63 were retrieved for full-text screening. An additional 10 publications were identified from grey literature sources or from hand-searching. Twelve publications were included in this report, and the remaining 61 articles were excluded. A flow diagram of the study selection is provided in Appendix 2. Two additional articles of potential interest have been included in Appendix 3. Both of these articles made comparisons between multiple assays (PCR, IHC, and in situ hybridization) rather than comparing PCR results to those obtained by direct sequencing. These two articles did not meet the inclusion criteria of this review.

Eleven observational studies and one evidence-based guideline were included in this review. Characteristics of the included primary studies, including pertinent methodological details, are provided in Appendix 4, Table A1.

4.1 Observational Studies

In 2008, Dahse et al.¹¹ used a PCR-based approach to test formalin-fixed tumour samples from patients with NSCLC for two common EGFR mutations (a deletion in exon 19 and a point mutation in exon 21). To detect the deletion in exon 19, a bidirectional PCR amplification of specific alleles was performed. To detect the point mutation in exon 21, an allele-specific PCR reaction (also known as amplification refractory mutation system [ARMS]) was used. A standard PCR reaction was performed using 80 ng to 100 ng of tumour sample DNA. DNA was extracted from 35 formalin-fixed, paraffin-embedded tissue samples. Normal control DNA, pooled from the

oral mucosa of five healthy volunteers, and positive control DNA from human lung cancer cell lines known to harbour the two EGFR mutations were used. The PCR reaction products were analyzed using gel electrophoresis. Three mutations were identified from the sample set, and all three mutations corresponded to deletions in exon 19. The authors concluded that their approach was a rapid and sensitive method that could be used to detect two common mutations in the EGFR gene and that this approach would be amenable to the clinical laboratory to identify patients with NSCLC who may respond to TKI therapy. The three mutations that were identified were confirmed by direct sequencing of the exons. It was unclear, however, if direct sequencing was performed only on samples that tested positive for a mutation by PCR or if direct sequencing was performed on all 35 samples. If the former is true, then it is unknown if additional mutations would have been identified using direct sequencing. Allele-specific PCR can be used to identify known mutations, but unlike direct sequencing, it cannot be used to identify new mutations. However, given the relatively simple and inexpensive methodology, allele-specific PCR can be easily adapted to detect additional mutations.

In 2008, Do et al.¹² published findings from a study in which they evaluated the use of high-resolution melting (HRM) to analyze EGFR and Kirsten rat sarcoma viral oncogene homolog (KRAS) gene mutations. The authors reported that KRAS gene mutations were assessed because studies have demonstrated that mutations in the KRAS gene are associated with a lack of responsiveness to TKIs. HRM is a method that is used to detect small differences in DNA sequences, such as small deletions or point mutations, based on the melting curve of the DNA. A DNA fragment with a mutation or a deletion would have a different melting curve from a DNA fragment with a normal sequence.¹³ The authors analyzed 200 lung cancer biopsy specimens, including some that were referred for EGFR-testing, based on clinical and patient characteristics (e.g., adenocarcinoma, Asian ethnicity, female, and non-smoker status). Of the 200 samples, 141 were adenocarcinomas, 24

were large cell carcinomas, 10 were squamous cell carcinomas, and 25 were of other or unknown histologies. DNA was extracted from formalin-fixed, paraffin embedded tissues, specifically from areas that were marked by a pathologist to be “tumour-rich” and were manually microdissected using a 21-gauge needle. Exons 18 to 21 of the EGFR gene and exon 2 of the KRAS gene were amplified using PCR, with a total of 5 ng of genomic DNA from tumour-rich areas. All samples were prepared in duplicate. HRM of PCR amplicons was performed using the Rotor-Gene 6000 real-time rotary analyzer. Wild-type DNA (DNA samples not containing mutations in the EGFR gene) was used as a control. All 200 samples underwent DNA sequencing of the specific exons for comparison. A summary of the mutational analyses detected by sequencing and by HRM is provided in Table 1.

Twenty-five mutations in the KRAS gene were detected using direct sequencing and HRM. A total of 70 false-positives were positive by HRM only. The authors reported that 45 clinical samples were HRM-positive in two or more HRM assays, leading the authors to suggest that these results were likely to be false-positives due to degradation of the sample DNA. The authors noted that the lowest number of discrepant results occurred in the exon 20 assay, which had the shortest DNA amplicons (121 base pairs [bp] and 146 bp), suggesting that shorter amplicons may be associated with improved HRM

performance. There were no discrepant results with the KRAS HRM assay, which has the shortest amplicon (92 bp). All results that were found to be positive for an EGFR mutation by sequencing were also found to be positive by HRM. Table 2 shows the sensitivity and specificity of using HRM to detect EGFR mutations when the results of DNA sequencing are taken as true positives.

The authors offered explanations for the discrepancy between sequencing results and HRM results. First, it was suggested that the formalin fixation process that was used in tissue preservation could have lead to PCR artifacts. An examination of these methods using fresh or frozen tissue may answer this question, although the availability of such tissue samples in a clinical laboratory may be limited. An alternative explanation is that some samples may have had levels of mutated DNA that were below the detection limit of sequencing, but that were correctly identified using HRM. The EGFR mutation rate in this study was high at 37.5% (75/200). This is likely due to selection bias because patients were being referred based on characteristics that were associated with EGFR mutation. The authors concluded that this method is an effective and rapid method of screening patients for EGFR and KRAS gene mutations using tissues sample typically available to a clinical laboratory. The authors did not discuss whether a positive HRM result should be confirmed by using direct sequencing.

Table 1: Number of EGFR Mutations Detected Using Sequencing and HRM¹²

| | Direct Sequencing | HRM | Mutations Detected Using HRM Only |
|-------------|--------------------------|------------|--|
| EGFR | | | |
| Exon 18 | 5 | 23 | 18 |
| Exon 19 | 46 | 67 | 21 |
| Exon 20 | 10 | 23 | 13 |
| Exon 21 | 23 | 41 | 18 |

EGFR = epidermal growth factor receptor; HRM = high resolution melting.

| Table 2: Sensitivity and Specificity of Using HRM for Detecting EGFR Mutations¹² | | |
|--|------------------------|-------------------------|
| | Sensitivity (%) | Specificity* (%) |
| EGFR | | |
| Exon 18 | 100 | 91 |
| Exon 19 | 100 | 88 |
| Exon 20 | 100 | 93 |
| Exon 21 | 100 | 91 |

EGFR = epidermal growth factor receptor; HRM = high resolution melting.

*These values were reported by Do et al.¹² It is unclear how these values were calculated.

Molina-Vila et al.¹⁴ evaluated a PCR-based method that was used to detect EGFR mutations in NSCLC samples. The objective of the study was to validate the use of this method for clinical samples containing less than 150 tumour cells. A total of 268 patients with NSCLC were eligible for assessment. There were 223 paraffin-embedded samples and 45 fresh samples. The tumour cells were microdissected using laser capture on all samples. Sufficient tumour cell material was available for 217 of the 268 samples. Two rounds of PCR amplification were needed. First, exons 19 and 21 were amplified using nested PCR. The PCR product for exon 19 was then subjected to a second round of PCR using primers with a fluorescent label. The length of the PCR amplicon was analyzed using a capillary electrophoresis system to detect fluorescence. The presence of point mutations in exon 21 was evaluated using the TaqMan real-time PCR assay, which can be used to discriminate between two alleles: one with a normal DNA sequence and one with a mutation in the sequence. All 217 samples were also assessed using direct sequencing.

Overall, EGFR mutations (confirmed by direct sequencing) were detected in 37 of the 217 samples (17%). Most of the patients with mutations were women or non-smokers. The results from using a PCR were identical to those obtained by sequencing in 86.2% of cases for exon 19 and in 78.3% of cases for exon 21. Thirty-five patients with sequencing-confirmed EGFR mutations were treated with erlotinib. Data on the clinical outcomes were available for 14 treated patients. Two patients had a complete response, five had a partial response, six had stable disease, and one had disease progression.

The authors reported that the minimum amount of DNA that could be evaluated was approximately 5 picograms (pg) per microlitre (µL). The method was validated in samples from paraffin-blocks, fresh biopsies, fresh pleural fluid, fresh pericardial fluid, and paraffin-embedded pleural fluid. The authors concluded that the use of this method eliminates the need for a large sample size and that this method can be applied to multiple specimen types. This may be particularly useful when the supply of clinical specimen material is limited or when the tumour is inoperable and tissue samples cannot be obtained. More time is required for the necessary two rounds of PCR, which will incur additional costs. The specialized equipment and expertise that are needed for laser capture microdissection would also add to the cost.

Smith et al. (2008)¹⁵ used HRM analysis to detect mutations in fine needle aspirate samples from 11 patients with NSCLC. The study was retrospective in design. DNA was isolated by manually scraping a slide of cellular material. The areas that were suggested for analysis were marked by a pathologist. Standard PCR of EGFR exons 18 to 21 was performed, and all samples contained a double-stranded DNA binding dye for HRM analysis. All reactions were done in triplicate. The concentration of DNA that was added to the PCR was not reported. Following PCR, HRM analysis was performed after PCR. DNA sequencing was performed on all samples with abnormal melt profiles. A mutation (L747-P753insS) in exon 19 was identified in three of the 11 patient samples. A point mutation in exon 19 (A755D) was also identified in one patient. The authors concluded that HRM analysis of cytological specimens can be used to identify EGFR mutations and may be useful when tissue

samples are unavailable. Additional expertise was needed for the required manual microdissection of tumour cell-rich areas of the cytological specimen. In addition, DNA sequencing was not performed on all samples, and some mutations may have been missed during HRM analysis.

Hoshi et al. (2007)¹⁶ reported their findings using a rapid assay for the detection of mutations in the EGFR gene. The SMart Amplification Process (SMAP) involves a set of five primers that are designed to amplify known mutant sequences. Non-specific amplification (i.e., non-mutant sequence) is minimized by using an asymmetrical primer design and by the addition of a binding protein into the reaction mix. This prevents mismatched primer-template pairing. The SMAP approach is similar to strand displacement amplification in which multiple copies of the DNA fragment containing a mutation are produced. A DNA-binding dye, SYBR Green I, was used to allow for the real-time monitoring of amplification. A fluorescent signal indicated the amplification of mutant DNA. The authors reported that the SMAP was previously shown to detect single nucleotide polymorphisms within 30 minutes.

A total of 45 samples were evaluated using SMAP and direct sequencing. Nine mutations (five in exon 19 and four in exon 21) were identified using both methods. SMAP was used to identify an additional mutation in one sample that was not identified using sequencing. This additional mutation was confirmed using a third technique (PCR-restriction, fragment length polymorphism). The authors suggested that the tenth mutation was not detected by using direct sequencing because the amount of mutant-containing tumour cells in the sample was low. This is supported by the SMAP data because the appearance of this amplification product was delayed during real-time analysis compared to other amplification products, indicating that the starting amount of mutant DNA was low. The authors concluded that the SMAP is a rapid method of testing for EGFR mutations in clinical samples and that it has a higher sensitivity and specificity than direct sequencing. The sensitivity and specificity were not reported. The improved performance may be particularly important if the

microdissection of tumour cell-rich areas from samples is not possible. The combination of normal and tumour cells may “dilute” the sample and hinder the ability of a test to detect a mutation. The SMAP is reportedly able to detect mutations that are present in low abundance.

Kimura et al. (2006)¹⁷ evaluated the use of a combined Scorpions-amplification refractory mutation system (Scorpions-ARMS) to detect EGFR gene mutations in the serum of patients with NSCLC. Blood samples were taken from 27 patients with confirmed NSCLC before they started treatment with gefitinib. EGFR mutations were detected in the serum of 13 of 27 patients using the Scorpions-ARMS method (exon 19, n=12; exon 21, n=1) and in 10 of 27 patients (exon 19, n=10) by direct sequencing. The additional three mutations that were identified using Scorpions-ARMS were not confirmed using direct sequencing. Paraffin-embedded tissue samples from a subset of the 27 patients were also obtained, and paired tissue or serum samples were available for 11 patients. The mutational status (as assessed exclusively using Scorpions-ARMS for the tissue samples) was consistent between tumour samples and serum samples in 72.7% of cases. The authors proposed that the discrepancy between results in serum DNA and tissue DNA may have been due to a low level of circulating tumour cells. This may have resulted in a false-negative finding (a negative serum result and a positive tissue result), which occurred in two of 11 cases. Patients who were treated with gefitinib and who had EGFR mutations had a statistically significantly longer median progression-free survival compared with treated patients without EGFR mutations (200 days compared to 46 days; $P = 0.005$). The overall survival between mutation-positive and mutation-negative groups was not statistically significantly different (611 days compared to 232 days; $P = 0.078$). The predictability of response to gefitinib was statistically significant when mutations were detected using Scorpions-ARMS ($P = 0.046$), but not when direct sequencing was performed ($P = 0.683$). The authors concluded that two common mutations in two exons of the EGFR gene were detected in the DNA from the serum of patients with NSCLC, and that patients with identified mutations had improved clinical

outcomes when treated with gefitinib. The evaluation of serum DNA may be particularly useful when the availability of tumour tissue is limited.

Asano et al. (2006)¹⁸ reported their findings regarding the use of a mutant-enriched PCR assay (ME-PCR) to detect mutations in the EGFR gene in clinical samples from patients with NSCLC. A total of 146 samples (108 surgically-resected frozen specimens, 18 samples from computed tomography-guided needle lung biopsies of primary tumours, and 20 samples of pleural fluid from patients with recurrent or advanced NSCLC) were analyzed for EGFR mutations. The ME-PCR approach involves two rounds of PCR on approximately 5 nanograms (ng) to 100 ng of DNA. After the first PCR reaction, the PCR amplification products are treated with a restriction enzyme that cuts the wild-type (non-mutated) DNA at a specific sequence. The wild-type DNA contains this recognition sequence, but the mutated DNA does not and will not be cut. After treatment with the restriction enzyme, the samples are re-amplified using a second round of PCR. The fragments are separated by polyacrylamide gel electrophoresis and visualized by silver staining. The authors compared the ME-PCR assay to a non-enriched PCR assay (NE-PCR). The NE-PCR method involved only the PCR reaction, and there was no treatment with a restriction enzyme. Direct sequencing of the NE-PCR-amplified product was performed.

Among the 146 samples, ME-PCR was used to detect mutations in 51 cases, and NE-PCR was used to detect mutations in 43 cases. The additional eight cases were confirmed to be mutation-positive using direct sequencing of the ME-PCR product. The authors concluded that ME-PCR may be used to detect EGFR mutations in a variety of clinical samples and that it is more

sensitive than standard NE-PCR, followed by sequencing. This approach required two rounds of PCR, which would incur more time and cost and may require a DNA sample that is of high quality.

In 2006, Ohnishi et al.¹⁹ reported on the use of a mutation-specific PCR-based method (MS-PCR) to identify EGFR gene mutations in patients with NSCLC. A total of 62 surgically resected tumour samples were analyzed for mutations in exons 19 and 21. Direct sequencing was performed on all samples. For direct sequencing and MS-PCR, 100 ng of sample DNA was amplified. The results from the mutational analysis of the 62 samples are provided in Table 3.

The authors did not explain the discrepancy between methods that were used to identify the mutations in exon 19. If the results of direct sequencing are taken as true positive, then MS-PCR may be less sensitive for detecting mutations in exon 19. Conversely, MS-PCR was used to detect three mutations that were not identified using direct sequencing. These mutations were confirmed by sequencing the MS-PCR-amplified products. The authors noted that these mutations were present in low abundance and were difficult to distinguish from the background signal during sequence analysis. The authors also performed a dual, MS-PCR (an assay for detecting mutations in both exons in one reaction mix). All the samples that were positive in the dual assay were also positive in the single MS-PCR. It was not reported if there were samples that were found to be positive by using single MS-PCR that were not identified in the dual assay. The authors concluded that the MS-PCR assay was a reliable method for detecting EGFR mutations in NSCLC tumour samples.

Table 3: EGFR Mutational Analysis of 62 Samples Using Direct Sequencing and MS-PCR¹⁹

| | DS | MS-PCR |
|---------|-------|--------|
| Exon 19 | 12/62 | 8/62 |
| Exon 21 | 11/62 | 14/62 |

DS = direct sequencing; EGFR = epidermal growth factor receptor; MS-PCR = mutation-specific PCR.

Yatabe et al. (2006)²⁰ described a rapid assay that was used to detect EGFR gene mutations in clinical samples from patients with NSCLC. A total of 195 samples from a section of tissue microarray blotted with 0.6 mm tissue cores were analyzed using direct sequencing and the test method (cycleave PCR). Cycleave PCR involves the use of two ribonucleic acid (RNA) probes that are labeled with two different fluorescent dyes. One probe is specific to the mutated sequence, and one is specific to the wild-type sequence. In a given sample, the RNA probes align with their complementary DNA sequence. An enzyme that cleaves the RNA-DNA complex is added to the reaction mix. During cleavage, a fluorescent signal is emitted. The assay was used to detect point mutations in exons 20 and 21. A standard analysis of fragment length was performed to detect the deletion in exon 19. Tumour cell-rich regions were scraped from the slide of paraffin-embedded tissue; microdissection was unnecessary. The concentration of DNA that was added to the reaction mixture was unspecified.

The reported concordance between direct sequencing and cycleave PCR was more than 95% (186 of 195 samples). Seven discordant samples were found to have mutations in other gene regions that were not targets of the cycleave assay. A second objective of the study was to evaluate the use of the assay in predicting responsiveness to gefitinib. The tissues from 29 individuals who were treated with gefitinib were examined for EGFR gene mutation status. The tissue samples were obtained prior to the initiation of gefitinib treatment. Ten of the 11 cases that had a partial response to treatment were positive for EGFR gene mutation (five cases of exon 19 deletion; five cases of exon 21 L858R point mutation); two of 13 cases with stable disease had EGFR mutations (both exon 19 deletions); and all five patients who had progressive disease tested negative for EGFR mutations. The authors concluded that the cycleave PCR assay was sensitive in detecting EGFR mutation and that it could be completed in approximately four to five hours. The assay results of mutational status also correlated

positively with the expected findings for TKI responders.

In 2005, Marchetti et al²¹ evaluated the use of single-strand conformation polymorphism (SSCP) PCR to screen samples from patients with NSCLC for mutations in the EGFR gene. A total of 860 surgical resection samples were obtained from consecutive patients. The specimens were snap-frozen and stored. SSCP-PCR is based on the principle²² that slight changes in DNA sequence (such as a deletion or a point mutation) can have profound effects on the conformation of single-strand DNA. The authors amplified the target regions of exons 18, 19, and 21 that are known to harbour EGFR mutations using PCR and 10 ng of genomic DNA. The PCR products were then denatured, separated by polyacrylamide gel electrophoresis, and silver stained.

Among the 860 samples, no EGFR gene mutations were found in the 454 squamous carcinomas and 31 large-cell carcinomas. Of the remaining 375 adenocarcinomas, 39 were found to have mutations using SSCP (8% exon 18, 46% exon 19, and 46% exon 21). Direct sequencing was used to identify mutations in 31 cases. The additional eight cases that were identified using SSCP were confirmed using direct sequencing of the SSCP-PCR products. Overall, the authors concluded that the SSCP-PCR assay was a rapid and sensitive method for the detection of EGFR mutation and that it had a greater sensitivity than direct sequencing. A sensitivity value was not reported. For all the tissue samples, the tumour cell content was 80% or greater. Therefore, the sensitivity of the SSCP-PCR assay to detect EGFR gene mutations in tissues samples with greater cellular heterogeneity (mixture of normal cells and tumour cells) is unknown.

Pan et al. (2005)²³ reported their findings using a PCR-based approach to test for EGFR mutations in clinical samples from patients with lung cancer (adenocarcinomas). An assay for mutations in exons 19 and 21 was developed and validated using the test PCR method and direct sequencing. Thirty-nine samples were studied. The PCR-based assay for an exon 19 deletion

involved the use of a fluorescently labeled primer. After PCR, the length of PCR amplicons was analyzed using a capillary electrophoresis system capable of measuring fluorescence. The exon 21-point mutation assay is based on the fact that samples with mutations have a DNA sequence that is recognized and cleaved by a restriction enzyme. Briefly, the standard PCR reaction was performed using fluorescently-labeled primers and the resultant PCR products are treated with the restriction enzyme. Length analysis is subsequently performed using capillary electrophoresis.

In the 39 samples, the PCR-based method was used to detect mutations in 29 samples compared with direct sequencing in 25 samples. The four additional samples were validated using sequencing of the PCR products. The authors concluded that the method was rapid and sensitive, and could be performed in one day compared to two days for sequencing. No information was provided on the sample preparation (whether the method requires microdissection of a tumour cell-rich population). The mutation rate of the sample set was higher (74%) than that of other studies in this review. This high rate does not reflect the prevalence of the mutation in cases of NSCLC, although the rate may be more comparable to that expected in a population that was selected based on clinical and patient characteristics (adenocarcinoma, Asian ethnicity, female, non-smoker, etc).

4.2 Guidelines and Recommendations

One consensus statement from Canada²⁴ regarding the role of TKI therapy for the treatment of patients with advanced, metastatic, or recurrent NSCLC was identified.

The quality of the included guideline was appraised using the AGREE instrument,¹⁰ which evaluates the guideline by addressing 23 questions through six domains (scope and purpose, stakeholder involvement, rigour of development, clarity and presentation, applicability, and editorial independence). Each item was rated on a four-point scale as follows:

4=Strongly Agree, 3=Agree, 2=Disagree, and 1=Strongly Disagree. The overall assessment of the guideline is rated using the following parameters as suggested in the instrument:

- Strongly recommend: The majority of items and most domain scores are above 60%, indicating high overall quality.
- Recommend with provisos: Most domain scores are between 30% and 60%, indicating that the guideline has a moderate overall quality, possibly due to insufficient information provided in the guideline.
- Would not recommend: The majority of items and most domain scores are less than 30%, indicating low overall quality and serious shortcomings.¹⁰

The included guideline received an overall AGREE assessment of “strongly recommend,” scoring highly on the domains of scope and purpose, rigour of development, and clarity of presentation.

The main recommendations regarding the use of TKI therapy in selected (based on EGFR gene mutation analysis or clinical characteristics) patients with advanced, metastatic, or recurrent NSCLC were:

- Evidence is currently insufficient to recommend single-agent EGFR-TKIs as first-line therapy either in unselected populations or in populations selected on the basis of molecular or clinical characteristics. (p29)²⁴
- The evidence is currently insufficient to select patients based on molecular markers predictive of improved survival with an EGFR-TKI. (p43)²⁴
- Based on available data, molecular markers and clinical characteristics should not be used to exclude patients from receiving EGFR-TKI therapy. (p43)²⁴

4.3 Limitations

A limited literature search was conducted for this report. Potentially relevant evidence that was published before 2005 and of non-English language was excluded using this approach. The eligibility of identified studies for inclusion in this CADTH rapid review was assessed exclusively based on methodological details in

the published article, and additional information was not sought from study authors.

All the primary studies meeting the inclusion criteria for this review were observational studies. Observational studies lack a control group and, as such, may be subject to bias. All the studies that were included in this review evaluated a PCR-based technology and made a comparison with direct sequencing. A limitation of several included studies was that the confirmation of a PCR result using direct sequencing was typically only performed in cases of positive test results. In these cases, given that the negative test results did not receive confirmation, it was not possible to calculate sensitivity, and most studies did not report sensitivity and specificity. Direct sequencing, which is often considered to be the gold-standard in EGFR mutation analysis, has limited sensitivity arising from the need for adequate amounts of mutant DNA to be present in the clinical specimen for detection. Most studies reported single-test performance characteristics; performing tests in duplicate or triplicate as is often done in PCR reactions may increase accuracy. Such an approach would add to the cost and may require additional clinical sample material.

Most of the studies analyzed a tumour cell-rich population, and microdissection techniques were used to isolate the cellular material. In several included studies, microdissection was performed using a laser capture microdissection microscope. This would incur additional cost and require expertise. Several studies evaluated PCR-based approaches in a patient population that was selected based on clinical and patient characteristics known to be associated with EGFR gene mutations including a diagnosis of adenocarcinoma, Asian ethnicity, female gender, and non-smoker status. These studies may be subject to selection bias because of the high mutation rate. The results of such studies may be of limited value when considering an unselected population.

One study¹⁷ evaluated the effectiveness of a commercial kit, the EGFR Scorpions Kit, which is manufactured by DxS Ltd. Most of the included studies did not use commercial kits for EGFR mutational analysis. Rather, individual reagents were used for the “in-house” PCR-based processes. Although this approach is justified, it may pose problems for clinical laboratories with respect to quality control and quality assurance.

The one guideline that was identified, a Canadian consensus statement, was published in 2009. The literature search for the guideline included studies that were published from 2000 to 2007. Preliminary data from three trials that were ongoing at the time of publication were discussed, but these data may have not been reflected in the recommendations.

5 CONCLUSIONS AND IMPLICATIONS FOR DECISION- OR POLICY-MAKING

Eleven observational studies evaluated the use of PCR-based strategies to detect mutations in the EGFR gene. While there were differences in the methods used between studies, several themes in mutational analysis could be observed. These themes included analysis of fragment length comparing the size of the PCR amplification products to what would have been expected for a non-mutated sequence; mutation-specific PCR assay using the presence of known regions in the DNA sequence that were sites for restriction enzyme cleavage (the restriction-enzyme-treated products were then visualized using gel electrophoresis, and the fragments could be identified); and high resolution melting analysis, which is used to distinguish between two DNA sequences based on how the DNA melts.

Some of the approaches can be used to detect only known mutations. Therefore, the assay would be adapted when new, clinically relevant mutations are identified. The adaptations, which are easily performed, require the design of new primers to amplify target sequences. The direct sequencing of DNA has the advantage of being able to detect new and known mutations.

For some of the assays proposed, two rounds of PCR were required which would add to the time required to evaluate samples. One of the assays, SMAP, which was a real-time assay that is used to evaluate samples within 30 minutes, had an improved sensitivity compared to direct sequencing. Most studies analyzed PCR products using an automated capillary electrophoresis system instead of traditional non-automated gel electrophoresis. This automated approach may improve reliability and decrease errors that are associated with sample loading. Most studies used tissues that had been formalin-fixed and paraffin-embedded; however, some analyses were performed on other specimens such as cytological samples or serum. In general, all the clinical specimens were adequate sources of DNA for analysis. This finding may be particularly important when there is a limited supply of clinical material; for example, in cases of inoperable tumours.

The one guideline that was identified for inclusion in this review, a Canadian consensus statement published in January 2009,²⁴ deemed there to be insufficient evidence for the use of TKIs as first-line therapies for the treatment of advanced, metastatic, or recurrent NSCLC. In March 2010, a national consensus meeting of Canadian medical oncologists and pathologists reviewed the topic of biomarker testing in patients with NSCLC. The report from this meeting is still in draft format; however, one recommendation regarding routine EGFR mutational analysis states:

“It is recommended that diagnostic lung cancer samples of patients with non-small cell lung cancer be routinely tested for activating mutations of the EGFR. Given the available clinical data, this testing should be limited to patients with advanced NSCLC and non-squamous histology. Testing should be

completed in a licensed clinical molecular genetics laboratory. Mutation testing is most relevant to treatment decisions in the first-line therapy setting and may also be relevant whenever a choice between chemotherapy and EGFR TKIs is to be made. Clinical data for EGFR protein expression using immunohistochemistry or gene copy using FISH testing is somewhat inconsistent. Therefore, such testing is not routinely recommended.” (Dr. Peter Ellis, Juravinski Cancer Centre, Hamilton, ON: personal communication, 2010 June)

In December 2009, with evidence from the IRESSA Pan-Asia Study,²⁵ Health Canada approved gefitinib as a first-line treatment for patients with locally advanced or metastatic NSCLC who have activating mutations in the EGFR gene.⁶ Guidelines from the American Society of Clinical Oncology support the use of gefitinib as a first-line treatment for patients with EGFR mutation-positive advanced NSCLC.²⁶

Overall, the evidence from observational studies suggests that PCR-based approaches are capable of identifying mutations in the EGFR gene with a similar sensitivity to that of direct sequencing. Therefore, PCR assays may be useful in selecting a population of patients with NSCLC who are likely to respond to treatment with a TKI. Most studies did not discuss whether direct sequencing is to be used to confirm a positive PCR result, if a PCR-based assay is used for screening. The use of direct sequencing would add to the cost. In addition, few included studies discussed the potential impact of a false-negative result found by using PCR. The potential reasons for the limitations of the included studies may be a consideration when deciding to use a PCR-based test. Other considerations such as the type of PCR-based assay, the costs of performing the assay (reagents and equipment), the technical expertise needed for microdissection, and the need for a rapid turnaround may also influence decision-making.

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APPENDIX 1: LITERATURE SEARCH STRATEGY

| OVERVIEW | |
|-----------------|--|
| Interface: | Ovid |
| Databases: | EMBASE <1996 to 2010 Week 10> Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <March 16, 2010> BIOSIS Previews <1989 to 2010 Week 14> Note: Subject headings have been customized for each database. Duplicates between databases were removed in Ovid. |
| Date of Search: | March 16, 2010 |
| Alerts: | Biweekly search updates began March 16, 2010 and ran until May 10, 2010. |
| Study Types: | Health technology assessments, systematic reviews, meta-analyses, randomized controlled trials, controlled clinical trials, observational studies and guidelines. |
| Limits: | Publication years 2005 – March 2010 English |
| SYNTAX GUIDE | |
| / | At the end of a phrase, searches the phrase as a subject heading |
| exp | Explode a subject heading |
| * | Before a word, indicates that the marked subject heading is a primary topic; or, after a word, a truncation symbol (wildcard) to retrieve plurals or varying endings |
| ? | Truncation symbol for one or no characters only |
| .ti | Title |
| .ab | Abstract |
| .hw | Heading Word; usually includes subject headings and controlled vocabulary |
| .pt | Publication type |
| .rn | CAS registry number |
| use prmz | Select only Medline results |
| use emef | Select only EMBASE results |
| use b10o89 | Select only Biosis results |

| Multi-database Strategy | |
|--------------------------------|--|
| Line # | Search Strategy |
| 1 | Carcinoma, Non-Small-Cell Lung/ or NSCLC.ti,ab. |
| 2 | (exp Adenocarcinoma/ or Carcinoma, Large Cell/ or exp Carcinoma, Squamous Cell/ or adenocarcinoma*.ti,ab.) and (lung* or pulmonary).ti,ab. |
| 3 | ((non-small cell lung or nonsmall cell lung) and (neoplasm* or cancer* or carcinoma* or tumor* or tumour*)).ti,ab. |
| 4 | ((large cell or squamous or bronchoalveolar or bronchiolo alveolar) and (neoplasm* or cancer* or carcinoma* or tumor* or tumour*) and (lung* or pulmonary)).ti,ab. |
| 5 | or/1-4 |
| 6 | *Lung non small cell cancer/ or NSCLC.ti,ab. |
| 7 | (*Adenocarcinoma/ or *Large cell carcinoma/ or *Squamous cell carcinoma/ or adenocarcinoma*.ti,ab.) and (lung* or pulmonary).ti,ab. |
| 8 | 6 or 7 or 3 or 4 |
| 9 | NSCLC.ti,ab,hw. |
| 10 | ((non-small cell lung or nonsmall cell lung) and (neoplasm* or cancer* or carcinoma* or tumor* or tumour*)).ti,ab,hw. |
| 11 | (adenocarcinoma* and (lung* or pulmonary)).ti,ab,hw. |
| 12 | ((large cell or squamous or bronchoalveolar or bronchiolo-alveolar) and (neoplasm* or cancer* or carcinoma* or tumor* or tumour*) and (lung* or pulmonary)).ti,ab,hw. |
| 13 | or/9-12 |
| 14 | Receptor, Epidermal Growth Factor/ or exp Genes, erbB/ or EC 2-7-1-112.rm. or (EGFR or epidermal growth factor receptor* or EGF receptor* or urogastrone receptor* or transforming growth factor alpha receptor* or TGF alpha receptor* or erbB or erbB1 or erbB-1 or erbB2 or erb 1 or erb 2 or HER1 or HER 1 or HER 2 or HER2 or neu).ti,ab. |
| 15 | *Epidermal growth factor receptor/ or EC 2-7-1-112.rm. or (EGFR or epidermal growth factor receptor* or EGF receptor* or urogastrone receptor* or transforming growth factor alpha receptor* or TGF alpha receptor* or erbB or erbB1 or erbB-1 or erbB2 or erb 1 or erb 2 or HER1 or HER 1 or HER 2 or HER2 or neu).ti,ab. |
| 16 | EC 2-7-1-112.cb. or (EGFR or epidermal growth factor receptor* or EGF receptor* or urogastrone receptor* or transforming growth factor alpha receptor* or TGF alpha receptor* or erbB or erbB1 or erbB-1 or erbB2 or erb 1 or erb 2 or HER1 or HER 1 or HER 2 or HER2 or neu).ti,ab,hw. |
| 17 | *Pharmacogenetics/ or (pharmacogenomic* or pharmacogenetic* or personali?e* medicine or personali?e* treatment* or personali?e* therap* or individuali?e* treatment* or individuali?e* therap* or target* therap*).ti. |
| 18 | *Pharmacogenetics/ or *Pharmacogenomics/ or (pharmacogenomic* or pharmacogenetic* or personali?e* medicine or personali?e* treatment* or personali?e* therap* or individuali?e* treatment* or individuali?e* therap* or target* therap*).ti. |
| 19 | (pharmacogenomic* or pharmacogenetic* or personali?e* medicine or personali?e* treatment* or personali?e* therap* or individuali?e* treatment* or individuali?e* therap* or target* therap*).ti. |
| 20 | 5 and (14 or 17) |
| 21 | 8 and (15 or 18) |
| 22 | 13 and (16 or 19) |
| 23 | 20 use prmz |
| 24 | 21 use emef |

| Multi-database Strategy | |
|---|---|
| Line # | Search Strategy |
| 25 | 22 use b10o89 |
| 26 | or/23-25 |
| 27 | limit 26 to english language |
| 28 | limit 27 to yr="2005 -Current" |
| 29 | 28 |
| 30 | limit 29 to yr="2005 - 2007" (results set split in order to remove duplicates) |
| 31 | remove duplicates from 30 |
| 32 | 28 |
| 33 | limit 32 to yr="2008 -Current" |
| 34 | remove duplicates from 33 |
| 35 | 31 or 34 |
| Systematic Review search filter: | |
| 36 | meta-analysis.pt. |
| 37 | meta-analysis/ or systematic review/ or meta-analysis as topic/ or exp technology assessment, biomedical/ |
| 38 | ((systematic* adj3 (review* or overview*)) or (methodologic* adj3 (review* or overview*))).ti,ab. |
| 39 | ((quantitative adj3 (review* or overview* or synthes*)) or (research adj3 (integrati* or overview*))).ti,ab. |
| 40 | ((integrative adj3 (review* or overview*)) or (collaborative adj3 (review* or overview*)) or (pool* adj3 analy*)).ti,ab. |
| 41 | (data synthes* or data extraction* or data abstraction*).ti,ab. |
| 42 | (handsearch* or hand search*).ti,ab. |
| 43 | (mantel haenszel or peto or der simonian or dersimonian or fixed effect* or latin square*).ti,ab. |
| 44 | (met analy* or metanaly* or health technology assessment* or HTA or HTAs).ti,ab. |
| 45 | (meta regression* or metaregression* or mega regression*).ti,ab. |
| 46 | (meta-analy* or metaanaly* or systematic review* or biomedical technology assessment* or bio-medical technology assessment*).mp,hw. |
| 47 | (medline or Cochrane or pubmed or medlars).ti,ab,hw. |
| 48 | (cochrane or health technology assessment or evidence report).jw. |
| 49 | (meta-analysis or systematic review).md. |
| 50 | or/36-49 |
| 51 | 35 and 50 |
| Randomized Controlled Trial search filter: | |
| 52 | Randomized Controlled Trial.pt. |
| 53 | Randomized Controlled Trials as Topic/ |
| 54 | Randomized Controlled Trial/ |
| 55 | Randomization/ |
| 56 | Random Allocation/ |
| 57 | Double-Blind Method/ |
| 58 | Double Blind Procedure/ |
| 59 | Double-Blind Studies/ |

| Multi-database Strategy | |
|---|---|
| Line # | Search Strategy |
| 60 | Single-Blind Method/ |
| 61 | Single Blind Procedure/ |
| 62 | Single-Blind Studies/ |
| 63 | Placebos/ |
| 64 | Placebo/ |
| 65 | (random* or sham or placebo*).ti,ab,hw. |
| 66 | ((singl* or doubl*) adj (blind* or dumm* or mask*)).ti,ab,hw. |
| 67 | ((tripl* or trebl*) adj (blind* or dumm* or mask*)).ti,ab,hw. |
| 68 | or/52-67 |
| 69 | 35 and 68 |
| Controlled Clinical Trial search filter: | |
| 70 | (Randomized Controlled Trial or Controlled Clinical Trial).pt. |
| 71 | Randomized Controlled Trial/ |
| 72 | Randomized Controlled Trials as Topic/ |
| 73 | Controlled Clinical Trial/ |
| 74 | Controlled Clinical Trials as Topic/ |
| 75 | Randomization/ |
| 76 | Random Allocation/ |
| 77 | Double-Blind Method/ |
| 78 | Double Blind Procedure/ |
| 79 | Double-Blind Studies/ |
| 80 | Single-Blind Method/ |
| 81 | Single Blind Procedure/ |
| 82 | Single-Blind Studies/ |
| 83 | Placebos/ |
| 84 | Placebo/ |
| 85 | Control Groups/ |
| 86 | Control Group/ |
| 87 | (random* or sham or placebo*).ti,ab,hw. |
| 88 | ((singl* or doubl*) adj (blind* or dumm* or mask*)).ti,ab,hw. |
| 89 | ((tripl* or trebl*) adj (blind* or dumm* or mask*)).ti,ab,hw. |
| 90 | (control* adj3 (study or studies or trial*)).ti,ab. |
| 91 | (Nonrandom* or non random* or non-random* or quasi-random* or quasirandom*).ti,ab,hw. |
| 92 | (allocated adj1 to).ti,ab,hw. |
| 93 | ((open label or open-label) adj5 (study or studies or trial*)).ti,ab,hw. |
| 94 | or/70-93 |
| 95 | 35 and 94 |
| Guideline search filter: | |
| 96 | Guidelines as topic/ |
| 97 | Guideline/ |
| 98 | Practice guideline/ |

| Multi-database Strategy | |
|---|--|
| Line # | Search Strategy |
| 99 | exp Consensus Development Conference/ |
| 100 | Consensus Development.sh. |
| 101 | Health Planning Guidelines/ |
| 102 | Practice Guidelines as Topic/ |
| 103 | Clinical Protocols/ |
| 104 | (Guideline or Practice Guideline or Consensus Development Conference).pt. |
| 105 | Standards.fs. |
| 106 | Practice Guideline/ |
| 107 | Clinical Practice/ |
| 108 | Clinical Protocol/ |
| 109 | Health Care Planning/ |
| 110 | (guideline* or standards or best practice).ti. |
| 111 | [(guideline* or standards or best practice).hw. use b9o89] |
| 112 | (expert consensus or consensus statement or consensus conference* or practice parameter* or position statement* or policy statement* or CPG or CPGs).ti,ab. |
| 113 | [(expert consensus or consensus statement or consensus conference* or practice parameter* or position statement* or policy statement* or CPG or CPGs).hw. use b9o89] |
| 114 | or/96-113 |
| 115 | 35 and 114 |
| Observational studies search filter: | |
| 116 | epidemiologic methods.sh. |
| 117 | epidemiologic studies.sh. |
| 118 | cohort studies/ |
| 119 | cohort analysis/ |
| 120 | longitudinal studies/ |
| 121 | longitudinal study/ |
| 122 | prospective studies/ |
| 123 | prospective study/ |
| 124 | follow-up studies/ |
| 125 | follow up/ |
| 126 | followup studies/ |
| 127 | retrospective studies/ |
| 128 | retrospective study/ |
| 129 | case-control studies/ |
| 130 | exp case control study/ |
| 131 | cross-sectional study/ |
| 132 | observational study/ |
| 133 | quasi experimental methods/ |
| 134 | quasi experimental study/ |
| 135 | (observational adj3 (study or studies or design or analysis or analyses)).ti,ab,hw. |
| 136 | (cohort adj7 (study or studies or design or analysis or analyses)).ti,ab,hw. |
| 137 | (prospective adj7 (study or studies or design or analysis or analyses or cohort)).ti,ab,hw. |

| Multi-database Strategy | |
|--------------------------------|--|
| Line # | Search Strategy |
| 138 | ((follow up or followup) adj7 (study or studies or design or analysis or analyses)).ti,ab,hw. |
| 139 | ((longitudinal or longterm or (long adj term)) adj7 (study or studies or design or analysis or analyses or data or cohort)).ti,ab,hw. |
| 140 | (retrospective adj7 (study or studies or design or analysis or analyses or cohort or data or review)).ti,ab,hw. |
| 141 | ((case adj control) or (case adj comparison) or (case adj controlled)).ti,ab. |
| 142 | (case-referent adj3 (study or studies or design or analysis or analyses)).ti,ab,hw. |
| 143 | (population adj3 (study or studies or analysis or analyses)).ti,ab. |
| 144 | (descriptive adj3 (study or studies or design or analysis or analyses)).ti,ab,hw. |
| 145 | ((multidimensional or (multi adj dimensional)) adj3 (study or studies or design or analysis or analyses)).ti,ab,hw. |
| 146 | (cross adj sectional adj7 (study or studies or design or research or analysis or analyses or survey or findings)).ti,ab,hw. |
| 147 | ((natural adj experiment) or (natural adj experiments)).ti,ab,hw. |
| 148 | (quasi adj (experiment or experiments or experimental)).ti,ab,hw. |
| 149 | ((non experiment or nonexperiment or non experimental or nonexperimental) adj3 (study or studies or design or analysis or analyses)).ti,ab,hw. |
| 150 | (prevalence adj3 (study or studies or analysis or analyses)).ti,ab,hw. |
| 151 | case series.ti,ab,hw. |
| 152 | case reports.pt. |
| 153 | case report/ |
| 154 | case study/ |
| 155 | (case adj3 (report or reports or study or studies or histories)).ti,ab,hw. |
| 156 | organizational case studies.sh. |
| 157 | or/116-156 |
| 158 | 35 and (50 or 68 or 94 or 114 or 157) |

| OTHER DATABASES | |
|-----------------------------------|---|
| PubMed | Same MeSH, keywords, limits, and study types used as per Medline search, with appropriate syntax used. |
| Cochrane Library Issue 2, 2010 | Same MeSH, keywords, and date limits used as per Medline search, excluding study types. Syntax adjusted for Cochrane Library databases. |

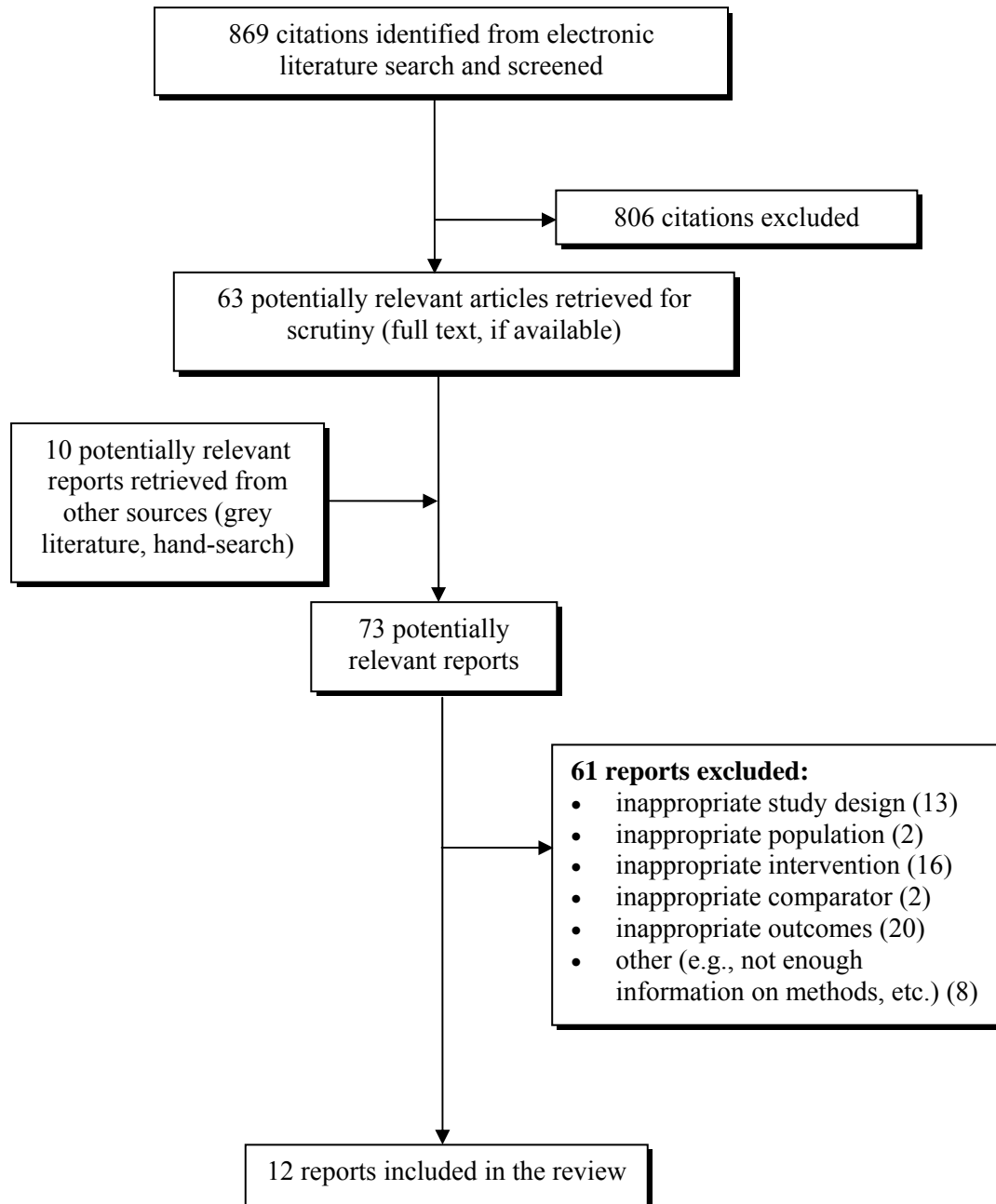
Grey Literature

| | |
|-------------------|--|
| Dates for Search: | March 15 — 19, 2010 |
| Keywords: | Non small cell lung cancer, epidermal growth factor receptor, polymerase chain reaction. |
| Limits: | Publication years 2005 — March 19, 2010 |

The following sections of the CADTH grey literature checklist, *Grey matters: a practical tool for evidence-based searching* (<http://www.cadth.ca/index.php/en/cadth/products/grey-matters>), were searched:

- Health Technology Assessment Agencies
- Clinical Practice Guidelines
- Databases (free)
- Internet Search
- Open Access Journals.

APPENDIX 2: SELECTION OF PUBLICATIONS



APPENDIX 3: ARTICLES OF POTENTIAL INTEREST — NOT MEETING INCLUSION CRITERIA

Gupta R, Dastane AM, McKenna R Jr, Marchevsky AM. The predictive value of epidermal growth factor receptor tests in patients with pulmonary adenocarcinoma: review of current "best evidence" with meta-analysis. *Hum Pathol*. 2009 Mar;40(3):356-65. [PubMed: PM18976796](#)

Li AR, Chitale D, Riely GJ, Pao W, Miller VA, Zakowski MF, et al. EGFR mutations in lung adenocarcinomas: clinical testing experience and relationship to EGFR gene copy number and immunohistochemical expression. *J Mol Diagn* [Internet]. 2008 May [cited 2010 Jun 14];10(3):242-8. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2329789> [PubMed: PM18403609](#)

APPENDIX 4: SUMMARY OF INCLUDED STUDIES

| Table A1: Characteristics of Included Primary Studies | | | | |
|---|---|--|---|--|
| Study, Year, Country | Patient and Tissue Sample Characteristics | Intervention (Techniques and Methods) | Comparator | Summary of Main Findings |
| Dahse et al., ¹¹ 2008 Germany | Women 16/35 (45.7%); men 19/35 (54.3%); median age at diagnosis 68.8 years; stage of disease not reported. <i>Specimens:</i> Formalin-fixed paraffin embedded tissues. <i>Chemotherapy history:</i> Not reported | <i>Bidirectional PCR amplification of specific alleles:</i> Requires 4 primers. <i>Allele-specific PCR:</i> Requires 3 primers. | Follow-up for mutation-positive PCR by direct sequencing performed; however, unclear if direct sequencing was done on all 35 samples. | Samples from 3 patients found to have exon 19 deletion. |
| Do et al., ¹² 2008 Australia | Patient characteristics not reported; stage of disease not reported. <i>Histology:</i> Adenocarcinomas (n = 141), large cell carcinomas (n = 24), squamous cell carcinomas (n = 10), and other or unknown histology (n = 25) <i>Specimens:</i> Formalin-fixed paraffin embedded tissues. <i>Chemotherapy history:</i> Not reported. | <i>High resolution melting analysis (HRM):</i> Tumour cell-rich areas microdissected from slides; 1 primer set for each exon except exon 20, which had 2 primer sets; reference dye SYTO 9; HRM analysis conducted using Rotor-Gene 6000. | Direct sequencing. | <i>Sensitivity (%):</i> Exon 18: 100 Exon 19: 100 Exon 20: 100 Exon 21: 100 <i>Specificity (%):</i> Exon 18: 91 Exon 19: 88 Exon 20: 93 Exon 21: 91 |

Table A1: Characteristics of Included Primary Studies

| Study, Year, Country | Patient and Tissue Sample Characteristics | Intervention (Techniques and Methods) | Comparator | Summary of Main Findings |
|---|---|--|--|---|
| <p>Molina-Vila et al.,¹⁴ 2008 Spain</p> | <p>Complete patient characteristics available for 182 patients: women — 85/182 (46.7%), men — 97/182 (53.3%); stage: I to II (n = 10), III to IV (n = 162), not reported (n = 10).</p> <p><i>Specimens:</i> Formalin-fixed paraffin embedded tissues.</p> <p><i>Chemotherapy history:</i> Reported that treatment was performed after EGFR mutational analysis.</p> | <p><i>Length analysis of PCR products Exon 19:</i> Fluorescent-labelled (6-FAM) primers; separation using 4-colour laser-induced capillary system (ABI Prism 3130xl Genetic Analyzer, Applied Biosystems).</p> <p><i>TaqMan Assay exon 21:</i> Fluorescent-labelled (6-FAM) primers; Analysis on Applied Biosystems 7000 real-time thermocycler.</p> | <p>Direct sequencing.</p> | <p>Overall mutation rate 37/217 samples (17%). <i>Concordance between PCR and direct sequencing (%):</i> Exon 19: 86.2 Exon 21: 78.3.</p> |
| <p>Smith et al.,¹⁵ 2008 USA</p> | <p>Women 4/11 (36.4%); men 7/11 (63.4%); median age 66 years; stage of disease not reported.</p> <p><i>Histology:</i> Poorly differentiated carcinoma (n = 5), adenocarcinoma (n = 4), squamous cell carcinoma (n = 1), non-small cell carcinoma (n = 1).</p> <p><i>Specimen:</i> Fine needle aspirates.</p> | <p><i>HRM:</i> DNA prepared from cellular material scraped from slide of specimen; PCR performed on LightCycler (Roche); Double-stranded DNA binding dye LCGreen I (Idaho Technology); HR-1 (Idaho Technology) instrument used for HRM analysis.</p> | <p>Direct sequencing on all samples with abnormal melt profiles.</p> | <p>Exon 19 mutation (deletion) 3/11 (27.3%).</p> |

Table A1: Characteristics of Included Primary Studies

| Study, Year, Country | Patient and Tissue Sample Characteristics | Intervention (Techniques and Methods) | Comparator | Summary of Main Findings |
|--|---|--|---------------------------|--|
| | <p><i>Chemotherapy history:</i> Reported that clinical specimens obtained before treatment.</p> | | | |
| <p>Hoshi et al.,¹⁶ 2007 Japan</p> | <p>Women 13/45 (28.9%); men 32/45 (71.1%); median age at diagnosis 65.6 years; stage of disease not reported.</p> <p><i>Histology:</i> Adenocarcinoma (n = 28), squamous cell carcinoma (n = 10), adenosquamous (n = 3), other types (n = 4).</p> <p><i>Specimen:</i> Fresh-frozen tumour samples.</p> <p><i>Chemotherapy history:</i> 15/45 patients were treated with gefitinib after obtaining clinical specimens.</p> | <p><i>SMAP:</i> Set of 5 primers used to amplify known target sequences in exons 19 and 21; process specifically amplifies mutant sequences.</p> | <p>Direct sequencing.</p> | <p><i>Mutations identified:</i> SMAP 10/45; direct sequencing 9/45.</p> |
| <p>Kimura et al.,¹⁷ 2006 Japan</p> | <p>Women 10/27 (37%); men 17/27 (63%); median age 64 years; stage of disease not reported.</p> <p><i>Histology:</i> Adenocarcinoma (n = 23), squamous cell carcinoma (n = 2), large cell carcinoma (n = 2).</p> | <p><i>Scorpions-ARMS:</i> Uses EGFR Scorpions Kit (DxS Ltd); primer sets designed by DxS to detect mutations in exons 19 and 21; real-time PCR performed using SmartCycler II.</p> | <p>Direct sequencing.</p> | <p><i>Mutations identified:</i> Scorpions-ARMS 13/27; direct sequencing 10/27. <i>Progression-free survival in patients treated with gefitinib (days; median):</i> Mutation-positive 200; mutation-negative 46 (P = 0.046). <i>Overall survival in</i></p> |

Table A1: Characteristics of Included Primary Studies

| Study, Year, Country | Patient and Tissue Sample Characteristics | Intervention (Techniques and Methods) | Comparator | Summary of Main Findings |
|---|---|--|---------------------------------------|---|
| | <p><i>Specimen:</i> Peripheral blood; serum.</p> <p><i>Chemotherapy history:</i> No chemotherapy.</p> | | | <p><i>patients treated with gefitinib (days; median):</i> Mutation-positive 611; mutation-negative 232 (P = 0.078).</p> |
| <p>Asano et al.,¹⁸ 2006</p> <p>Japan</p> | <p>Patient characteristics not reported; stage of disease not reported.</p> <p><i>Histology:</i> Adenocarcinoma (n = 72), squamous cell carcinoma (n = 33), adenosquamous (n = 2), large cell carcinoma (n = 1).</p> <p><i>Specimen:</i> Surgically resected, lung biopsies, and pleural fluid.</p> <p><i>Chemotherapy history:</i> Not reported.</p> | <p><i>ME-PCR:</i> 2 rounds of PCR separated by intervening step of restriction enzyme digest; restriction enzyme digest targeted to non-mutated sequences.</p> | <p>NE-PCR; and direct sequencing.</p> | <p><i>Mutations identified:</i> ME-PCR 51/146; NE-PCR (confirmed by direct sequencing) 43/146.</p> |
| <p>Ohnishi et al.,¹⁹ 2006</p> <p>Japan</p> | <p>Patient characteristics not reported; stage of disease not reported.</p> <p><i>Histology:</i> Adenocarcinoma (n = 54), squamous cell carcinoma (n = 5), adenosquamous (n = 1), large cell carcinoma (n = 2).</p> | <p><i>MS-PCR:</i> Standard PCR reaction based on known mutations in exon 19 and 21.</p> | <p>Direct sequencing.</p> | <p><i>Mutations identified:</i> MS-PCR 22/62; direct sequencing 23/62.</p> |

Table A1: Characteristics of Included Primary Studies

| Study, Year, Country | Patient and Tissue Sample Characteristics | Intervention (Techniques and Methods) | Comparator | Summary of Main Findings |
|--|--|---|--------------------|---|
| | <p><i>Specimen:</i> Surgically resected, fresh-frozen.</p> <p><i>Chemotherapy history:</i> Not reported.</p> | | | |
| Yatabe et al., ²⁰ 2006 Japan | <p>Patient characteristics not reported; stage of disease not reported.</p> <p><i>Histology:</i> Not reported.</p> <p><i>Specimen:</i> Tissue cores (0.6 mm) blotted on tissue array; fresh-frozen; paraffin-embedded.</p> <p><i>Chemotherapy history:</i> 29 patients treated with gefitinib after failure of first- or second-line chemotherapy.</p> | <p><i>Cycleave real-time PCR (Takara):</i> Based on use of fluorescent-labelled RNA probe that forms complexes with complementary DNA sequence in PCR mix; mutant and wild-type probes labelled with different dyes; targets mutations in exons 19, 20, and 21.</p> | Direct sequencing. | <p>Concordance between Cycleave PCR and direct sequencing 95% (186/195). Clinical outcomes of 29 patients treated with gefitinib PR 11 (10 mutation positive), SD 13 (2 mutation positive), PD 5 (0 mutation positive).</p> |
| Marchetti et al., ²¹ 2005 Italy | <p>Women 112/860 (13%); men 748/860 (87%); median age 62.7 years; stage I (57%), II (14%), III (29%).</p> <p><i>Histology:</i> Adenocarcinoma (n = 289), squamous cell carcinoma (n = 454), large cell</p> | <p><i>SSCP-PCR:</i> Based on conformation changes in DNA structure caused by slight changes in DNA sequences (point mutations or deletions); standard PCR is performed and amplification products denatured; denatured</p> | Direct sequencing. | <p>No mutations were identified in the small cell carcinomas or large cell carcinomas (N=485)</p> <p>Mutations identified in adenocarcinomas and bronchioloalveolar carcinomas (N=375) by</p> |

Table A1: Characteristics of Included Primary Studies

| Study, Year, Country | Patient and Tissue Sample Characteristics | Intervention (Techniques and Methods) | Comparator | Summary of Main Findings |
|---|--|---|---------------------------|---|
| | <p>carcinoma (n = 31), bronchioloalveolar carcinomas (n = 86).</p> <p><i>Specimen:</i> Fresh-frozen tissue.</p> <p><i>Chemotherapy history:</i> Not reported.</p> | <p>products separated by polyacrylamide gel electrophoresis and differences in fragment length detected using silver staining.</p> | | <p>SSCP-PCR: 39</p> <p>These mutations were confirmed by direct sequencing</p> |
| <p>Pan et al.,²³ 2005</p> <p>USA</p> | <p>39 samples from patients with lung cancer; patient characteristics not reported; stage of disease not reported.</p> <p><i>Histology:</i> Not reported.</p> <p><i>Specimen:</i> Tissue samples preservation method not reported.</p> <p><i>Chemotherapy history:</i> Not reported.</p> | <p><i>Exon 19 deletion assay:</i> Standard PCR reaction using fluorescent-labelled primers; analysis of fragment length performed using capillary electrophoresis.</p> <p><i>Exon 21 mutation assay:</i> Standard PCR reaction using fluorescent-labelled primers; after PCR, samples are treated with a restriction enzyme; only the mutated DNA sequence contains target sight of the restriction enzyme, where the DNA is cleaved; analysis of fragment length is performed using capillary electrophoresis.</p> | <p>Direct sequencing.</p> | <p><i>Mutations identified:</i> PCR 29/39 (74.4%); direct sequencing 25/39 (64.1%).</p> |

DNA = deoxyribonucleic acid; EGFR = epidermal growth factor receptor; 6-FAM = 6-carboxyfluorescein; HRM = high resolution melting; IHC = immunohistochemistry; ME-PCR = mutation-enriched polymerase chain reaction; mm = millimeter; MS-PCR = mutation-specific polymerase chain reaction; n = number; NE-PCR = non-enriched polymerase chain reaction; PCR = polymerase chain reaction; PD = progressive disease; PR = partial response; Scorpions-ARMS = Scorpions-amplification refractory mutation system; SD = stable disease; SMAP = smart amplification process; SSCP-PCR = single-strand conformation polymorphism polymerase chain reaction.

APPENDIX 5: ASSESSMENT OF INCLUDED GUIDELINES

| Table A2: AGREE Domain Scores and Overall Assessment | | | | | | | |
|---|------------------------------|------------------------------------|----------------------------------|-------------------------------------|--------------------------|-----------------------------------|-------------------------------|
| Study, Year, Country | Scope and Purpose (%) | Stakeholder Involvement (%) | Rigour of Development (%) | Clarity and Presentation (%) | Applicability (%) | Editorial Independence (%) | Overall Assessment (%) |
| Ellis et al., ²⁴ 2009 Canada | 88 | 42 | 71 | 63 | 6 | 33 | Strongly recommend. |

AGREE = Appraisal of Guidelines for Research & Evaluation.