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Horizon scanning prioritising summary

Update Number 3

Epidermal growth factor receptor mutational assay for patients with non small-cell lung carcinoma

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UPDATE PRIORITISING SUMMARY

REGISTER ID: **000148**

NAME OF TECHNOLOGY: **EPIDERMAL GROWTH FACTOR RECEPTOR MUTATION TEST**

PURPOSE AND TARGET GROUP: **TO IDENTIFY POTENTIAL RESPONDERS TO THE DRUG GEFITINIB ("IRESSA") AMONGST PATIENTS WITH NON-SMALL CELL LUNG CARCINOMA**

STAGE OF DEVELOPMENT (IN AUSTRALIA):

☐	Yet to emerge	☐	Established
☐	Experimental	☐	Established *but* changed indication or modification of technique
☐	Investigational	☐	Should be taken out of use
☒	Nearly established		

AUSTRALIAN THERAPEUTIC GOODS ADMINISTRATION APPROVAL

☐	Yes	ARTG number
☐	No	
☒	Not applicable	

INTERNATIONAL UTILISATION:

COUNTRY	LEVEL OF USE		
	Trials Underway or Completed	Limited Use	Widely Diffused
United States		✓	
Australia	✓		

IMPACT SUMMARY:

The Pharmaceutical Benefits Advisory Committee (PBAC) recently approved the use of gefitinib or IRESSA® for the treatment of patients with locally advanced or metastatic non-small cell lung carcinoma who have previously received chemotherapy. A proviso was added that only patients with a proven somatic mutation in the kinase domain of the epidermal growth factor receptor (EGFR) would be eligible to receive gefitinib. Mutation analysis of the tyrosine kinase domain of the EGFR is currently not funded by the Medicare Benefits Schedule (MBS).

BACKGROUND

Tyrosine kinases regulate signalling pathways, controlling critical cellular activity and when over-expressed they may contribute to the development of cancers. Protein kinase over-expression may occur by a somatic mutation or chromosomal alteration in the tyrosine kinase domain (Pao et al 2004; Paez et al 2004). EGFR is a member of the four cell surface membrane receptors, the ErbB-3 family. The activation of EGFR leads to tyrosine kinase activation. This results in a cascade of signalling events, mediating an increase in cellular proliferation, motility, adhesion, blocking of apoptosis, increased angiogenesis and a resistance to chemotherapy. Any of these factors may contribute to the development of a

malignancy. Increased activity at the EGFR has been shown to occur with a variety of solid tumours. The EGFR is highly expressed in 88-99 per cent of non-small cell lung carcinomas, with high expression more common in squamous cell cancers (57-92%) than in adenocarcinomas (33-58%) (Janne et al 2004).

Developing tumours depend on the mutated tyrosine kinase for their continued survival, and therefore cancer therapy is aimed at inhibiting this dependency. The EGFR is the presumptive target for the tyrosine kinase inhibitor, gefitinib or IRESSA®. Gefitinib is an anilinoquiazoline, which reversibly competes with ATP[1] at a critical binding site within the EGFR protein, inhibiting its activity (Pao et al 2004). Several studies have assessed the effectiveness of gefitinib in patients with non-small cell lung carcinoma who have failed other forms of chemotherapy (Pao et al 2004; Paez et al 2004; Lynch et al 2004; Janne et al 2004). Patients with a mutation in the tyrosine kinase domain of the EGFR may demonstrate an increased sensitivity to inhibition of tumour growth, demonstrated by non-progression or stabilisation of the disease, to treatment with gefitinib. However, not all patients diagnosed with non-small cell carcinoma will have a mutation in the EGFR (Lynch et al 2004). Jänne et al (2004) described a case series of 200 consecutive patients (level IV intervention evidence) with advanced non-small cell lung carcinoma. Of these patients, 172 were prescribed gefitinib for a median duration of two months (range 0-22+ months). Twenty-three patients died prior to treatment and five withdrew their consent. Median follow-up was 13 months (range 2-23 months). Patients were not screened for mutations of the EGFR either before or after treatment. Of the 172 patients available for evaluation only seven (4.1%, 95%CI; 1.7, 8.2%) experienced a partial response to gefitinib and 60 patients (35%) had stable, non-progressive disease.[2]

In addition, not all mutations of the EGFR are affected by gefitinib. Missense mutations in the extracellular domain of EGFR and deletions in the regulatory intracellular domain will not result in an EGFR mutation capable of being targeted by gefitinib. Two classes of somatic mutations were identified in the kinase domain of the EGFR, which were associated with sensitivity to gefitinib. The first class comprises amino acid substitutions in the P-loop at exon 18 or in the activation domain (exon 21). The second class comprises in-frame deletions within exon 19, which has the effect of altering the structure of the αC helix. All of these mutations affect ATP binding (Minna et al 2004).

Prior to EGFR mutational analysis, a biopsy sample of the tumour must first be obtained from the patient. Tissue specimens may be frozen or paraffin embedded. DNA is extracted from the biopsy samples and exons 18 through 24 of the EGFR (the region associated with responsiveness to gefitinib) are amplified by nested PCR[3] reactions. The second round PCR products are sequenced bi-directionally to identify any mutations. EGFR mutational analysis takes approximately two weeks for a diagnostic laboratory to complete. Mutations in the 18- to 24-exon region of the EGFR may confer responsiveness to treatment with gefitinib resulting in non-progression or stabilisation of the disease (Laboratory for Molecular Medicine 2004).

CLINICAL NEED AND BURDEN OF DISEASE

In Australia, there were 8,275 registered cases of lung cancer in the year 2001. Of these registered cases, 5,384 were males and 2,891 were females (AIHW and AACR 2004). Lung cancer is the leading cause of male cancer death with 53 deaths per 100,000 males in 2002.

[1] ATP = adenosine triphosphate

[2] Note: The United States Food and Drug Administration made a statement on 17th December 2004, to the effect that a large clinical trial conducted by AstraZeneca, comparing gefitinib with placebo in patients with non-small cell lung carcinoma, showed no survival benefit from taking gefitinib. Source: http://www.fda.gov/bbs/topics/news/2004/new01145.html

[3] PCR = polymerase chain reaction

The death rate for females from lung cancer is 25 deaths per 100,000 females. Overall, in the year 2002, lung cancer as the underlying cause of death was responsible for 8,110 (6.1%) of all deaths (AIHW 2004).

A cross-sectional survey of Victorian doctors caring for patients diagnosed with lung cancer was conducted during 1996-97. Of the 1054 patients diagnosed with lung cancer, 635 (73%) were diagnosed with non-small cell lung carcinoma (Richardson et al 2000). If these results were translated to the Australia population diagnosed with lung cancer, approximately 6,040 individuals would have been diagnosed with non-small cell lung carcinoma in the year 2001. Projection figures based on data from the Australian Institute of Health and Welfare used in the submission to PBAC, estimate that in the year 2005 there would be a total of 7,655 individuals diagnosed with lung cancer and 5,588 of these patients would have non-small cell lung carcinoma.

DIFFUSION

In Australia there are three NATA[4] accredited laboratories offering EGFR mutation testing commencing from January 2005. These laboratories are the Peter McCullum Cancer Institute in Melbourne, Network Pathology Austin Health in Melbourne and the Institute of Medical and Veterinary Science in Adelaide. This test is currently not offered on the MBS and therefore costs would be borne by the patient.

COMPARATORS

PCR followed by sequencing is the gold standard for the detection of mutations in the tyrosine kinase domain of the EGFR, however immunohistochemistry may be used to detect abnormal activity of the EGFR. Several commercial kits are currently available including those manufactured by Zymed Laboratories Inc and Biomeda Corporation. Formalin fixed, paraffin-embedded tissue samples are treated and sectioned before incubation with a primary antibody (anti-EGFR). The sequential application of conjugate followed by chromogen enables localisation and visualisation, under the microscope, of the bound primary antibody, indicating the presence or absence of EGFR protein expression. No staining indicates an absence of EGFR protein over-expression, with strong staining indicating over-expression of the EGFR protein. Studies have reported EGFR over-expression in pulmonary neoplasms (Han et al 2005; Zymed Laboratories Inc 2004). Although increased EGFR expression is common in lung cancers it does not correlate with a favourable clinical response to gefitinib (Minna et al 2004). Immunohistochemistry is a multi-step process, dependent on correct handling and tissue preparation and results may be open to interpretation. Immunohistochemistry is offered on the MBS, depending on the complexity of the biopsy sample, by item numbers 72813 to 72836 and 72844 to 72857.

EFFECTIVENESS AND SAFETY ISSUES

There are no published studies available that describe the effectiveness of the mutational analysis of the EGFR. However, PCR and sequencing are established diagnostic tools and when offered by NATA accredited laboratories are performed by technically qualified personnel following specific protocols of quality assurance, sample preparation, amplification, detection and interpretation of results (White et al 1992). The advantages of PCR is that it has high sensitivity, high specificity and good reproducibility. Its limitations are: the potential for false-positive results from contaminating DNA; the potential for false-negatives due to the presence of PCR inhibitors; it is expensive and it is technically complex (Louie et al 2000).

The Harvard Medical School Partners Healthcare Center for Genetics and Genomics conduct EGFR mutational analysis and estimate the assay has greater than 99.9 per cent accuracy to

[4] NATA is the National Association of Testing Authorities, Australia

detect mutations in the sequence analysed (source: assay protocol sheet). Sensitivity of the test is dependent on the preparation and the quality of the DNA obtained from the biopsy sample. Inadequate DNA extraction may occur in 25 per cent of paraffin embedded samples. In addition the biopsy sample may contain a mixture of cell types. A minimum of 50 per cent tumour cells is required to ensure the accuracy of EGFR sequencing (Laboratory for Molecular Medicine 2004).

Two poor quality tudies by Lynch et al (2004) and Paez et al (2004) have described somatic mutations in the tyrosine kinase domain of the EGFR in patients with non-small cell lung carcinoma who responded positively to treatment with gefitinib.

A poorly conducted and low quality retrospective cohort study by Paez et al (2004) found that of 119 patients diagnosed with non-small cell lung carcinoma, only 16 (13%) had a mutation in the tyrosine kinase domain of the EGFR. Mutations were more frequent in patients with adenocarcinomas (15/70 or 21%) than in other non-small cell lung carcinomas (1/49 or 2%). To investigate whether EGFR mutations might determine gefitinib sensitivity, pre-treatment samples were obtained from nine patients. Of the five patients who responded to gefitinib with either partial radiographic responses (4/5 patients) or a dramatic improvement in symptoms (1/5 patients), all had mutations in the tyrosine kinase domain of EGFR. Four patients with disease progression revealed no mutations in the EGFR. The difference between the two groups was statistically significant ($p = 0.003$) (level III-3 prognostic evidence) (Paez et al 2004).

Lynch et al (2004) described 275 patients treated with gefitinib (median duration of therapy was > 16 months) (level IV prognostic evidence). Of these patients, 25 responded well to treatment. EGFR mutational analysis was then conducted on a small sub-group of responders (n=9) who had a biopsy sample available for analysis. The remaining 16 patients had only fine needle aspirates available, which contain insufficient tumour material for EGFR mutational analysis. EGFR mutations were found in 8/9 (89%) of responding patients. Of these eight patients, three had a major, four had a partial and one had a minor response to treatment, as determined by established guidelines on evaluating the response to treatment in solid tumours. The median duration of survival in these patients was 18 months, however the median survival time of patients who did not respond to gefitinib was not stated. The one patient without an EGFR mutation had a partial response to treatment. Mutational analysis was not conducted on the remaining patients who did not respond to gefitinib and therefore their mutational status remains unknown (Lynch et al 2004).

Mutational analysis of the EGFR is used as a prognostic marker. PCR may be effective at determining whether a mutation is present in the EGFR, however it is unclear from the available published evidence whether the mutation is a good prognostic marker for successful treatment of patients with gefitinib.

COST IMPACT

Of the three laboratories offering EGFR mutation analysis, commencing January 2005, two laboratories (Peter McCallum Cancer Institute and Network Pathology Austin Health) are offering two tests: either a 4-(exons 18 to 21) or 7-exon (exons 18 to 24) mutation analysis. Prices quoted for the 4-exon test range from $700 to $850. However, all three laboratories will be offering the 7-exon analysis costing between $900 to $1000 (personal communication Barney Rudzki, Molecular Pathology, Institute of Medical and Veterinary Science).

Based on data from the Australian Institute of Health and Welfare supplied in the gefinitib submission to PBAC, it is estimated that in the year 2005 there would be 5,588 patients diagnosed with non-small cell lung carcinoma. If all of these patients were screened for

EGFR mutations this would cost between $3.9 million (4-exon analysis) and $5.6 million (7-exon analysis).

ETHICAL, CULTURAL OR RELIGIOUS CONSIDERATIONS

No issues were identified in the sources examined.

OTHER ISSUES

Several authors involved in studies of gefitinib have received lecture fees or honorariums from AstraZeneca.

The EGFR mutation analysis is currently being validated at the Institute of Medical Veterinary Science, in South Australia. It will be several months before any data on test accuracy are available comparing EGFR mutational analysis of material from patients with non-small cell lung carcinoma to normal tissue. The test is more difficult than a standard pathology test due to the complexity of extracting the tumour sample from formalin fixed material, however the sequencing side of the test appears uncomplicated (personal communication Barney Rudzki, Molecular Pathology, Institute of Medical and Veterinary Science).

JANUARY 2005 – CONCLUSION:

There is no available published evidence in respect to the diagnostic accuracy of the mutational analysis of the EGFR, however validation data will be made available from Australian laboratories during the early part of 2005. In addition, at this stage there is insufficient evidence to support the use of a mutation in the tyrosine kinase domain of the EGFR as a prognostic marker for effective treatment of patients with non-small cell lung carcinoma with gefitinib. Therefore it is recommended that the be monitored.

JANUARY 2005 - SOURCES OF FURTHER INFORMATION:

AIHW (2004). *Australia's Health 2004,* Australian Institute of Health and Welfare (AIHW), Canberra.

AIHW and AACR (2004). *Cancer in Australia 2001,* Australian Institute of Health and Welfare (AIHW) and Australasian Association of Cancer Registries (AACR), Canberra.

Han, S. W., Hwang, P. G. et al (2005). 'Epidermal growth factor receptor (EGFR) downstream molecules as response predictive markers for gefitinib (Iressa, ZD1839) in chemotherapy-resistant non-small cell lung cancer', *Int J Cancer,* 113 (1), 109-115.

Janne, P. A., Gurubhagavatula, S. et al (2004). 'Outcomes of patients with advanced non-small cell lung cancer treated with gefitinib (ZD1839, "Iressa") on an expanded access study', *Lung Cancer,* 44 (2), 221-230.

Laboratory for Molecular Medicine (2004). *EGFR sequencing test* [Internet]. Harvard Medical School - Partners Healthcare Center for Genetics and Genomics. Available from: http://www.hpcgg.org/LMM/comment/EGFR_info_101404.html [Accessed 11th January 2005].

Louie, M., Louie, L. & Simor, A. E. (2000). 'The role of DNA amplification technology in the diagnosis of infectious diseases', *Cmaj,* 163 (3), 301-309.

Lynch, T. J., Bell, D. W. et al (2004). 'Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib', *N Engl J Med,* 350 (21), 2129-2139.

Minna, J. D., Gazdar, A. F. et al (2004). 'Cancer. A bull's eye for targeted lung cancer therapy', *Science,* 304 (5676), 1458-1461.

Paez, J. G., Janne, P. A. et al (2004). 'EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy', *Science,* 304 (5676), 1497-1500.

Pao, W., Miller, V. et al (2004). 'EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib', *Proc Natl Acad Sci U S A,* 101 (36), 13306-13311.

Richardson, G. E., Thursfield, V. J. & Giles, G. G. (2000). 'Reported management of lung cancer in Victoria in 1993: comparison with best practice. Anti-Cancer Council of Victoria Lung Cancer Study Group', *Med J Aust,* 172 (7), 321-324.

White, T. J., Madej, R. & Persing, D. H. (1992). 'The polymerase chain reaction: clinical applications', *Adv Clin Chem,* 29, 161-196.

Zymed Laboratories Inc (2004). *EGFr Kit product sheet* [Internet]. Zymed Laboratories Inc. Available from: http://www.zymed.com/pdf/28-xxxx/28-8763.pdf [Accessed 12th January 2004].

SEARCH CRITERIA TO BE USED:

Adenocarcinoma
Carcinoma, Non-Small-Cell Lung
Lung Neoplasms
Receptor, Epidermal Growth Factor
Protein-Tyrosine Kinase
Amino Acid Sequence
Base Sequence
DNA Mutational Analysis
Genes, erbB-1
Molecular Sequence Data
Mutation
Sequence Deletion
Amino Acid Motifs
Amino Acid Sequence
Amino Acid Substitution
Quinazolines/pharmacology/*therapeutic use

JUNE 2006 UPDATE - EFFECTIVENESS AND SAFETY ISSUES

Since January 2005, numerous studies have been published describing the detection of mutations in the EGFR, using PCR techniques, in patients with non-small cell lung carcinoma.

Endo et al (2005) (level IV diagnostic evidence) and others had previously established that there are at least 11 mutations in the EGFR gene. A small sub-group of non-small cell lung carcinoma (NSCLC) patients being treated with gefitinib (n=27) had their genomic DNA sequenced in the EGFR gene region (exons 18, 19, 20 and 21). These DNA sequences were examined and a further two mutations were identified, bringing the total number of known mutations to 13. Thirteen specific DNA probes were designed and used to conduct TaqMan[1] PCR to determine the mutational status of two groups of NSCLC patients (n=94 and n=182). Results from the TaqMan PCR matched DNA sequencing, the standard method for detecting mutations, in 93/94 (98.9%) of cases, however in the one mismatched case TaqMan PCR detected a mutation that was undetected by direct sequencing. In group one and group two, 27/94 (28.7%) and 46/182 (25.3%) patients, respectively, were found to have a mutation in the EGFR region. The authors conclude that although the TaqMan PCR is highly accurate, it is only detecting the *13 known mutations* and that probes cannot be designed to the sequences of *unknown mutations*, thus there may be a high rate of undetected false negatives.

Similar results were reported by Zhou et al (2006) (level IV diagnostic evidence), however this study only used 10 specific DNA probes designed from genes coding for EGFR in exons 18, 19 and 21. Of the unselected patients (mutational status unknown) with NSCLC, 21/80 (26.3%) had a mutation detected in the EGFR region. When compared to direct DNA sequencing, the sensitivity and specificity for detecting a mutation in the EGFR region was 100 per cent using real time PCR with TaqMan probes. In addition, the study by Pan et al (2005) obtained DNA from 39 lung cancer patients and ran a PCR using 10 specific probes from exons 19 and 21 (level IV diagnostic evidence). Direct DNA sequencing detected 25/39 (64%) mutations, whereas PCR found 29/39 (74%) mutations, demonstrating that PCR is highly specific and sensitive when compared to the standard method. A reduced number of exons were used in this study approximately 90 per cent of mutated cases are accounted for by two mutations: short in-frame deletions in exon 19 and a point mutation in exon 21.

In conclusion, PCR for the detection of mutations in the EGFR region is highly specific and sensitive, however, the number of false negatives is dependent on the number of specific probes used in the PCR assay. PCR is more rapid and accurate than direct sequencing.

Controversy still surrounds the clinical efficacy of gefitinib, a tyrosine kinase inhibitor, for the treatment of patients with NSCLC based on the mutational status of the EGFR gene. A recent review of the clinical experience of gefitinib was conducted by Cappuzzo et al (2006). Initial studies, as discussed in the original summary, demonstrated a short-term response (shrinkage of tumour) to gefitinib may be conferred by the presence of mutations in the tyrosine kinase domain of the EGFR. These mutations were associated with females of East Asian ethnicity who had never smoked and had adenocarcinoma histology. However, recent studies have demonstrated that there is a significant portion of patients with EGFR mutations who do not respond to gefitinib. In a number of studies described in the review, patients carrying the EGFR mutation had a higher response rate to gefitinib, an elongated time to disease progression, but there was no overall survival benefit when compared to patients without the mutation.

A recent study by Cappuzzo et al (2005) found that EGFR mutations were absent from patients with stable NSCLC disease and the survival benefit conferred by treatment with another tyrosine kinase inhibitor, erlotinib, was not confined to patients with EGFR mutations. This finding has led to the suggestion that an increased copy number of the EGFR gene may be critical for sensitivity or responsiveness to tyrosine kinase inhibitors such as gefitinib. Cappuzzo et al (2005) reported on 102 NSCLC patients treated with gefitinib (250 mg daily) (level IV diagnostic evidence). Patients with an *increased EGFR gene copy number* had a significantly higher response rate to gefitinib (36% versus 3%, $p<0.001$), a longer median time to disease progression (9 months versus 2.5 months, $p<0.001$) and longer median survival and higher 1-year survival rate (18.7 months versus 7 months, and 57% versus 33%, respectively, $p=0.03$) when compared to patients with a *low gene copy number*. These patients were more likely to be female ($p=0.04$) and never smokers ($p=0.001$). Some studies have reported a significant response rate to tyrosine kinase inhibitors and longer survival in patients with EGFR mutations *and* a high copy number of the EGFR gene, compared to patients with the EGFR mutation and a low copy number of the EGFR gene (Tsao et al 2005, Hirsch et al (in press) and Cappuzzo et al 2005). These results were conflicted by the retrospective analysis of two RCTs which reported that there was no significant association with EGFR gene copy number and response to inhibitors, time to disease progression or survival. However, numbers of patients in these trials were small.

Other data suggests that there may be an association with over expression of the HER2 gene and sensitivity of tumours to gefitinib (Cappuzzo et al 2006). In 102 patients with NSCLC, those with a high copy number of the HER2 gene had a better response rate to gefitinib and longer time to disease progression when compared to patients with a low copy number of the HER2 gene. In addition, those patients with a high copy number of *both* the EGFR and HER2 genes and EGFR mutations had a better response rate to gefitinib, longer time to disease progression and an increased survival benefit. All three of these factors were associated with a never smoking history.

Gefitinib is associated with adverse events including diarrhoea, rash, fatigue, dyspnoea and in some cases, interstitial ling disease (Nagaria et al 2006), therefore should be administered with caution to the appropriate patient group.

JUNE 2006 -CONCLUSION:

The mutational analysis of the EGFR gene by PCR assay is highly sensitive and specific when compared to standard DNA sequencing. Most of the studies included in this assessment have been of poor quality (level IV diagnostic evidence) and have been "hypothesis generating"; that is the same patient group with NSCLC have had their DNA trawled to find potential differences to explain their response to gefitinib. Gefitinib may result in an increased survival benefit in a select group of patients; however it is unclear from the literature exactly what the characteristics of this patient group should be.

JUNE 2006 - HEALTHPACT ACTION:

It is still unclear from the available published evidence whether EGFR mutations are a good prognostic marker for successful treatment of patients with gefitinib. As a result it is recommended that this technology be archived.

JUNE 2006 - SOURCES OF FURTHER INFORMATION:

Cappuzzo, F., Finocchiaro, G. et al (2006). 'Clinical experience with gefitinib: An update', *Crit Rev Oncol Hematol,* 58 (1), 31-45.

Cappuzzo, F., Hirsch, F. R. et al (2005). 'Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer', *J Natl Cancer Inst,* 97 (9), 643-655.

Endo, K., Konishi, A. et al (2005). 'Epidermal growth factor receptor gene mutation in non-small cell lung cancer using highly sensitive and fast TaqMan PCR assay', *Lung Cancer,* 50 (3), 375-384.

Hirsch, F. R., Varella-Garcia, M. et al (2005). 'Increased epidermal growth factor receptor gene copy number detected by fluorescence in situ hybridization associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes: a Southwest Oncology Group Study', *J Clin Oncol,* 23 (28), 6838-6845.

Nagaria, N. C., Cogswell, J. et al (2005). 'Side effects and good effects from new chemotherapeutic agents. Case 1. Gefitinib-induced interstitial fibrosis', *J Clin Oncol,* 23 (10), 2423-2424.

Pan, Q., Pao, W. & Ladanyi, M. (2005). 'Rapid polymerase chain reaction-based detection of epidermal growth factor receptor gene mutations in lung adenocarcinomas', *J Mol Diagn,* 7 (3), 396-403.

Tsao, M. S., Sakurada, A. et al (2005). 'Erlotinib in lung cancer - molecular and clinical predictors of outcome', *N Engl J Med,* 353 (2), 133-144.

Zhou, C., Ni, J. et al (2006). 'Rapid detection of epidermal growth factor receptor mutations in non-small cell lung cancer using real-time polymerase chain reaction with TaqMan-MGB probes', *Cancer J,* 12 (1), 33-39.

LIST OF STUDIES INCLUDED

Level IV diagnostic evidence	3