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Epidermal growth factor receptor (EGFR) mutation assay.

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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 CARCINOMA**

STAGE OF DEVELOPMENT (IN AUSTRALIA):

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

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:

Patients with locally advanced or metastatic non-small cell lung carcinoma and a proven somatic mutation in the kinase domain of the epidermal growth factor receptor (EGFR) would be suitable candidates to receive gefitinib or IRESSA®. 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. 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

[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

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

See complete volume of Prioritising Summaries for definitions of Levels of Evidence.
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 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

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

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 studies 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, 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.

SOURCES OF FURTHER INFORMATION:
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, Institute of Medical and Veterinary Science).

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.

HEALTHPACT ACTION:
Therefore it is recommended that this technology be monitored.

SOURCES OF FURTHER INFORMATION:
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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