

TITLE Could molecular pathology testing in lung cancer be more cost effective?

AUTHORS Kathy Walsh¹, Yuan Kheng^{1,2}, Anca Oniscu¹, David Harrison^{1,2}, William Wallace¹

1. Molecular Pathology, Dept. Laboratory Medicine, Royal Infirmary of Edinburgh, Little France Crescent, Old Dalkeith Road, Edinburgh, EH16 4SA, UK.
2. School of Medicine, University of St Andrews, North Haugh, St Andrews KY16 9TF

Correspondence to: Kathy Walsh, Molecular Pathology, Dept. Laboratory Medicine, Royal Infirmary of Edinburgh, Little France Crescent, Old Dalkeith Road, Edinburgh, EH16 4SA, UK. Kathy.walsh@nhslothian.scot.nhs.uk

ABSTRACT

Aims: *EGFR* and *ALK* analysis is routinely undertaken prior to targeted treatment of non-squamous non-small cell lung carcinoma (NSCLC). Increasingly limited resources require molecular pathology services to be cost effective without detriment to patient care.

Methods: Data from an audit of molecular pathology testing in the South East of Scotland Cancer network has been used to explore different testing strategies with the aim of reducing costs; including investigation of TTF1 expression as a negative predictor for *EGFR* mutations.

Results: TTF1 immunohistochemistry had a high negative predictive value for *EGFR* mutations (99%). Reflex testing all non-squamous NSCLC had the highest costs whereas limiting testing to those who might be considered for treatment would save 7.5%; the serial model could save 32.7%.

Conclusions: Testing only patients being considered for *EGFR* and *ALK* inhibitors represented small savings; more significant savings would be achievable if testing algorithms utilized known associations between clinical biomarkers.

INTRODUCTION

Current UK and USA recommendations for the treatment of locally advanced or metastatic non-squamous non-small cell lung cancer (non-squamous NSCLC) include first line *EGFR* tyrosine kinase inhibitors (gefitinib, erlotinib and afatinib) for tumours with activating *EGFR* mutations; and *ALK* inhibitors (crizotinib and ceritinib) for previously treated patients whose tumours harbour *ALK* gene rearrangements.¹⁻³ As a result, predictive *EGFR* mutation analysis has been carried out by clinical laboratories since 2009 and in 2013 *ALK* rearrangement analysis was added to the testing algorithm. Studies have shown that in a Caucasian population approximately 10% of NSCLC have *EGFR* mutations,⁴ 2 to 5% have *ALK* rearrangements,⁵ and 35% have *KRAS* mutations.⁶ Although there is no direct therapeutic value in the detection of somatic *KRAS* mutations it is performed in many laboratories carry as *KRAS* mutations are, in the vast majority of samples, mutually exclusive with *EGFR* and *ALK* mutations.⁷

Thyroid transcription factor 1 (TTF1) has, for many years, been used as an immunohistochemical marker to aid the diagnosis of lung adenocarcinoma. Approximately 80% of non-squamous NSCLC requested for molecular pathology testing show positive nuclear staining with TTF1 antibodies.⁸ Several studies have

shown a correlation between TTF1 protein expression and the presence of *EGFR* mutations (see table 1), indeed TTF1 IHC has been shown to be a good negative predictor of *EGFR* mutations in western populations; however, this association appears to be less strong in East Asian populations.

Table 1: Studies comparing TTF1 IHC with the presence of *EGFR* mutations

Study	No. samples with TTF1 and <i>EGFR</i> status	Proportion TTF1 positive	No. <i>EGFR</i> mut TTF1 neg	NPV	Population (geographic)
Vallee <i>et al.</i> ⁸	1038	79.0%	3	98.6%	France
Vincenten <i>et al.</i> ⁹	797	67.9%	9	96.5%	Netherlands
Krawczyk <i>et al.</i> ¹⁰	727	80.4%	10	93.0%	Poland
Chatziandreou <i>et al.</i> ¹¹	595	70.4%	2	98.9%	Greece
Sheffield <i>et al.</i> ¹²	306	77.1%	4	94.3%	Canada
Somaiah <i>et al.</i> ¹³ (pilot and validation)	301	90%	2	93.3%	America
	131	72.5%	1	97.2%	America
Leary <i>et al.</i> ¹⁴	70	78.6%	0	100%	Britain
Zhang <i>et al.</i> ¹⁵	1042	87.2%	50	62.4%	China
Shanzhi <i>et al.</i> ¹⁶	660	98.5%	1	90%	China
Chung <i>et al.</i> ¹⁷	496	89.3%	17	67.92%	Taiwan
Sun <i>et al.</i> ¹⁸	190	79.5%	6	84.6%	Korea
Yatabe <i>et al.</i> ¹⁹	95	57.9%	7	82.5%	Japan

There is still debate as to the best approach for molecular pathology testing of lung cancers; some laboratories favour testing all patients with a histologically or cytologically diagnosed non-squamous non-small cell lung cancer, whereas others test only samples from patients being considered for *EGFR* or *ALK* targeted therapy. Although a request based model may require rapid turnaround times in order to meet the clinical need it would be likely to reduce costs by avoiding testing unnecessary cases. This report explores options for improving cost efficiency in lung cancer molecular pathology without impacting patient care.

METHODS

The pathology laboratory based at the Royal Infirmary of Edinburgh provides molecular testing in lung cancer for the South East of Scotland cancer network covering NHS Lothian, NHS Borders, NHS Fife and NHS Dumfries and Galloway. A clinical audit was carried out of lung cancer Molecular Pathology requests across the network between January 2011 and March 2014. After histopathology assessment and macrodissection (as required) DNA was extracted from FFPE tissue using the QIAamp DNA FFPE tissue Kit (Qiagen). *EGFR* mutation analysis was carried out using the Therascreen *EGFR* RGQ PCR kit (Qiagen); mutations in *KRAS* codons 12, 13 and 61 were detected using an in-house Pyrosequencing assay. Samples requiring *ALK* rearrangement analyses were initially screened for *ALK* protein expression by immunohistochemistry (IHC) using the D5F3 clone (1 in 200 dilution) on a Bond-III system (Leica, UK). Samples positive by *ALK* IHC were tested for *ALK* gene rearrangements by fluorescence in situ hybridisation (FISH) using the Vysis *ALK*

Break Apart FISH Probe Kit (Abbott Molecular). Pearson's chi-squared test and Fisher's Exact test were used to explore associations between clinicopathological and molecular parameters. TTF1 IHC was performed, if not already carried out for histological diagnosis, on samples with *EGFR* mutations. TTF1 IHC was performed on a Bond-III using the 8G7G3/1 antibody (M3575, Dako) with 20 minutes retrieval and Leica solution ER2. Sections stained with TTF1 were assessed for the presence of any nuclear staining by a consultant histopathologist specialising in respiratory pathology.

The number and diagnoses of all lung cancer patients registered in the Borders, Dumfries & Galloway, Fife and Lothian Health Boards between April 2013 and March 2014 were supplied by the South East Scotland Cancer Network (SCAN). These data, and the results of the clinical audit, were used to develop models of testing using four algorithms. 1. In the reflex model samples from all patients diagnosed with non-squamous NSCLC, regardless of intention to treat with *EGFR* or *ALK* targeted therapies, would be simultaneously tested for *EGFR*, *KRAS* and *ALK* mutations. 2. In the request model only samples from patients with metastatic or locally advanced (stage III and IV) non-squamous NSCLC, i.e. those eligible for treatment with *EGFR* TKIs or *ALK* inhibitors, would be simultaneously tested for *EGFR*, *KRAS* and *ALK* mutations. 3. The serial testing model was designed to utilise the mutually exclusive relationship between *EGFR*, *KRAS* and *ALK* mutations in order to minimise testing. All patients eligible for targeted therapy would be tested for *KRAS* mutations; only those with no mutations would have *EGFR* mutation analysis and only samples with no *KRAS* or *EGFR* mutations would have *ALK* rearrangement analysis. 4. Finally, in the TTF1-serial model all patients eligible for targeted therapy would be initially tested for TTF1 and *ALK* rearrangements; given the limited data available this model does not assume a correlation between TTF1 expression and *ALK* rearrangements. Only TTF1 positive *ALK* negative samples would have *KRAS* mutation analysis and only those with no *KRAS* mutations would be tested for *EGFR* mutations. The cost of testing using each model was estimated using the CMD Impact Business Planning Tool developed by the Royal College of Pathologists, Cancer Research UK and the Association of the British Pharmaceutical Industry (<https://www.rcpath.org/cmd-impact.html>). The estimated annual cost of each model was represented as a proportion of the cost of the request model.

RESULTS

Between January 2011 and March 2014 there were 710 requests for lung cancer molecular pathology testing; of those suitable for testing 10.5% had *EGFR* mutations, 36.5% had *KRAS* mutations, 2.3% had *ALK* gene rearrangements, and 79.4% showed positive staining for TTF1. No samples were found to have co-occurring *EGFR*, *KRAS* or *ALK* mutations. Although positive TTF1 IHC was not predictive for the presence of *EGFR* mutations (positive predictive value 13.4%, n= 461) no samples with *EGFR* mutations were negative for TTF1 expression. After additional TTF1 IHC 117 specimens with *EGFR* mutations were positive for TTF1 nuclear staining and 1 showed only cytoplasmic staining and was therefore considered negative; equating to a negative predictive value (NPV) of 99.05%

Four hundred patients with histopathology/ cytopathological samples were diagnosed with adenocarcinoma or non-small cell lung carcinoma between April 2013 and March 2014; of those 370 were stage III or IV and therefore eligible for *EGFR* TKI or *ALK* inhibitor therapy. Based on these figures, the annual cost of lung cancer

molecular pathology testing by the request model was estimated to be 92.5% of that of the reflex model. Testing by the serial model was estimated to cost only 67.3% of the request model. If TTF1 were fully validated as a negative predictive biomarker the cost of testing could be reduced to 62.5% of the reflex model (summarised in table 2).

Table 2: Summary of models

Model	Cohort	Tests carried out	Cost relative to reflex model
Reflex	All non-squamous NSCLC	<i>EGFR, ALK & KRAS</i> simultaneously	100%
Request	Stage III or IV non-squamous NSCLC	<i>KRAS;</i> <i>KRAS</i> neg > <i>EGFR</i> ; <i>KRAS & EGFR</i> neg > <i>ALK</i>	92.5%
Serial		<i>TTF1 & ALK;</i> <i>TTF1</i> pos & <i>ALK</i> neg > <i>KRAS</i> ; <i>TTF1</i> pos & <i>KRAS</i> neg > <i>EGFR</i>	67.3%
TTF1-serial			62.5%

Neg= no mutation detected, Pos= positive expression of protein

DISCUSSION

In our cohort TTF1 IHC had a high negative predictive value (NPV) for *EGFR* mutations supporting the association previously reported in several studies with Caucasian populations,^{8 9 11 14} although this correlation seems to be weaker in East Asian populations.^{15 17-19} Two studies used a scoring system to determine TTF1 status which may have reduced the number of positive samples and therefore lowered the NPV.^{9 18} Our study was carried out in a clinical diagnostic setting and any nuclear staining, even focal staining, was considered positive for TTF1. One sample from a total of 118 *EGFR* mutation positive patients showed only cytoplasmic staining with the TTF1 antibody; upon further investigation this biopsy, which had a p.(Gly719X) *EGFR* mutation, was taken post chemo-radiation. A previous specimen taken pre-treatment was TTF1 positive, however since several years had elapsed between the two samples it was not possible to determine if the latter biopsy was a recurrence or a second primary tumour. Unfortunately, there was too little tissue remaining in the pre-treatment sample to perform *EGFR* mutation analysis.

Many clinical laboratories are experiencing increasing pressures to reduce costs; where funding is restricted algorithms must supply the most cost effective use of limited resources without compromising clinical utility and the welfare of patients. The saving represented by only testing patients eligible for treatment with targeted therapies (the request model) would be easily achievable in most clinical laboratories without any detrimental effect on patient care; a proposal supported by data from another institution.²⁰ In reality NHS Lothian employs a system between the reflex and request models and accepts requests from oncologists if a patient is being considered for treatment or from histopathologists if the diagnostic sample confirms distant metastatic disease.

Currently there is no direct therapeutic impact of *KRAS* nevertheless *KRAS* mutation status in non-squamous NSCLC does have some value, since a large proportion of tumours carry mutations their detection ensures, particularly in samples with a low proportion of neoplastic cells, that the appropriate tissue has been tested. Withdrawing

KRAS analysis would reduce costs by 18% compared to the reflex model. However, stratifying the cohort using the serial model, including *KRAS* analysis, would allow a much greater saving; a 32.7% reduction compared to the reflex model. Laboratories whose primary method is a multiplex assay, for example next generation sequencing (NGS), would not benefit from the serial model; but for many labs NGS is not an option and will, for the foreseeable future, continue to carry out single genes analyses. The value of TTF1 IHC may not be limited to cost efficiency, if an initial specimen was considered insufficient for molecular analysis the TTF1 status may help determine the value of subjecting a patient to a procedure to obtain a repeat sample. Although promising there is, as yet, too little data on the association between TTF1 IHC and *EGFR* to fully support its use as a negative predictor for *EGFR* mutations. Further audits by molecular pathology laboratories could elucidate this relationship and help confirm or refute the use of this readily available histopathology biomarker as a screening tool prior to *EGFR* mutation analysis.

REFERENCES

1. Lindeman NI, Cagle PT, Beasley MB, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. *J Thorac Oncol* 2013;**8**(7):823-59.
2. SMC. Scottish Medicines Consortium: NHS Scotland; [Available from: www.scottishmedicines.org.uk/Home].
3. NICE. National Institute for Health and Care Excellence [Available from: www.nice.org.uk].
4. Sharma SV, Bell DW, Settleman J, et al. Epidermal growth factor receptor mutations in lung cancer. *Nature reviews Cancer* 2007;**7**(3):169-81.
5. Gainor JF, Varghese AM, Ou SH, et al. ALK rearrangements are mutually exclusive with mutations in EGFR or KRAS: an analysis of 1,683 patients with non-small cell lung cancer. *Clin Cancer Res* 2013;**19**(15):4273-81.
6. Rekhtman N, Ang DC, Riely GJ, et al. KRAS mutations are associated with solid growth pattern and tumor-infiltrating leukocytes in lung adenocarcinoma. *Mod Pathol* 2013;**26**(10):1307-19.
7. Dearden S, Stevens J, Wu YL, et al. Mutation incidence and coincidence in non small-cell lung cancer: meta-analyses by ethnicity and histology (mutMap). *Ann Oncol* 2013;**24**(9):2371-6.
8. Vallee A, Sagan C, Le Loupp AG, et al. Detection of EGFR gene mutations in non-small cell lung cancer: lessons from a single-institution routine analysis of 1,403 tumor samples. *Int J Oncol* 2013;**43**(4):1045-51.
9. Vincenten J, Smit EF, Vos W, et al. Negative NKX2-1 (TTF-1) as temporary surrogate marker for treatment selection during EGFR-mutation analysis in patients with non-small-cell lung cancer. *J Thorac Oncol* 2012;**7**(10):1522-7.
10. Krawczyk P, Ramlau R, Chorostowska-Wynimko J, et al. The efficacy of EGFR gene mutation testing in various samples from non-small cell lung cancer patients: a multicenter retrospective study. *J Cancer Res Clin Oncol* 2015;**141**(1):61-8.

11. Chatziandreu I, Tsioli P, Sakellariou S, et al. Comprehensive Molecular Analysis of NSCLC; Clinicopathological Associations. *PLoS One* 2015;**10**(7):e0133859.
12. Sheffield BS, Bosdet IE, Ali RH, et al. Relationship of thyroid transcription factor 1 to EGFR status in non-small-cell lung cancer. *Curr Oncol* 2014;**21**(6):305-8.
13. Somaiah N, Fidler MJ, Garrett-Mayer E, et al. Epidermal growth factor receptor (EGFR) mutations are exceptionally rare in thyroid transcription factor (TTF-1)-negative adenocarcinomas of the lung. *Oncoscience* 2014;**1**(8):522-8.
14. Leary AF, de Castro DG, Nicholson AG, et al. Establishing an EGFR mutation screening service for non-small cell lung cancer - Sample quality criteria and candidate histological predictors. *European Journal of Cancer* 2012;**48**(1):61-67.
15. Zhang Y, Wang R, Li Y, et al. Negative Thyroid Transcription Factor 1 Expression Defines an Unfavorable Subgroup of Lung Adenocarcinomas. *J Thorac Oncol* 2015;**10**(10):1444-50.
16. Shanzhi W, Yiping H, Ling H, et al. The relationship between TTF-1 expression and EGFR mutations in lung adenocarcinomas. *PLoS One* 2014;**9**(4):e95479.
17. Chung KP, Huang YT, Chang YL, et al. Clinical significance of thyroid transcription factor-1 in advanced lung adenocarcinoma under epidermal growth factor receptor tyrosine kinase inhibitor treatment. *Chest* 2012;**141**(2):420-8.
18. Sun PL, Seol H, Lee HJ, et al. High incidence of EGFR mutations in Korean men smokers with no intratumoral heterogeneity of lung adenocarcinomas: correlation with histologic subtypes, EGFR/TTF-1 expressions, and clinical features. *J Thorac Oncol* 2012;**7**(2):323-30.
19. Yatabe Y, Kosaka T, Takahashi T, et al. EGFR mutation is specific for terminal respiratory unit type adenocarcinoma. *Am J Surg Pathol* 2005;**29**(5):633-9.
20. Sauter JL, Butnor KJ. Clinical and Cost Implications of Universal Versus Locally Advanced-Stage and Advanced-Stage-Only Molecular Testing for Epidermal Growth Factor Receptor Mutations and Anaplastic Lymphoma Kinase Rearrangements in Non-Small Cell Lung Carcinoma: A Tertiary Academic Institution Experience. *Arch Pathol Lab Med* 2016;**140**(4):358-61.