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Establishing an *EGFR* mutation screening service for non-small cell lung cancer – Sample quality criteria and candidate histological predictors

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ABSTRACT

Introduction: EGFR screening requires good quality tissue, sensitivity and turn-around time (TAT). We report our experience of routine screening, describing sample type, TAT, specimen quality (cellularity and DNA yield), histopathological description, mutation result and clinical outcome.

Methods: Non-small cell lung cancer (NSCLC) sections were screened for *EGFR* mutations (M+) in exons 18–21. Clinical, pathological and screening outcome data were collected for year 1 of testing. Screening outcome alone was collected for year 2.

Results: In year 1, 152 samples were tested, most (72%) were diagnostic. TAT was 4.9 days (95% confidence interval (CI) = 4.5–5.5). *EGFR*-M+ prevalence was 11% and higher (20%) among never-smoking women with adenocarcinomas (ADCs), but 30% of mutations occurred in current/ex-smoking men. *EGFR*-M+ tumours were non-mucinous ADCs and 100% thyroid transcription factor (TTF1+). No mutations were detected in poorly differentiated NSCLC-not otherwise specified (NOS). There was a trend for improved overall survival (OS) among *EGFR*-M+ versus *EGFR*-M– patients (median OS = 78 versus 17 months). In year 1, test failure rate was 19%, and associated with scant cellularity and low DNA concentrations. However 75% of samples with poor cellularity but representative of tumour were informative and mutation prevalence was 9%. In year 2, 755 samples were tested; mutation prevalence was 13% and test failure only 5.4%. Although samples with low DNA concentration (<2 ng/μL) had more test failures (30% versus 3.9% for [DNA] > 2.2 ng/μL), the mutation rate was 9.2%.

Conclusion: Routine epidermal growth factor receptor (*EGFR*) screening using diagnostic samples is fast and feasible even on samples with poor cellularity and DNA content. Mutations tend to occur in better-differentiated non-mucinous TTF1+ ADCs. Whether these histological criteria may be useful to select patients for *EGFR* testing merits further investigation.

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1. Introduction

Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors such as gefitinib or erlotinib have proven activity in non-small cell lung cancer (NSCLC). Clinical phenotype has been shown to correlate with benefit from these drugs (female, non-smoker, East-Asian ethnicity and adenocarcinoma (ADC)).[1] A number of somatic *EGFR* mutations (*EGFR*-M+) have been identified between exons 18 and 21, the commonest being exon 19 deletions (comprising 45–55% of *EGFR* mutations) and the exon 21 missense mutation L858R (35–45%).[2] *EGFR* genotype is the strongest predictor of clinical benefit. Five randomised Phase III trials have compared gefitinib or erlotinib monotherapy to first-line chemotherapy, all in East-Asian NSCLC patients. Two initial trials used clinically selected criteria to enrich for *EGFR*-M+ NSCLC randomising patients to gefitinib (G) or chemotherapy (C).[3,4] Among *EGFR*-M+ NSCLC both response rate and progression-free survival (PFS) significantly favored gefitinib ($p < 0.001$), while the converse was true for *EGFR*-M– patients. A significant interaction was observed between treatment and *EGFR* mutation ($p < 0.001$) validating *EGFR* genotype as a relevant biomarker.[4] Three subsequent trials in proven *EGFR*-M+ patients[5–7] confirmed a significant PFS improvement for the EGFR tyrosine kinase inhibitors (TKI) compared to chemotherapy.

Thus, *EGFR* mutational screening is relevant if first-line treatment with an EGFR TKI is being considered. This requires access to quality tissue with sufficient tumour cellularity to allow reliable somatic DNA extraction. Unfortunately, the diagnosis of NSCLC is frequently made on cytological specimens or small core biopsies with scanty tissue. The 'gold-standard' for *EGFR* analysis has been direct sequencing of exons 18–21 using surgical specimens. It offers the advantage of detecting all mutations, known and unknown, but is time-consuming and has low sensitivity due to wild-type germline admixture requiring tissue microdissection to minimise normal tissue contamination.[8] Therefore increasingly, more sensitive mutation-targeted screening approaches are being used such as mutant-enriched sequencing or amplification refractory mutation system (ARMS). These technologies are faster but will only identify known mutations.[9]

Real-time, high-throughput routine *EGFR* mutation testing began at the Royal Marsden Hospital (RMH) in January 2009. Screening was initially conducted using the TheraScreen EGFR29 ARMS system (DxS Ltd., Manchester, United Kingdom (UK)) which detects 29 common somatic mutations identified from COSMIC (catalogue of somatic mutations in cancer, http://www.sanger.ac.uk/genetics/CGP/cosmic/).

There are currently no clear guidelines defining the sample quality criteria required for targeted mutational analyses. In addition there is no consensus on whether *EGFR* testing in non-Asian populations should be limited to NSCLC patients with certain demographic and/or pathological characteristics. Information obtained in routine histology i.e. morphology and immunohistochemistry could enrich the population for testing. We therefore aimed to determine the feasibility of prospective *EGFR* testing in routine clinical practice and describe demographics, clinical outcome, histological subtype, testing turn-around time (TAT), screening outcome (including test failure rates) and sample quality (cellularity, DNA yield) in our series of prospectively screened NSCLC patients.

2. Methods

We reviewed the first year of screening (January 2009 to January 2010) for which detailed clinical and pathological data were available. Screening outcome was also collected for the second year (January 2010 to January 2011).

2.1. Patients

Tumour samples (formalin fixed, paraffin-embedded (FFPE) blocks, unstained sections or FFPE cell pellets) from patients with NSCLC were received in the RMH Department of Molecular Diagnostics. Prior to DNA extraction, unstained sections were reported by a histopathologist as (1) good cellularity and >30% tumour cells, (2) good cellularity but <30% tumour cells, (3) poor cellularity but representative of tumour, (4) scanty cellularity, necrotic or unrepresentative. However, some sections sent from external hospitals had not undergone pathological review. Demographic and clinical data were available for year 1 patients from the RMH Lung Unit Research Database. In addition, histopathological subtyping was available on samples from one referring institution ($N = 96$, Royal Brompton Harefield Trust Hospital (RBHFT)); all cases were examined by a thoracic pathologist (A.G.N.) and classified using the 2004 World Health Organisation (WHO) classification[10] and the updated International Association for the Study of Lung Cancer (IASLC)/American Thoracic Society (ATS)/European Respiratory Society (ERS) ADC classification of 2011.[11] Data were also collected on TTF-1 (Dako Ltd., UK) and cytokeratin 7 (CK7) (Dako Ltd., UK) positivity. All data were de-identified to ensure patient confidentiality. The Clinical Audit Committee approved the study. Patient consent was not required as this was an approved audit of routine clinical care, with all data anonymised.

2.2. Mutation testing

Genomic DNA was extracted from all specimens regardless of sample cellularity from 10 μm sections (QIAamp DNA FFPE kit, Qiagen, UK) and concentration was determined (ND-1000 spectrophotometer, Nano-Drop Technologies). For samples analysed between January and December 2009, *EGFR* mutational screening was conducted using the TheraScreen EGFR29 ARMS mutation kit (DxS Ltd., Manchester, UK) according to manufacturer's instructions. All testing was conducted in the Department of Molecular Diagnostics at the Royal Marsden Hospital and the Institute of Cancer Research, a CPA (Clinical Pathology Accreditation) accredited laboratory. The EGFR29 ARMS method has a sensitivity limit of 1%. Positive controls for the most common mutations were included in every run. After January 2010, *EGFR* mutation screening was performed by triple assessment using previously internally validated methods: a combination of ARMS (for L858R and T790M mutations), fragment analysis (for exon 19 deletions and exon 20 insertions) and direct sequencing (for the rarer exon 18 or exon 21 mutations). Mutation testing was

defined as successful if the cycle threshold (Ct) value for the control gene was <33. The TAT was examined and defined as the number of working days between the date the sample was received in the Molecular Diagnostics Laboratory and the date results were available for the clinician.

2.3. Statistical methods

Differences between means were assessed by unpaired two-tailed *t*-test. Where data were not normally distributed, medians and interquartile range (IQR) or geometric means and 95% confidence intervals (95% confidence interval (CI)) were used and differences assessed using a Mann–Whitney test. Overall survival was measured from date of diagnosis until death or last follow-up and was analysed using Kaplan–Meier estimates and log-rank testing.

3. Results

3.1. Mutation prevalence – year 1 of testing

One hundred and fifty-two NSCLC samples were received during the first year of screening. The sample source was a core biopsy of the primary or metastatic site in the majority of cases (72%; Table 1). These 152 samples belonged to 144 patients, eight samples being duplicates from the same patient (Fig. 1). Sixteen of 144 patients tested were *EGFR* mutant (11%). Fifty percent (8/16) of *EGFR* mutations were exon 19 deletions, 38% (6/16) were exon 21 L858R point mutations, and 12% (2/16) were exon 20 insertions.

The mean TAT from the time the sample was received in RMH/Molecular Diagnostics to genotyping result was 4.9 business days (95% CI = 4.5–5.5). However, from the perspective of the oncologist, the actual time from request to result was significantly longer at 17.8 days (95% CI = 16.4–19.4), as this interval takes into account the time to request sample from referring hospital, sample identification and retrieval, section cutting, shipping and pathological review.

3.2. Patient demographics, treatment and clinical outcome

Demographic and clinical data were available on 121 patients tested during year 1. Patients screened tended to be Caucasian–European (82%), female (59%) with an ADC (67%). The majority had advanced disease (III/IV) at presentation (87%). Only 9% were stage I/II and 4% unknown. Among *EGFR*-M+ patients, 100% had an ADC and 64% were female. Seventy percent were Caucasian, 7% Chinese and 23% non-disclosed. While the majority were never smokers or past smokers (all >20 years since cessation), 14% were current smokers with a 50 and 70 pack year history, respectively. Mutation rate among female never-smokers with an ADC was slightly higher at 20%. However five (31%) *EGFR* mutations were detected in tumours from current or ex-smoking male patients.

Among patients who received first-line systemic treatment for metastatic disease ($N = 99$) regardless of *EGFR* status, the majority (84/99) were treated with chemotherapy with a response rate of 33%. Only 15% (15/99) of patients received an EGFR TKI in the first-line setting with a response rate of 47%. Almost two thirds (65%) of patients proceeded to second line therapy, 52% of which received an EGFR TKI with a response rate of 15%. During most of the 1st year of screening, EGFR TKIs were available in the UK in the second line setting only, regardless of mutational status. All *EGFR*-M+ patients received or were planned for treatment with a TKI except for one patient who refused. In this series of patients with metastatic NSCLC, including 87% with stage III/IV disease at presentation, the median survival from diagnosis was 19 months, with a non-significant trend in favour of *EGFR*-M+ versus *EGFR*-M– patients (median OS = 78 months versus 17 months; $p = 0.086$, Fig. 2).

3.3. Association between sample cellularity, DNA yield and mutation testing outcome – year 1

DNA extraction and mutation screening were performed on all samples received regardless of the outcome of pathological assessment. Among 152 samples received during year 1 of *EGFR* mutation screening, suboptimal DNA samples were obtained for 29 cases (test failure rate of 19%). Over time, testing failure rate improved markedly from 33% during the first 3 months to 13% in the last 3 months of year 1 of testing.

Sample cellularity and DNA yield were examined next in an effort to assess the suitability of this series of routine diagnostic samples for genomic analyses. Among the subset of samples examined, 56% of the samples demonstrated good cellularity and >30% tumour content, another 11% had good

Table 1 – Sample source during first year of *EGFR* testing.

Sample source		Percent of samples ($N = 152$)
Surgical resection	Lung	7% (11)
	Excisional biopsy (bone, brain or lymph node)	7% (11)
Total surgical resections		**14% (22)**
Pulmonary core biopsy	computed tomography guided biopsy	38% (57)
	Transbronchial biopsy	16% (24)
	Pleural biopsy	8% (12)
	Lymph node biopsy	2% (3)
Extrapulmonary core biopsy	Bone	3% (4)
	Other (liver, adrenal, soft tissue)	6% (9)
Total core biopsies		**72% (109)**
Cell pellet from cytological specimen		**4% (6)**
Unknown		**10% (15)**

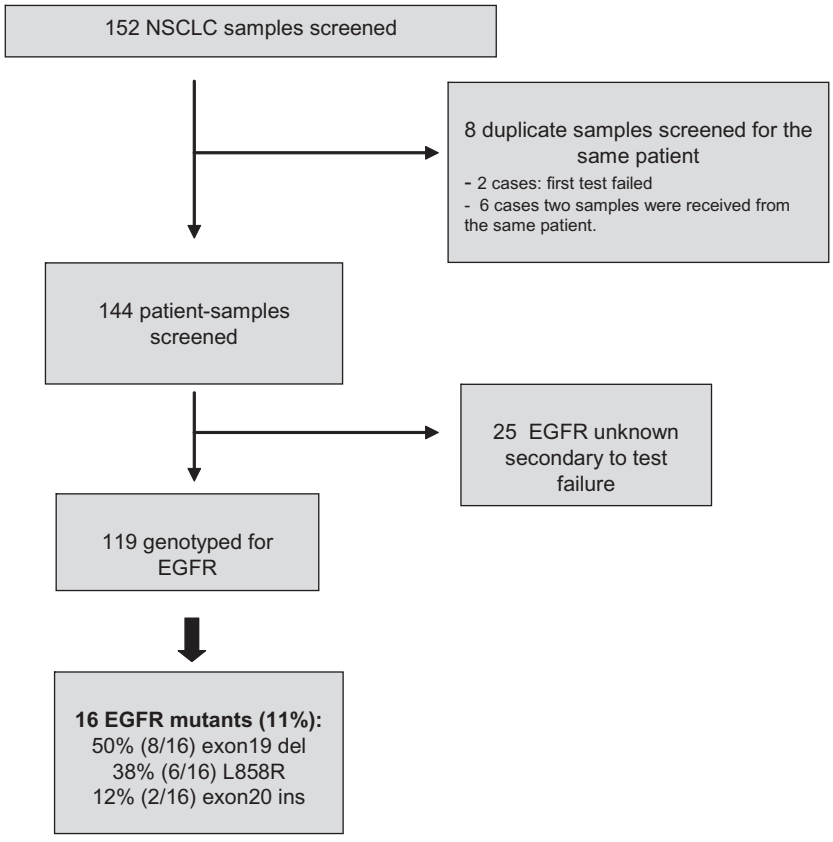

Fig. 1 – Non-small cell lung cancer (NSCLC) samples submitted to epidermal growth factor receptor (EGFR) testing at the Royal Marsden Hospital during year 1 of routine screening from January 2009 to January 2010.

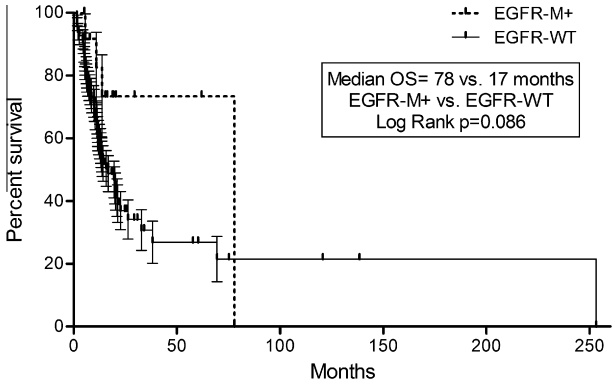

Fig. 2 – Kaplan–Meier curves for overall survival from diagnosis. Survival data were available for 119 year 1 patients, including 14 epidermal growth factor receptor (EGFR) mutants.

cellularity but less than 30% tumour content. Over a quarter of samples (28%) pathologically examined demonstrated poor cellularity but were representative of the tumour. DNA yield in individual samples was highly variable ranging from none detected to 1627 ng/μL with a median of 10.2 ng/μL (95% CI:7.7–14.9 ng/μL).

Samples with the best tumour cellularity (good cellularity and >30% tumour content) were associated with high DNA yield, 91% testing success rate and an *EGFR* mutation rate of 9% (Fig. 3A–C). Although samples with good cellularity but <30% tumour content yielded less DNA (mean = 8.1 versus 25.7 ng/μL, $p = 0.1$, Fig 3A) the difference was not significant and the genotyping success and mutation rates remained comparable to the best quality samples (Fig. 3B and C). Samples with poor cellularity but representative of the tumour yielded significantly less DNA (2.8 ng/μL) but demonstrated a test success rate of 77% and a mutation prevalence of 9% comparable to the mutation rate in the group of samples with the best tumour cellularity. In contrast, among the worst quality samples (scanty or necrotic) only two of six could be genotyped and no mutations were detected.

3.4. *Year 2 of EGFR testing*

There was a marked increase in the number of samples screened from 33 in the first 6 months of year 1 to 473 in the last 6 months of year 2 (Fig. 4). *EGFR* mutation rate during year 2 was 13% (99/755). Among the 99 *EGFR* mutations, the distribution in decreasing order of frequency was as follows: exon19 deletions ($N = 46$), L858R ($N = 36$), exon20 insertions ($N = 9$), exon18 mutations ($N = 5$), other exon21 mutations ($N = 3$). Test failure rates dropped significantly from 19% in the first year (27% and 16% for 1st and 2nd 6-months, Fig 4) to 5% in year 2 (4% and 7% for 1st and 2nd 6-months, Fig 4) resulting in an informative testing rate of 92% for the first two years of *EGFR* screening.

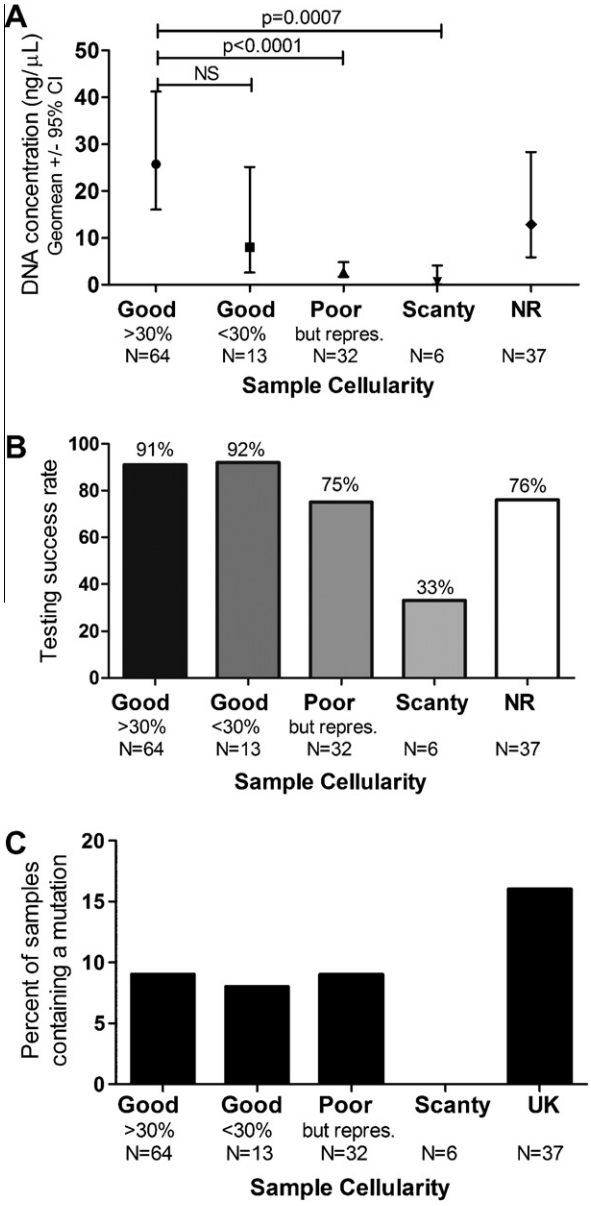

Fig. 3 – DNA yield, test failure rate and mutation prevalence according to sample cellularity. One hundred and fifteen of the 152 samples were submitted to pathological review prior to DNA extraction. Sample cellularity was ascribed to one of four categories: (1) good cellularity and >30% tumour content (*N* = 64); (2) good cellularity but <30% tumour content (*N* = 13); (3) poor cellularity but representative of tumour (*N* = 32); (4) scanty or necrotic cellularity (*N* = 6).

Reliable source data on specimen type and cellularity was not available for year 2 samples because the majority of requests were external referrals, but DNA concentration was measured for year 2 samples. Using a DNA concentration cut-off of 2 ng/μL (the lower limit of detection for DNA by spectrophotometry), 13% (97/755) of year 2 samples contained low levels of DNA (<2 ng/μL), compared to 22% (34/152) in year 1. As expected, samples yielding <2 ng/μL DNA were associated with a higher failure rate of 30% (versus 4% among samples with >2 ng/μL DNA), but the mutation detection rate was

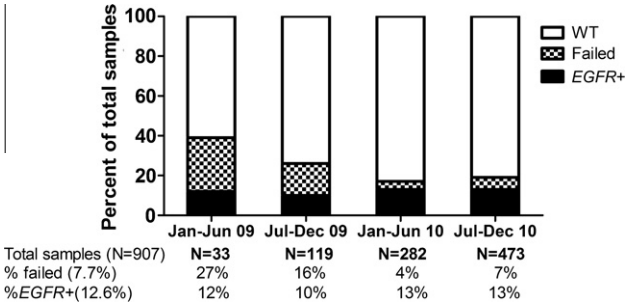

Fig. 4 – Testing outcome of samples analysed in the first 2 years and proportion of wild-type *EGFR* (WT), failed test and *EGFR* mutated (*EGFR*+).

9%, only slightly lower than the mutation rate in samples with >2 ng/μL DNA (Table 2).

3.5. *Correlation between EGFR mutation status and histopathological subtype*

Detailed histological descriptions were available on a further subset of samples referred by a single external institution (RBHFT, *N* = 96), during the second year of testing. In this selected group which only included 5% of squamous NSCLC, the mutation rate was higher at 21%. *EGFR* mutations were limited to tumours identifiable as non-mucinous ADCs histologically (Table 3); no mutations were detected among mucinous ADCs or among the NSCLC-NOS refined to ADC by immunohistochemistry (e.g. TTF1 and/or CK7-positive). *EGFR*-M+ tumours were also 100% TTF1-positive, compared to only 73% for EGFR wild-type. In *EGFR*-M+ resections, 50% had acinar pattern (2/4), and one each had a predominant papillary or micropapillary pattern. In EGFR wild-type resections, 17% had a predominantly solid subtype compared to 0% in *EGFR*-M+ ADCs.

4. Discussion

Given the strong evidence for a clinical benefit of EGFR tyrosine kinase inhibitors in patients with *EGFR*-M+ NSCLC, systematic molecular profiling should be integrated early in the diagnostic pathway. High-throughput *EGFR* mutation screening for clinical practice requires tumour tissue of adequate quality, a sensitive assay and acceptable laboratory turn-around time. Results of prospective mutational testing at our institution illustrate the feasibility of incorporating *EGFR* screening into routine patient management, with a testing TAT of 4.9 days. Including years 1 and 2, we report an *EGFR* mutation prevalence of 13% among a selected population, comparable to other series in Europe and the United States.[12,13]

Although access to first-line EGFR TKIs was somewhat limited at the time of this series, over 80% of *EGFR*-M+ treated received an EGFR TKI in the first or second line. Overall survival was 19 months, with a trend for an OS advantage for *EGFR* mutation+ compared to *EGFR* wild-type patients. Our data are comparable to trial datasets[4] and to the largest prospective European screening programme conducted to date in Spain.[13]

Table 2 – Test Failure rate according to DNA concentration. During the first two years of screening, 907 samples were tested, 14% (131/907) demonstrated low DNA concentrations, e.g. <2 ng/μL.

DNA concentration	Sample tested N	Failed tests N (%)	Mutated N (%)
<2 ng/μL	131	40 (30%)	12 (9%)
>2 ng/μL	776	30 (4%)	103 (13%)

Given the low prevalence of *EGFR* mutations in Caucasian populations, selection criteria to identify those tumours most likely to harbour the mutation would be useful. Although targeting testing to non-smoking women with an adenocarcinoma would have increased mutation detection rate from 11% to 20% in the series of patients tested during year 1, over 30% of mutant tumours (found in male current or ex-smokers) would have been missed. Detailed immuno-histopathological review of 96 cases, demonstrated that *EGFR*-M+ tumours were all non-mucinous ADCs and tended to be well differentiated: they were all TTF1-positive and half *EGFR*-positive resections were papillary or micropapillary. In contrast, none of the NSCLC-NOS were mutated, not even those re-classified as ADC on the basis of adenocarcinomatous features (e.g. TTF1 and/or CK7-positive). This is in line with published observations that *EGFR*-mutated cancers tend to be low grade, papillary, well-differentiated ADCs.[14] Further investigations are required to determine whether pathological features may be predictive of *EGFR* mutation in a non-Asian population.

One of our objectives was to audit the suitability of routine diagnostic samples for high-throughput *EGFR* testing and contribute towards establishing a quality assurance (QA) benchmark. In an effort to gain a greater understanding of the sample quality criteria for optimal genomic analyses in routine clinical practice, all samples were tested regardless of histological assessment or DNA yield, giving a clearer estimate of the denominator for mutation testing success. The testing failure rate improved steadily from 27% in the first 6 months to less than 6% in year 2, likely attributable to increased technical expertise. In contrast to direct sequencing where the commonly accepted threshold to define an adequate sample is 50% tumour cellularity on the basis of a sensitivity to detect at least 25% mutant DNA admixed with normal DNA for a heterozygous mutation,[9,15] no such cut-off has been defined for targeted mutational analyses which usually have sensitivities below 5%.[9] Although samples with >30% tumour cellularity yielded the highest DNA concentrations and a successful test in 91% of cases, samples with poor cellularity but representative of tumour resulted in an informative result in 75% of cases suggesting that low cellularity may be acceptable for targeted mutational analysis after careful pathological review. DNA obtained from FFPE specimens can be highly degraded, a sample with high concentration

Table 3 – Histopathological analysis of 96 samples from Royal Brompton Harefield Trust Hospital (RBHFT). Between January 2010 and January 2011 there were 96 requests for (*EGFR*) mutation analysis from the RBHFT on 94 patients. These samples were reviewed by a single pathologist (A.G.N.) and classified as squamous non-small cell lung cancer (NSCLC), adenocarcinoma (ADC) or NSCLC-not otherwise specified (NOS) based on the World Health Organisation (WHO) 2004 classification. For those classified as NSCLC-NOS on H&E examination alone, further immunochemical staining was performed, and tumours re-classified as NSCLC-ADC if demonstrating adenocarcinomatous differentiation, e.g. TFF1 and/or cytokeratin 7 (CK7)-positivity.

	EGFR mutation positive	N = 20	*EGFR* mutation negative	N = 76
Tumour subtype (N = 96)	Non-mucinous ADC	20	Non-mucinous ADC	48
			Mucinous ADC	7
			Squamous cell cancer (SQCC)	3
			Thymoma	2
			NSCLC	16
			NSCLC-ADC	10
			NSCLC-squamous differentiation (SQ)	2
			NSCLC-pleomorphic	1
			NSCLC-NOS	3
Tumour pattern (resections only, N = 27)	Resections	N = 4	Resections	N = 23
	Acinar predominant	2	Acinar predominant	12
	Papillary predominant	1	Papillary predominant	2
	Micropapillary predominant	1	Micropapillary predominant	5
	Lepidic predominant	0	Lepidic predominant	0
	Solid predominant	0	Solid predominant	4
TFF1-positive		15/15 (100%)		40/55 (73%)

Abbreviations: ADC, adenocarcinoma; SQCC, squamous cell cancer; NSCLC, non-small cell lung cancer; NOS, not otherwise specified; SQ, squamous differentiation.

of degraded DNA, may not be suitable for mutation analysis, whilst a small DNA sample from a well-preserved specimen can be optimal. In year 2, out of 97 samples with <2 ng/μL DNA, 12 *EGFR* mutations were detected supporting that even samples with low levels of DNA may be appropriate as long as the specimens are rigorously processed and fixed to minimise DNA degradation.[16]

The debate is ongoing regarding the most appropriate *EGFR* testing methodology for diagnostic application.[17] The EGFR29 ARMS was the screening test used in trial Iressa Pan-Asia Study (IPASS) trial and during our first year of testing. A recent report suggested that mutation specific polymerase chain reaction (PCR) may miss a significant proportion of mutations detected by sequencing.[18] We have compared ARMS and sequencing on the same samples and shown that 10–20% of mutations are missed by ARMS, but that 20% of mutations detected by ARMS at low levels are missed by direct sequencing (unpublished data). The best way forward may therefore be a combination of screening technologies. In January 2010, the screening protocol for *EGFR* at the Royal Marsden Hospital/Institute of Cancer Research was changed to a triple assessment (a combination of ARMS, fragment analysis and direct sequencing for exons 18–21).

We have shown that routine targeted *EGFR* screening using diagnostic samples is fast and feasible, with over 900 samples now screened, and a test failure rate dropping to 6%. Given the respective limitations of individual currently available *EGFR* testing methodologies, we now use a triple assessment for optimal *EGFR*-M+ detection. While pathological review remains critical, even small samples with poor cellularity and low DNA yield may be successfully tested as long as they are representative of tumour and have been fixed and processed to maximise DNA integrity.

Selecting patients for *EGFR* screening on the basis of phenotype (non-smoking female ADC) may not be appropriate in a (UK) population as a significant proportion of mutations may be missed. Mutations tended to occur in better-differentiated non-mucinous TTF-1-positive ADCs. Whether these histological criteria may be more useful than smoking and gender in selecting patients for *EGFR* testing in a non-Asian population merits further investigation.

Conflict of interest statement

A.L.: none. D.G. discloses honoraria from Astra Zeneca and GSK. A.W.: none. S.A.: none. A.G.N. for advisory boards for GSK, AZ, Eli Lilly and Oncimmune Ltd. in relation to management of patients with lung cancer. M.O.B advisory board work for AZ, Roche, Boeringher, and two grants from Roche. S.P.: none.

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REFERENCES

1. Tsao MS, Sakurada A, Cutz JC, et al. Erlotinib in lung cancer – molecular and clinical predictors of outcome. *N Engl J Med* 2005;**353**:133–44.
2. Sharma SV, Bell DW, Settleman J, et al. Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer* 2007;**7**:169–81.
3. Lee DH, Park K, Kim JH, et al. Randomized Phase III trial of gefitinib versus docetaxel in non-small cell lung cancer patients who have previously received platinum-based chemotherapy. *Clin Cancer Res* 2010;**16**:1307–14.
4. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009;**361**:947–57.
5. Zhou C, Wu YL, Chen G, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol* 2011;**12**:735–42.
6. Mitsudomi T, Morita S, Yatabe Y, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol* 2010;**11**:121–8.
7. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* 2010;**362**:2380–8.
8. Molina-Vila MA, Bertran-Alamillo J, Reguart N, et al. A sensitive method for detecting EGFR mutations in non-small cell lung cancer samples with few tumor cells. *J Thorac Oncol* 2008;**3**:1224–35.
9. Pao W, Ladanyi M. Epidermal growth factor receptor mutation testing in lung cancer: searching for the ideal method. *Clin Cancer Res* 2007;**13**:4954–5.
10. Travis WD, Brambilla E, Muller-Hermelink H-K, et al. *WHO classification: pathology and genetics. Tumours of the lung, pleura, thymus and heart*. Lyon, France: IARC Press; 2004.
11. Travis WD, Brambilla E, Noguchi M, et al. International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. *J Thorac Oncol* 2011;**6**:244–85.
12. D'Angelo SP, Park B, Azzoli CG, et al. Reflex testing of resected stage I through III lung adenocarcinomas for EGFR and KRAS mutation: report on initial experience and clinical utility at a single center. *J Thorac Cardiovasc Surg* 2010.
13. Rosell R, Moran T, Queralt C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. *N Engl J Med* 2009;**361**:958–67.
14. Motoi N, Szoke J, Riely GJ, et al. Lung adenocarcinoma: modification of the 2004 WHO mixed subtype to include the major histologic subtype suggests correlations between papillary and micropapillary adenocarcinoma subtypes, EGFR mutations and gene expression analysis. *Am J Surg Pathol* 2008;**32**:810–27.
15. Smouse JH, Cibas ES, Janne PA, et al. EGFR mutations are detected comparably in cytologic and surgical pathology specimens of nonsmall cell lung cancer. *Cancer Cytopathol* 2009;**117**:67–72.
16. von Ahlfen S, Missel A, Bendrat K, et al. Determinants of RNA quality from FFPE samples. *PLoS One* 2007;**2**:e1261.
17. Pirker R, Herth FJ, Kerr KM, et al. Consensus for EGFR mutation testing in non-small cell lung cancer: results from a European workshop. *J Thorac Oncol* 2010;**5**:1706–13.
18. Penzel R, Sers C, Chen Y, et al. EGFR mutation detection in NSCLC-assessment of diagnostic application and recommendations of the German Panel for Mutation Testing in NSCLC. *Virchows Arch* 2011;**458**:95–8.