

Accepted Manuscript

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PII: S1556-0864(17)32760-0

DOI: [10.1016/j.jtho.2017.09.1958](https://doi.org/10.1016/j.jtho.2017.09.1958)

Reference: JTHO 743

To appear in: *Journal of Thoracic Oncology*

Received Date: 17 May 2017

Revised Date: 13 September 2017

Accepted Date: 22 September 2017

Please cite this article as: Tokaca N, Barth S, O'Brien M, Bhosle J, Fotiadis N, Wotherspoon A, Thompson L, Popat S, Molecular adequacy of image-guided rebiopsies for molecular retesting in advanced non-small cell lung cancer: a single centre experience, *Journal of Thoracic Oncology* (2017), doi: 10.1016/j.jtho.2017.09.1958.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Molecular adequacy of image-guided rebiopsies for molecular retesting in advanced non-small cell lung cancer: a single centre experience

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Disclosure: No conflicts of interest are declared by the authors.

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Keywords: lung cancer; biopsy; genotype; non-small cell lung cancer

Word count: 3233 (excluding title page, abstract, tables and references).

ACCEPTED MANUSCRIPT

ABSTRACT

Word count = 228/250

Introduction

In the era of biomarker-driven systemic therapy for advanced non-small cell lung cancer (NSCLC), the role of routine repeated biopsies for decision-making, outside EGFR mutant disease, remains unproven. We report our centre's experience of safety and adequacy for molecular retesting of tumour material obtained from image-guided lung rebiopsies in NSCLC.

Methods

We performed a retrospective case-note analysis of patients undergoing image-guided lung rebiopsies at a single cancer centre between 2011-14. The primary objective was the pathological success rate. Secondary and exploratory objectives were technical success rate, histological concordance, molecular adequacy, genotypes identified and complication rate.

Results

103 patients underwent transthoracic image-guided procedures. 66 rebiopsies in NSCLC were identified and analysed. Pathological success rate was 87.1%. A high histological discordance rate was observed (12/52 evaluable cases, 23.1%). Pre-test molecular adequacy as determined by the lung pathologist was 78.8% (52/66). 51 out of 52 adequate samples were sent for molecular analysis with a total of 209 genes analysed including *EGFR*, *ALK*, *KRAS*, *BRAF*, *DDR2*, *NRAS*, *ROS1* and *RET*. Post-genotyping molecular adequacy was 87.1% (182/209). 20 new potentially actionable mutations were identified, with 13/66 (19.7%) patients commencing new targeted treatment as a result. Overall, rebiopsies informed clinical decision-making in 63.6%. Rates of complications were pneumothorax 15%, pneumothorax requiring chest drain 3% and haemoptysis 8%.

Conclusion

- 30 We validate the pathological and molecular adequacy rates of rebiopsies and demonstrate
31 clinical utility in routine decision-making.

1 INTRODUCTION

2 Lung cancer is the commonest cause of cancer related mortality in men and women
3 worldwide,^{1, 2} with more than 80% classified as non-small cell lung cancer (NSCLC).
4 Identification of driver somatic aberrations in advanced NSCLC has led to rational
5 implementation of genotype-directed therapy, with international guidelines recommending
6 molecular testing^{3, 4} since EGFR and ALK kinase inhibitors have demonstrated marked
7 superior efficacy over chemotherapy in those harbouring activating *EGFR* mutations and *ALK*
8 rearrangements, respectively, and are licensed for 1st line therapy, alongside ROS1 kinase
9 inhibitors.^{5, 6, 7, 8, 9, 10} However, multiple mechanisms of acquired resistance to molecular-
10 directed therapy have been identified including emergence of additional somatic mutations
11 with reduced affinity for drug, for instance the *EGFR* T790M gatekeeper,¹¹ but also other less
12 common mechanisms such as histological non-concordance^{12, 13, 14} or bypass track activation
13 e.g. through gene amplification.¹⁵

14

15 Therapeutic strategies to overcome mechanisms of acquired resistance are being developed,
16 and in some cases licensed. For example, the EGFR mutation-specific kinase inhibitor
17 osimertinib is active both against classical activating *EGFR* mutations (e.g. L858R or exon 19
18 deletion) and the resistance mutation T790M, resulting in FDA and EMA licenses for NSCLC
19 progressing on or after first-line EGFR-TKI (afatinib/erlotinib/gefitinib) and with evidence of
20 T790M.^{16, 17}

21

22 Other potentially targetable somatic aberrations have been identified in up to 70% of patients
23 with adenocarcinoma sub-type NSCLC¹⁸ and in more than 50% of squamous NSCLC¹⁹ and a
24 variety of global efforts are underway to identify and validate the efficacy of genotype-directed
25 therapy in relapsed NSCLC through the multi-arm multi-agent (MAMA) designed trials, such
26 as the NCI-MATCH trial (NCT02465060) and the UK National Lung MATRIX Trial
27 (NCT02664935). Whilst circulating tumour DNA (ctDNA) genotyping is an effective and
28 validated technology for some alleles (e.g. *EGFR*-T790M), contingent on clinical setting, the
29 low specificity of some genotyping technologies coupled with the low ctDNA shedding rate for
30 M1a NSCLC may limit clinical interpretation.

Therefore, repeated biopsies for molecular characterisation purposes may be indicated for the optimal management of patients with relapsed advanced NSCLC, and are recommended especially in tumours with oncogene addiction to identify resistance-associated genotypes and guide therapy choice.^{3, 20}

Image guided percutaneous transthoracic core needle biopsies are a standard diagnostic tool used to obtain tumour tissue at point of diagnosis or relapse. Safety and tissue diagnostic yields of biopsies at first diagnosis of lung cancer are well established.^{21, 22, 23} However, data remain limited on the adequacy of tumour material obtained by repeat image-guided percutaneous biopsies in order to molecularly characterise tumours for clinical decision making. Here, we report our centre's experience of safety and adequacy for molecular testing of tumour material obtained from image-guided transthoracic rebiopsies in NSCLC patients.

METHODS

This is a retrospective analysis of patients undergoing image-guided lung rebiopsies at a single cancer centre between 2011 and 2014. Rebiopsy was defined as biopsy after cancer progression following anti-cancer therapy (any line) or repeated biopsy where initial histological or molecular analysis was inadequate or incomplete for clinical decision-making. This study was approved by the local audit committee.

Patients

Patients were identified through search of electronic patient records for those with diagnosis of NSCLC undergoing image-guided lung biopsies between November 2011 and April 2014. Patients with other primary thoracic malignancies (e.g. small cell lung cancer, mesothelioma, thymic malignancies, carcinoid tumours) were excluded.

Individual case notes were hand-searched for pre-defined data items including fields on demography (age, gender, smoking history, pulmonary comorbidities, history of other malignancies), lung cancer (diagnosis, disease stage, number of previous lines of systemic

anti-cancer treatment, somatic mutational status at biopsy time), rebiopsy data (biopsy indication, image guidance mode, number of passes, needle gauge, number of cores obtained), post-procedure complications (pneumothorax, haemoptysis, hospitalization), rebiopsy tissue sample (presence/absence of malignancy, histological subtype, molecular analysis performed, mutations identified, molecular success, molecular failure reasons). A validated data capture spreadsheet was created and populated by two independent investigators (NT, SB) who reviewed case-notes, identified and entered data. Disagreements were reviewed and consensus sought with arbitration by a third reviewer (SP).

Objectives

Primary objective was to determine the pathological success rate, defined as proportion of rebiopsy cases confirmed to contain malignant cells (as documented in the pathology reports).

Secondary and exploratory objectives included: technical success rate; concordance of pre- and post-biopsy histological subtype; adequacy of rebiopsy material for molecular analysis; number and nature of new mutations identified; and incidence of complications.

Definitions

Technical success was defined as successful insertion of biopsy needle into target lesion and cells or lung tissues were present in specimen, as documented in the pathology reports. Histological concordance was determined by comparison of original histological diagnoses, as documented in case-notes, with histological diagnoses on rebiopsy specimens, which were reviewed and classified by a dedicated lung pathologist using the 2015 WHO classification. Diagnostic biopsies were re-reviewed by a dedicated thoracic pathologist where possible. Molecular analysis of rebiopsy material was performed as clinically indicated for individual cases. Adequacy of rebiopsy material for molecular analysis was defined as minimum 30% viable tumour cells in sample, as assessed by a dedicated thoracic pathologist as per routine practice. Reasons for inadequacy as reported by the pathologist were identified by case notes review and grouped into consistent themes. Post-test molecular success rate

was defined as the proportion of successfully informative individual gene analyses out of the total number of genes analysed.

Statistical analysis

Differences in inter-gene failure rates were tested using the chi-square test for comparing multiple proportions with a significance level of $\alpha=0.05$, with Bonferroni correction for multiple pairwise comparisons. The relationship between number of cores (<3 versus ≥ 3 cores) and molecular adequacy was tested using the Fisher's exact test,

RESULTS

Patients

One hundred and three patients were identified from case-notes searching with a diagnosis of thoracic malignancy undergoing image-guided percutaneous transthoracic procedures between November 2011 and April 2014. 7 patients had pleural drain insertion or pleural fluid aspiration and were excluded from analysis. 16 out of 103 patients underwent an initial diagnostic biopsy for suspected lung cancer (14 to obtain a histological diagnosis and 2 for completion of staging at diagnosis), and were excluded from further analysis, as this was an initial biopsy as opposed to a rebiopsy. 14 patients with a diagnosis of other thoracic malignancy including 10 mesotheliomas, 2 SCLC, and 2 thymic malignancies, were excluded from further analysis.

66 patients with NSCLC rebiopsy were included in final analysis. Patient characteristics are summarised in Table 1.

Procedures

Mode of image guidance was computed tomography (CT) in 60 out of 66 cases (91%) and ultrasound (US) in 6 cases (9%). Four patients had a CT-guided chest wall biopsy. All procedures were performed by an experienced interventional radiologist using dedicated CT-guided biopsy software (i-sequence and i-spiral) on a Somatom Definition Edge CT scanner (Siemens, Erlangen, Germany). Rapid on-site evaluation (ROSE) was not used for any of the procedures.

Although all rebiopsies were considered for molecular analysis, primary indications for rebiopsy varied. Majority of patients underwent rebiopsy primarily for molecular testing (41/66, 62.1%), including 11 patients for first-time molecular analysis, 13 patients for repeat analysis due to previous failure, 11 for expanded molecular profiling and 6 for EGFR T790M mutation detection. In 12 patients documented primary indication for repeat biopsy was histological confirmation of disease relapse, in 4 patients primary indication was to exclude clinical suspicion of high grade neuroendocrine transformation, while in 2 patients it was disease restaging. Seven out of 66 patients had a rebiopsy in the context of a research protocol.

Technical success was achieved in all 66 patients (100% rate). Mean target lesion size was 40.7mm (95% CI: 35.9–45.5), with mean distance to pleura of 15mm (95% CI: 11.35–18.55). A range of needle gauge sizes was used, from 14G to 18G, with majority procedures performed using an 18G needle (86% or 45/52 cases where needle gauge size was documented). Median number of cores obtained was 3 (range: 1 to 6), in one case reported as “multiple”, and not documented in 3 cases. Target lesion locations were evenly distributed between all lobes of the lung (53% in upper and 45% in lower lobes), with one lesion located in the right middle lobe.

Pathological findings

Pathological success was achieved in 54 out of all 66 patients (81.8%). In 8 patients no malignant cells were found in the sample. Presence or absence of malignant cells was non-evaluable in 4 cases, when rebiopsy was performed as part of a research protocol. These 4 cases were not evaluated for histopathology and were therefore excluded from further analyses. Therefore the pathological success rate for evaluable cases was 54/62 (87.1%).

Histological concordance was evaluable in 52 cases (in 2 out of 54 cases containing malignant cells histological subtype was not reported on rebiopsy tissue). Concordance of pre- and post-rebiopsy histological subtype was observed in 40/52 (76.9%). Discordance was observed in 12 (23.1%) cases as detailed in Table 2. In one case, rebiopsy sample histopathology was consistent with thymoma, in a patient with known synchronous diagnoses of NSCLC adenocarcinoma and thymoma.

Molecular analysis

Fifty two cases were adequate for further molecular analysis as subjectively determined by the lung pathologist, resulting in pre-test molecular adequacy of 78.8% of all rebiopsy cases. 2 cases containing malignant cells (pathologically successful) were inadequate for molecular analysis due to "poor sample quality".

Molecular analysis was performed in 51/66 patients, resulting in a total number of 209 genes analysed. In one patient whose rebiopsy sample showed NSCLC with rhabdoid differentiation, tissue was subjectively adequate for molecular analysis, but molecular testing was not requested as not clinically indicated.

Genes analysed on at least one occasion were *EGFR*, *ALK*, *KRAS*, *NRAS*, *BRAF*, *DDR2*, *ROS1* and *RET*. Individual PCR-based gene assays were performed including: cobas 480®(Roche) for *EGFR* and *KRAS* mutations, capillary electrophoresis single-strand conformation analysis (CE-SSCA) for *EGFR*, *BRAF* exon 15 mutation and *NRAS* mutations, and direct sequencing for *BRAF* exon 11 and *DDR2* as next generation sequencing (NGS) was not routinely implemented during this period. Fluorescence in situ hybridisation (FISH) was used to detect *ALK* and *ROS1* rearrangements.

One hundred and eighty two genes out of 209 genes were analysed successfully (evaluable), with post-test molecular success rate of 87.1% (Figure 1).

There was significant inter-gene variation in molecular failure rates ($p=0.005$). For instance, *EGFR* analysis was performed in 50 and *ALK* analysis in 40 patients, with molecular failure rates of 4% and 2.5% respectively, while *KRAS* was analysed 41 times with a failure rate of 24.4% ($p=0.04$ and $p=0.04$, respectively). Rates of molecular success and failure by gene are shown in Table 3. The observed inter-gene variation in failure rates is likely due to sequential nature of individual gene tests performed, with less material available for each subsequent analysis.

Reason for molecular analysis failure, where recorded, was always poor sample quality. We explored a possible relationship between number of cores obtained and molecular adequacy and found no significant difference in molecular failure rates between cases where fewer than

3 cores were obtained and those with 3 or more cores ($p=0.185$). There did not appear to be any clear links between incidence of molecular test failure and patient characteristics or technical aspects of rebiopsy.

Twenty four genetic aberrations were identified, including 20 new previously unknown potentially targetable mutations including: activating mutations in *EGFR* in two patients in whom molecular testing had previously failed (one *EGFR* exon 19 deletion and one S768I point mutation); two *EGFR* T790M acquired resistance mutations; one *EGFR* primary resistance mutation (exon 20 deletion). *ALK* rearrangements were identified in 2 patients. 11 patients were found to have a *KRAS* mutation, 1 patient had a *NRAS* Q61L mutation and 1 had a *DDR2* mutation.

Safety

Rate of all complications was 25.7% (17 out of 66 patients). Presence of pneumothorax was assessed in all patients by post-procedure plain chest radiograph or limited post-procedure chest computed tomography (CT) and confirmed in 12/66 cases (18.2%). However, only 2 out of 12 cases required intervention with chest drain insertion (3.0%). Median age of patients suffering a pneumothorax was similar to that of overall study population (63 (range 37-76) versus 67 (37-84)). Rate of ex or current smoking was slightly higher in the pneumothorax group than in the overall population (83.3% vs. 71.2%), but none had a history of significant pulmonary comorbidities compared with 13% in the overall group.

Haemoptysis was reported in 5 out of 66 cases (7.6%), and not recorded in 2 patients. All cases were categorised as mild haemoptysis (<30ml over 24hrs) not requiring further intervention. 2 patients (3.0%) required prolonged hospitalisation post-procedure (>48 hours) for management of pneumothorax requiring chest drain insertion. Three patients required a prolonged admission for unrelated reasons.

109 Post-rebiopsy clinical outcomes

110 We extracted data on post-rebiopsy clinical treatment pathways, to explore the ways in which
111 rebiopsy affected clinical decision-making. This data is summarised in Table 4. In 42 out of 66
112 patients (63.6%), rebiopsy had a direct impact on the choice of subsequent treatment,
113 including 13 (19.7%) who commenced licensed targeted therapies for newly identified
114 somatic mutations (7, 54% in clinical trial setting) or histology-specific chemotherapy. Four
115 patients (6%) were too unwell for further systemic therapy following rebiopsy.

116

DISCUSSION

We report a retrospective study of adequacy of image-guided transthoracic rebiopsies in 66 patients in terms of safety, technical success rates, and adequacy for pathological and molecular analysis.

With 100% technological success rate, 87.1% pathological adequacy and 78.8% molecular adequacy as subjectively assessed by a lung pathologist, we show that image guided lung rebiopsies are feasible and can yield tissue adequate for analysis of multiple biomarkers in the setting of standard clinical practice. We report rates of pneumothorax (18%), chest drain insertion (3%) and mild haemoptysis (8%) which are similar to those previously reported in large series of percutaneous transthoracic biopsies in primary diagnostic setting^{24, 25, 26, 27} and therefore conclude that rebiopsy is not at any increased risk compared to primary biopsies.

We observed a relatively high rate of histological discordance of 23% between rebiopsy material and prior diagnostic biopsies. In cases where histological discrepancy was observed, initial diagnostic biopsies were re-reviewed where available to explore possible causes for the differences. In two cases where squamous cell carcinoma at initial biopsy was reclassified as adenocarcinoma on rebiopsy, and where diagnostic biopsy material was available for review, rebiopsy tumour material showed some features of overlap between adenocarcinoma and squamous cell carcinoma. The discordance between biopsies may therefore reflect sampling of different components of the same tumour with both adenocarcinoma and squamous cell carcinoma features. Another possible explanation for the observed differences may be sampling bias, with patients whose initial samples were inadequate for optimal histological assessment and diagnosis selected for rebiopsy, leading to higher rates of histological discordance in our cohort (e.g. 3 instances of NSCLC NOS were reclassified as squamous cell carcinoma).

Overall 182 of 209 (87.1%) individual gene tests were performed successfully in 51 patients. Molecular success rates varied significantly between individual gene assays. *EGFR* testing

was completed successfully in 48 out of 50 cases (96%), in line with rates reported in several previous studies of adequacy of rebiopsy tissue for *EGFR* testing.^{14, 28, 29, 30, 31} Two prospective studies of rebiopsies in 121³⁰ and 162¹⁴ patients with acquired resistance to *EGFR*-TKIs reported rates of 86% and 95.6% respectively. Another recent prospective study enrolled 24 *EGFR* mutant patients commencing afatinib therapy with a view to rebiopsy for *EGFR* T790M analysis at progression. Out of 23 patients who developed progressive disease, only 14 completed a rebiopsy, with 11 samples (78.6%) sufficient for molecular analysis.³¹

Most studies of rebiopsies have focused on mechanisms of acquired resistance to *EGFR*-TKI and in particular detection of T790M mutation, and few studies have evaluated adequacy for multiple biomarker testing on rebiopsy tissue outside of this context.^{32, 33, 34} Tam et al have reported a retrospective analysis of adequacy of percutaneous transthoracic core needle biopsies for the evaluation of multiple molecular biomarkers within the context of the genotype-directed BATTLE trial.³³ 170 biopsies were performed in 151 NSCLC patients screened for the trial. Specimens of 82.9% of patients were found to have adequate tumour tissue for analysis of 11 different biomarkers within *EGFR*, *KRAS*, *BRAF*, *VEGFR*, *RXR* and *Cyclin D* genes. Pneumothorax and chest tube insertion rates were 15.3% and 9.4%, respectively. In our study, rates of pre-test (87.1%) and post-test molecular adequacy (78.8%) are similar to those reported in the BATTLE trial despite our relatively unselected patient cohort in the setting of standard clinical practice.

The main limitation of this study is that this is a retrospective observational study based on clinical experience of a single oncology centre. As a tertiary referral centre and an institution with well-established infrastructure and experience in this area, our experience may not be representative of the patient profile and resources available in other community-based centres. Secondly, the discrepancy between subjective pathologist assessed pre-test molecular adequacy and post-test molecular success rate has been difficult to explore in absence of complete data on reasons for test failure. Thirdly, incomplete data on technical aspects of each procedure precluded analysis of potential relationship between incidence of

molecular analysis failure and the way procedures were performed, which would help define optimal conditions to obtain adequate tissue samples. Finally, instead of single-gene tests performed in parallel or sequentially, many centres have now moved to implementing NGS-based molecular genotyping,^{35, 36, 37, 38} and so the individual molecular success rate at individual genes may not reflect changes in gene-testing methodologies.

Choice of optimal treatment and development of treatment strategies in NSCLC are predicated by tumour histological and molecular characterisation. Repeated molecular profiling is likely to be required at multiple time points during the treatment pathway, as is already the case for *EGFR T790M* mutation detection,²⁰ given inter-patient and intra-patient molecular heterogeneity identified from sequencing studies,³⁹ and evolutionary pressures of molecular selection from targeted therapy in oncogene-addicted NSCLCs. Nevertheless, in a real world setting, our data has identified the clinical utility and limitations of rebiopsies in advanced NSCLCs, demonstrating a clinically important utility in decision-making and for molecular characterization. Improvements in the histological yield and molecular adequacy of rebiopsies may be achieved by implementation of standardised protocols and algorithms in radiology departments and laboratories to ensure optimal handling of samples for molecular analyses as highlighted in the CAP/IASCL/AMP Guideline.⁴⁰ Use of rapid on-site evaluation (ROSE) of specimens at time of procedure has been shown to improve diagnostic yield, decrease the need for repeat procedures and facilitate collection of sufficient material for molecular testing,⁴¹ although resource considerations are likely to affect wide-spread use of this technique.

Validation of circulating tumour DNA (ctDNA) for genotyping is facilitating a less invasive approach for detection of *EGFR T790M* at point of progression,⁴² but tissue based verification remains an important strategy to identify patients suitable for *EGFR T790M* inhibitors, especially due to the low sensitivity of some ctDNA testing methods. It is also important to verify other resistance mechanism such as histological non-concordance and to stratify patients for other systemic therapies within clinical trials. In our study rebiopsies produced clinically relevant information, helping to guide the choice of treatment in nearly two thirds of

91 patients, through identification of new actionable driver and resistance mutations, change in
92 histological classification, and confirmation or exclusion of recurrent disease.

93

94 Our study provides valuable data on the role and utility of rebiopsy for molecular analysis of
95 multiple molecular markers in a heterogeneous group of NSCLC patients in the setting of
96 standard clinical practice. We validate the pathological and molecular adequacy rates of
97 rebiopsies and demonstrate clinical utility in routine decision making.

1 **Acknowledgments:**

- 2 We acknowledge NHS funding to the Royal Marsden Hospital, RM NIHR-Biomedical
3 Research Centre and the Institute of Cancer Research.

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TABLES

Demographic variable		No. out of 66 (%)
Median Age	67 (IQR 60-71)	
Sex		
Male		35 (53)
Female		31 (47)
Smoking (at time of diagnosis)		
Ex-smoker		35 (53)
Never smoker		18 (27)
Active smoker		12 (18)
Unknown		1 (2)
Pulmonary Comorbidities		
None		57 (86)
COPD		5 (7)
Previous pulmonary TB		2 (3)
Asthma		1 (2)
Emphysema		1 (2)
Other malignancy		
Yes*		4 (6)
No		62 (94)
Histological subtype at time of biopsy		
Adenocarcinoma		45 (68)
Squamous cell carcinoma		14 (21)
Adenosquamous		1 (2)
NSCLC NOS		6 (9)
Stage at diagnosis		
II		6 (9)
III		7 (11)

IV		53 (80)
Previous lines of systemic treatment		
0		16 (24)
1		24 (36)
2		14 (21)
3		7 (11)
4		5 (8)
Mutational status at time of biopsy		
EGFR		
Unknown		37 (56)
EGFR WT		20 (30)
EGFR mutation present		9 (14)
ALK		
Unknown		51 (77)
No rearrangement		14 (21)
Rearrangement present		1 (2)

Table 1. Patient characteristics

*Other malignancies: 3 patients had past history of endometrial cancer (1), breast cancer (1) and basal cell carcinoma lip (1). 1 patient had a concurrent diagnosis of thymoma.

Original histology	n	Rebiopsy histology	Number (%)
Adenocarcinoma	38	Adenocarcinoma	36 (94.8)
		NSCLC NOS	1 (2.6)
		Poorly differentiated TTF-1 negative ca.	1 (2.6)
Squamous cell carcinoma	9	Squamous cell carcinoma	3 (33.3)
		Adenocarcinoma	4 (44.5)
		NSCLC NOS	1 (11.1)
		Pleomorphic ca. rhabdoid subtype	1 (11.1)
NSCLC NOS	4	NSCLC NOS	1 (25.0)
		Squamous cell carcinoma	3 (75.0)
Adenosquamous carcinoma	1	Adenocarcinoma	1 (100)
Total*	52	Concordant	40 (76.9)
		Discordant	12 (23.1)

Table 2. Histological discordance rates

*Total of 52 cases were evaluable for histological concordance. 14 cases were non-evaluable including: 8 cases with no malignant cells in sample (pathological fail), 4 cases sent to research laboratory, 2 cases histological subtype not reported. NOS, not otherwise specified.

Gene	No. analysed	No. failed	Wild type	Mutation/ rearrangement present	Failure rate
EGFR	50	2	39	9	4%
ALK	40	1	37	2	2.5%
KRAS	41	10	20	11	24.4%
BRAF Exon 11	27	6	21	0	22.2%
BRAF Exon 15	40	7	33	0	17.5%
DDR2	5	1	3	1	20%
ROS1	3	0	3	0	0%
RET	2	0	2	0	0%
NRAS	1	0	0	1	0%
TOTAL	209	27	158	24	12.9%

Table 3. Molecular analysis results by gene

Post-Rebiopsy Clinical Outcomes		No. of patients
Potentially actionable genetic mutation identified		20
	Patients started licenced TKI*	6
	Patients entered clinical trial of targeted therapy*	7
	Patients started chemotherapy but potentially eligible for future clinical trial*	4
	Patients too unwell for further systemic therapy	3
Activating mutation confirmed/no acquired resistance mutation		4
	Patients switched to chemotherapy	2
	Patients switched to second generation TKI	1
	Patients too unwell for systemic therapy	1
Mandatory biopsy within research protocol – patients entered clinical trial*		6
Histological discordance identified – new treatment paradigm*		4
Histological confirmation of NSCLC recurrence*		12
	Patients started palliative treatment*	10
	Patients started radical treatment*	2
NSCLC recurrence ruled out – patients continued surveillance*		3
Pathological or molecular failure		13
No actionable mutations identified		4
Total		66

Table 4. Rebiopsy outcomes and post-biopsy patient pathways.

*indicates patients in whom rebiopsy informed subsequent choice of treatment.

FIGURE LEGEND

Figure 1. Consort diagram.

